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ABOUT COVER

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AIMS AND SCOPE

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EDITORIAL

Balancing act: The dilemma of rapid hyperglycemia correction in diabetes management

Ke-Xin Zhang, Cheng-Xia Kan, Xiao-Dong Sun

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Abstract

The global diabetes surge poses a critical public health challenge, emphasizing the need for effective glycemic control. However, rapid correction of chronic hyperglycemia can unexpectedly trigger microvascular complications, necessitating a reevaluation of the speed and intensity of glycemic correction. Theories suggest swift blood sugar reductions may cause inflammation, oxidative stress, and neurovascular changes, resulting in complications. Healthcare providers should cautiously approach aggressive glycemic control, especially in long-standing, poorly controlled diabetes. Preventing and managing these complications requires a personalized, comprehensive approach with education, monitoring, and interdisciplinary care. Diabetes management must balance short and long-term goals, prioritizing overall well-being. This editorial underscores the need for a personalized, nuanced approach, focusing on equilibrium between glycemic control and avoiding overcorrection.

Key Words: Diabetes; Hyperglycemia correction; Management; Microvascular complications; Glucose control

Core Tip: Rapid glycemia corrections may unexpectedly lead to microvascular complications in diabetes. Balancing glycemic control is crucial in diabetes management. Prioritizing an individualized, comprehensive care approach is essential to ensure long-term well-being.

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INTRODUCTION

The global increase in diabetes prevalence poses an ongoing challenge to public health[1,2]. Despite the well-demonstrated benefits of maintaining blood glucose levels close to normal in preventing or slowing the development of diabetes-related complications, a significant portion of those affected by diabetes struggle to reach their glycemic target goals[3,4]. A recent case report by Huret *et al*[5] discussed a 25-year-old woman who has lived with type 1 diabetes since the age of 9. Initially, her diabetes was unstable but without complications. During an unplanned pregnancy, her hyperglycemia was intensively managed. However, its consequences became evident over the subsequent two years as the patient developed a cascade of microvascular complications, including Charcot neuroarthropathy, proliferative diabetic retinopathy, gastroparesis, bladder voiding disorders, and end-stage renal failure requiring hemodialysis.

This case highlights an infrequently discussed issue in diabetes management: The ramifications of aggressive hyperglycemia correction. While preventing complications and maintaining glycemic control is crucial, the rate and intensity of correction, particularly for patients with a history of chronic hyperglycemia, demand equal consideration. This case highlights the complexity of diabetes management. Patients must navigate between preventing complications and avoiding the perils of overcorrection, which paradoxically leads to a cascade of microvascular complications.

Diabetes management is a multifaceted challenge affecting millions worldwide[6,7]. Prolonged hyperglycemia is closely associated with the development of numerous diabetes-related complications, such as cardiovascular disease, retinopathy, neuropathy, and nephropathy[8,9]. These complications represent the darker aspects of diabetes, impacting both the individual's well-being and healthcare resources. The primary goal is to correct and control chronic hyperglycemia, essential for individuals with diabetes. Naturally, healthcare providers and patients aim for tight glycemic control to reduce complications. However, a paradoxical situation may arise when attempting to correct hyperglycemia too rapidly and intensively. What if this pursuit takes an unexpected turn, yielding paradoxical outcomes? This case reveals a perplexing scenario where rapid correction of chronic hyperglycemia unexpectedly leads to the emergence of microvascular complications.

Microvascular complications following rapid glycemic correction in diabetes are complex and not fully understood [10]. Several theories shed light on this phenomenon. Swift reductions in blood sugar levels can lead to hypoglycemia, potentially damaging small blood vessels and nerves while triggering the release of stress hormones, inflammation, and oxidative stress[11-13]. This neurovascular theory suggests that rapid improvements in blood glucose levels affect the autonomic nervous system, increasing blood flow to extremities, leading to localized inflammation and vascular changes contributing to neuroarthropathy[13,14]. Diabetic neuropathy, commonly affecting the feet and reducing protective sensation and proprioception, raises the risk of unnoticed injury or trauma, especially when exacerbated by rapid glycemic correction. In addition, reperfusion injury can occur when high blood sugar levels are rapidly corrected, causing a sudden increase in blood flow to previously poorly perfused tissues, potentially leading to vascular hyperpermeability [15,16].

It is important to note that the relationship between rapid glycemic correction and these complications is not fully understood, and not all individuals with diabetes who experience rapid improvements in blood glucose control will develop these complications. However, healthcare providers should exercise caution when implementing aggressive glycemic control regimens, particularly in individuals with longstanding poorly controlled diabetes or during the perioperative period[16,17].

Preventing and managing these complications involves a comprehensive approach that includes careful glycemic control, regular medical check-ups, and addressing other risk factors like hypertension, hyperlipidemia, and smoking[18,19]. Diabetes care should be individualized, recognizing the unique needs of each patient[20]. Regular monitoring of blood glucose levels and overall health is essential to make timely adjustments to the management plan while avoiding abrupt corrections[18]. Patient education is crucial to help patients understand the potential consequences of rapid hyperglycemia correction and actively engage in their care. A collaborative approach involving endocrinologists, nutritionists, diabetes educators, and mental health professionals is necessary to provide comprehensive care. Diabetes management should consider both immediate and long-term goals, striking a balance between short-term and long-term objectives, given the lifelong nature of the condition[21,22].

CONCLUSION

Therefore, diabetes management is an ongoing process, and this case highlights the complexity of diabetes management. Pursuing rapid correction of hyperglycemia, while crucial, may lead to unexpected consequences. A balanced and personalized approach, including patient education, interdisciplinary care, and long-term considerations, is the key to effective diabetes control. Diabetes management is, in fact, a delicate equilibrium between glycemic control and avoiding overcorrection.

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REFERENCES

1 **GBD 2021 Diabetes Collaborators**. Global, regional, and national burden of diabetes from 1990 to 2021, with projections of prevalence to 2050: a systematic analysis for the Global Burden of Disease Study 2021. *Lancet* 2023; **402**: 203-234 [PMID: 37356446 DOI: 10.1016/S0140-6736(23)01301-6]
2 **Zhang K**, Kan C, Han F, Zhang J, Ding C, Guo Z, Huang N, Zhang Y, Hou N, Sun X. Global, Regional, and National Epidemiology of Diabetes in Children From 1990 to 2019. *JAMA Pediatr* 2023; **177**: 837-846 [PMID: 37399036 DOI: 10.1001/jamapediatrics.2023.2029]
3 **van Dieren S**, Beulens JW, van der Schouw YT, Grobbee DE, Neal B. The global burden of diabetes and its complications: an emerging pandemic. *Eur J Cardiovasc Prev Rehabil* 2010; **17** Suppl 1: S3-S8 [PMID: 20489418 DOI: 10.1097/01.hjr.0000368191.86614.5a]
4 Evidence review for blood glucose control management: Perioperative care in adults: Evidence review K. London: National Institute for Health and Care Excellence (NICE); 2020 Aug- [PMID: 32931169]
5 **Huret P**, Lopes P, Dardari R, Penfornis A, Thomas C, Dardari D. Rapid correction of hyperglycemia: A necessity but at what price? A brief report of a patient living with type 1 diabetes. *World J Diabetes* 2023; **14**: 1710-1716 [PMID: 38077801 DOI: 10.4239/wjd.v14.i11.1710]
6 **Yari Z**, Behrouz V, Zand H, Pourvali K. New Insight into Diabetes Management: From Glycemic Index to Dietary Insulin Index. *Curr Diabetes Rev* 2020; **16**: 293-300 [PMID: 31203801 DOI: 10.2174/1573399815666190614122626]
7 **Lee SH**. The Growing Challenge of Diabetes Management in an Aging Society. *Diabetes Metab J* 2023; **47**: 630-631 [PMID: 37793980 DOI: 10.4093/dmj.2023.0279]
8 **Zhou R**, Cui Y, Zhang Y, De J, An X, Duan Y, Kang X, Lian F. The Long-Term Effects of Non-Pharmacological Interventions on Diabetes and Chronic Complication Outcomes in Patients With Hyperglycemia: A Systematic Review and Meta-Analysis. *Front Endocrinol (Lausanne)* 2022; **13**: 838224 [PMID: 35370954 DOI: 10.3389/fendo.2022.838224]
9 **Zheng Y**, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nat Rev Endocrinol* 2018; **14**: 88-98 [PMID: 29219149 DOI: 10.1038/nrendo.2017.151]
10 **Gibbons CH**, Goebel-Fabbri A. Microvascular Complications Associated With Rapid Improvements in Glycemic Control in Diabetes. *Curr Diab Rep* 2017; **17**: 48 [PMID: 28526993 DOI: 10.1007/s11892-017-0880-5]
11 **Jain E**, Kotwal S, Gnanaraj J, Khaliq W. Osmotic Demyelination After Rapid Correction of Hyperosmolar Hyperglycemia. *Cureus* 2023; **15**: e34551 [PMID: 36874309 DOI: 10.7759/cureus.34551]
12 **Gibbons CH**. Treatment induced neuropathy of diabetes-Long term implications in type 1 diabetes. *J Diabetes Complications* 2017; **31**: 715-720 [PMID: 28159476 DOI: 10.1016/j.jdiacomp.2017.01.010]
13 **Boavida L**, Carvalho J, Oliveira S, Delgado Alves J. Muscle Infarction Following Rapid Glycemic Control in a Patient With Diabetes-Associated Microvascular Disease. *Cureus* 2021; **13**: e17182 [PMID: 34540416 DOI: 10.7759/cureus.17182]
14 **Hyun U**, Sohn JW. Autonomic control of energy balance and glucose homeostasis. *Exp Mol Med* 2022; **54**: 370-376 [PMID: 35474336 DOI: 10.1038/s12276-021-00705-9]
15 **Hjelm LR**. Diabetes Mellitus: An Overview in Relationship to Charcot Neuroarthropathy. *Clin Podiatr Med Surg* 2022; **39**: 535-542 [PMID: 36180186 DOI: 10.1016/j.cpm.2022.05.001]
16 **Suto C**, Hori S, Kato S, Muraoka K, Kitano S. Effect of perioperative glycemic control in progression of diabetic retinopathy and maculopathy. *Arch Ophthalmol* 2006; **124**: 38-45 [PMID: 16401783 DOI: 10.1001/archopht.124.1.38]
17 **Suto C**, Hori S. Rapid preoperative glycemic correction to prevent progression of retinopathy after phacoemulsification in diabetic patients with poor glycemic control. *J Cataract Refract Surg* 2003; **29**: 2034-2035 [PMID: 14604734 DOI: 10.1016/j.jcrs.2003.08.003]
18 **Skoufalos A**, Thomas R, Patel R, Mei C, Clarke JL. Continuous Glucose Monitoring: An Opportunity for Population-Based Diabetes Management. *Popul Health Manag* 2022; **25**: 583-591 [PMID: 36154298 DOI: 10.1089/pop.2022.0196]
19 **Zhai Z**, Yang Y, Lin G, Lin W, Wu J, Liu X, Zhang S, Zhou Q, Liu H, Hao G. The hypertension and hyperlipidemia status among type 2 diabetic patients in the community and influencing factors analysis of glycemic control. *Diabetol Metab Syndr* 2023; **15**: 73 [PMID: 37046317 DOI: 10.1186/s13098-023-01013-0]
20 **Kalra S**, Bantwal G, Sahay RK, Bhattacharya S, Baruah MP, Sheikh S, Lathia T. Incorporating Integrated Personalised Diabetes Management (iPDM) in Treatment Strategy: A Pragmatic Approach. *Indian J Endocrinol Metab* 2022; **26**: 106-110 [PMID: 35873934 DOI: 10.4103/ijem.ijem_478_21]

21 **Nam S**, Chesla C, Stotts NA, Kroon L, Janson SL. Barriers to diabetes management: patient and provider factors. *Diabetes Res Clin Pract* 2011; **93**: 1-9 [PMID: 21382643 DOI: 10.1016/j.diabres.2011.02.002]

22 **Ahmed S**, Styers JP. Inpatient Diabetes Management. *Prim Care* 2022; **49**: 339-349 [PMID: 35595487 DOI: 10.1016/j.pop.2021.11.006]

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EDITORIAL

Glucagon-like peptide-1 receptor agonists as a possible intervention to delay the onset of type 1 diabetes: A new horizon

Mahmoud Nassar, Ajay Chaudhuri, Husam Ghanim, Paresh Dandona

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Abstract

Type 1 diabetes (T1D) is a chronic autoimmune condition that destroys insulin-producing beta cells in the pancreas, leading to insulin deficiency and hyperglycemia. The management of T1D primarily focuses on exogenous insulin replacement to control blood glucose levels. However, this approach does not address the underlying autoimmune process or prevent the progressive loss of beta cells. Recent research has explored the potential of glucagon-like peptide-1 receptor agonists (GLP-1RAs) as a novel intervention to modify the disease course and delay the onset of T1D. GLP-1RAs are medications initially developed for treating type 2 diabetes. They exert their effects by enhancing glucose-dependent insulin secretion, suppressing glucagon secretion, and slowing gastric emptying. Emerging evidence suggests that GLP-1RAs may also benefit the treatment of newly diagnosed patients with T1D. This article aims to highlight the potential of GLP-1RAs as an intervention to delay the onset of T1D, possibly through their potential immunomodulatory and anti-inflammatory effects and preservation of beta-cells. This article aims to explore the potential of shifting the paradigm of T1D management from reactive insulin replacement to proactive disease modification, which should open new avenues for preventing and treating T1D, improving the quality of life and long-term outcomes for individuals at risk of T1D.

Key Words: Type 1 diabetes; Semaglutide; Glucagon-like peptide-1 receptor agonists; Insulin therapy; Autoimmune response; Blood glucose monitoring; B-cell preservation; Early screening; Teplizumab; Randomized controlled trials

Core Tip: New research suggests a novel approach to treating type 1 diabetes (T1D) by using glucagon-like peptide-1 receptor agonists, specifically semaglutide, to significantly improve blood glucose control and potentially slow the progression of the disease in newly diagnosed patients. This strategy, which leads to less insulin dependence and better metabolic markers, could change the way T1D is managed in a big way. At the same time, the study supports early T1D risk screening, especially in groups with high risk, so that early interventions can be made, evaluating the benefits against the possible emotional and financial effects. This dual approach shows that there are bright futures for improving the lives of patients with T1D.

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INTRODUCTION

Type 1 diabetes (T1D) is a chronic disease that has long posed therapeutic challenges. This ailment, rooted in the autoimmune destruction of pancreatic β-cells by T-cells, results in a severe decline of β-cell activity and an eventual complete lack of insulin[1-3]. The only treatment for this disease is intensive insulin therapy, which requires multiple daily injections or continuous subcutaneous insulin infusion with frequent monitoring of blood glucose. Despite advances in closed-loop hybrid pumps and continuous glucose monitoring devices, 75% of subjects with T1D maintain an A1c above 7%. Moreover, there is a significant disease burden and emotional burden associated with the diagnosis and management of T1D. Even with modern medical breakthroughs, many T1D sufferers still grapple with maintaining optimal blood sugar levels. Intensive insulin therapies, though advantageous, can sometimes lead to hypoglycemia, presenting a therapeutic conundrum[4,5].

THE POTENTIAL OF GLUCAGON-LIKE PEPTIDE-1 RECEPTOR AGONISTS

Researchers observed promising results in a study examining the potential benefits of Glucagon-like peptide-1 receptor agonists (GLP-1RAs) for T1D patients with positive C-peptide levels. Our recent exploration, as published in the New England Journal of Medicine, sheds light on a hopeful path. We delved into the impact of semaglutide, a GLP-1RA, within three months on ten newly diagnosed T1D patients. These individuals began with an average glycated hemoglobin of 11.7% ± 2.1% and a fasting C-peptide of 0.65% ± 0.33% ng/mL, all undergoing standard insulin treatments [6]. Introducing semaglutide and dietary modifications led to the discontinuation of prandial insulin for all participants within a quarter year. Impressively, by half a year, seven had ceased using basal insulin. A year later, the average glycated hemoglobin decreased to 5.7% ± 0.4%, while the fasting C-peptide surged to an average of 1.05 ± 0.40 ng/mL. Continuous glucose assessments revealed an 89% ± 3% time-in-range[6].

The study entailed a retrospective analysis of 11 normal-weight T1D patients treated with GLP-1RA in conjunction with insulin. Notable findings included a significant reduction in HbA1c levels from 10.74% ± 0.96% to 7.4% ± 0.58% after 12 ± 1 wk of GLP-1RA therapy. Additionally, there was a noteworthy decline in total insulin dose by 64% and a minor weight reduction. Importantly, C-peptide concentrations, indicative of endogenous insulin production, surged significantly, enhancing pancreatic beta-cell function. Remarkably, 50% of the study participants achieved freedom from insulin therapy while on GLP-1RA therapy over the study duration[7].

In the Adjunct One Treat-To-Target Randomized Trial, the addition of liraglutide to insulin therapy in T1D was assessed over 52 wk in 1398 adults. Participants were administered liraglutide (at concentrations of 1.8, 1.2, or 0.6 mg) or a placebo in conjunction with insulin. The study found that HbA1c levels reduced by 0.34%–0.54% from an initial 8.2%, insulin doses diminished more with liraglutide compared to the placebo, and there was a notable weight reduction in the liraglutide cohorts. However, liraglutide recipients experienced elevated rates of symptomatic hypoglycemia, and the 1.8 mg liraglutide group saw a significant rise in hyperglycemia with ketosis. Consequently, despite its benefits, the increased adverse events suggest caution in the broader clinical application of liraglutide for T1D[8].

IMMUNOTHERAPY: A SPECTRUM OF OUTCOMES

Various immunotherapies, including Teplizumab, Otelixizumab, and Abatacept, have displayed promise but are not without complications. For example, Otelixizumab users have reported headaches, fevers, and rashes, typical reactions to anti-CD3 antibodies[9,10]. Teplizumab has been linked to skin issues, leukopenia, respiratory infections, and lymphopenia[11-13]. Most issues with Abatacept were related to the infusion process[14,15].

THE DEBATE ON SCREENING

The question of T1D risk screening remains contentious, especially for those without familial ties to the condition. A study by Ziegler *et al*[5] in Bavaria showcased the viability of screening children during standard pediatric appointments, pinpointing 280 children with multiple autoantibodies, 43 of whom later developed T1D[5,16]. The means of early identification and action are clear. Yet, the financial and emotional tolls of screening warrant consideration. Nevertheless, research indicates that psychosocial screenings can pinpoint vulnerable families[17]. Moreover, regions with a high prevalence of diabetic ketoacidosis could economically justify presymptomatic T1D screenings[18,19]. The timing and approach to screening are debated, focusing on the balance between cost and comprehensive detection[18,20,21].

CONCLUSION

Our findings suggest that early T1D screening, combined with interventions such as GLP-1RA, could significantly impede the progression of the disease, especially in high-risk obese individuals. Pediatric professionals should exercise heightened caution with patients prone to T1D due to genetic or autoimmune factors. As we venture further into this realm, the prospect of an enhanced quality of life for T1D patients becomes increasingly tangible.

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REFERENCES

1 **Araujo DB**, Dantas JR, Silva KR, Souto DL, Pereira MFC, Moreira JP, Luiz RR, Claudio-Da-Silva CS, Gabbay MAL, Dib SA, Couri CEB, Maiolino A, Rebelatto CLK, Daga DR, Senegaglia AC, Brofman PRS, Baptista LS, Oliveira JEP, Zajdenverg L, Rodacki M. Allogenic Adipose Tissue-Derived Stromal/Stem Cells and Vitamin D Supplementation in Patients With Recent-Onset Type 1 Diabetes Mellitus: A 3-Month Follow-Up Pilot Study. *Front Immunol* 2020; **11**: 993 [PMID: 32582156 DOI: 10.3389/fimmu.2020.00993]

2 **Curtin F**, Champion B, Davoren P, Duke S, Ekinci EI, Gilfillan C, Morbey C, Nathow T, O'Moore-Sullivan T, O'Neal D, Roberts A, Stranks S, Stuckey B, Vora P, Malpass S, Lloyd D, Maëstracci-Beard N, Buffet B, Kornmann G, Bernard C, Porchet H, Simpson R. A safety and pharmacodynamics study of temelimab, an antipathogenic human endogenous retrovirus type W envelope monoclonal antibody, in patients with type 1 diabetes. *Diabetes Obes Metab* 2020; **22**: 1111-1121 [PMID: 32077207 DOI: 10.1111/dom.14010]

3 **Ehlers MR**. Strategies for clinical trials in type 1 diabetes. *J Autoimmun* 2016; **71**: 88-96 [PMID: 27068279 DOI: 10.1016/j.jaut.2016.03.008]

4 **Cai J**, Wu Z, Xu X, Liao L, Chen J, Huang L, Wu W, Luo F, Wu C, Pugliese A, Pileggi A, Ricordi C, Tan J. Umbilical Cord Mesenchymal Stromal Cell With Autologous Bone Marrow Cell Transplantation in Established Type 1 Diabetes: A Pilot Randomized Controlled Open-Label Clinical Study to Assess Safety and Impact on Insulin Secretion. *Diabetes Care* 2016; **39**: 149-157 [PMID: 26628416 DOI: 10.2337/dc15-0171]

5 **Ziegler AG**, Kick K, Bonifacio E, Haupt F, Hippich M, Dunstheimer D, Lang M, Laub O, Warncke K, Lange K, Assfalg R, Jolink M, Winkler C, Achenbach P; Fr1da Study Group. Yield of a Public Health Screening of Children for Islet Autoantibodies in Bavaria, Germany. *JAMA* 2020; **323**: 339-351 [PMID: 31990315 DOI: 10.1001/jama.2019.21565]

6 **Dandona P**, Chaudhuri A, Ghanim H. Semaglutide in Early Type 1 Diabetes. *N Engl J Med* 2023; **389**: 958-959 [PMID: 37672701 DOI: 10.1056/NEJMc2302677]

7 **Kuhadiya ND**, Prohaska B, Ghanim H, Dandona P. Addition of glucagon-like peptide-1 receptor agonist therapy to insulin in C-peptide-positive patients with type 1 diabetes. *Diabetes Obes Metab* 2019; **21**: 1054-1057 [PMID: 30536789 DOI: 10.1111/dom.13609]

8 **Mathieu C**, Zinman B, Hemmingsson JU, Woo V, Colman P, Christiansen E, Linder M, Bode B; Adjunct One Investigators. Efficacy and Safety of Liraglutide Added to Insulin Treatment in Type 1 Diabetes: The Adjunct One Treat-To-Target Randomized Trial. *Diabetes Care* 2016; **39**: 1702-1710 [PMID: 27506222 DOI: 10.2337/dc16-0691]

9 **Aronson R**, Gottlieb PA, Christiansen JS, Donner TW, Bosi E, Bode BW, Pozzilli P; Defend Investigator Group. Low-dose otelixizumab anti-

CD3 monoclonal antibody Defend-1 study: results of the randomized phase III study in recent-onset human type 1 diabetes. *Diabetes Care* 2014; **37**: 2746-2754 [PMID: 25011949 DOI: 10.2337/dc13-0327]

10 **Keymeulen B**, van Maurik A, Inman D, Oliveira J, McLaughlin R, Gittelman RM, Roep BO, Gillard P, Hilbrands R, Gorus F, Mathieu C, Van de Velde U, Wisniacki N, Napolitano A. A randomised, single-blind, placebo-controlled, dose-finding safety and tolerability study of the anti-CD3 monoclonal antibody otelixizumab in new-onset type 1 diabetes. *Diabetologia* 2021; **64**: 313-324 [PMID: 33145642 DOI: 10.1007/s00125-020-05317-y]

11 **Hagopian W**, Ferry RJ Jr, Sherry N, Carlin D, Bonvini E, Johnson S, Stein KE, Koenig S, Daifotis AG, Herold KC, Ludvigsson J; Protégé Trial Investigators. Teplizumab preserves C-peptide in recent-onset type 1 diabetes: two-year results from the randomized, placebo-controlled Protégé trial. *Diabetes* 2013; **62**: 3901-3908 [PMID: 23801579 DOI: 10.2337/db13-0236]

12 **Herold KC**, Gitelman SE, Willi SM, Gottlieb PA, Waldron-Lynch F, Devine L, Sherr J, Rosenthal SM, Adi S, Jalaludin MY, Michels AW, Dziura J, Bluestone JA. Teplizumab treatment may improve C-peptide responses in participants with type 1 diabetes after the new-onset period: a randomised controlled trial. *Diabetologia* 2013; **56**: 391-400 [PMID: 23086558 DOI: 10.1007/s00125-012-2753-4]

13 **Herold KC**, Bundy BN, Long SA, Bluestone JA, DiMeglio LA, Dufort MJ, Gitelman SE, Gottlieb PA, Krischer JP, Linsley PS, Marks JB, Moore W, Moran A, Rodriguez H, Russell WE, Schatz D, Skyler JS, Tsalikian E, Wherrett DK, Ziegler AG, Greenbaum CJ; Type 1 Diabetes TrialNet Study Group. An Anti-CD3 Antibody, Teplizumab, in Relatives at Risk for Type 1 Diabetes. *N Engl J Med* 2019; **381**: 603-613 [PMID: 31180194 DOI: 10.1056/NEJMoa1902226]

14 **Orban T**, Bundy B, Becker DJ, DiMeglio LA, Gitelman SE, Goland R, Gottlieb PA, Greenbaum CJ, Marks JB, Monzavi R, Moran A, Raskin P, Rodriguez H, Russell WE, Schatz D, Wherrett D, Wilson DM, Krischer JP, Skyler JS; Type 1 Diabetes TrialNet Abatacept Study Group. Co-stimulation modulation with abatacept in patients with recent-onset type 1 diabetes: a randomised, double-blind, placebo-controlled trial. *Lancet* 2011; **378**: 412-419 [PMID: 21719096 DOI: 10.1016/S0140-6736(11)60886-6]

15 **Russell WE**, Bundy BN, Anderson MS, Cooney LA, Gitelman SE, Goland RS, Gottlieb PA, Greenbaum CJ, Haller MJ, Krischer JP, Libman IM, Linsley PS, Long SA, Lord SM, Moore DJ, Moore WV, Moran AM, Muir AB, Raskin P, Skyler JS, Wentworth JM, Wherrett DK, Wilson DM, Ziegler AG, Herold KC; Type 1 Diabetes TrialNet Study Group. Abatacept for Delay of Type 1 Diabetes Progression in Stage 1 Relatives at Risk: A Randomized, Double-Masked, Controlled Trial. *Diabetes Care* 2023; **46**: 1005-1013 [PMID: 36920087 DOI: 10.2337/dc22-2200]

16 **Greenbaum CJ**. A Key to T1D Prevention: Screening and Monitoring Relatives as Part of Clinical Care. *Diabetes* 2021; **70**: 1029-1037 [PMID: 33931405 DOI: 10.2337/db20-1112]

17 **Schwartz DD**, Cline VD, Axelrad ME, Anderson BJ. Feasibility, acceptability, and predictive validity of a psychosocial screening program for children and youth newly diagnosed with type 1 diabetes. *Diabetes Care* 2011; **34**: 326-331 [PMID: 21216856 DOI: 10.2337/dc10-1553]

18 **McQueen RB**, Geno Rasmussen C, Waugh K, Frohnert BI, Steck AK, Yu L, Baxter J, Rewers M. Cost and Cost-effectiveness of Large-scale Screening for Type 1 Diabetes in Colorado. *Diabetes Care* 2020; **43**: 1496-1503 [PMID: 32327420 DOI: 10.2337/dc19-2003]

19 **Sims EK**, Besser REJ, Dayan C, Geno Rasmussen C, Greenbaum C, Griffin KJ, Hagopian W, Knip M, Long AE, Martin F, Mathieu C, Rewers M, Steck AK, Wentworth JM, Rich SS, Kordonouri O, Ziegler AG, Herold KC; NIDDK Type 1 Diabetes TrialNet Study Group. Screening for Type 1 Diabetes in the General Population: A Status Report and Perspective. *Diabetes* 2022; **71**: 610-623 [PMID: 35316839 DOI: 10.2337/dbi20-0054]

20 **Chmiel R**, Giannopoulou EZ, Winkler C, Achenbach P, Ziegler AG, Bonifacio E. Progression from single to multiple islet autoantibodies often occurs soon after seroconversion: implications for early screening. *Diabetologia* 2015; **58**: 411-413 [PMID: 25409656 DOI: 10.1007/s00125-014-3443-1]

21 **Parikka V**, Näntö-Salonen K, Saarinen M, Simell T, Ilonen J, Hyöty H, Veijola R, Knip M, Simell O. Early seroconversion and rapidly increasing autoantibody concentrations predict prepubertal manifestation of type 1 diabetes in children at genetic risk. *Diabetologia* 2012; **55**: 1926-1936 [PMID: 22441569 DOI: 10.1007/s00125-012-2523-3]

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EDITORIAL

Elucidating the cardioprotective mechanisms of sodium-glucose cotransporter-2 inhibitors beyond glycemic control

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Abstract

Sodium-glucose cotransporter-2 (SGLT2) inhibitors have emerged as a pivotal intervention in diabetes management, offering significant cardiovascular benefits. Empagliflozin, in particular, has demonstrated cardioprotective effects beyond its glucose-lowering action, reducing heart failure hospitalizations and improving cardiac function. Of note, the cardioprotective mechanisms appear to be independent of glucose lowering, possibly mediated through several mechanisms involving shifts in cardiac metabolism and anti-fibrotic, anti-inflammatory, and anti-oxidative pathways. This editorial summarizes the multifaceted cardiovascular advantages of SGLT2 inhibitors, highlighting the need for further research to elucidate their full therapeutic potential in cardiac care.

Key Words: Diabetes; Sodium-glucose cotransporter-2; Cardiovascular diseases; Empagliflozin

Core Tip: Sodium-glucose cotransporter-2 inhibitors like empagliflozin offer cardioprotective benefits that extend beyond blood glucose control, improving heart function and reducing failure-related hospitalizations. Ongoing research is essential to elucidate the underlying mechanisms, potentially revolutionizing heart failure treatment across various patient profiles.

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INTRODUCTION

The global increase in diabetes represents a significant public health challenge and is closely associated with an increased risk for cardiovascular diseases (CVD)[1]. The lack of specific treatments to prevent its progression has left a significant gap in therapeutic strategies. Consequently, there is an urgent need for novel approaches to prevent and manage diabetes-related cardiac complications. Sodium-glucose cotransporter-2 (SGLT2) inhibitors (*e.g.*, empagliflozin), primarily known for their glucose-lowering capability, have emerged as unexpected protective agents against CVD in patients with diabetes. SGLT2 inhibitors may have beneficial effects on heart failure, including cases with dilated cardiomyopathy, by improving cardiac function and reducing hospitalization rates for heart failure[2]. However, the unresolved cardioprotective mechanisms of these inhibitors have stimulated considerable scientific interest. The study by Li *et al*[3] provides an interesting insight into the molecular dynamics through which empagliflozin may exert its therapeutic effects on the diabetic heart.

Clinical trials have demonstrated that SGLT2 inhibitors significantly reduce the risk of hospitalization for heart failure and cardiovascular mortality. Notably, the DAPA-HF and EMPEROR-Reduced trials highlighted the positive effects of SGLT2 inhibition in patients with heart failure with a reduced ejection fraction, including those with and without diabetes[4-10]. A comprehensive meta-analysis further reinforced these findings, indicating that SGLT2 inhibitors decrease the risk of cardiovascular mortality or first hospitalization for heart failure across a broad spectrum of left ventricular ejection fractions[2,4]. Additionally, a meta-analysis involving over 21000 participants revealed consistent reductions in the risk of composite cardiovascular mortality or hospitalization for heart failure, as well as all-cause mortality[11]. Evidence from clinical studies also indicated that SGLT2 inhibitors can improve diastolic function, particularly in heart failure with a preserved ejection fraction, a condition commonly observed in diabetic heart disease[12].

Animal studies have similarly provided evidence to support the cardioprotective role of SGLT2 inhibitors. A meta-analysis of preclinical animal models found that SGLT2 inhibitors reduced myocardial infarct size independent of diabetes, indicating a potential for broad cardioprotective applications beyond glucose-lowering effects[13]. Our studies demonstrated that empagliflozin could also alleviate obesity-related cardiac dysfunction and attenuate ischemia/reperfusion injury[14,15]. These studies provided evidence that SGLT2 inhibitors could benefit a wide population of heart failure patients, not just those with a reduced ejection fraction.

On the basis of the experimental data provided by Li *et al*[3], empagliflozin treatment displays therapeutic potential in mitigating diabetic cardiomyopathy in db/db mice. The treatment improved cardiac function, reduces myocardial apoptosis, and beneficially modulates signaling pathways associated with cardiac health, such as increased adenosine monophosphate-activated protein kinase (AMPK)/peroxisome proliferator-activated receptor gamma coactivator-1alpha (PGC-1) protein phosphorylation and decreased myosin phosphatase target subunit 1 phosphorylation. Furthermore, *in vitro* studies supported these findings, demonstrating that empagliflozin protects cardiomyocytes from high-glucose-induced mitochondrial damage, oxidative stress, and apoptosis, effects that were partly reversed by the addition of compound C, an AMPK inhibitor. The results were corroborated by the use of Rho kinase inhibitors and PGC-1α overexpression, which further validates the role of these pathways in cardiac protection. Interestingly, no SGLT2 protein expression was detected in cardiomyocytes, suggesting that the cardioprotective effects of empagliflozin may be independent of its glucose-lowering action and possibly mediated by AMPK/PGC-1α pathways. This indicates a potential non-glycemic beneficial effect of SGLT2 inhibitors on cardiac function in the context of diabetes, meriting further investigation. This study highlights novel mechanisms regarding the effectiveness of SGLT2 inhibitors in treating diabetic cardiomyopathy.

SGLT2 inhibitors, beyond their role in glucose excretion, confer cardiac protection through several mechanisms[16,17] (Figure 1). Primarily, they act as mild diuretics, which reduce cardiac preload and afterload by promoting natriuresis and osmotic diuresis, thereby lessening the cardiac load[18]. They also beneficially shift cardiac metabolism away from fatty acid oxidation, which is less oxygen-efficient, towards glucose utilization and potentially towards ketone body utilization, thus improving the heart's energy efficiency[19]. These drugs may also protect against cardiac fibrosis by several means. They reduce hyperglycemia-related advanced glycation end-products, downregulate transforming growth factor-beta, and inhibit the cardiac sodium-hydrogen exchanger, which together help to prevent hypertrophy and fibrosis [20,21].

Moreover, SGLT2 inhibitors contribute to reducing arrhythmia risks and modulate ion homeostasis within the heart, suggesting a role in improving myocardial cell function and calcium handling[22]. Their cardioprotective effects extend to anti-inflammatory and antioxidant actions, because they diminish nuclear factor-kappaB activity and enhance antioxidant system activity (*e.g.*, Sestrin2, nuclear factor erythroid 2-related factor 2, heme oxygenase-1)[14,23]. This contributes to decreasing oxidative stress, another risk factor for heart failure. In addition, these drugs improve endothelial function and arterial compliance, partly through increased nitric oxide production, and affect the secretion of adipokines, which are involved in the pathophysiology of heart failure[24-26]. This endothelial protection was confirmed by studies showing that empagliflozin suppresses endothelial apoptosis and maintains capillarization through the protein kinase B/endothelial nitric oxide synthase/nitric oxide pathway, thereby enhancing heart performance[27]. Cai *et al*[28] further

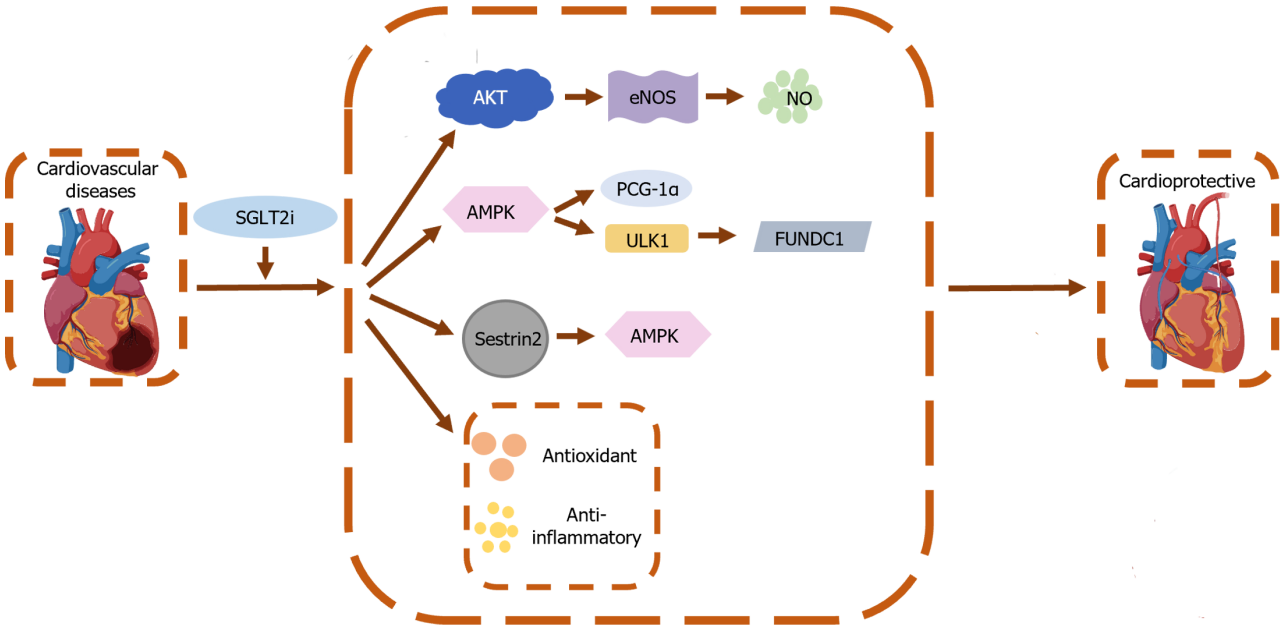

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Figure 1 The cardioprotective mechanisms of sodium-glucose cotransporter-2 inhibitors beyond glycemic control. SGLT2i: Sodium-glucose cotransporter-2 inhibitor; AMPK: Adenosine monophosphate-activated protein kinase; Akt: Protein kinase B; eNOS: Endothelial nitric oxide synthase; NO: Nitric oxide; PGC-1α: Peroxisome proliferator-activated receptor gamma coactivator-1alpha; ULK1: Unc-51 like autophagy activating kinase 1; FUNDC1: FUN14 domain containing 1.

demonstrated that empagliflozin mitigates endothelial oxidative stress and inhibits mitochondrial apoptosis *via* the AMPK/unc-51 like autophagy activating kinase 1/FUN14 domain containing 1/mitophagy axis, thereby improving cardiac microvascular structure and endothelial function. SGLT2 inhibitors also induce protective autophagy and reduce apoptosis in cardiac cells, and they are being investigated for their potential effects on specific molecular pathways such as Sestrin2-AMPK, which are associated with heart failure management[14,23,29]. Overall, the multifaceted approach to SGLT2 inhibitors highlight their potential as a therapeutic strategy for cardiovascular health, with ongoing research continuing to elucidate their complex mechanisms and benefits.

Nevertheless, the exact mechanisms by which SGLT2 inhibitors exert their cardioprotective effects remain under investigation, and it is likely that multiple mechanisms act in concert. Perhaps the most striking finding was empagliflozin's effectiveness in the absence of SGLT2 expression in cardiomyocytes. This clearly demonstrated the diabetes-independent action of this drug, highlighting its potential as a targeted therapy for CVD. The cardioprotective effects observed in patients with heart failure, including those with CVD, have led to an expansion of the indications for SGLT2 inhibitors beyond diabetes to include the treatment of heart failure with a reduced ejection fraction, with ongoing research potentially further broadening their therapeutic applications. Despite these promising findings, further research is necessary to fully elucidate the extent to which these mechanisms contribute to the cardiovascular benefits of SGLT2 inhibitors, the understanding of which will enhance the clinical application of these agents and potentially lead to more targeted treatments for patients with diabetic heart disease.

CONCLUSION

SGLT2 inhibitors have become an essential therapeutic advancement in diabetes management due to their low risk of hypoglycemia and notable cardiovascular benefits. In addition to their glucose-lowering effects, SGLT2 inhibitors are recognized for their efficacy in treating heart failure through various non-glycemic mechanisms. These include hemodynamic changes and anti-inflammatory, anti-fibrotic, antioxidant, and metabolic effects, which together contribute to the cardiovascular advantages observed with SGLT2 inhibitor use. Further research is ongoing to fully understand the mechanisms through which these inhibitors exert their cardioprotective effects.

FOOTNOTES

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REFERENCES

1 **Zhang X**, Zhu J, Kim JH, Sumerlin TS, Feng Q, Yu J. Metabolic health and adiposity transitions and risks of type 2 diabetes and cardiovascular diseases: a systematic review and meta-analysis. *Diabetol Metab Syndr* 2023; **15**: 60 [PMID: 36973730 DOI: 10.1186/s13098-023-01025-w]

2 **Vaduganathan M**, Docherty KF, Claggett BL, Jhund PS, de Boer RA, Hernandez AF, Inzucchi SE, Kosiborod MN, Lam CSP, Martinez F, Shah SJ, Desai AS, McMurray JJV, Solomon SD. SGLT-2 inhibitors in patients with heart failure: a comprehensive meta-analysis of five randomised controlled trials. *Lancet* 2022; **400**: 757-767 [PMID: 36041474 DOI: 10.1016/S0140-6736(22)01429-5]

3 **Li N**, Zhu Q, Li G, Wang T, Zhou H. Empagliflozin ameliorates diabetic cardiomyopathy probably *via* activating AMPK/PGC-1α and inhibiting the RhoA/ROCK pathway. *World J Diabetes* 2023; **14**: 1862-1876 [DOI: 10.4239/wjd.v14.i12.1862]

4 **Zannad F**, Ferreira JP, Pocock SJ, Anker SD, Butler J, Filippatos G, Brueckmann M, Ofstad AP, Pfarr E, Jamal W, Packer M. SGLT2 inhibitors in patients with heart failure with reduced ejection fraction: a meta-analysis of the EMPEROR-Reduced and DAPA-HF trials. *Lancet* 2020; **396**: 819-829 [PMID: 32877652 DOI: 10.1016/S0140-6736(20)31824-9]

5 **Rossing P**, Inzucchi SE, Vart P, Jongs N, Docherty KF, Jhund PS, Køber L, Kosiborod MN, Martinez FA, Ponikowski P, Sabatine MS, Solomon SD, DeMets DL, Bengtsson O, Lindberg M, Langkilde AM, Sjöstrand M, Stefansson BV, Karlsson C, Chertow GM, Hou FF, Correa-Rotter R, Toto RD, Wheeler DC, McMurray JJV, Heerspink HJL; DAPA-CKD and DAPA-HF Trial Committees and Investigators. Dapagliflozin and new-onset type 2 diabetes in patients with chronic kidney disease or heart failure: pooled analysis of the DAPA-CKD and DAPA-HF trials. *Lancet Diabetes Endocrinol* 2022; **10**: 24-34 [PMID: 34856173 DOI: 10.1016/S2213-8587(21)00295-3]

6 **McMurray JJV**, Solomon SD, Docherty KF, Jhund PS. The Dapagliflozin and Prevention of Adverse outcomes in Heart Failure trial (DAPA-HF) in context. *Eur Heart J* 2021; **42**: 1199-1202 [PMID: 31898736 DOI: 10.1093/eurheartj/ehz916]

7 **Vaduganathan M**, Claggett BL, Jhund PS, Cunningham JW, Pedro Ferreira J, Zannad F, Packer M, Fonarow GC, McMurray JJV, Solomon SD. Estimating lifetime benefits of comprehensive disease-modifying pharmacological therapies in patients with heart failure with reduced ejection fraction: a comparative analysis of three randomised controlled trials. *Lancet* 2020; **396**: 121-128 [PMID: 32446323 DOI: 10.1016/S0140-6736(20)30748-0]

8 **McMurray JJV**, Solomon SD, Inzucchi SE, Køber L, Kosiborod MN, Martinez FA, Ponikowski P, Sabatine MS, Anand IS, Bělohlávek J, Böhm M, Chiang CE, Chopra VK, de Boer RA, Desai AS, Diez M, Drozdz J, Dukát A, Ge J, Howlett JG, Katova T, Kitakaze M, Ljungman CEA, Merkely B, Nicolau JC, O'Meara E, Petrie MC, Vinh PN, Schou M, Tereshchenko S, Verma S, Held C, DeMets DL, Docherty KF, Jhund PS, Bengtsson O, Sjöstrand M, Langkilde AM; DAPA-HF Trial Committees and Investigators. Dapagliflozin in Patients with Heart Failure and Reduced Ejection Fraction. *N Engl J Med* 2019; **381**: 1995-2008 [PMID: 31535829 DOI: 10.1056/NEJMoa1911303]

9 **Packer M**, Anker SD, Butler J, Filippatos G, Pocock SJ, Carson P, Januzzi J, Verma S, Tsutsui H, Brueckmann M, Jamal W, Kimura K, Schnee J, Zeller C, Cotton D, Bocchi E, Böhm M, Choi DJ, Chopra V, Chuquiure E, Giannetti N, Janssens S, Zhang J, Gonzalez Juanatey JR, Kaul S, Brunner-La Rocca HP, Merkely B, Nicholls SJ, Perrone S, Pina I, Ponikowski P, Sattar N, Senni M, Seronde MF, Spinar J, Squire I, Taddei S, Wanner C, Zannad F; EMPEROR-Reduced Trial Investigators. Cardiovascular and Renal Outcomes with Empagliflozin in Heart Failure. *N Engl J Med* 2020; **383**: 1413-1424 [PMID: 32865377 DOI: 10.1056/NEJMoa2022190]

10 **Verma S**, Dhingra NK, Butler J, Anker SD, Ferreira JP, Filippatos G, Januzzi JL, Lam CSP, Sattar N, Peil B, Nordaby M, Brueckmann M, Pocock SJ, Zannad F, Packer M; EMPEROR-Reduced trial committees and investigators. Empagliflozin in the treatment of heart failure with reduced ejection fraction in addition to background therapies and therapeutic combinations (EMPEROR-Reduced): a post-hoc analysis of a randomised, double-blind trial. *Lancet Diabetes Endocrinol* 2022; **10**: 35-45 [PMID: 34861154 DOI: 10.1016/S2213-8587(21)00292-8]

11 **Zelniker TA**, Wiviott SD, Raz I, Im K, Goodrich EL, Bonaca MP, Mosenzon O, Kato ET, Cahn A, Furtado RHM, Bhatt DL, Leiter LA, McGuire DK, Wilding JPH, Sabatine MS. SGLT2 inhibitors for primary and secondary prevention of cardiovascular and renal outcomes in type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials. *Lancet* 2019; **393**: 31-39 [PMID: 30424892 DOI: 10.1016/S0140-6736(18)32590-X]

12 **Nassif ME**, Windsor SL, Borlaug BA, Kitzman DW, Shah SJ, Tang F, Khariton Y, Malik AO, Khumri T, Umpierrez G, Lamba S, Sharma K, Khan SS, Chandra L, Gordon RA, Ryan JJ, Chaudhry SP, Joseph SM, Chow CH, Kanwar MK, Pursley M, Siraj ES, Lewis GD, Clemson BS, Fong M, Kosiborod MN. The SGLT2 inhibitor dapagliflozin in heart failure with preserved ejection fraction: a multicenter randomized trial. *Nat Med* 2021; **27**: 1954-1960 [PMID: 34711976 DOI: 10.1038/s41591-021-01536-x]

13 **Sayour AA**, Celeng C, Oláh A, Ruppert M, Merkely B, Radovits T. Sodium-glucose cotransporter 2 inhibitors reduce myocardial infarct size in preclinical animal models of myocardial ischaemia-reperfusion injury: a meta-analysis. *Diabetologia* 2021; **64**: 737-748 [PMID: 33483761 DOI: 10.1007/s00125-020-05359-2]

14 **Sun X**, Han F, Lu Q, Li X, Ren D, Zhang J, Han Y, Xiang YK, Li J. Empagliflozin Ameliorates Obesity-Related Cardiac Dysfunction by Regulating Sestrin2-Mediated AMPK-mTOR Signaling and Redox Homeostasis in High-Fat Diet-Induced Obese Mice. *Diabetes* 2020; **69**: 1292-1305 [PMID: 32234722 DOI: 10.2337/db19-0991]

15 **Lu Q**, Liu J, Li X, Sun X, Zhang J, Ren D, Tong N, Li J. Empagliflozin attenuates ischemia and reperfusion injury through LKB1/AMPK signaling pathway. *Mol Cell Endocrinol* 2020; **501**: 110642 [PMID: 31759100 DOI: 10.1016/j.mce.2019.110642]

16 **Huang K**, Luo X, Liao B, Li G, Feng J. Insights into SGLT2 inhibitor treatment of diabetic cardiomyopathy: focus on the mechanisms. *Cardiovasc Diabetol* 2023; **22**: 86 [PMID: 37055837 DOI: 10.1186/s12933-023-01816-5]

17 **Li N**, Zhou H. SGLT2 Inhibitors: A Novel Player in the Treatment and Prevention of Diabetic Cardiomyopathy. *Drug Des Devel Ther* 2020; **14**: 4775-4788 [PMID: 33192053 DOI: 10.2147/DDDT.S269514]

18 **Packer M**, Anker SD, Butler J, Filippatos G, Zannad F. Effects of Sodium-Glucose Cotransporter 2 Inhibitors for the Treatment of Patients With Heart Failure: Proposal of a Novel Mechanism of Action. *JAMA Cardiol* 2017; **2**: 1025-1029 [PMID: 28768320 DOI: 10.1001/jamacardio.2017.2275]

19 **Yurista SR**, Silljé HHW, Oberdorf-Maass SU, Schouten EM, Pavez Giani MG, Hillebrands JL, van Goor H, van Veldhuisen DJ, de Boer RA, Westenbrink BD. Sodium-glucose co-transporter 2 inhibition with empagliflozin improves cardiac function in non-diabetic rats with left ventricular dysfunction after myocardial infarction. *Eur J Heart Fail* 2019; **21**: 862-873 [PMID: 31033127 DOI: 10.1002/ejhf.1473]

20 **Li C**, Zhang J, Xue M, Li X, Han F, Liu X, Xu L, Lu Y, Cheng Y, Li T, Yu X, Sun B, Chen L. SGLT2 inhibition with empagliflozin attenuates myocardial oxidative stress and fibrosis in diabetic mice heart. *Cardiovasc Diabetol* 2019; **18**: 15 [PMID: 30710997 DOI: 10.1186/s12933-019-0816-2]

21 **Uthman L**, Li X, Baartscheer A, Schumacher CA, Baumgart P, Hermanides J, Preckel B, Hollmann MW, Coronel R, Zuurbier CJ, Weber NC. Empagliflozin reduces oxidative stress through inhibition of the novel inflammation/NHE/[Na(+)](c)/ROS-pathway in human endothelial cells. *Biomed Pharmacother* 2022; **146**: 112515 [PMID: 34896968 DOI: 10.1016/j.biopha.2021.112515]

22 **Cardoso R**, Graffunder FP, Ternes CMP, Fernandes A, Rocha AV, Fernandes G, Bhatt DL. SGLT2 inhibitors decrease cardiovascular death and heart failure hospitalizations in patients with heart failure: A systematic review and meta-analysis. *EClinicalMedicine* 2021; **36**: 100933 [PMID: 34308311 DOI: 10.1016/j.eclinm.2021.100933]

23 **Quagliariello V**, De Laurentiis M, Rea D, Barbieri A, Monti MG, Carbone A, Paccone A, Altucci L, Conte M, Canale ML, Botti G, Maurea N. The SGLT-2 inhibitor empagliflozin improves myocardial strain, reduces cardiac fibrosis and pro-inflammatory cytokines in non-diabetic mice treated with doxorubicin. *Cardiovasc Diabetol* 2021; **20**: 150 [PMID: 34301253 DOI: 10.1186/s12933-021-01346-y]

24 **Ugusman A**, Kumar J, Aminuddin A. Endothelial function and dysfunction: Impact of sodium-glucose cotransporter 2 inhibitors. *Pharmacol Ther* 2021; **224**: 107832 [PMID: 33662450 DOI: 10.1016/j.pharmthera.2021.107832]

25 **Navodnik MP**, Janež A, Žuran I. The Effect of Additional Treatment with Empagliflozin or Semaglutide on Endothelial Function and Arterial Stiffness in Subjects with Type 1 Diabetes Mellitus-ENDIS Study. *Pharmaceutics* 2023; **15** [PMID: 37514131 DOI: 10.3390/pharmaceutics15071945]

26 **Mone P**, Varzideh F, Jankauskas SS, Pansini A, Lombardi A, Frullone S, Santulli G. SGLT2 Inhibition *via* Empagliflozin Improves Endothelial Function and Reduces Mitochondrial Oxidative Stress: Insights From Frail Hypertensive and Diabetic Patients. *Hypertension* 2022; **79**: 1633-1643 [PMID: 35703100 DOI: 10.1161/HYPERTENSIONAHA.122.19586]

27 **Nakao M**, Shimizu I, Katsuumi G, Yoshida Y, Suda M, Hayashi Y, Ikegami R, Hsiao YT, Okuda S, Soga T, Minamino T. Empagliflozin maintains capillarization and improves cardiac function in a murine model of left ventricular pressure overload. *Sci Rep* 2021; **11**: 18384 [PMID: 34526601 DOI: 10.1038/s41598-021-97787-2]

28 **Cai C**, Guo Z, Chang X, Li Z, Wu F, He J, Cao T, Wang K, Shi N, Zhou H, Toan S, Muid D, Tan Y. Empagliflozin attenuates cardiac microvascular ischemia/reperfusion through activating the AMPKα1/ULK1/FUNDC1/mitophagy pathway. *Redox Biol* 2022; **52**: 102288 [PMID: 35325804 DOI: 10.1016/j.redox.2022.102288]

29 **Ala M**. SGLT2 Inhibition for Cardiovascular Diseases, Chronic Kidney Disease, and NAFLD. *Endocrinology* 2021; **162** [PMID: 34343274 DOI: 10.1210/endocr/bqab157]

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REVIEW

Genotype-based precision nutrition strategies for the prediction and clinical management of type 2 diabetes mellitus

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Abstract

Globally, type 2 diabetes mellitus (T2DM) is one of the most common metabolic disorders. T2DM physiopathology is influenced by complex interrelationships between genetic, metabolic and lifestyle factors (including diet), which differ between populations and geographic regions. In fact, excessive consumptions of high fat/high sugar foods generally increase the risk of developing T2DM, whereas habitual intakes of plant-based healthy diets usually exert a protective effect. Moreover, genomic studies have allowed the characterization of sequence DNA variants across the human genome, some of which may affect gene expression and protein functions relevant for glucose homeostasis. This comprehensive literature review covers the impact of gene-diet interactions on T2DM susceptibility and disease progression, some of which have demonstrated a value as biomarkers of personal responses to certain nutritional interventions. Also, novel genotype-based dietary strategies have been developed for improving T2DM control in comparison to general lifestyle recommendations. Furthermore, progresses in other omics areas (epigenomics, metagenomics, proteomics, and metabolomics) are improving current understanding of genetic insights in T2DM clinical outcomes. Although more investigation is still needed, the analysis of the genetic make-up may help to decipher new paradigms in the pathophysiology of T2DM as well as offer further opportunities to personalize the screening, prevention, diagnosis, management, and prognosis of T2DM through precision nutrition.

Key Words: Type 2 diabetes mellitus; Nutrigenetics; Single nucleotide polymorphism; Genotype; Diet; Precision nutrition

Core Tip: The onset and progression of type 2 diabetes mellitus (T2DM) is influenced by complex interrelationships between genetic and dietary factors. Indeed, a number of nutrigenetic studies have identified significant gene-diet interactions related to T2DM predisposition, nutrient metabolic status, and dietary intervention responsiveness. Moreover, this knowledge has motivated the interest for the design and implementation of genotype-based dietary strategies for improving glycemic outcomes compared to conventional nutritional advice. Although more investigation is required, these insights may help to explain disease phenotype heterogeneity, with relevance in precision nutrition for the personalized prevention and clinical management of T2DM.

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a metabolic disease caused by insufficient pancreatic insulin secretion or defective hormone actions in target tissues[1]. T2DM is recognized as a major public health concern due to rising global prevalence and negative impact on human wellbeing and life expectancy, being significantly associated with morbidity burden and premature mortality[2].

Several factors have been identified to contribute to the prevalence of T2DM including the genetic background[3]. Accordingly, a number of sequence DNA variants across the human genome have been characterized, some of which may affect gene expression and protein functions relevant for maintaining glucose homeostasis[3-5]. Largely, single nucleotide polymorphisms (SNPs) have been the most prevalent studied genetic variations in the field of precision medicine, with applications in T2DM prevention and personalized management[6-8]. Moreover, genetic risk scores (GRS) have been developed to assess the additive effect of SNPs[9-11].

Of note, the genetic contribution to T2DM status may depend on interactions with environmental issues including diet, which may explain some of the inconsistencies reported among epidemiological studies relating diet to chronic diseases [12]. Thus, interrelationships between genetic variants and dietary features (*i.e.*, intakes of macro and micronutrients, eating behaviors, nutritional patterns, and the consumption of particular foods) may influence T2DM risk or disease complications by affecting critical pathways involved in glucose signaling, insulin secretion, β-cell function, gluco-lipotoxicity, inflammation and oxidative stress[12-14]. Therefore, people with higher genetic predisposition should avoid certain harmful foods or adopt healthy dietary patterns to delay T2DM onset.

In this context, it has been illustrated that the combination of genetic (52 SNPs in 37 genes) and dietary data (food with high sugar content) using machine learning approaches may improve the prediction of T2DM incidence[15]. Likewise, high genetic (48 SNPs) and dietary risk scores (based on sugar-sweetened beverages, processed meat, whole grains and coffee) were associated with increased incidence of T2DM[16].

In this document, potential interactions between genetic polymorphisms and dietary factors concerning T2DM susceptibility and disease progression are reviewed, some of which have demonstrated a value as biomarkers of personal responses to nutritional interventions. Also, novel genotype-based dietary strategies for the prevention and clinical management of T2DM are documented. Future directions comprising the integration of genetics with another omics tools are also postulated. These insights may help to explain heterogeneity in predisposition to T2DM and the development of related systemic complications, with relevance in disease stratification and precision nutrition through the study of the human genome.

GENETIC BACKGROUND, DIETARY INTAKE, AND T2DM RISK

A relevant precision nutrition approach in T2DM risk prediction/prevention include the analysis of associations between genetic polymorphisms and T2DM that are modulated by dietary features. Indeed, a number of nutrigenetic studies have identified significant gene-diet interactions related to T2DM predisposition (Table 1). These include single SNPs mapped to genes involved in pivotal physiological processes such as energy breakdown, nutrient utilization, insulin signaling, circadian rhythm, cell cycle regulation, pancreatic function, hypothalamic food intake control, neuronal synapse, signal transduction, and taste perception, which interact with nutritional factors to influence T2DM risk (Table 1). Among them, the consumption of particular foods (vegetables, whole grains, coffee, olive oils, alcoholic beverages, and dairy products), macronutrients (carbohydrates, fatty acids, protein, fiber) and micronutrients (iron, folate) intakes, adherence to dietary patterns, and eating time schedules (Table 1).

In addition, GRS have been constructed to evaluate the cumulative effects of SNPs on T2DM susceptibility, where dietary factors are implicated. For instance, and obesity GRS positively interacted with dietary intake of cholesterol to affect insulin resistance in overweight/obese Spanish individuals[17]. Of note, Brazilian subjects with high GRS for metabolic disease and total fat intakes had increased blood glucose and insulin-related traits than those with low GRS [18]. Conversely, lower serum levels of glycated hemoglobin were found in Ghanaian adults with low total fat intake (≤

Table 1 Gene-diet interactions concerning the risk of developing type 2 diabetes mellitus and individual responses to nutritional interventions

SNP reference	Gene symbol	Gene function	Risk allele	Dietary interaction	Main outcome	Population	Ref.
rs7903146	*TCF7L2*	Wnt signaling pathway	T	High dessert and milk intakes (above median)	Higher T2DM risk	Algerian	[83]
rs7903146	*TCF7L2*	Wnt signaling pathway	C	Fiber intake	Inversely associated with T2DM incidence	Swedish	[84]
rs7903146 and rs4506565	*TCF7L2*	Wnt signaling pathway	rs7903146 (C) and rs4506565 (A)	Per daily 30-g increased intake of whole grain and per daily 5-g increased intake of cereal fiber	Decreased risk of developing T2DM	Swedish men	[85]
rs7901695	*TCF7L2*	Wnt signaling pathway	T	Upper protein intake quantiles	Higher HbA1c, HOMA-IR, blood glucose, and insulin levels	Polish	[86]
rs6696797, rs4244372, and rs10881197	*AMY1*	Carbohydrate digestion	rs6696797 (A), rs4244372 (A), rs10881197 (G)	Carbohydrate intake > 65% of total energy	Higher T2DM incidence	Korean women	[87]
rs2233998	*TAS2R4*	Bitter taste perception	T	High intakes of carbohydrates or sugars (highest tertile) and low intakes of fruits or vegetables (lowest tertile)	Higher T2DM incidence	Korean women	[88]
rs1801282 and rs3856806	*PPARG*	Fatty acid storage and glucose metabolism	rs1801282 (Pro12), rs3856806 (C)	High fat consumption (the third sex-specific tertile of fat intake	Increased T2DM risk	French	[89]
rs7756992	*CDKAL1*	Beta cells function	G	First tertiles of protein and fat intakes	Higher T2DM risk	Korean	[90]
rs7754840	*CDKAL1*	Pancreatic beta cells function	G	Habitual coffee intake	Lower risk of prediabetes and T2DM	East Asians	[91]
rs5215	*KCNJ11*	Formation of ATP-sensitive potassium (K-ATP) channels in pancreatic beta cells	C	Habitual coffee intake	Lower risk of prediabetes and T2DM	East Asians	[91]
rs4402960	*IGF2BP2*	Cellular metabolism modulation by post transcriptional regulation	T	Habitual coffee intake	Lower risk of prediabetes and T2DM	East Asians	[91]
rs10517030	*PGC-1α*	Regulation of genes involved in energy metabolism	C	Low-energy diet (daily consumption less than estimated energy intake)	Positively associated with T2DM prevalence and insulin resistance and negatively associated with beta cell function	Koreans	[92]
rs6265	*BDNF*	Survival and growth of neurons, and synaptic efficiency and plasticity	Met	Low-energy (daily consumption less than estimated daily energy intake), low-protein (< 13% daily energy), and high-carbohydrate (70% daily energy)	Lower risk for T2DM	Koreans	[93]
rs161364 and rs8065080	*TRPV1*	Receptor for capsaicin, non-selective cation channel, and participates in transduction of painful thermal stimuli	rs161364 (T) and rs8065080 (C)	High preference for oily foods and high fat intake from oily foods	Lower risk for T2DM	Koreans	[94]
rs77768175, rs2074356 and rs11066280	*HECTD4*	Glucose homeostasis and glucose metabolic process	rs77768175 (A), rs2074356 (G), rs11066280 (T)	Alcohol consumption (> 5 g/d)	Significantly increased risks of T2DM	East Asians	[95]
rs10830963	*MTNR1B*	Regulation of the	G	Increasing dietary iron	Increased risk of	Chinese	[96]

		circadian actions of melatonin		intake	elevated fasting glucose, higher fasting glucose, and higher HbA1c		
rs10830963	*MTNR1B*	Regulation of the circadian actions of melatonin	G	Late dinner	Impaired glucose tolerance	European	[97]
rs10830963	*MTNR1B*	Regulation of the circadian actions of melatonin	G	Late eating	Impaired glucose tolerance and insulin secretion defects	European	[98]
rs2943641	*IRS1*	Insulin signaling	T	Lower tertiles of carbohydrate intake (women) and lowest tertile of fat intake (men)	Decreased risk of T2DM	Swedish	[99]
rs7578326 and rs2943641	*IRS1*	Insulin signaling	rs7578326 (G) and rs2943641 (T)	Low SFA-to-carbohydrate ratio (≤ 0.24)	Lower risk of insulin resistance and metabolic syndrome	American	[100]
rs10423928	*GIPR*	Insulin release stimulation	T	Highest carbohydrate quintile	Decreased T2DM risk	Swedish	[101]
rs3014866	*S100A9*	Cell cycle progression and differentiation	C	High dietary SFA: Carbohydrate ratio intake	Higher insulin resistance	Spanish white adults, North American non-Hispanic white adults, and Hispanic adults	[102]
rs709592	*PSMD3*	Maintenance of protein homeostasis	T	Low carbohydrate intake (≤ 49.1% energy)	Higher insulin resistance	Americans	[103]
rs8065443	*PSMD3*	Maintenance of protein homeostasis	A	Low (n-3):(n-6) PUFA ratio (≤ 0.11)	Higher insulin resistance	Americans	[103]
rs7645550	*KCNMB3*	Control of smooth muscle tone and neuronal excitability	T	Low (n-3):(n-6) PUFA ratio (≤ 0.11)	Lower insulin resistance	Americans	[104]
rs1183319	*KCNMB3*	Control of smooth muscle tone and neuronal excitability	G	High (n-3):(n-6) PUFA ratio (> 0.09)	Higher HbA1c levels	Hispanics	[104]
rs2270188	*CAV2*	Signal transduction, lipid metabolism, cellular growth control and apoptosis	T	Increase of daily fat intake from 30% to 40% energy	Greater risk of T2DM	European	[105]
rs10923931	*NOTCH2*	Wnt signaling pathway	T	Increasing fiber intake	Lower T2DM risk	Swedish	[106]
rs4457053	*ZBED3*	Wnt signaling pathway	G	Increasing fiber intake	Lower T2DM risk	Swedish	[106]
rs3765467	*GLP1R*	Insulinotropic action of GLP-1 in β-cells	G	Highest tertiles of energy, protein and carbohydrate consumption	Higher risk for decreased insulin secretion	Japanese men	[107]
rs9939609	*FTO*	Regulation of energy intake	A	Low adherence to the Mediterranean diet (≤ 9 points)	Higher risk of prevalent T2DM	Spanish	[108]
rs9939609	*FTO*	Regulation of energy intake	A	Low folate intake (< 406 μg/d)	Higher fasting plasma glucose concentrations	Spanish	[108]
rs17782313	*MC4R*	Hypothalamic leptin-melanocortin signaling pathway	C	Low adherence to the Mediterranean diet (≤ 9 points)	Higher risk of prevalent T2DM	Spanish	[108]

SNP: Single nucleotide polymorphism; T2DM: Type 2 diabetes mellitus; SFA: Saturated fatty acids; PUFA: Polyunsaturated fatty acids; HbA1c: Glycosylated hemoglobin; HOMA-IR: Homeostasis model assessment-estimated insulin resistance; GLP-1: Glucagon-like peptide-1; TCF7L2: Transcription factor 7 like 2; AMY1: Amylase 1; PPARG: Peroxisome proliferator-activated receptor gamma; IGF2BP2: Insulin-like growth factor 2 binding protein 2; PGC-1α: Proliferator-activated receptor-gamma coactivator-1alpha; BDNF: Brain-derived neurotrophic factor; TRPV1: Transient receptor potential vanilloid-1 channel; HECTD4: HECT domain E3 ubiquitin protein ligase 4; MTNR1B: Melatonin receptor 1B; IRS1: Insulin receptor substrate-1; GIPR: Glucose-dependent insulinotropic polypeptide receptor; CAV2: Caveolin-2; ZBED3: Zinc finger BED-type containing 3; FTO: Fat mass and obesity associated; MC4R: Melanocortin 4 receptor.

36.5 g/d) despite carrying more than two risk alleles of vitamin D-related genetic variants[19]. Also, associations between a GRS related to insufficient glucose-stimulated insulin secretion and T2DM risk was accentuated in Asian individuals with high energy and calcium intakes[20]. Moreover, Korean subjects carrying polygenic variants linked to oxidative stress had increased risk of T2DM, which was lowered the by the intakes of dietary antioxidants[21]. Besides, the genetic predisposition to T2DM was exacerbated with higher intakes of dietary branched-chain amino acids in Chinese[22].

Regarding specific foods, it was reported that middle-aged Korean adults with high GRS affecting insulin signaling presented more instances of insulin resistance when combined with high coffee (≥ 10 cups/wk) or caffeine (≥ 220 mg/d) intakes[23]. Likewise, alcohol consumption significantly increased the risk of T2DM especially in Chinese men with low genetic predisposition to insulin secretion deterioration[24]. In the same way, the association between the consumption of sugar-sweetened beverages and serum glucose abnormalities was stronger in Chileans with high T2DM genetic susceptibility[25]. Conversely, augmented genetic risk for T2DM was ameliorated by increasing the consumption of fruits in Chinese population[26]. In line with this finding, lower plant protein intake (< 39 g/d) was identified as a factor contributing to increase the risk of T2DM in genetically predisposed Asian Indians[27].

Furthermore, a high GRS for impaired insulin secretion increased the risk of T2DM by consuming a low-carbohydrate Western dietary pattern in Korean adults[28]. In Asians, higher fasting serum glucose concentrations were found in participants with high T2DM-linked GRS who adopted a Western dietary pattern[29]. On the contrary, it was reported that Koreans with high GRS for insulin resistance may be benefited by consuming a plant-based diet with high amounts of fruits, vitamin C, and flavonoids[30].

These studies show evidence concerning interactions between genetic variants and T2DM risk depending on dietary intakes, which may be useful for the design of nutritional therapies aimed to control the burden of T2DM, although more research is needed in populations with different genetic ancestries including Hispanics and Africans.

GENE-DIET INTERACTIONS AFFECTING METABOLIC STATUS IN T2DM PATIENTS

Once T2DM has established, several physiopathological processes affecting glucose/lipid metabolism homeostasis, immune function, adipokine secretion, and gut microbiota dysbiosis play a critical role in the development of vascular injuries including diabetic heart disease and stroke[31]. Thus, it is important to monitor the metabolic status in T2DM in order to prevent or delay the progression of complications associated with this disease.

Accordingly, some studies have analyzed the effect of gene-diet interactions on glycemic, lipid, and inflammatory features in T2DM patients, with relevance in clinical disease management. In this regard, studies in Mexican population have evidenced relevant gene-nutrient interactions concerning glycemic control and lipid profile in T2DM. For example, positive correlations were found between calcium intake and glycated hemoglobin and potassium intake and triglyceride-glucose index only in carriers of the 408 Val risk allele of the *SLC22A1/OCT1* Met408Val polymorphism[32]. Also, higher blood concentrations of total cholesterol, non-high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol were found in carriers of the *APOE* ε2 allele with low consumption of monounsaturated fatty acids (MUFA), whereas carriers of the apolipoprotein E (*APOE*) ε4 allele with high dietary ω-6:ω-3 polyunsaturated fatty acids (PUFA) ratio presented higher glycated hemoglobin levels[33]. Likewise, A1 allele carriers of the *DRD2/ANKK1* TaqIA polymorphism were protected from serum triglyceride increases by maltose intake, but A2A2 homozygotes were susceptible to triglyceride rises through excessive consumptions of total fat, MUFA, and dietary cholesterol[34].

In Iranians with T2DM, Met allele carriers of the brain-derived neurotrophic factor (*BDNF*) Val66Mat polymorphism with high scores of dietary indices showed lower blood levels of triglycerides ((healthy eating index and diet quality index), total cholesterol, and interleukin-18 (phytochemical index) than Val/Val homozygotes[35]. Meanwhile, C-allele carriers of the *APOA2*-265 T>C polymorphism had highest means of body mass index, waist circumference, blood cholesterol and serum ghrelin and leptin levels when dietary acid load (either potential renal acid load or net endogenous acid production) values were high[36]. Of note, higher inflammatory and antioxidant markers including C-reactive protein, total antioxidant capacity, superoxide dismutase, and 8-isoprostaneF2alpha were found in B2B2 homozygotes of the *CETP* TaqB1 polymorphism when they consumed diets with high dietary insulin index[37]. Similarly, risk-allele carriers (CG, GG) of the peroxisome proliferator-activated receptor (*PPAR*)-γ Pro12Ala polymorphism who consumed a diet with high dietary insulin load and insulin indexes were more likely to be obese and have increased inflammatory markers (*i.e.*, interleukin-18, isoprostaneF2α, and pentraxin-3) compared to individuals with the CC genotype[38]. Moreover, worse plasma lipid profile was found in participants carrying the AA/AG genotype of the *ApoB* EcoRI polymorphism when increasing the percentage of energy derived from dietary fat, carbohydrates, protein, saturated fatty acids (SFA), and cholesterol in comparison to GG homozygotes[39]. In the same way, Del-allele carries of the *ApoB* Ins/Del genetic variant who consumed high amounts of MUFA (≥ 12% E) and carbohydrates (≥ 54% E) had higher blood levels of triglycerides and low density lipoprotein-cholesterol, while low carbohydrate (< 54% E) intakes were associated with raised serum concentrations of leptin and ghrelin in T2DM patients with this same genetic profile compared to Ins/Ins homozygotes[40]. In addition, an increased risk of obesity was found in carriers of the Del allele of *ApoB* gene when combined with a low consumption of dietary ω-3 PUFA (< 0.6% E) in T2DM subjects[41]. Taken together, these results could be useful to prevent cardiometabolic risk factors and later complications in T2DM patients *via* manipulation of dietary intakes of selected nutrients mainly in genetically susceptible individuals. However, more investigation is needed in other populations with diverse ancestries and exposed to different environments in order to regionalize antidiabetic nutritional treatments.

GENETIC POLYMORPHISMS AS BIOMARKERS OF GLYCEMIC RESPONSES TO DIETARY ADVICE

Dietary strategies aimed to achieve or improve glucose homeostasis not always have a positive impact in all individuals, which can be due to genetic factors. In this sense, some trials have evaluated the value of SNPs as potential biomarkers of glycemic outcomes in response to different nutritional interventions. For instance, the variant rs3071 of the *SCD* gene modified blood glucose response to dietary oils varying in MUFA content in adults with obesity, where CC genotype carriers showed an increase in blood glucose levels with a high SFA/low MUFA control oil, but reductions in this outcome with both high MUFA oil diets[42]. Within the multicenter NUGENOB study, the T allele of the protein phosphatase Mg(2+)/Mn(2+)-dependent 1K (*PPM1K*) rs1440581 genetic variant was associated with higher reductions of serum insulin and homeostasis model assessment (HOMA)-B after a high-fat (40%-45% E) diet, whereas an opposite effect was found in the low-fat (20%-25% E) diet group[43]. Also, obese individuals who were homozygous for the T-risk allele of the transcription factor 7 like 2 (*TCF7L2*) rs7903146 polymorphism and consumed a high-fat (40%-45% E) diet, underwent smaller reductions in HOMA-estimated insulin resistance (HOMA-IR)[44].

Findings from the POUNDS lost trial revealed greater decreases in fasting glucose, serum insulin, and HOMA-IR in T-allele participants of the glucose-dependent insulinotropic polypeptide receptor (*GIPR*) rs2287019 variant who were assigned to low-fat (20%-25% E) diets[45]. In addition, subjects with the risk-conferring CC genotype of the insulin receptor substrate-1 (*IRS1*) rs2943641 SNP had greater decreases in insulin and HOMA-IR than those without this genetic profile in the highest-carbohydrate (65% E) dietary group[46]. Whereas, the T allele of deficient activity of 7-dehydrocholesterol reductase (*DHCR7*) rs12785878 polymorphism was associated with higher decreases in serum insulin and HOMA-IR only in high-protein (25% E) diets[47]. Similarly, greater drops in fasting insulin levels were related to the *PCSK7* rs236918 G allele in high-dietary carbohydrate (65% E) intakes, especially in white Americans[48]. Of note, carriers of the risk allele (A) of the Fat mass and obesity associated (*FTO*) rs1558902 variant benefited more in improving insulin sensitivity by consuming high-fat (40%-45% E) diets rather than low-fat (20%-25% E) regimens[49].

In a Spanish cohort with obesity, improvements in serum insulin levels and HOMA-IR were associated with the *ADRB3* Trp64Trp genotype after hypocaloric diet with high protein (34% E) content[50]. Besides, AA genotype carries of the *BDNF* rs10767664 variant underwent reductions in insulin resistance markers when consumption of MUFA (67.5%) was high[51]. Likewise, *TNFA*-308GG homozygotes had a better glycemic response after high (22.7%) dietary intakes of PUFA[52]. In the same say, *UCP3* 55CC genotype carriers benefited more (more decreases in blood glucose, serum insulin, and HOMA-IR) when consumed a high-protein (34% E) diet[53]. Interestingly, it was suggested that the T allele of the *ADIPOQ* rs1501299 SNP was related to a lack of response of fasting glucose/insulin and HOMA-IR secondary to a Mediterranean-style diet in Spanish obese individuals[54]. Insulin resistance was ameliorated after the consumption of this same dietary pattern in T allele carries of the *RETN* rs10401670 gene polymorphism[55]. Comparable results were reported concerning insulin resistance reductions in CC genotype carries of the melatonin receptor 1B (*MTNR1B*) rs10830963 variant but not in GC + GG groups after following a hypocaloric diet with Mediterranean pattern[56].

Some studies have evaluated the cumulative effect of multiple SNPs (by calculating GRS) instead of single variants. In this context, participants with high genetic risk of glucose abnormalities showed increased fasting glucose after consuming a high-fat diet (40%-45% E), which was not observed in subjects assigned to the low-fat (20%-25% E) group [57]. A lower GRS for diabetes was associated with higher reductions in fasting insulin, glycated hemoglobin, and HOMA-IR, and a lesser increase in HOMA-B only when the consumption of dietary protein (15% E) was low[58]. In the meantime, insulin resistance improvements were limited to individuals with a higher GRS of habitual coffee consumption following a low-fat (20%-25% E) dietary intervention[59].

The influence of the genetic background on metabolic outcomes after dietary treatments have also been assessed in T2DM patients. For example, a dietary intervention based on increased intakes of whole grains, vegetables, and legumes was able to prevent an age-related increase in blood triglyceride concentrations in Koreans with impaired fasting glucose or new-onset of T2DM carrying the TT genotype of the *APOA5*-1131 T>C SNP[60]. Accordingly, low glycemic index diets induced significant decreases of serum lipids, fasting blood glucose, and glycated albumin only in Chinese women with T2DM who were *FABP2* Ala54 homozygotes[61]. Furthermore, carriers of the *FTO* rs9939609 risk allele (A) underwent a better response in improving body mass index and diastolic blood pressure in response to supplementation with epigallocatechin-3-gallate (300 mg/d) in Iranian patients with T2DM[62].

Overall, current evidence suggests a role of selected genetic polymorphisms in modulating the individual metabolic responses to some dietary treatments. However, available studies have been performed mainly in Europeans/Caucasians, with particular genetic backgrounds; therefore, additional studies in different populations are required including Latin Americans, Africans, and Asians. Also, the analysis of the effects of supplementation with antioxidant micronutrients and bioactive compounds with anti-inflammatory properties is warranted.

GENOTYPE-BASED DIETARY INTERVENTIONS AND GLYCEMIC OUTCOMES

The knowledge about the implication of genetic variants and dietary factors in the onset and progression of T2DM has motivated the interest for the design and implementation of genotype-based intervention strategies for improving glycemic/metabolic outcomes compared to traditional nutritional prescriptions. For instance, it was evidenced that a personalized low-glycemic index nutrigenetic diet (utilizing 28 SNPs with evidence of gene-diet/lifestyle interactions) induced higher fasting glucose reductions than a Ketogenic diet in overweight/obese individuals[63]. Likewise, healthier effects in HOMA-IR and insulin serum levels were observed in MTHFR 677T allele carriers consuming a GENOMEX diet comprising of diet-related adaptive gene polymorphisms highly prevalent in Mexicans[64]. However, no differences were

detected regarding glucose homeostasis outcomes at 24 wk of follow-up between a nutrigenetic-guided diet (using genetic information of a proprietary algorithm) and a standard balanced diet in obese or overweight American veterans [65].

In T2DM patients, a case study based on the N-of-1 approach revealed better glycemic control when adhered to a genetically-guided Mediterranean diet (high-quality foods rich in fiber and antioxidants that have been proven to exert beneficial glycaemia effects) considering genetic variants guiding the personalized selection of macronutrients for the nutritional management of T2DM[66]. Similarly, greater improvements in fasting plasma glucose and glycosylated hemoglobin concentrations were found in patients with pre-diabetes or T2DM following a personalized nutritional plan (taking in consideration SNPs associated with individual responses to macronutrient intakes) compared to conventional medical nutrition therapy[67].

Furthermore, some studies have evaluated the utility of genetic disclosure as a tool for T2DM prevention and disease control. For example, participants who received diabetes genetic risk counseling together with general education about modifiable risk factors and personal stimulus to adopt diabetes lifestyle prevention behaviors reported high levels of support, perceived personal control and satisfaction with the genetic counseling sessions[68]. Nevertheless, diabetes genetic risk testing and counseling did not necessarily improved disease prevention behaviors such as self-reported motivation or prevention program adherence among overweight individuals at increased phenotypic risk for T2DM[69]. Moreover, comparison analyzes did not revealed significant differences between genetic testing results and traditional risk counseling concerning behavior changes to reduce the risk of T2DM in non-diabetic overweight/obese veterans[70]. Given inconsistences in available evidence, more research is needed to translate this knowledge into clinical care in T2DM. Further investigation should contemplate information that could interfere with the results including the prevalence and metabolic effects of selected SNPs, cultural level of populations, compatibility of dietary plans with genotypic characteristics, and the quality of nutritional/lifestyle advice.

FUTURE DIRECTIONS

In addition to genetics, progresses in other omics areas are improving current understanding of the biological/molecular mechanisms involved in T2DM pathogenesis and clinical outcomes[71]. Similar to the influence of the genetic background, it has been evidenced that epigenetic modifications may alter transcriptional activity resulting in different T2DM traits and phenotypes; certainly, different genes responsible for the interindividual variability in responses to antidiabetic treatments (including dietary advice) are subjected to epigenetic regulation[72]. More importantly, interactions among polymorphisms in key metabolic genes (*i.e.*, *TCF7L2*), related methylation status, and environmental factors have been suggested as a possible etiologic pattern for T2DM[73]. Besides, SNPs in microRNA (miRNA) genes may change the structure of miRNAs and their target gene expressions to influence T2DM risk[74].

Also, metagenomic and metabolomic methodologies have emerged to investigate the interrelationships between the gut microbiota dysbiosis and their related metabolites (affecting critical metabolic pathways in the host such as immunity and nutrient metabolism) in the development of T2DM[75]. Of note, characterization of gut microbiota of individuals carrying the risk alleles of the *PPARGC1A* (rs8192678) and *PPARD* (rs2267668) variants revealed some taxa (with overrepresentation of ABC sugar transporters) putatively associated with insulin resistance and T2DM[76]. Correspondingly, the *MMP27* rs7129790 polymorphism was strongly associated with high gut abundance of Proteobacteria in Mexican Americans with a high prevalence of obesity and T2DM[77].

Moreover, high-throughput proteomics assays have allowed the discovery and representation of potential protein-T2DM links, providing novel intervention targets in this disease[78]. Interestingly, a set of circulating proteins causally associated with T2DM were identified using two-sample Mendelian randomization approaches, which is a validated method to examine the causal effect of variation in genes of known function on disease[79]. Also, Mendelian randomization analyses did not uncover significant causal effects between proteins (*i.e.*, retinal dehydrogenase 1, galectin-4, cathepsin D, and lipoprotein lipase) and diabetes, suggesting that identified proteins are expected to be biomarkers for T2DM, rather than demonstrating causal pathways[80].

Additionally, coupling genomic data (*i.e.*, GRS) with conventional phenotypical information (*i.e.*, age, sex, body composition, medication use, and vital signs) is being useful for enhancing individual T2DM risk stratification and disease prediction[81,82]. Advances in next-generation sequencing technologies and the use of machine learning and other artificial intelligence methods became fundamental to analyze these T2DM-associated multiomics datasets.

CONCLUSION

Current evidence support the impact of genetic variation on the risk of developing blood glucose/insulin alterations and subsequent T2DM as well as its implication in affecting the lipid, inflammatory, and carbohydrate status in T2DM patients through interactions with dietary factors. These include SNPs and other structural variants mapped to metabolically active genes such as *TCF7L2*, amylase 1, *TAS2R4*, *PPARG*, *CDKAL1*, *KCNJ11*, insulin-like growth factor 2 binding protein 2, proliferator-activated receptor-gamma coactivator-1alpha, *BDNF*, transient receptor potential vanilloid-1 channel, HECT domain E3 ubiquitin protein ligase 4, *MTNR1B*, *IRS1*, *GIPR*, *S100A9*, *PSMD3*, *KCNMB3*, Caveolin-2, *NOTCH2*, zinc finger BED-type containing 3, *GLP1R*, *FTO*, melanocortin 4 receptor, *SLC22A1/OCT1*, *APOE*, *DRD2/ANKK1*, *APOA2*, *CETP*, *PPAR-γ*, and *ApoB*, which have been analyzed using single and cumulative approaches. Moreover, some genetic polymorphisms have been identified as putative biomarkers of individual responses to energy-

restricted nutritional prescriptions aimed to glucose control including those located in *SCD*, *PPM1K*, *FTO*, *TCF7L2*, *GIPR*, *IRS1*, *DHCR7*, *PCSK7*, *ADRB3*, *BDNF*, *TNFA*, *UCP3*, *ADIPOQ*, *RETN*, *MTNR1B*, *APOA5*, and *FABP2* genes. Furthermore, some genotype-based dietary strategies have been developed for improving T2DM control in comparison to general lifestyle recommendations for all people. However, more research is needed in order to expand and confirm these findings in other populations less explored such as Latin Americans and Africans considering some sources of variability (*i.e.*, allele frequency, quantitative trait locus, and gender influence) incorporating the assessment of the role of food bioactive compounds and micronutrients in prospective dietary interventions. In any case, the analysis of the genetic make-up may help to decipher new paradigms in the pathophysiology of T2DM as well as offer further opportunities to personalize the screening, prevention, diagnosis, management, and prognosis of T2DM.

FOOTNOTES

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REFERENCES

1 **Galicia-Garcia U**, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, Ostolaza H, Martín C. Pathophysiology of Type 2 Diabetes Mellitus. *Int J Mol Sci* 2020; **21** [PMID: 32872570 DOI: 10.3390/ijms21176275]

2 **Khan MAB**, Hashim MJ, King JK, Govender RD, Mustafa H, Al Kaabi J. Epidemiology of Type 2 Diabetes - Global Burden of Disease and Forecasted Trends. *J Epidemiol Glob Health* 2020; **10**: 107-111 [PMID: 32175717 DOI: 10.2991/jegh.k.191028.001]

3 **Laakso M**, Fernandes Silva L. Genetics of Type 2 Diabetes: Past, Present, and Future. *Nutrients* 2022; **14** [PMID: 35956377 DOI: 10.3390/nu14153201]

4 **Kaul N**, Ali S. Genes, Genetics, and Environment in Type 2 Diabetes: Implication in Personalized Medicine. *DNA Cell Biol* 2016; **35**: 1-12 [PMID: 26495765 DOI: 10.1089/dna.2015.2883]

5 **Mambiya M**, Shang M, Wang Y, Li Q, Liu S, Yang L, Zhang Q, Zhang K, Liu M, Nie F, Zeng F, Liu W. The Play of Genes and Non-genetic Factors on Type 2 Diabetes. *Front Public Health* 2019; **7**: 349 [PMID: 31803711 DOI: 10.3389/fpubh.2019.00349]

6 **Shoily SS**, Ahsan T, Fatema K, Sajib AA. Common genetic variants and pathways in diabetes and associated complications and vulnerability of populations with different ethnic origins. *Sci Rep* 2021; **11**: 7504 [PMID: 33820928 DOI: 10.1038/s41598-021-86801-2]

7 **Sikhayeva N**, Iskakova A, Saigi-Morgui N, Zholdybaeva E, Eap CB, Ramanculov E. Association between 28 single nucleotide polymorphisms and type 2 diabetes mellitus in the Kazakh population: a case-control study. *BMC Med Genet* 2017; **18**: 76 [PMID: 28738793 DOI: 10.1186/s12881-017-0443-2]

8 **Chen M**, Zhang X, Fang Q, Wang T, Li T, Qiao H. Three single nucleotide polymorphisms associated with type 2 diabetes mellitus in a Chinese population. *Exp Ther Med* 2017; **13**: 121-126 [PMID: 28123479 DOI: 10.3892/etm.2016.3920]

9 **Hubacek JA**, Dlouha L, Adamkova V, Dlouha D, Pacal L, Kankova K, Galuska D, Lanska V, Veleba J, Pelikanova T. Genetic risk score is associated with T2DM and diabetes complications risks. *Gene* 2023; **849**: 146921 [PMID: 36174902 DOI: 10.1016/j.gene.2022.146921]

10 **Shitomi-Jones LM**, Akam L, Hunter D, Singh P, Mastana S. Genetic Risk Scores for the Determination of Type 2 Diabetes Mellitus (T2DM) in North India. *Int J Environ Res Public Health* 2023; **20** [PMID: 36834424 DOI: 10.3390/ijerph20043729]

11 **Duschek E**, Forer L, Schönherr S, Gieger C, Peters A, Kronenberg F, Grallert H, Lamina C. A polygenic and family risk score are both independently associated with risk of type 2 diabetes in a population-based study. *Sci Rep* 2023; **13**: 4805 [PMID: 36959271 DOI: 10.1038/s41598-023-31496-w]

12 **Ramos-Lopez O**, Milagro FI, Allayee H, Chmurzynska A, Choi MS, Curi R, De Caterina R, Ferguson LR, Goni L, Kang JX, Kohlmeier M, Marti A, Moreno LA, Pérusse L, Prasad C, Qi L, Reifen R, Riezu-Boj JI, San-Cristobal R, Santos JL, Martínez JA. Guide for Current Nutrigenetic, Nutrigenomic, and Nutriepigenetic Approaches for Precision Nutrition Involving the Prevention and Management of Chronic Diseases Associated with Obesity. *J Nutrigenet Nutrigenomics* 2017; **10**: 43-62 [PMID: 28689206 DOI: 10.1159/000477729]

13 **Ortega Á**, Berná G, Rojas A, Martín F, Soria B. Gene-Diet Interactions in Type 2 Diabetes: The Chicken and Egg Debate. *Int J Mol Sci* 2017; **18** [PMID: 28574454 DOI: 10.3390/ijms18061188]

14 **Dietrich S**, Jacobs S, Zheng JS, Meidtner K, Schwingshackl L, Schulze MB. Gene-lifestyle interaction on risk of type 2 diabetes: A systematic review. *Obes Rev* 2019; **20**: 1557-1571 [PMID: 31478326 DOI: 10.1111/obr.12921]

15 **Sorgini C**, Christensen J, Parnell L, Tucker K, Ordovas JM, Lai CQ. Predicting Type 2 Diabetes Incidence with Genome-wide Gene-gene and

Gene-diet Interactions (OR31-08-19). *Curr Dev Nutr* 2019; **3**: nzz037.OR31-08 [DOI: 10.1093/cdn/nzz037.OR31-08-19]

16 **Ericson U**, Hindy G, Drake I, Schulz CA, Brunkwall L, Hellstrand S, Almgren P, Orho-Melander M. Dietary and genetic risk scores and incidence of type 2 diabetes. *Genes Nutr* 2018; **13**: 13 [PMID: 29796113 DOI: 10.1186/s12263-018-0599-1]

17 **Ramos-Lopez O**, Riezu-Boj JI, Milagro FI, Cuervo M, Goni L, Martinez JA. Interplay of an Obesity-Based Genetic Risk Score with Dietary and Endocrine Factors on Insulin Resistance. *Nutrients* 2019; **12** [PMID: 31877696 DOI: 10.3390/nu12010033]

18 **Alsulami S**, Cruvinel NT, da Silva NR, Antoneli AC, Lovegrove JA, Horst MA, Vimaleswaran KS. Effect of dietary fat intake and genetic risk on glucose and insulin-related traits in Brazilian young adults. *J Diabetes Metab Disord* 2021; **20**: 1337-1347 [PMID: 34900785 DOI: 10.1007/s40200-021-00863-7]

19 **Alathari BE**, Nyakotey DA, Bawah AM, Lovegrove JA, Annan RA, Ellahi B, Vimaleswaran KS. Interactions between Vitamin D Genetic Risk and Dietary Factors on Metabolic Disease-Related Outcomes in Ghanaian Adults. *Nutrients* 2022; **14** [PMID: 35807945 DOI: 10.3390/nu14132763]

20 **Hong KW**, Kim SH, Zhang X, Park S. Interactions among the variants of insulin-related genes and nutrients increase the risk of type 2 diabetes. *Nutr Res* 2018; **51**: 82-92 [PMID: 29673546 DOI: 10.1016/j.nutres.2017.12.012]

21 **Choi Y**, Kwon HK, Park S. Polygenic Variants Linked to Oxidative Stress and the Antioxidant System Are Associated with Type 2 Diabetes Risk and Interact with Lifestyle Factors. *Antioxidants (Basel)* 2023; **12** [PMID: 37372010 DOI: 10.3390/antiox12061280]

22 **Wang W**, Jiang H, Zhang Z, Duan W, Han T, Sun C. Interaction between dietary branched-chain amino acids and genetic risk score on the risk of type 2 diabetes in Chinese. *Genes Nutr* 2021; **16**: 4 [PMID: 33663374 DOI: 10.1186/s12263-021-00684-6]

23 **Daily JW**, Liu M, Park S. High genetic risk scores of SLIT3, PLEKHA5 and PPP2R2C variants increased insulin resistance and interacted with coffee and caffeine consumption in middle-aged adults. *Nutr Metab Cardiovasc Dis* 2019; **29**: 79-89 [PMID: 30454882 DOI: 10.1016/j.numecd.2018.09.009]

24 **Yu H**, Wang T, Zhang R, Yan J, Jiang F, Li S, Jia W, Hu C. Alcohol consumption and its interaction with genetic variants are strongly associated with the risk of type 2 diabetes: a prospective cohort study. *Nutr Metab (Lond)* 2019; **16**: 64 [PMID: 31528183 DOI: 10.1186/s12986-019-0396-x]

25 **López-Portillo ML**, Huidobro A, Tobar-Calfucoy E, Yáñez C, Retamales-Ortega R, Garrido-Tapia M, Acevedo J, Paredes F, Cid-Ossandon V, Ferreccio C, Verdugo RA. The Association between Fasting Glucose and Sugar Sweetened Beverages Intake Is Greater in Latin Americans with a High Polygenic Risk Score for Type 2 Diabetes Mellitus. *Nutrients* 2021; **14** [PMID: 35010944 DOI: 10.3390/nu14010069]

26 **Jia X**, Xuan L, Dai H, Zhu W, Deng C, Wang T, Li M, Zhao Z, Xu Y, Lu J, Bi Y, Wang W, Chen Y, Xu M, Ning G. Fruit intake, genetic risk and type 2 diabetes: a population-based gene-diet interaction analysis. *Eur J Nutr* 2021; **60**: 2769-2779 [PMID: 33399975 DOI: 10.1007/s00394-020-02449-0]

27 **Alsulami S**, Bodhini D, Sudha V, Shanthi Rani CS, Pradeepa R, Anjana RM, Radha V, Lovegrove JA, Gayathri R, Mohan V, Vimaleswaran KS. Lower Dietary Intake of Plant Protein Is Associated with Genetic Risk of Diabetes-Related Traits in Urban Asian Indian Adults. *Nutrients* 2021; **13** [PMID: 34578944 DOI: 10.3390/nu13093064]

28 **Kim DS**, Kim BC, Daily JW, Park S. High genetic risk scores for impaired insulin secretory capacity doubles the risk for type 2 diabetes in Asians and is exacerbated by Western-type diets. *Diabetes Metab Res Rev* 2018; **34** [PMID: 29048714 DOI: 10.1002/dmrr.2944]

29 **Hur HJ**, Yang HJ, Kim MJ, Lee KH, Kim MS, Park S. Association of Polygenic Variants with Type 2 Diabetes Risk and Their Interaction with Lifestyles in Asians. *Nutrients* 2022; **14** [PMID: 35956399 DOI: 10.3390/nu14153222]

30 **Park S**. Association of polygenic risk scores for insulin resistance risk and their interaction with a plant-based diet, especially fruits, vitamin C, and flavonoid intake, in Asian adults. *Nutrition* 2023; **111**: 112007 [PMID: 37116407 DOI: 10.1016/j.nut.2023.112007]

31 **Khamis AM**. Pathophysiology, Diagnostic Criteria, and Approaches to Type 2 Diabetes Remission. *Cureus* 2023; **15**: e33908 [PMID: 36819346 DOI: 10.7759/cureus.33908]

32 **Zepeda-Carrillo EA**, Ramos-Lopez O, Martínez-López E, Barrón-Cabrera E, Bernal-Pérez JA, Velasco-González LE, Rangel-Rios E, Bustamante Martínez JF, Torres-Valadez R. Effect of Metformin on Glycemic Control Regarding Carriers of the SLC22A1/OCT1 (rs628031) Polymorphism and Its Interactions with Dietary Micronutrients in Type 2 Diabetes. *Diabetes Metab Syndr Obes* 2022; **15**: 1771-1784 [PMID: 35711690 DOI: 10.2147/DMSO.S354579]

33 **Torres-Valadez R**, Ramos-Lopez O, Frías Delgadillo KJ, Flores-García A, Rojas Carrillo E, Aguiar-García P, Bernal Pérez JA, Martinez-Lopez E, Martínez JA, Zepeda-Carrillo EA. Impact of APOE Alleles-by-Diet Interactions on Glycemic and Lipid Features- A Cross-Sectional Study of a Cohort of Type 2 Diabetes Patients from Western Mexico: Implications for Personalized Medicine. *Pharmgenomics Pers Med* 2020; **13**: 655-663 [PMID: 33273843 DOI: 10.2147/PGPM.S277952]

34 **Ramos-Lopez O**, Mejia-Godoy R, Frías-Delgadillo KJ, Torres-Valadez R, Flores-García A, Sánchez-Enríquez S, Aguiar-García P, Martínez-López E, Zepeda-Carrillo EA. Interactions between DRD2/ANKK1 TaqIA Polymorphism and Dietary Factors Influence Plasma Triglyceride Concentrations in Diabetic Patients from Western Mexico: A Cross-sectional Study. *Nutrients* 2019; **11** [PMID: 31766642 DOI: 10.3390/nu11122863]

35 **Naeini Z**, Abaj F, Rafiee M, Koohdani F. Interactions of BDNF Val66met and dietary indices in relation to metabolic markers among patient with type 2 diabetes mellitus: a cross-sectional study. *J Health Popul Nutr* 2023; **42**: 34 [PMID: 37072879 DOI: 10.1186/s41043-023-00375-5]

36 **Abaj F**, Esmaeily Z, Naeini Z, Rafiee M, Koohdani F. Dietary acid load modifies the effects of ApoA2-265 T > C polymorphism on lipid profile and serum leptin and ghrelin levels among type 2 diabetic patients. *BMC Endocr Disord* 2022; **22**: 190 [PMID: 35883173 DOI: 10.1186/s12902-022-01083-7]

37 **Abaj F**, Rafiee M, Koohdani F. Interaction between CETP polymorphism and dietary insulin index and load in relation to cardiovascular risk factors in diabetic adults. *Sci Rep* 2021; **11**: 15906 [PMID: 34354158 DOI: 10.1038/s41598-021-95359-y]

38 **Abaj F**, Rafiee M, Koohdani F. A personalised diet approach study: Interaction between PPAR-γ Pro12Ala and dietary insulin indices on metabolic markers in diabetic patients. *J Hum Nutr Diet* 2022; **35**: 663-674 [PMID: 35560467 DOI: 10.1111/jhn.13033]

39 **Abaj F**, Koohdani F. Macronutrient intake modulates impact of EcoRI polymorphism of ApoB gene on lipid profile and inflammatory markers in patients with type 2 diabetes. *Sci Rep* 2022; **12**: 10504 [PMID: 35732646 DOI: 10.1038/s41598-022-13330-x]

40 **Rafiee M**, Sotoudeh G, Djalali M, Alvandi E, Eshraghian M, Javadi F, Doostan F, Koohdani F. The interaction between apolipoprotein B insertion/deletion polymorphism and macronutrient intake on lipid profile and serum leptin and ghrelin levels in type 2 diabetes mellitus patients. *Eur J Nutr* 2019; **58**: 1055-1065 [PMID: 29374794 DOI: 10.1007/s00394-018-1621-5]

41 **Rafiee M**, Sotoudeh G, Djalali M, Alvandi E, Eshraghian M, Sojoudi F, Koohdani F. Dietary ω-3 polyunsaturated fatty acid intake modulates impact of Insertion/Deletion polymorphism of ApoB gene on obesity risk in type 2 diabetic patients. *Nutrition* 2016; **32**: 1110-1115 [PMID: 27210509 DOI: 10.1016/j.nut.2016.03.012]

42 **Mutch DM**, Lowry DE, Roth M, Sihag J, Hammad SS, Taylor CG, Zahradka P, Connelly PW, West SG, Bowen K, Kris-Etherton PM, Lamarche B, Couture P, Guay V, Jenkins DJA, Eck P, Jones PJH. Polymorphisms in the stearoyl-CoA desaturase gene modify blood glucose response to dietary oils varying in MUFA content in adults with obesity. *Br J Nutr* 2022; **127**: 503-512 [PMID: 33829984 DOI: 10.1017/S0007114521001264]

43 **Goni L**, Qi L, Cuervo M, Milagro FI, Saris WH, MacDonald IA, Langin D, Astrup A, Arner P, Oppert JM, Svendstrup M, Blaak EE, Sørensen TI, Hansen T, Martínez JA. Effect of the interaction between diet composition and the PPM1K genetic variant on insulin resistance and β cell function markers during weight loss: results from the Nutrient Gene Interactions in Human Obesity: implications for dietary guidelines (NUGENOB) randomized trial. *Am J Clin Nutr* 2017; **106**: 902-908 [PMID: 28768654 DOI: 10.3945/ajcn.117.156281]

44 **Grau K**, Cauchi S, Holst C, Astrup A, Martinez JA, Saris WH, Blaak EE, Oppert JM, Arner P, Rössner S, Macdonald IA, Klimcakova E, Langin D, Pedersen O, Froguel P, Sørensen TI. TCF7L2 rs7903146-macronutrient interaction in obese individuals' responses to a 10-wk randomized hypoenergetic diet. *Am J Clin Nutr* 2010; **91**: 472-479 [PMID: 20032493 DOI: 10.3945/ajcn.2009.27947]

45 **Qi Q**, Bray GA, Hu FB, Sacks FM, Qi L. Weight-loss diets modify glucose-dependent insulinotropic polypeptide receptor rs2287019 genotype effects on changes in body weight, fasting glucose, and insulin resistance: the Preventing Overweight Using Novel Dietary Strategies trial. *Am J Clin Nutr* 2012; **95**: 506-513 [PMID: 22237064 DOI: 10.3945/ajcn.111.025270]

46 **Qi Q**, Bray GA, Smith SR, Hu FB, Sacks FM, Qi L. Insulin receptor substrate 1 gene variation modifies insulin resistance response to weight-loss diets in a 2-year randomized trial: the Preventing Overweight Using Novel Dietary Strategies (POUNDS LOST) trial. *Circulation* 2011; **124**: 563-571 [PMID: 21747052 DOI: 10.1161/CIRCULATIONAHA.111.025767]

47 **Qi Q**, Zheng Y, Huang T, Rood J, Bray GA, Sacks FM, Qi L. Vitamin D metabolism-related genetic variants, dietary protein intake and improvement of insulin resistance in a 2 year weight-loss trial: POUNDS Lost. *Diabetologia* 2015; **58**: 2791-2799 [PMID: 26416604 DOI: 10.1007/s00125-015-3750-1]

48 **Huang T**, Huang J, Qi Q, Li Y, Bray GA, Rood J, Sacks FM, Qi L. PCSK7 genotype modifies effect of a weight-loss diet on 2-year changes of insulin resistance: the POUNDS LOST trial. *Diabetes Care* 2015; **38**: 439-444 [PMID: 25504030 DOI: 10.2337/dc14-0473]

49 **Zheng Y**, Huang T, Zhang X, Rood J, Bray GA, Sacks FM, Qi L. Dietary Fat Modifies the Effects of FTO Genotype on Changes in Insulin Sensitivity. *J Nutr* 2015; **145**: 977-982 [PMID: 25761503 DOI: 10.3945/jn.115.210005]

50 **de Luis DA**, Aller R, Izaola O, de la Fuente B, Romero E. GENETIC VARIATION IN THE BETA-3-ADRENORECEPTOR GENE (TRP64ARG POLYMORPHISM) AND THEIR INFLUENCE ON ANTHROPOMETRIC PARAMETERS AND INSULIN RESISTANCE AFTER A HIGH PROTEIN/LOW CARBOHYDRATE VERSUS A STANDARD HYPOCALORIC DIET. *Nutr Hosp* 2015; **32**: 487-493 [PMID: 26268075 DOI: 10.3305/nh.2015.32.2.9293]

51 **de Luis DA**, Romero E, Izaola O, Primo D, Aller R. Cardiovascular Risk Factors and Insulin Resistance after Two Hypocaloric Diets with Different Fat Distribution in Obese Subjects: Effect of the rs10767664 Gene Variant in Brain-Derived Neurotrophic Factor. *J Nutrigenet Nutrigenomics* 2017; **10**: 163-171 [PMID: 29339649 DOI: 10.1159/000485248]

52 **de Luis DA**, Aller R, Izaola O, Gonzalez Sagrado M, Conde R. Role of G308 promoter variant of tumor necrosis factor alpha gene on weight loss and metabolic parameters after a high monounsaturated versus a high polyunsaturated fat hypocaloric diets. *Med Clin (Barc)* 2013; **141**: 189-193 [PMID: 23601741 DOI: 10.1016/j.medcli.2012.12.021]

53 **de Luis DA**, Aller R, Izaola O, Romero E. Effect of -55CT Polymorphism of UCP3 on Insulin Resistance and Cardiovascular Risk Factors after a High Protein/Low Carbohydrate versus a Standard Hypocaloric Diet. *Ann Nutr Metab* 2016; **68**: 157-163 [PMID: 26848765 DOI: 10.1159/000444150]

54 **de Luis DA**, Izaola O, Primo D, Gómez-Hoyos E, Ortola A, López-Gómez JJ, Aller R. Role of rs1501299 variant in the adiponectin gene on total adiponectin levels, insulin resistance and weight loss after a Mediterranean hypocaloric diet. *Diabetes Res Clin Pract* 2019; **148**: 262-267 [PMID: 29154912 DOI: 10.1016/j.diabres.2017.11.007]

55 **de Luis D**, Aller R, Izaola O, Primo D. Role of the rs10401670 variant in the resistin gene on the metabolic response after weight loss secondary to a high-fat hypocaloric diet with a Mediterranean pattern. *J Hum Nutr Diet* 2022; **35**: 722-730 [PMID: 34907604 DOI: 10.1111/jhn.12975]

56 **de Luis DA**, Izaola O, Primo D, Aller R. Association of the rs10830963 polymorphism in melatonin receptor type 1B (MTNR1B) with metabolic response after weight loss secondary to a hypocaloric diet based in Mediterranean style. *Clin Nutr* 2018; **37**: 1563-1568 [PMID: 28869073 DOI: 10.1016/j.clnu.2017.08.015]

57 **Wang T**, Huang T, Zheng Y, Rood J, Bray GA, Sacks FM, Qi L. Genetic variation of fasting glucose and changes in glycemia in response to 2-year weight-loss diet intervention: the POUNDS LOST trial. *Int J Obes (Lond)* 2016; **40**: 1164-1169 [PMID: 27113490 DOI: 10.1038/ijo.2016.41]

58 **Huang T**, Ley SH, Zheng Y, Wang T, Bray GA, Sacks FM, Qi L. Genetic susceptibility to diabetes and long-term improvement of insulin resistance and β cell function during weight loss: the Preventing Overweight Using Novel Dietary Strategies (POUNDS LOST) trial. *Am J Clin Nutr* 2016; **104**: 198-204 [PMID: 27281308 DOI: 10.3945/ajcn.115.121186]

59 **Han L**, Ma W, Sun D, Heianza Y, Wang T, Zheng Y, Huang T, Duan D, Bray JGA, Champagne CM, Sacks FM, Qi L. Genetic variation of habitual coffee consumption and glycemic changes in response to weight-loss diet intervention: the Preventing Overweight Using Novel Dietary Strategies (POUNDS LOST) trial. *Am J Clin Nutr* 2017; **106**: 1321-1326 [PMID: 28931532 DOI: 10.3945/ajcn.117.156232]

60 **Kim M**, Chae JS, Kim M, Lee SH, Lee JH. Effects of a 3-year dietary intervention on age-related changes in triglyceride and apolipoprotein A-V levels in patients with impaired fasting glucose or new-onset type 2 diabetes as a function of the APOA5 -1131 T > C polymorphism. *Nutr J* 2014; **13**: 40 [PMID: 24775272 DOI: 10.1186/1475-2891-13-40]

61 **Liu PJ**, Liu YP, Qin HK, Xing T, Li SS, Bao YY. Effects of polymorphism in FABP2 Ala54Thr on serum lipids and glycemic control in low glycemic index diets are associated with gender among Han Chinese with type 2 diabetes mellitus. *Diabetes Metab Syndr Obes* 2019; **12**: 413-421 [PMID: 30988637 DOI: 10.2147/DMSO.S196738]

62 **Hosseini S**, Alipour M, Zakerkish M, Cheraghian B, Ghandil P. Effects of epigallocatechin gallate on total antioxidant capacity, biomarkers of systemic low-grade inflammation and metabolic risk factors in patients with type 2 diabetes mellitus: the role of FTO-rs9939609 polymorphism. *Arch Med Sci* 2021; **17**: 1722-1729 [PMID: 34900054 DOI: 10.5114/aoms.2020.95903]

63 **Vranceanu M**, Pickering C, Filip L, Pralea IE, Sundaram S, Al-Saleh A, Popa DS, Grimaldi KA. A comparison of a ketogenic diet with a LowGI/nutrigenetic diet over 6 months for weight loss and 18-month follow-up. *BMC Nutr* 2020; **6**: 53 [PMID: 32983551 DOI: 10.1186/s40795-020-00370-7]

64 **Ojeda-Granados C**, Panduro A, Rivera-Iñiguez I, Sepúlveda-Villegas M, Roman S. A Regionalized Genome-Based Mexican Diet Improves Anthropometric and Metabolic Parameters in Subjects at Risk for Obesity-Related Chronic Diseases. *Nutrients* 2020; **12** [PMID: 32121184

DOI: 10.3390/nu12030645]
65 **Frankwich KA**, Egnatios J, Kenyon ML, Rutledge TR, Liao PS, Gupta S, Herbst KL, Zarrinpar A. Differences in Weight Loss Between Persons on Standard Balanced vs Nutrigenetic Diets in a Randomized Controlled Trial. *Clin Gastroenterol Hepatol* 2015; **13**: 1625-1632.e1 [PMID: 25769412 DOI: 10.1016/j.cgh.2015.02.044]
66 **Gkouskou K**, Lazou E, Skoufas E, Eliopoulos AG. Genetically Guided Mediterranean Diet for the Personalized Nutritional Management of Type 2 Diabetes Mellitus. *Nutrients* 2021; **13** [PMID: 33503923 DOI: 10.3390/nu13020355]
67 **Gkouskou KK**, Grammatikopoulou MG, Lazou E, Sanoudou D, Goulis DG, Eliopoulos AG. Genetically-Guided Medical Nutrition Therapy in Type 2 Diabetes Mellitus and Pre-diabetes: A Series of n-of-1 Superiority Trials. *Front Nutr* 2022; **9**: 772243 [PMID: 35265654 DOI: 10.3389/fnut.2022.772243]
68 **Waxler JL**, O'Brien KE, Delahanty LM, Meigs JB, Florez JC, Park ER, Pober BR, Grant RW. Genetic counseling as a tool for type 2 diabetes prevention: a genetic counseling framework for common polygenetic disorders. *J Genet Couns* 2012; **21**: 684-691 [PMID: 22302620 DOI: 10.1007/s10897-012-9486-x]
69 **Grant RW**, O'Brien KE, Waxler JL, Vassy JL, Delahanty LM, Bissett LG, Green RC, Stember KG, Guiducci C, Park ER, Florez JC, Meigs JB. Personalized genetic risk counseling to motivate diabetes prevention: a randomized trial. *Diabetes Care* 2013; **36**: 13-19 [PMID: 22933432 DOI: 10.2337/dc12-0884]
70 **Voils CI**, Coffman CJ, Grubber JM, Edelman D, Sadeghpour A, Maciejewski ML, Bolton J, Cho A, Ginsburg GS, Yancy WS Jr. Does Type 2 Diabetes Genetic Testing and Counseling Reduce Modifiable Risk Factors? A Randomized Controlled Trial of Veterans. *J Gen Intern Med* 2015; **30**: 1591-1598 [PMID: 25876740 DOI: 10.1007/s11606-015-3315-5]
71 **Wang S**, Yong H, He XD. Multi-omics: Opportunities for research on mechanism of type 2 diabetes mellitus. *World J Diabetes* 2021; **12**: 1070-1080 [PMID: 34326955 DOI: 10.4239/wjd.v12.i7.1070]
72 **Raciti GA**, Nigro C, Longo M, Parrillo L, Miele C, Formisano P, Béguinot F. Personalized medicine and type 2 diabetes: lesson from epigenetics. *Epigenomics* 2014; **6**: 229-238 [PMID: 24811791 DOI: 10.2217/epi.14.10]
73 **Qie R**, Han M, Huang S, Li Q, Liu L, Zhang D, Cheng C, Zhao Y, Liu D, Qin P, Guo C, Zhou Q, Tian G, Zhang Y, Wu X, Wu Y, Li Y, Yang X, Feng Y, Hu F, Zhang M, Hu D, Lu J. Association of TCF7L2 gene polymorphisms, methylation, and gene-environment interaction with type 2 diabetes mellitus risk: A nested case-control study in the Rural Chinese Cohort Study. *J Diabetes Complications* 2021; **35**: 107829 [PMID: 33419631 DOI: 10.1016/j.jdiacomp.2020.107829]
74 **Gong W**, Xiao D, Ming G, Yin J, Zhou H, Liu Z. Type 2 diabetes mellitus-related genetic polymorphisms in microRNAs and microRNA target sites. *J Diabetes* 2014; **6**: 279-289 [PMID: 24606011 DOI: 10.1111/1753-0407.12143]
75 **Safari-Alighiarloo N**, Emami Z, Rezaei-Tavirani M, Alaei-Shahmiri F, Razavi S. Gut Microbiota and Their Associated Metabolites in Diabetes: A Cross Talk Between Host and Microbes-A Review. *Metab Syndr Relat Disord* 2023; **21**: 3-15 [PMID: 36301254 DOI: 10.1089/met.2022.0049]
76 **Bailén M**, Tabone M, Bressa C, Lominchar MGM, Larrosa M, González-Soltero R. Unraveling Gut Microbiota Signatures Associated with PPARD and PARGC1A Genetic Polymorphisms in a Healthy Population. *Genes (Basel)* 2022; **13** [PMID: 35205333 DOI: 10.3390/genes13020289]
77 **Kwan SY**, Sabotta CM, Joon A, Wei P, Petty LE, Below JE, Wu X, Zhang J, Jenq RR, Hawk ET, McCormick JB, Fisher-Hoch SP, Beretta L. Gut Microbiome Alterations Associated with Diabetes in Mexican Americans in South Texas. *mSystems* 2022; **7**: e0003322 [PMID: 35477306 DOI: 10.1128/msystems.00033-22]
78 **Chen ZZ**, Gerszten RE. Metabolomics and Proteomics in Type 2 Diabetes. *Circ Res* 2020; **126**: 1613-1627 [PMID: 32437301 DOI: 10.1161/CIRCRESAHA.120.315898]
79 **Ghanbari F**, Yazdanpanah N, Yazdanpanah M, Richards JB, Manousaki D. Connecting Genomics and Proteomics to Identify Protein Biomarkers for Adult and Youth-Onset Type 2 Diabetes: A Two-Sample Mendelian Randomization Study. *Diabetes* 2022; **71**: 1324-1337 [PMID: 35234851 DOI: 10.2337/db21-1046]
80 **Beijer K**, Nowak C, Sundström J, Ärnlöv J, Fall T, Lind L. In search of causal pathways in diabetes: a study using proteomics and genotyping data from a cross-sectional study. *Diabetologia* 2019; **62**: 1998-2006 [PMID: 31446444 DOI: 10.1007/s00125-019-4960-8]
81 **Rohde PD**, Nyegaard M, Kjolby M, Sørensen P. Multi-Trait Genomic Risk Stratification for Type 2 Diabetes. *Front Med (Lausanne)* 2021; **8**: 711208 [PMID: 34568370 DOI: 10.3389/fmed.2021.711208]
82 **Timasheva Y**, Balkhiyarova Z, Avzaletdinova D, Rassoleeva I, Morugova TV, Korytina G, Prokopenko I, Kochetova O. Integrating Common Risk Factors with Polygenic Scores Improves the Prediction of Type 2 Diabetes. *Int J Mol Sci* 2023; **24** [PMID: 36674502 DOI: 10.3390/ijms24020984]
83 **Ouhaibi-Djellouli H**, Mediene-Benchekor S, Lardjam-Hetraf SA, Hamani-Medjaoui I, Meroufel DN, Boulenouar H, Hermant X, Saidi-Mehtar N, Amouyel P, Houti L, Goumidi L, Meirhaeghe A. The TCF7L2 rs7903146 polymorphism, dietary intakes and type 2 diabetes risk in an Algerian population. *BMC Genet* 2014; **15**: 134 [PMID: 25491720 DOI: 10.1186/s12863-014-0134-3]
84 **Hindy G**, Sonestedt E, Ericson U, Jing XJ, Zhou Y, Hansson O, Renström E, Wirfält E, Orho-Melander M. Role of TCF7L2 risk variant and dietary fibre intake on incident type 2 diabetes. *Diabetologia* 2012; **55**: 2646-2654 [PMID: 22782288 DOI: 10.1007/s00125-012-2634-x]
85 **Wirström T**, Hilding A, Gu HF, Östenson CG, Björklund A. Consumption of whole grain reduces risk of deteriorating glucose tolerance, including progression to prediabetes. *Am J Clin Nutr* 2013; **97**: 179-187 [PMID: 23235198 DOI: 10.3945/ajcn.112.045583]
86 **Bauer W**, Adamska-Patruno E, Krasowska U, Moroz M, Fiedorczuk J, Czajkowski P, Bielska D, Gorska M, Kretowski A. Dietary Macronutrient Intake May Influence the Effects of TCF7L2 rs7901695 Genetic Variants on Glucose Homeostasis and Obesity-Related Parameters: A Cross-Sectional Population-Based Study. *Nutrients* 2021; **13** [PMID: 34200102 DOI: 10.3390/nu13061936]
87 **Shin D**, Lee KW. Dietary carbohydrates interact with AMY1 polymorphisms to influence the incidence of type 2 diabetes in Korean adults. *Sci Rep* 2021; **11**: 16788 [PMID: 34408213 DOI: 10.1038/s41598-021-96257-z]
88 **Lee KW**, Shin D. Interactions between Bitter Taste Receptor Gene Variants and Dietary Intake Are Associated with the Incidence of Type 2 Diabetes Mellitus in Middle-Aged and Older Korean Adults. *Int J Mol Sci* 2023; **24** [PMID: 36768516 DOI: 10.3390/ijms24032199]
89 **Lamri A**, Abi Khalil C, Jaziri R, Velho G, Lantieri O, Vol S, Froguel P, Balkau B, Marre M, Fumeron F. Dietary fat intake and polymorphisms at the PPARG locus modulate BMI and type 2 diabetes risk in the D.E.S.I.R. prospective study. *Int J Obes (Lond)* 2012; **36**: 218-224 [PMID: 21540831 DOI: 10.1038/ijo.2011.91]
90 **Choi WJ**, Jin HS, Kim SS, Shin D. Dietary Protein and Fat Intake Affects Diabetes Risk with CDKAL1 Genetic Variants in Korean Adults. *Int J Mol Sci* 2020; **21** [PMID: 32764395 DOI: 10.3390/ijms21165607]
91 **Lee JK**, Kim K, Ahn Y, Yang M, Lee JE. Habitual coffee intake, genetic polymorphisms, and type 2 diabetes. *Eur J Endocrinol* 2015; **172**:

595-601 [PMID: 25755232 DOI: 10.1530/EJE-14-0805]
92 **Park S**, Kim BC, Kang S. Interaction effect of PGC-1α rs10517030 variants and energy intake in the risk of type 2 diabetes in middle-aged adults. *Eur J Clin Nutr* 2017; **71**: 1442-1448 [PMID: 28488691 DOI: 10.1038/ejcn.2017.68]
93 **Daily JW**, Park S. Interaction of BDNF rs6265 variants and energy and protein intake in the risk for glucose intolerance and type 2 diabetes in middle-aged adults. *Nutrition* 2017; **33**: 187-194 [PMID: 27553771 DOI: 10.1016/j.nut.2016.07.001]
94 **Park S**, Zhang X, Lee NR, Jin HS. TRPV1 Gene Polymorphisms Are Associated with Type 2 Diabetes by Their Interaction with Fat Consumption in the Korean Genome Epidemiology Study. *J Nutrigenet Nutrigenomics* 2016; **9**: 47-61 [PMID: 27287034 DOI: 10.1159/000446499]
95 **Lee YJ**, Lee H, Jang HB, Yoo MG, Im S, Koo SK, Lee HJ. The potential effects of HECTD4 variants on fasting glucose and triglyceride levels in relation to prevalence of type 2 diabetes based on alcohol intake. *Arch Toxicol* 2022; **96**: 2487-2499 [PMID: 35713687 DOI: 10.1007/s00204-022-03325-y]
96 **Shen L**, Wang Z, Zang J, Liu H, Lu Y, He X, Wu C, Su J, Zhu Z. The Association between Dietary Iron Intake, SNP of the MTNR1B rs10830963, and Glucose Metabolism in Chinese Population. *Nutrients* 2023; **15** [PMID: 37111205 DOI: 10.3390/nu15081986]
97 **Lopez-Minguez J**, Saxena R, Bandín C, Scheer FA, Garaulet M. Late dinner impairs glucose tolerance in MTNR1B risk allele carriers: A randomized, cross-over study. *Clin Nutr* 2018; **37**: 1133-1140 [PMID: 28455106 DOI: 10.1016/j.clnu.2017.04.003]
98 **Garaulet M**, Lopez-Minguez J, Dashti HS, Vetter C, Hernández-Martínez AM, Pérez-Ayala M, Baraza JC, Wang W, Florez JC, Scheer FAJL, Saxena R. Interplay of Dinner Timing and MTNR1B Type 2 Diabetes Risk Variant on Glucose Tolerance and Insulin Secretion: A Randomized Crossover Trial. *Diabetes Care* 2022; **45**: 512-519 [PMID: 35015083 DOI: 10.2337/dc21-1314]
99 **Ericson U**, Rukh G, Stojkovic I, Sonestedt E, Gullberg B, Wirfält E, Wallström P, Orho-Melander M. Sex-specific interactions between the IRS1 polymorphism and intakes of carbohydrates and fat on incident type 2 diabetes. *Am J Clin Nutr* 2013; **97**: 208-216 [PMID: 23221578 DOI: 10.3945/ajcn.112.046474]
100 **Zheng JS**, Arnett DK, Parnell LD, Smith CE, Li D, Borecki IB, Tucker KL, Ordovás JM, Lai CQ. Modulation by dietary fat and carbohydrate of IRS1 association with type 2 diabetes traits in two populations of different ancestries. *Diabetes Care* 2013; **36**: 2621-2627 [PMID: 23596181 DOI: 10.2337/dc12-2607]
101 **Sonestedt E**, Lyssenko V, Ericson U, Gullberg B, Wirfält E, Groop L, Orho-Melander M. Genetic variation in the glucose-dependent insulinotropic polypeptide receptor modifies the association between carbohydrate and fat intake and risk of type 2 diabetes in the Malmo Diet and Cancer cohort. *J Clin Endocrinol Metab* 2012; **97**: E810-E818 [PMID: 22399504 DOI: 10.1210/jc.2011-2444]
102 **Blanco-Rojo R**, Delgado-Lista J, Lee YC, Lai CQ, Perez-Martinez P, Rangel-Zuñiga O, Smith CE, Hidalgo B, Alcala-Diaz JF, Gomez-Delgado F, Parnell LD, Arnett DK, Tucker KL, Lopez-Miranda J, Ordovas JM. Interaction of an S100A9 gene variant with saturated fat and carbohydrates to modulate insulin resistance in 3 populations of different ancestries. *Am J Clin Nutr* 2016; **104**: 508-517 [PMID: 27440084 DOI: 10.3945/ajcn.116.130898]
103 **Zheng JS**, Arnett DK, Parnell LD, Lee YC, Ma Y, Smith CE, Richardson K, Li D, Borecki IB, Ordovas JM, Tucker KL, Lai CQ. Genetic variants at PSMD3 interact with dietary fat and carbohydrate to modulate insulin resistance. *J Nutr* 2013; **143**: 354-361 [PMID: 23303871 DOI: 10.3945/jn.112.168401]
104 **Zheng JS**, Arnett DK, Parnell LD, Lee YC, Ma Y, Smith CE, Richardson K, Li D, Borecki IB, Tucker KL, Ordovás JM, Lai CQ. Polyunsaturated Fatty Acids Modulate the Association between PIK3CA-KCNMB3 Genetic Variants and Insulin Resistance. *PLoS One* 2013; **8**: e67394 [PMID: 23826284 DOI: 10.1371/journal.pone.0067394]
105 **Fisher E**, Schreiber S, Joost HG, Boeing H, Döring F. A two-step association study identifies CAV2 rs2270188 single nucleotide polymorphism interaction with fat intake in type 2 diabetes risk. *J Nutr* 2011; **141**: 177-181 [PMID: 21178094 DOI: 10.3945/jn.110.124206]
106 **Hindy G**, Mollet IG, Rukh G, Ericson U, Orho-Melander M. Several type 2 diabetes-associated variants in genes annotated to WNT signaling interact with dietary fiber in relation to incidence of type 2 diabetes. *Genes Nutr* 2016; **11**: 6 [PMID: 27551309 DOI: 10.1186/s12263-016-0524-4]
107 **Nishiya Y**, Daimon M, Mizushiri S, Murakami H, Tanabe J, Matsuhashi Y, Yanagimachi M, Tokuda I, Sawada K, Ihara K. Nutrient consumption-dependent association of a glucagon-like peptide-1 receptor gene polymorphism with insulin secretion. *Sci Rep* 2020; **10**: 16382 [PMID: 33009421 DOI: 10.1038/s41598-020-71853-7]
108 **Ortega-Azorín C**, Sorlí JV, Asensio EM, Coltell O, Martínez-González MÁ, Salas-Salvadó J, Covas MI, Arós F, Lapetra J, Serra-Majem L, Gómez-Gracia E, Fiol M, Sáez-Tormo G, Pintó X, Muñoz MA, Ros E, Ordovás JM, Estruch R, Corella D. Associations of the FTO rs9939609 and the MC4R rs17782313 polymorphisms with type 2 diabetes are modulated by diet, being higher when adherence to the Mediterranean diet pattern is low. *Cardiovasc Diabetol* 2012; **11**: 137 [PMID: 23130628 DOI: 10.1186/1475-2840-11-137]

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REVIEW

Emerging and multifaceted potential contributions of polyphenols in the management of type 2 diabetes mellitus

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Abstract

Type 2 diabetes mellitus (T2DM) is recognized as a serious public health concern with a considerable impact on human life, long-term health expenditures, and substantial health losses. In this context, the use of dietary polyphenols to prevent and manage T2DM is widely documented. These dietary compounds exert their beneficial effects through several actions, including the protection of pancreatic islet β-cell, the antioxidant capacities of these molecules, their effects on insulin secretion and actions, the regulation of intestinal microbiota, and their contribution to ameliorate diabetic complications, particularly those of vascular origin. In the present review, we intend to highlight these multifaceted actions and the molecular mechanisms by which these plant-derived secondary metabolites exert their beneficial effects on type 2 diabetes patients.

Key Words: Polyphenols; Antioxidants; Oxidative stress; Type 2 diabetes mellitus; Health benefits

Core Tip: At present, a compelling body of evidence suggests that dietary polyphenols may represent an important alternative source to the management of type 2 diabetes mellitus due to their multifaceted actions on glucose homeostasis as well as in attenuating many diabetes complications raised because of the hyperglycemic condition. Additionally, new data derived from either clinical trials or meta-analyses have started to figure out the usefulness of these bioactive compounds thus providing solid clinical shreds of evidence.

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INTRODUCTION

Diabetes mellitus (DM) is a heterogeneous group of chronic metabolic disorders characterized by hyperglycemia resulting from defects of insulin action, insulin secretion, or both[1]. This metabolic disease is a global health issue, which has been increasing from time to time and it is now considered as one of the most important disorders worldwide. According to International Diabetes Federation, 10.5% of adults of the world population are currently living with diabetes and this alarming indicator is predicted to rise to 11.3 % (643 million people) by 2030 and to 12.2 % (783 million) by 2045 [2].

Noteworthy, a considerable proportion of the world's burden of diabetes is caused by type 2 DM (T2DM). In this regard, T2DM is recognized as a serious public health concern with a considerable impact on human life and health expenditures[3]. The onset and progression of T2DM are determined by a complex pathophysiological basis where oxidative stress is a crucial contributor not only involved in the disease development but also to diabetes complications, particularly those associated with both microvascular (retinopathy, nephropathy, and neuropathy) and macrovascular complications (ischemic heart disease, peripheral vascular disease, and cerebrovascular disease[4].

Acute or chronic hyperglycemia upregulates reactive oxygen species (ROS) production in the mitochondrial electron transfer chain. This excessive production of superoxide mediates the downregulation of glyceraldehyde-3-phosphate dehydrogenase levels, which in turn activates the major pro-oxidative pathways involved in the pathogenesis of diabetes complications, such as the activation of protein kinase C, the polyol and hexosamine pathways, the formation of advanced glycation end products productions (AGEs), as well as the increased expression of the receptor for AGEs[5-7]. On the other hand, antioxidant mechanisms are diminished in diabetic patients, which may further augment oxidative stress[8-10].

During the last few years, compelling shreds of evidence have shed light on the usefulness of dietary antioxidants as an alternative option in the treatment of T2DM, considering both the adverse effects conferred by conventional pharmacological treatments as well as the enormous economic burden that lifelong treatments place on patients[11].

In this regard, dietary polyphenols have emerged as an option to manage T2DM[12]. These compounds are one of the most abundant secondary plant metabolites, which are grouped into four major families, flavonoids, ligands, stilbenes, and phenolic acids, and are widely found in fruits, vegetables, nuts, cereals, and in many beverages such as tea, coffee, and red wines. These bioactive phytochemicals can reach and act at several cellular compartment levels including cellular membranes by binding to the bilayer interface or by interacting with the hydrophobic fatty acid tails[13].

A growing body of experimental and clinical evidence supports the protective role of these compounds on several human diseases through their antioxidant activity and diverse molecular mechanisms[14-18] (Figure 1). This review aims to highlight the roles of this large and heterogeneous family of secondary metabolites of plants containing phenol rings, on pancreatic islet β-cell functioning and promotion of insulin production and signaling, protection against micro-and microvascular complications, protection against the progression of T2DM-associated obesity, management of dyslipidemia and gut microbiome dysbiosis. In addition, the capacity of polyphenols to reduce both the formation of advanced glycation products and their pathologic consequences is also addressed.

LITERATURE SEARCH

The literature search was conducted using Medline/PubMed, Embase, Cochrane, and *RCA*, databases. Search terms included "type-2 diabetes mellitus", "prediabetes", "polyphenols", "natural antioxidants", "oxidative stress" and "abnormal glucose homeostasis". Articles published between January 2013 to March 2023 and additional publications were retrieved by snowballing. Exclusion criteria included T1DM (autoimmune β-cell destruction), gestational DM, pancreatogenic diabetes, drug-induced diabetes, and the monogenic diabetes syndromes.

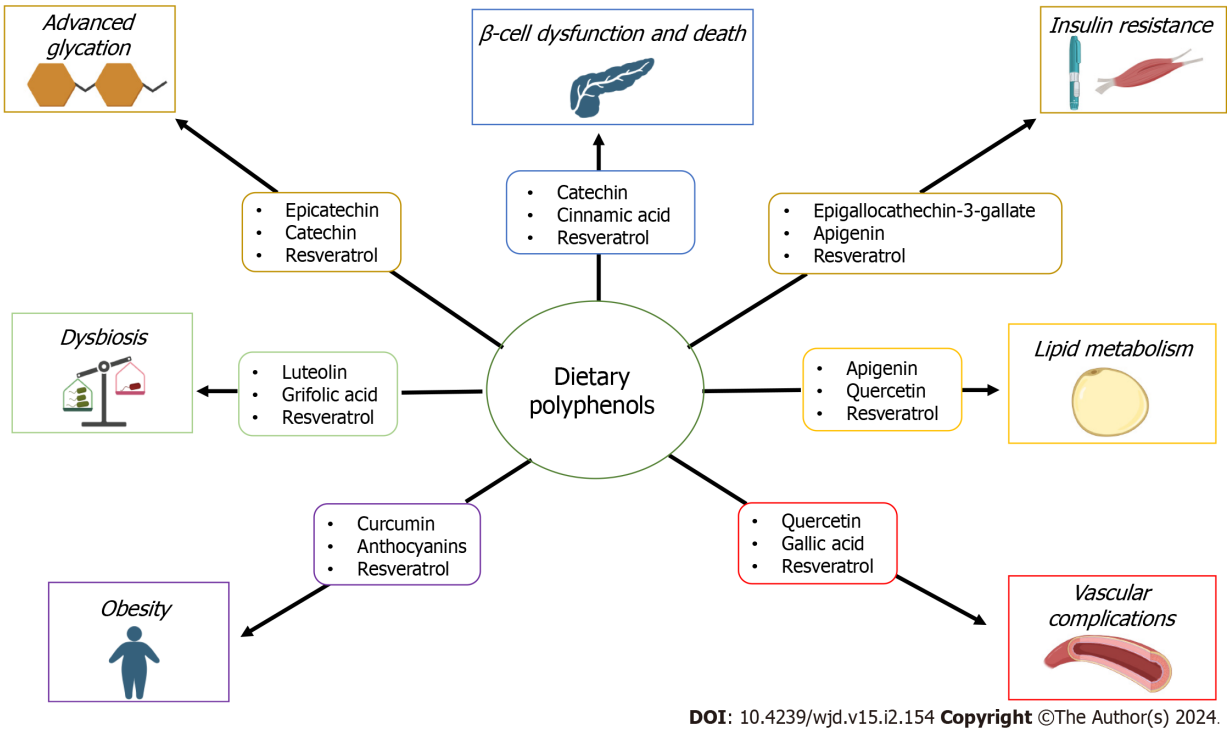

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Figure 1 Some polyphenols for which there is documented information about their beneficial properties in the management of the main alterations observed in type 2 diabetes mellitus.

B-CELL DYSFUNCTION AND DEATH

Currently, both clinical and experimental data support that during the development of T2DM, there is not only a progressive deterioration in β-cell functioning but also a marked reduction of the β-cell mass in the pancreatic islets of Langerhans[19-21]. Many factors such as the glucotoxicity associated with the hyperglycemic state, the oxidative and endoplasmic reticulum stresses, as well as the lipotoxicity due to chronic exposure to saturated free fatty acids, are crucial elements in decreased β-cell functioning and, eventually in β cell death through apoptosis[19,22,23].

Hyperglycemia is a crucial factor in the onset of oxidative stress in T2DM and it even correlates with the progression of disease[24]. Additionally, β-cells are very susceptible to oxidative damage, because of their low antioxidant capacity[25,26], and consequently, oxidative stress is a very important contributor to the impairment of β-cell functioning[23,27,28]. Furthermore, oxidative stress mediates the permeabilization of mitochondrial membranes, and consequently the release of cytochrome C and thus β-cell death by apoptosis[29].

Based on their antioxidant activities polyphenols are major regulators of oxidative stress and consequently the improvement of mitochondrial functions. At present, compelling pieces of evidence support that many metabolic disorders such as type 2 diabetes, are associated with impaired mitochondrial function such as diminished oxidative capacity and antioxidant defense, mainly due to the onset of an oxidative stress condition[30,31].

Oxidative stress condition is established by the imbalance between the production of ROS and antioxidant defense mechanisms, and where the detrimental ROS activities exceed the antioxidant capacities of the cell. Mitochondrial dysfunction is defined by several features including a diminished mitochondrial biogenesis, an altered membrane potential, a decrease in the mitochondrial number as well as by an altered activity of oxidative proteins due to the accumulation of ROS in cells and tissue[32,33].

Polyphenols can not only exert powerful antioxidant actions and thus protect against oxidative stress[34], they have additional capacities to modulate crucial pathways in mitochondrial functionality such as mitochondrial biogenesis, mitochondrial membrane potential, ATP synthesis, intra-mitochondrial oxidative status, and apoptosis cell death[35-38]. Cocoa catechins can also improve insulin secretion by increasing the expression of some genes involved in mitochondrial respiration[39].

Resveratrol is known for its remarkable activities in improving pancreatic β-cell function mainly by its effect on sirtuin 1 (SIRT1), a master regulator for β-cell function[40]. Cinnamic acid derivatives can improve the insulin-secreting capacity of β-cells, by raising the levels of intracellular calcium[41]. Noteworthy, compelling pieces of evidence support that the hyperglycemia-associated overexpression of human amylin, also known as islet amyloid polypeptide, can form aggregates to favor amylin fibril formation, and these fibrils evoke the activation of caspases cascade, and thus leading to β-cell death by apoptosis[42,43]. Several polyphenols such as rosmarinic acid, ferulic acid, epigallocatechin gallate, and resveratrol, among many others, can interfere with the formation of fibrillar structures and thus avoid β-cell death[44,45].

INSULIN RESISTANCE

Insulin receptor (IR) is a tyrosine kinase receptor, which is autophosphorylated upon insulin binding and it is expressed in all tissues. The major responders to IR engagement by insulin are the liver, skeletal muscle, and adipose tissue[46]. Upon insulin binding complex signaling is activated including several substrates such as insulin or insulin-like growth factor-1, IR, IR substrate (IRS)-1, and phosphatidylinositol-3 kinase (PI3K)/Akt or ERK kinases. The phosphorylation of IRS1 can recruit PI3K rendering Akt phosphorylated, which in turn can regulate crucial events such as the translocation of glucose transporter-4 (GLUT4) to the cell surface, promoting glycogen synthesis through inhibition of glycogen synthase kinase 3 activity, the induction of protein synthesis *via* activation of mammalian target of rapamycin and the inhibition of Forkhead transcription factors of the O class (FoxO) transcription factors[47,48].

The inactivation of Akt and activation of FoxO1, through the suppression of IRS1 and IRS2 in different organs following hyperinsulinemia, over-nutrition, and inflammation, represent crucial mechanisms for insulin resistance in humans[49,50]. Compelling shreds of evidence support that oxidative stress is an important contributor to insulin resistance in T2DM[51], and that the overproduction of mitochondrial H_2O_2[52,53], and the overactivation of NAPDPH oxidase, *via* angiotensin II/AT1 receptor can mediate skeletal muscle insulin resistance[54-56].

ROS are known to actively participate in several crucial physiological processes at the cellular level such as differentiation, cellular signaling, and phosphorylation/dephosphorylation events among many others[57]. The existence of various endogenous antioxidant systems is responsible for maintaining ROS at the low levels required to contribute to cellular homeostasis[58]. However, the hyperglycemia condition, which is a hallmark of T2DM, is crucial in the acquisition of a dysfunctional state of these antioxidant systems, thus favoring the onset of the oxidative stress condition [59,60]. Thus, this condition is a crucial element in the multifactorial etiology of insulin resistance. Oxidative stress impairs β-cell function, which markedly reduces not only insulin production but also its secretion into the circulation. Additionally, oxidative stress can reduce GLUT-4 gene expression and translocation to the membrane[61-63].

The c Jun-N-terminal kinases (JNKs) is major signal transducer driving the physio-logical response to several cellular stressors, including oxidative stress. Epigallocatechin gallate, the major green tea catechin can protect both the IR and IRS proteins from phosphorylation by JNKs, a crucial event in the onset of insulin resistance[63], as well as by reducing the expression of the negative regulator of IR protein tyrosine phosphatase 1B (PTP1B)[64].

Resveratrol, which is one of the main polyphenolic compounds of red wines, peanuts, and apples, is a potent activator of SIRT1, which is a potent intracellular inhibitor of oxidative stress, and thus attenuates insulin resistance and improves insulin signaling in the skeletal muscle cells[65,66]. Additionally, some polyphenols can also stimulate glucose uptake in both skeletal muscle and adipocytes by translocating GLUT4 to the plasma membrane through an adenosine monophosphate (AMP)-activated protein kinase (AMPK)-dependent pathway[67].

PTP1B is an intracellular enzyme responsible for the deactivation of the IR, resulting in insulin resistance in various tissues[68,69]. Hence, PTP1B has become an important target for controlling insulin resistance and T2DM. In this regard, many polyphenols have inhibitory activity on PTP1B as demonstrated either by screening platforms for detecting the inhibition activity or by Quantitative Structure-Activity Relationship analysis[70,71].

OBESITY

Obesity is the major driving factor of T2DM and it is characterized by chronic low-grade inflammation with permanently increased oxidative stress[72,73]. The onset of a chronic condition of oxidative stress in obesity is supported by different mechanisms implicated in the homeostasis of adipose tissue, which contributes to the development of pathological systemic consequences[74].

On one hand, those associated with increased ROS production such as the adipocytes-associated endoplasmic reticulum stress, a sustained increase of NOX activities, as well as the high level of post-prandial-associated ROS generation, and on the other, the altered antioxidant defenses observed in obese patients[75-78]. In addition to the antioxidant properties of polyphenols, they exert several beneficial effects on obesity far beyond their antioxidant capacity[79], such as the attenuation of obesity-linked inflammation[80-82], the beneficial regulation of several key obesity path-ways such as the modulation of food intake[81], the inhibition of pancreatic lipase[82,83], decreasing lipogenesis by inhibiting both fatty acid synthase activity and the activation of the AMP-AMPK[84,85], and by increasing thermogenesis and mitochondrial biogenesis[86].

Finally, some polyphenols have been reported to mediate the suppression of the conversion of preadipocytes into adipocytes, which can store an excessive lipid load. This polyphenols-mediated suppression of adipocyte differentiation occurs by the regulation of crucial factors such as the CCAAT/enhancer binding protein α, the nuclear receptor peroxisome proliferator-activated receptor γ 1 and 2, (PPARγ1, PPARγ2), and GLUT-4 in mature adipocytes[84,86-88].

DYSBIOSIS

Human gut microbiota is considered a complex microbial ecosystem composed of different microorganisms, including bacteria, archaea, viruses, fungi, and protists, which are involved in the regulation of many physiological processes and numerous diseases[89].

Firmicutes and Bacteroidetes are the main phyla that compose the adult gut flora, regulating the homeostatic production of microbiota-induced metabolites such as butyrate, which have anti-inflammatory and antioxidative properties, and the production of lipopolysaccharide (LPS), which can promote systemic inflammation and insulin resistance through induction of metabolic endotoxemia[90,91].

Growing data raised from both clinical and experimental evidence shows that T2DM patients have an altered gut microbiota, where the Bacterioidetes/Firmicutes ratio of the intestinal flora of diabetic patients significantly differs from non-T2DM adults[92,93]. A crucial consequence of the quantitative change in gut microbiota composition in T2DM patients is the impairment of the expression of gut-microbiota-related metabolites, which have crucial consequences in the metabolic regulation of glucose homeostasis, and insulin sensitivity[93].

Short-chain fatty acids (SCFAs) are considered one of the main microbial metabolites, that have crucial effects on the expression of glucagon-like peptide-1 (GLP-1) and GLP-2 *via* stimulating G-protein-coupled receptors, thus contributing to improving glucose homeostasis and amplification of insulin sensitivity[94].

Under this dysbiosis condition that affects T2DM patients, structural changes in the intestinal epithelium barrier allow LPS translocation into the bloodstream, resulting in increased plasmatic levels of LPS, which in consequence, activates Toll-like receptor-4 leading to the production of pro-inflammatory mediators, and sustaining low-grade systemic inflammation[95].

This condition known as metabolic endotoxemia induces a significant decrease in bacterial populations which are crucial producers of beneficial gut-derived metabolites such as SCFA, thus supporting the impairment of glucose metabolism and insulin resistance[96,97]. In addition, different studies have demonstrated that specific gut microbiota dysbiosis in mice models of T2DM, induces GLP-1 resistance and consequently, the impairment of GLP1-induced insulin secretion, which is crucial in the acquisition of the insulin resistance condition in diabetic individuals[98].

At present, polyphenols have emerged as novel compounds that could interact with microbiota and exert strong regulatory effects on intestinal bacteria, with subsequent regulation of gut microbiota and its derivate metabolites[99]. These interactions between polyphenols and gut microbiota can positively affect crucial metabolic markers of T2DM, improving systemic inflammation and insulin sensitivity[100,101].

Growing evidence reveals that distinct types of polyphenolic compounds, such as genistein, curcumin, and grifolic acid can increase GLP-1 secretion from L-cells *via* different mechanisms[102-105]. Besides their effect to directly stimulate GLP-1 secretion, some polyphenols, particularly luteolin, apigenin, and resveratrol may also naturally suppress DPP-IV activity, which potentially increases the half-life of GLP-1, thus stimulating glucose-dependent insulin secretion and regulating glycemia[106,107].

Different studies demonstrate that different doses of oral intake of polyphenols including catechins, and (-)-epigallocatechin-3-gallate, can also favor the increase of different microbial populations of SCFA-producing agents in fecal samples of human patients, thus improving the insulin sensitivity and glucose homeostasis of individuals[108,109].

In addition, other phenolic compounds including chlorogenic and ferulic acid can also act as antidiabetic agents, through significant upregulating of the expression of GLUT4 and PPAR-γ, thus favoring the uptake of 2-deoxyglucose in time- and dose-dependent manner, and improving the pathogenesis of T2DM progression[110-112]. Branched-chain amino acids (BCAAs) include leucine, isoleucine, and valine, which cannot be synthesized *de novo* by mammalians and consequently, they are acquired either from the diet or gut microbiota. Elevated plasma circulating levels of BCAAs and their ketoacids are associated with insulin resistance in obesity and T2DM[113-117].

Conversely, experimental results have demonstrated that lowering BCAA and branched-chain alpha-keto acid levels is associated with improved insulin sensitivity and reduced fat accumulation in mouse models[118]. Emerging studies have suggested that polyphenol administration may accelerate the catabolism of BCAA, inducing a lowering of circulating BCAA levels, thus improving glucose homeostasis and insulin sensitivity[119].

Additionally, some evidence also supports that intestinal catabolites of polyphenolic compounds by the action of the gut microbiota could act as a strong antiglycative agent[120,121]. In this sense, dietary polyphenolic intake may have a significant positive impact on the generation of glycation products and diabetes-related complications[122,123]. Taken together, those findings suggest that a polyphenols-enriched diet can strongly modulate the dysbiotic changes induced by hyperglycemia, improving the regulation of metabolites that mediate glucose homeostasis and insulin sensitivity in T2DM patients.

VASCULAR COMPLICATIONS

Vascular complications in T2DM are those long-term complications that affect the blood vessel network, and are responsible for most of the morbidity, and required hospitalization in these patients[124]. The vascular complications of diabetes are classified as either microvascular (retinopathy, nephropathy, and neuropathy) or macrovascular, which includes coronary artery, peripheral, and cerebral vascular diseases[125].

At present, a large body of compelling evidence supports that oxidative stress has a key role in the pathogenesis of vascular complications in diabetes[126-128]. As a major regulator of vascular homeostasis, the vascular endothelial cells play crucial roles by controlling vascular tone through a balance between vasodilation and vasoconstriction, fibrinolysis, platelet adhesion and aggregation, leukocyte activation, adhesion, and transmigration, smooth muscle cell proliferation, and modulating the growth of blood vessels[129,130].

The onset of an imbalanced vasodilation and vasoconstriction, elevated ROS, and proinflammatory factors, as well as a reduced nitric oxide (NO) bioavailability, are crucial elements in the onset of the systemic disorder known as endothelial dysfunction[131]. NO is produced in the endothelium by the endothelial NO synthase (eNOS), a Ca^{2+}-calmodulin-

dependent enzyme that can convert the L-arginine to NO plus citrulline. By activation of soluble guanylyl cyclase and modulation of cation channels, NO promotes vascular smooth muscle cells relaxation and thus regulates vascular tone. Additionally, NO is a crucial mediator in controlling platelet activation and aggregation[132].

When ROS bioavailability overtakes the antioxidant defenses due to the onset of oxidative stress, superoxide (O^{2-}) rapidly inactivates NO and forms peroxynitrite ($ONOO^-$). It is known that peroxynitrite inactivates prostacyclin synthase thus favoring the deterioration of vascular health due to the vasodilatory, growth-inhibiting, antithrombotic, and antiadhesive effects of prostacyclin. Additionally, peroxynitrite increases the release of prostaglandin H2 and thromboxane A2, which are potent vasoconstrictors, prothrombotic, growth- and adhesion-promoting agents[133-135]. A growing body of data supports the beneficial roles of polyphenols in protecting against endothelial dysfunction induced by oxidative stimuli[136-138].

Of note, some polyphenols, as reported for resveratrol and its derivatives show dual protecting activities, either by the expression of Nox4, a ROS-generating enzyme highly expressed in the endothelium, and by enhancing the expression of two crucial members of the antioxidant defense of the vascular wall, such as glutathione peroxidase 1 and superoxide dismutase 1[139]. Moreover, polyphenols seem to have peroxynitrite-scavenging activity[140]. Furthermore, different reports have demonstrated that some polyphenols such as resveratrol and others derived from strawberry and grape skin and seeds, can promote the phosphorylation of eNOS at Ser1177 by PI3K/Akt pathway, which is essential for NO production[141-143]. In addition, resveratrol is reported to increase both endothelial eNOS mRNA and protein levels[144-146]. This effect seems to be associated with the effects of resveratrol on SIRT1 and FOXO factors[147].

POLYPHENOLS AND ADVANCED GLYCATION

Advanced glycation is one of the major pathways involved in the onset and progression of T2DM complications, particularly those associated with the cardiovascular system[148]. Since the pioneering works of the Vlassara group[149,150], a huge and compelling body of evidence has demonstrated the paramount importance of AGEs in diabetes complications, due to the hyperglycemic condition[151,152].

The formation of AGEs involves the reaction of reducing sugars, such as glucose, with the terminal amino groups of proteins, nucleic acids, or phospholipids to initially form unstable Schiff bases, which evolve towards the formation of more stable compounds called Amadori products, which by a series of complex reaction yield the AGEs. Degradation of both Schiff bases and Amadori products rise to highly reactive short-chain carbonyl compounds, called α-dicarbonyls [153].

These highly reactive compounds can also be formed by hexose autoxidation, as well as by-products of either the glycolytic or polyol pathways and from lipid oxidation. Dicarbolyls can then react non-enzymatically with lysine or arginine residues to produce AGEs[154,155].

The AGEs exert their deleterious effects, either directly by cross-linking of proteins, thus disrupting protein functioning and turn-over[156,157], or indirectly by binding to a signaling receptor for AGE-modified proteins, known as the receptor of advanced glycation end-products (RAGE)[158,159]. Noteworthy, oxidative stress is an important contributor to the formation of endogenous eAGEs, by leading to the increased formation of endogenous reactive aldehydes such as glyoxal, methylglyoxal (MG), and thus favoring the formation of AGEs[160]. Additionally, when AGEs activate RAGE, NADPH oxidase is activated and thus increases ROS levels[161].

At present, compelling evidence derived from experimental and clinical data studies supports the role of different polyphenols as very active inhibitors of the deleterious effects of AGEs, through several mechanisms[162,163]. By their antioxidant activities, polyphenols are potent antiglycation compounds and antiglycation activity strongly correlates with the free radical scavenging activity and antiglycation activity[120], as reported catechins, proanthocyanidins, anthocyanin, stilbenoids, and flavonols[164,165]. Additionally, polyphenols have other properties, which are essential to reduce the formation of AGEs, such as the chelation of transition metal, as reported for chlorogenic and caffeic acids[166,167].

The capacity of trapping dicarbonyl compounds is another crucial activity reported for some polyphenols considering that dicarbonyls are one of the main precursors of AGEs[154], epigallocatechin-3-gallate, resveratrol, catechin, and epicatechin as well as different procyanidins can efficiently trap both glyoxal and MG[162,168,169]. Dicarbonyls are detoxified by the glyoxalase system a highly specific enzyme responsible for the detoxification of dicarbonyl species[170]. Some polyphenols can even stimulate this detoxifying system[171]. Finally, several reports have demonstrated that polyphenols can actively reduce the undesired consequences of the activation of RAGE, either by interfering with receptor signaling as well as by reducing its expression[172-174].

LIPID METABOLISM

T2DM has been widely associated with an increased risk for atherosclerotic cardiovascular disease, which is closely related to raised plasmatic low-density lipoprotein (LDL) levels with important oxidative changes[175], which support diabetic hyperlipidemia and accelerated atherosclerosis, increasing the risk of macrovascular complication and cardiovascular morbidity. Noteworthy, LDL is a highly sensitive molecule to hyperglycemia-induced hyperglycemia damage and modification, making it highly pathogenic and atherogenic[176,177]. Under hyperglycemic conditions, transition metals in the presence of oxygen catalyze the autoxidation of glucose or lipid peroxidation[178]. In addition, excess ROS formation in T2DM patients fuels vascular inflammation and mediates oxidized LDL (ox-LDL) formation,

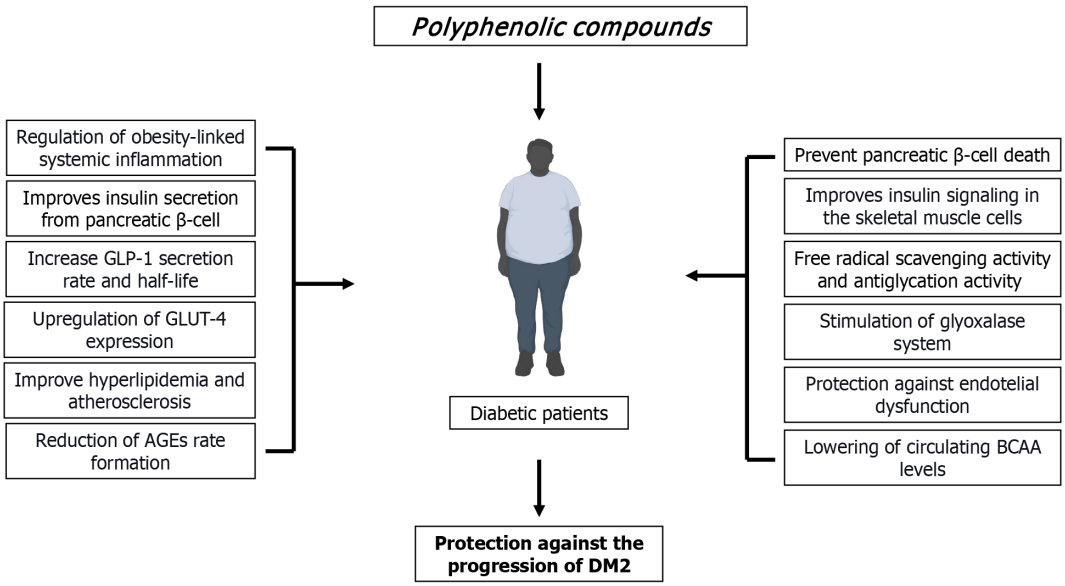

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Figure 2 Polyphenols have multifaceted actions to support their use in the management of type 2 diabetes mellitus. Due to their positive actions on multiple physiopathological mechanisms which are crucial not only in the onset of type 2 diabetes mellitus (T2DM) by protecting and supporting many functions of β-cells and insulin signaling, but also in those associated with common T2DM complications by improving dyslipidemia profiles, reducing systemic inflammation, dampening the deleterious consequences of the high rate formation of advanced glycation end products production, reducing oxidative stress, as well as by supporting vascular functionality. AGE: Advanced glycation end products production; GLP-1: Glucagon-like peptide-1; GLUT-4: Glucose transporter 4; BCAA: Branched-chain amino acid; DM: Diabetes mellitus.

which is considered a hallmark feature of atherosclerotic development due to the crucial induction of atherosclerotic plaque progression and destabilization in T2DM patients[179-181].

Besides the different pathways that conflux in activate NADPH oxidase and subsequent ROS production in T2DM patients, the increased expression of ox-LDL also stimulates NADPH oxidase, thus contributing to increment ROS formation and oxidative stress in T2DM patients[182]. In addition, hyperglycemia-mediated mitochondrial ROS production can also promote the nuclear factor kappa-beta-mediated entry of monocytes in atherosclerotic lesions, fueling the inflammation and progression of unstable plaques, and increasing the risk of macrovascular complication in T2DM patients[183], thus, sustaining a vicious cycle that perpetuating ROS production and ox-LDL formation, contributes to the progression of atherosclerosis unstable plaques on DM patients.

In recent years, polyphenols have been postulated to lower lipids through different mechanisms that imply beneficial effects on cardiovascular diseases of T2DM patients[184]. Based on their antioxidant effects, different studies have shown that many polyphenols including resveratrol, apigenin, and some synthetic polyphenol-like molecules can inhibit NAPDH oxidase activity, thus decreasing vascular oxidation and atherogenesis in nondiabetic apolipoprotein (apo) E–deficient mice[185], as well as improve hyperlipidemia and atherosclerosis in diabetic individuals[186].

Resveratrol based on its antioxidant activities can influence lipid metabolism and is considered an important protective compound against LDL oxidation and atherosclerosis progression[187]. In this sense, the free radical scavenging activity of resveratrol has been investigated, revealing that this polyphenol compound can interact with free radicals to form relatively stable free radicals and non-radicals, resulting in inhibition of lipid peroxidation by Fenton reaction products [188,189], which may decrease the progression of accelerated atherosclerosis through inhibition of oxidation in T2DM patients[190,191].

More recently, it was demonstrated that resveratrol can upregulate eNOS expression by increasing cAMP levels, and decreasing ox-LDL-induced oxidative stress in human endothelial cells, leading to a significant improvement of endothelial dysfunction and atherosclerosis in mice[192]. Similar results have been demonstrated for quercetin, an important flavonoid, which has demonstrated protective effects in diabetic individuals through significantly reversed dyslipidemia and hepatic steatosis in diabetic mice, including lowered liver cholesterol and triglycerides contents[193, 194]. Taken together, these findings suggest that dietary polyphenols may be crucial in the regulation of dysregulated lipid metabolism through the modulation of antioxidative mechanisms in T2DM patients.

CONCLUSION

A compelling body of evidence suggests that dietary polyphenols may represent an important alternative to the management of T2DM due to their multifaceted actions on glucose homeostasis as well as by attenuating many diabetes complications raised because of the hyperglycemic condition (Figure 2). Most of the pieces of evidence derived from animals and *in vitro* studies support these issues. However, new emerging data derived from either clinical trials or meta-

Table 1 Clinical trials and meta-analysis studies in the last five years supporting the roles of dietary polyphenols in the management of type 2 diabetes mellitus

Type of study	Beneficial effects	Ref.
Randomized clinical trial	Increased antioxidant capacity and antioxidant gap in T2DM patients	García-Martínez *et al*[195], 2023
Double-masked, cross-over, dietary intervention trial	Improvement of endothelial function in both healthy individuals and T2DM patients	Bapir *et al*[196], 2022
Meta-analysis	Improving HbA1c, and insulin levels in T2DM	García-Martínez *et al*[197], 2021
Randomized, clinical trial	Lowering fasting blood glucose levels in T2DM	Sirvent *et al*[198], 2022
Systemic review and meta-analysis	Reduction of systolic and diastolic blood pressure and fasting blood glucose levels in T2DM patients	Gu *et al*[199], 2022
Systematic review and meta-analysis	Reduction of fasting blood glucose and HbA1c levels	Delpino *et al*[200], 2021
Randomized clinical trial	Improvement of glycemic control by reducing insulin resistance	Mahjabeen *et al*[201], 2022
Randomized clinical trial	Lowering effects on inflammatory status and oxidative stress biomarkers in diabetic patients	Grabež *et al*[202], 2022
Randomized clinical trial	Improvement of glycaemia markers	Gómez-Martínez *et al*[203], 2021
Systematic review and meta-analysis	Improvement of glycemic control and cardiometabolic parameters in patients with T2DM	Abdelhaleem *et al*[68], 2022
Meta-analysis	Reduction of insulin resistance, HbA1c levels and fasting blood glucose	Delpino and Figueiredo [204], 2022
Meta-analysis	Improvement of glucose control and lowering blood pressure	Nyambuya *et al*[205], 2020
Randomized clinical trial	Improvement of postprandial dyslipidemia and inflammation following a high-fat dietary challenge in adults with T2D	Davis *et al*[206], 2020
Meta-analysis	Significant reduction in CRP level in patients with T2D	Hosseini *et al*[194], 2021
Meta-analysis	Combined effects with anti-diabetic medication to lowering serum glucose levels in individuals with T2D	Raimundo *et al*[207], 2020
Randomized clinical trial	Improvement of glycemic control and lipid profile	Hoseini *et al*[208], 2019
Meta-analysis	Lowering fasting blood glucose, HbA1c, and HOMA-IR	Huang *et al*[209], 2019
Randomized clinical trial	Improvement of lipid profile and lowering serum biomarkers of inflammation	Adibian *et al*[210], 2019
Randomized clinical trial	Lowering postprandial hyperglycemia and serum biomarkers of inflammation	Schell *et al*[211], 2019
Randomized clinical trial	Lowering fasting blood glucose and improvement of lipid profile	Mollace *et al*[212], 2019
Systematic review and meta-analysis	Lowering the risk of T2D	Rienks *et al*[213], 2018
Randomized clinical trial	Reduction of plasma protein carbonyl content and increasing plasma total antioxidant capacity	Seyyedebrahimi *et al*[214], 2018

T2D: Type 2 diabetes; HbA1c: Glycosylated hemoglobin; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance; CRP: C-reactive protein; T2DM: Type 2 diabetes mellitus.

analyses have started to figure out the usefulness of these bioactive compounds, and thus providing solid clinical shreds of evidence (Table 1). However, much more research is needed on some topics that may be crucial to explain the current controversial results in some clinical studies. In this regard, a full understanding of the metabolisms and bioavailability, the assessment of dietary intake by measuring urine or blood polyphenol metabolites, duration of exposure, delivery systems that guarantee high stability, as well as more efforts to understand the structure-activity relationship of polyphenols, are crucial elements to be considered in the design and execution of more double-blinded clinical trials.

FOOTNOTES

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REFERENCES

1 **Tomic D**, Shaw JE, Magliano DJ. The burden and risks of emerging complications of diabetes mellitus. *Nat Rev Endocrinol* 2022; **18**: 525-539 [PMID: 35668219 DOI: 10.1038/s41574-022-00690-7]
2 **Sun H**, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, Stein C, Basit A, Chan JCN, Mbanya JC, Pavkov ME, Ramachandaran A, Wild SH, James S, Herman WH, Zhang P, Bommer C, Kuo S, Boyko EJ, Magliano DJ. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Res Clin Pract* 2022; **183**: 109119 [PMID: 34879977 DOI: 10.1016/j.diabres.2021.109119]
3 **Khan MAB**, Hashim MJ, King JK, Govender RD, Mustafa H, Al Kaabi J. Epidemiology of Type 2 Diabetes - Global Burden of Disease and Forecasted Trends. *J Epidemiol Glob Health* 2020; **10**: 107-111 [PMID: 32175717 DOI: 10.2991/jegh.k.191028.001]
4 **Galicia-Garcia U**, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, Ostolaza H, Martín C. Pathophysiology of Type 2 Diabetes Mellitus. *Int J Mol Sci* 2020; **21** [PMID: 32872570 DOI: 10.3390/ijms21176275]
5 **Giacco F**, Brownlee M. Oxidative stress and diabetic complications. *Circ Res* 2010; **107**: 1058-1070 [PMID: 21030723 DOI: 10.1161/CIRCRESAHA.110.223545]
6 **Black HS**. A Synopsis of the Associations of Oxidative Stress, ROS, and Antioxidants with Diabetes Mellitus. *Antioxidants (Basel)* 2022; **11** [PMID: 36290725 DOI: 10.3390/antiox11102003]
7 **Yan LJ**. Pathogenesis of chronic hyperglycemia: from reductive stress to oxidative stress. *J Diabetes Res* 2014; **2014**: 137919 [PMID: 25019091 DOI: 10.1155/2014/137919]
8 **Rains JL**, Jain SK. Oxidative stress, insulin signaling, and diabetes. *Free Radic Biol Med* 2011; **50**: 567-575 [PMID: 21163346 DOI: 10.1016/j.freeradbiomed.2010.12.006]
9 **Maritim AC**, Sanders RA, Watkins JB 3rd. Diabetes, oxidative stress, and antioxidants: a review. *J Biochem Mol Toxicol* 2003; **17**: 24-38 [PMID: 12616644 DOI: 10.1002/jbt.10058]
10 **Sharifi-Rad M**, Anil Kumar NV, Zucca P, Varoni EM, Dini L, Panzarini E, Rajkovic J, Tsouh Fokou PV, Azzini E, Peluso I, Prakash Mishra A, Nigam M, El Rayess Y, Beyrouthy ME, Polito L, Iriti M, Martins N, Martorell M, Docea AO, Setzer WN, Calina D, Cho WC, Sharifi-Rad J. Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases. *Front Physiol* 2020; **11**: 694 [PMID: 32714204 DOI: 10.3389/fphys.2020.00694]
11 **Pasupuleti VR**, Arigela CS, Gan SH, Salam SKN, Krishnan KT, Rahman NA, Jeffree MS. A Review on Oxidative Stress, Diabetic Complications, and the Roles of Honey Polyphenols. *Oxid Med Cell Longev* 2020; **2020**: 8878172 [PMID: 33299532 DOI: 10.1155/2020/8878172]
12 **Golovinskaia O**, Wang CK. The hypoglycemic potential of phenolics from functional foods and their mechanisms. *Food Sci Hum Wellness* 2023; **12**: 986-1007 [DOI: 10.1016/j.fshw.2022.10.020]
13 **Meleleo D**, Avato P, Conforti F, Argentieri MP, Messina G, Cibelli G, Mallamaci R. Interaction of Quercetin, Cyanidin, and Their O-Glucosides with Planar Lipid Models: Implications for Their Biological Effects. *Membranes (Basel)* 2023; **13** [PMID: 37367804 DOI: 10.3390/membranes13060600]
14 **Cory H**, Passarelli S, Szeto J, Tamez M, Mattei J. The Role of Polyphenols in Human Health and Food Systems: A Mini-Review. *Front Nutr* 2018; **5**: 87 [PMID: 30298133 DOI: 10.3389/fnut.2018.00087]
15 **Tomás-Barberán FA**, Andrés-Lacueva C. Polyphenols and health: current state and progress. *J Agric Food Chem* 2012; **60**: 8773-8775 [PMID: 22578138 DOI: 10.1021/jf300671j]
16 **Tsao R**. Chemistry and biochemistry of dietary polyphenols. *Nutrients* 2010; **2**: 1231-1246 [PMID: 22254006 DOI: 10.3390/nu2121231]
17 **Pandey KB**, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. *Oxid Med Cell Longev* 2009; **2**: 270-278 [PMID: 20716914 DOI: 10.4161/oxim.2.5.9498]
18 **Rudrapal M**, Khairnar SJ, Khan J, Dukhyil AB, Ansari MA, Alomary MN, Alshabrmi FM, Palai S, Deb PK, Devi R. Dietary Polyphenols and Their Role in Oxidative Stress-Induced Human Diseases: Insights Into Protective Effects, Antioxidant Potentials and Mechanism(s) of Action. *Front Pharmacol* 2022; **13**: 806470 [PMID: 35237163 DOI: 10.3389/fphar.2022.806470]
19 **Cerf ME**. Beta cell dysfunction and insulin resistance. *Front Endocrinol (Lausanne)* 2013; **4**: 37 [PMID: 23542897 DOI: 10.3389/fendo.2013.00037]
20 **White MG**, Shaw JA, Taylor R. Type 2 Diabetes: The Pathologic Basis of Reversible β-Cell Dysfunction. *Diabetes Care* 2016; **39**: 2080-2088 [PMID: 27926891 DOI: 10.2337/dc16-0619]
21 **Ferrannini E**, Mari A. β-Cell function in type 2 diabetes. *Metabolism* 2014; **63**: 1217-1227 [PMID: 25070616 DOI: 10.1016/j.metabol.2014.05.012]
22 **Porte D Jr**, Kahn SE. beta-cell dysfunction and failure in type 2 diabetes: potential mechanisms. *Diabetes* 2001; **50** Suppl 1: S160-S163

[PMID: 11272181 DOI: 10.2337/diabetes.50.2007.S160]
23 **Cerf ME**. Beta Cell Physiological Dynamics and Dysfunctional Transitions in Response to Islet Inflammation in Obesity and Diabetes. *Metabolites* 2020; **10** [PMID: 33182622 DOI: 10.3390/metabo10110452]
24 **Bhatti JS**, Sehrawat A, Mishra J, Sidhu IS, Navik U, Khullar N, Kumar S, Bhatti GK, Reddy PH. Oxidative stress in the pathophysiology of type 2 diabetes and related complications: Current therapeutics strategies and future perspectives. *Free Radic Biol Med* 2022; **184**: 114-134 [PMID: 35398495 DOI: 10.1016/j.freeradbiomed.2022.03.019]
25 **Drews G**, Krippeit-Drews P, Düfer M. Oxidative stress and beta-cell dysfunction. *Pflugers Arch* 2010; **460**: 703-718 [PMID: 20652307 DOI: 10.1007/s00424-010-0862-9]
26 **Dludla PV**, Mabhida SE, Ziqubu K, Nkambule BB, Mazibuko-Mbeje SE, Hanser S, Basson AK, Pheiffer C, Kengne AP. Pancreatic β-cell dysfunction in type 2 diabetes: Implications of inflammation and oxidative stress. *World J Diabetes* 2023; **14**: 130-146 [PMID: 37035220 DOI: 10.4239/wjd.v14.i3.130]
27 **Dinić S**, Arambašić Jovanović J, Uskoković A, Mihailović M, Grdović N, Tolić A, Rajić J, Đorđević M, Vidaković M. Oxidative stress-mediated beta cell death and dysfunction as a target for diabetes management. *Front Endocrinol (Lausanne)* 2022; **13**: 1006376 [PMID: 36246880 DOI: 10.3389/fendo.2022.1006376]
28 **Eguchi N**, Vaziri ND, Dafoe DC, Ichii H. The Role of Oxidative Stress in Pancreatic β Cell Dysfunction in Diabetes. *Int J Mol Sci* 2021; **22** [PMID: 33546200 DOI: 10.3390/ijms22041509]
29 **Ma ZA**. The role of peroxidation of mitochondrial membrane phospholipids in pancreatic β -cell failure. *Curr Diabetes Rev* 2012; **8**: 69-75 [PMID: 22414059 DOI: 10.2174/157339912798829232]
30 **Blake R**, Trounce IA. Mitochondrial dysfunction and complications associated with diabetes. *Biochim Biophys Acta* 2014; **1840**: 1404-1412 [PMID: 24246956 DOI: 10.1016/j.bbagen.2013.11.007]
31 **Kowalczyk P**, Sulejczak D, Kleczkowska P, Bukowska-Ośko I, Kucia M, Popiel M, Wietrak E, Kramkowski K, Wrzosek K, Kaczyńska K. Mitochondrial Oxidative Stress-A Causative Factor and Therapeutic Target in Many Diseases. *Int J Mol Sci* 2021; **22** [PMID: 34948180 DOI: 10.3390/ijms222413384]
32 **Pieczenik SR**, Neustadt J. Mitochondrial dysfunction and molecular pathways of disease. *Exp Mol Pathol* 2007; **83**: 84-92 [PMID: 17239370 DOI: 10.1016/j.yexmp.2006.09.008]
33 **Hu F**, Liu F. Mitochondrial stress: a bridge between mitochondrial dysfunction and metabolic diseases? *Cell Signal* 2011; **23**: 1528-1533 [PMID: 21616143 DOI: 10.1016/j.cellsig.2011.05.008]
34 **Hussain T**, Tan B, Yin Y, Blachier F, Tossou MC, Rahu N. Oxidative Stress and Inflammation: What Polyphenols Can Do for Us? *Oxid Med Cell Longev* 2016; **2016**: 7432797 [PMID: 27738491 DOI: 10.1155/2016/7432797]
35 **Koh YC**, Ho CT, Pan MH. The Role of Mitochondria in Phytochemically Mediated Disease Amelioration. *J Agric Food Chem* 2023; **71**: 6775-6788 [PMID: 37125676 DOI: 10.1021/acs.jafc.2c08921]
36 **Sandoval-Acuña C**, Ferreira J, Speisky H. Polyphenols and mitochondria: an update on their increasingly emerging ROS-scavenging independent actions. *Arch Biochem Biophys* 2014; **559**: 75-90 [PMID: 24875147 DOI: 10.1016/j.abb.2014.05.017]
37 **Chodari L**, Dilsiz Aytemir M, Vahedi P, Alipour M, Vahed SZ, Khatibi SMH, Ahmadian E, Ardalan M, Eftekhari A. Targeting Mitochondrial Biogenesis with Polyphenol Compounds. *Oxid Med Cell Longev* 2021; **2021**: 4946711 [PMID: 34336094 DOI: 10.1155/2021/4946711]
38 **Bhagani H**, Nasser SA, Dakroub A, El-Yazbi AF, Eid AA, Kobeissy F, Pintus G, Eid AH. The Mitochondria: A Target of Polyphenols in the Treatment of Diabetic Cardiomyopathy. *Int J Mol Sci* 2020; **21** [PMID: 32674299 DOI: 10.3390/ijms21144962]
39 **Rowley TJ 4th**, Bitner BF, Ray JD, Lathen DR, Smithson AT, Dallon BW, Plowman CJ, Bikman BT, Hansen JM, Dorenkott MR, Goodrich KM, Ye L, O'Keefe SF, Neilson AP, Tessem JS. Monomeric cocoa catechins enhance β-cell function by increasing mitochondrial respiration. *J Nutr Biochem* 2017; **49**: 30-41 [PMID: 28863367 DOI: 10.1016/j.jnutbio.2017.07.015]
40 **Vetterli L**, Brun T, Giovannoni L, Bosco D, Maechler P. Resveratrol potentiates glucose-stimulated insulin secretion in INS-1E beta-cells and human islets through a SIRT1-dependent mechanism. *J Biol Chem* 2011; **286**: 6049-6060 [PMID: 21163946 DOI: 10.1074/jbc.M110.176842]
41 **Adisakwattana S**, Moonsan P, Yibchok-Anun S. Insulin-releasing properties of a series of cinnamic acid derivatives in vitro and in vivo. *J Agric Food Chem* 2008; **56**: 7838-7844 [PMID: 18651742 DOI: 10.1021/jf801208t]
42 **Zhang S**, Liu J, Dragunow M, Cooper GJ. Fibrillogenic amylin evokes islet beta-cell apoptosis through linked activation of a caspase cascade and JNK1. *J Biol Chem* 2003; **278**: 52810-52819 [PMID: 14532296 DOI: 10.1074/jbc.M308244200]
43 **Kanatsuka A**, Kou S, Makino H. IAPP/amylin and β-cell failure: implication of the risk factors of type 2 diabetes. *Diabetol Int* 2018; **9**: 143-157 [PMID: 30603362 DOI: 10.1007/s13340-018-0347-1]
44 **Sequeira IR**, Poppitt SD. Unfolding Novel Mechanisms of Polyphenol Flavonoids for Better Glycaemic Control: Targeting Pancreatic Islet Amyloid Polypeptide (IAPP). *Nutrients* 2017; **9** [PMID: 28754022 DOI: 10.3390/nu9070788]
45 **Mahboob A**, Senevirathne DKL, Paul P, Nabi F, Khan RH, Chaari A. An investigation into the potential action of polyphenols against human Islet Amyloid Polypeptide aggregation in type 2 diabetes. *Int J Biol Macromol* 2023; **225**: 318-350 [PMID: 36400215 DOI: 10.1016/j.ijbiomac.2022.11.038]
46 **Boucher J**, Kleinridders A, Kahn CR. Insulin receptor signaling in normal and insulin-resistant states. *Cold Spring Harb Perspect Biol* 2014; **6** [PMID: 24384568 DOI: 10.1101/cshperspect.a009191]
47 **Saltiel AR**. Insulin signaling in health and disease. *J Clin Invest* 2021; **131** [PMID: 33393497 DOI: 10.1172/JCI142241]
48 **Siddle K**. Signalling by insulin and IGF receptors: supporting acts and new players. *J Mol Endocrinol* 2011; **47**: R1-10 [PMID: 21498522 DOI: 10.1530/JME-11-0022]
49 **Taylor R**. Insulin resistance and type 2 diabetes. *Diabetes* 2012; **61**: 778-779 [PMID: 22442298 DOI: 10.2337/db12-0073]
50 **Wondmkun YT**. Obesity, Insulin Resistance, and Type 2 Diabetes: Associations and Therapeutic Implications. *Diabetes Metab Syndr Obes* 2020; **13**: 3611-3616 [PMID: 33116712 DOI: 10.2147/DMSO.S275898]
51 **Hurrle S**, Hsu WH. The etiology of oxidative stress in insulin resistance. *Biomed J* 2017; **40**: 257-262 [PMID: 29179880 DOI: 10.1016/j.bj.2017.06.007]
52 **Anderson EJ**, Lustig ME, Boyle KE, Woodlief TL, Kane DA, Lin CT, Price JW 3rd, Kang L, Rabinovitch PS, Szeto HH, Houmard JA, Cortright RN, Wasserman DH, Neufer PD. Mitochondrial H2O2 emission and cellular redox state link excess fat intake to insulin resistance in both rodents and humans. *J Clin Invest* 2009; **119**: 573-581 [PMID: 19188683 DOI: 10.1172/JCI37048]
53 **Henriksen EJ**, Diamond-Stanic MK, Marchionne EM. Oxidative stress and the etiology of insulin resistance and type 2 diabetes. *Free Radic Biol Med* 2011; **51**: 993-999 [PMID: 21163347 DOI: 10.1016/j.freeradbiomed.2010.12.005]

54 **Wei Y**, Sowers JR, Nistala R, Gong H, Uptergrove GM, Clark SE, Morris EM, Szary N, Manrique C, Stump CS. Angiotensin II-induced NADPH oxidase activation impairs insulin signaling in skeletal muscle cells. *J Biol Chem* 2006; **281**: 35137-35146 [PMID: 16982630 DOI: 10.1074/jbc.M601320200]

55 **Petersen MC**, Shulman GI. Mechanisms of Insulin Action and Insulin Resistance. *Physiol Rev* 2018; **98**: 2133-2223 [PMID: 30067154 DOI: 10.1152/physrev.00063.2017]

56 **Masenga SK**, Kabwe LS, Chakulya M, Kirabo A. Mechanisms of Oxidative Stress in Metabolic Syndrome. *Int J Mol Sci* 2023; **24** [PMID: 37175603 DOI: 10.3390/ijms24097898]

57 **Nolfi-Donegan D**, Braganza A, Shiva S. Mitochondrial electron transport chain: Oxidative phosphorylation, oxidant production, and methods of measurement. *Redox Biol* 2020; **37**: 101674 [PMID: 32811789 DOI: 10.1016/j.redox.2020.101674]

58 **He L**, He T, Farrar S, Ji L, Liu T, Ma X. Antioxidants Maintain Cellular Redox Homeostasis by Elimination of Reactive Oxygen Species. *Cell Physiol Biochem* 2017; **44**: 532-553 [PMID: 29145191 DOI: 10.1159/000485089]

59 **Choi SW**, Benzie IF, Ma SW, Strain JJ, Hannigan BM. Acute hyperglycemia and oxidative stress: direct cause and effect? *Free Radic Biol Med* 2008; **44**: 1217-1231 [PMID: 18226604 DOI: 10.1016/j.freeradbiomed.2007.12.005]

60 **Qasim N**, Arif A, Mahmood R. Hyperglycemia enhances the generation of ROS and RNS that impair antioxidant power and cause oxidative damage in human erythrocytes. *Biochem Cell Biol* 2023; **101**: 64-76 [PMID: 36379031 DOI: 10.1139/bcb-2022-0008]

61 **Yaribeygi H**, Sathyapalan T, Atkin SL, Sahebkar A. Molecular Mechanisms Linking Oxidative Stress and Diabetes Mellitus. *Oxid Med Cell Longev* 2020; **2020**: 8609213 [PMID: 32215179 DOI: 10.1155/2020/8609213]

62 **Copps KD**, White MF. Regulation of insulin sensitivity by serine/threonine phosphorylation of insulin receptor substrate proteins IRS1 and IRS2. *Diabetologia* 2012; **55**: 2565-2582 [PMID: 22869320 DOI: 10.1007/s00125-012-2644-8]

63 **Solinas G**, Becattini B. JNK at the crossroad of obesity, insulin resistance, and cell stress response. *Mol Metab* 2017; **6**: 174-184 [PMID: 28180059 DOI: 10.1016/j.molmet.2016.12.001]

64 **Mi Y**, Zhang W, Tian H, Li R, Huang S, Li X, Qi G, Liu X. EGCG evokes Nrf2 nuclear translocation and dampens PTP1B expression to ameliorate metabolic misalignment under insulin resistance condition. *Food Funct* 2018; **9**: 1510-1523 [PMID: 29423494 DOI: 10.1039/c7fo01554b]

65 **Vlavcheski F**, Den Hartogh DJ, Giacca A, Tsiani E. Amelioration of High-Insulin-Induced Skeletal Muscle Cell Insulin Resistance by Resveratrol Is Linked to Activation of AMPK and Restoration of GLUT4 Translocation. *Nutrients* 2020; **12** [PMID: 32230718 DOI: 10.3390/nu12040914]

66 **Zin CAJCM**, Mohamed WMIW, Khan NAK, Ishak WRW. Effects of Fruit and Vegetable Polyphenols on the Glycemic Control and Metabolic Parameters in Type 2 Diabetes Mellitus: A Review. *Prev Nutr Food Sci* 2022; **27**: 257-264 [PMID: 36313061 DOI: 10.3746/pnf.2022.27.3.257]

67 **Shahwan M**, Alhumaydhi F, Ashraf GM, Hasan PMZ, Shamsi A. Role of polyphenols in combating Type 2 Diabetes and insulin resistance. *Int J Biol Macromol* 2022; **206**: 567-579 [PMID: 35247420 DOI: 10.1016/j.ijbiomac.2022.03.004]

68 **Abdelhaleem IA**, Brakat AM, Adayel HM, Asla MM, Rizk MA, Aboalfetoh AY. The effects of resveratrol on glycemic control and cardiometabolic parameters in patients with T2DM: A systematic review and meta-analysis. *Med Clin (Barc)* 2022; **158**: 576-585 [PMID: 34666902 DOI: 10.1016/j.medcli.2021.06.028]

69 **Teimouri M**, Hosseini H, ArabSadeghabadi Z, Babaei-Khorzoughi R, Gorgani-Firuzjaee S, Meshkani R. The role of protein tyrosine phosphatase 1B (PTP1B) in the pathogenesis of type 2 diabetes mellitus and its complications. *J Physiol Biochem* 2022; **78**: 307-322 [PMID: 34988903 DOI: 10.1007/s13105-021-00860-7]

70 **Hussain H**, Green IR, Abbas G, Adekenov SM, Hussain W, Ali I. Protein tyrosine phosphatase 1B (PTP1B) inhibitors as potential anti-diabetes agents: patent review (2015-2018). *Expert Opin Ther Pat* 2019; **29**: 689-702 [PMID: 31402706 DOI: 10.1080/13543776.2019.1655542]

71 **Rath P**, Ranjan A, Ghosh A, Chauhan A, Gurnani M, Tuli HS, Habeeballah H, Alkhanani MF, Haque S, Dhama K, Verma NK, Jindal T. Potential Therapeutic Target Protein Tyrosine Phosphatase-1B for Modulation of Insulin Resistance with Polyphenols and Its Quantitative Structure-Activity Relationship. *Molecules* 2022; **27** [PMID: 35408611 DOI: 10.3390/molecules27072212]

72 **Furukawa S**, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, Shimomura I. Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest* 2004; **114**: 1752-1761 [PMID: 15599400 DOI: 10.1172/JCI21625]

73 **Keaney JF Jr**, Larson MG, Vasan RS, Wilson PW, Lipinska I, Corey D, Massaro JM, Sutherland P, Vita JA, Benjamin EJ; Framingham Study. Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham Study. *Arterioscler Thromb Vasc Biol* 2003; **23**: 434-439 [PMID: 12615693 DOI: 10.1161/01.ATV.0000058402.34138.11]

74 **Skalicky J**, Muzakova V, Kandar R, Meloun M, Rousar T, Palicka V. Evaluation of oxidative stress and inflammation in obese adults with metabolic syndrome. *Clin Chem Lab Med* 2008; **46**: 499-505 [PMID: 18298345 DOI: 10.1515/CCLM.2008.096]

75 **Chrysohoou C**, Panagiotakos DB, Pitsavos C, Skoumas I, Papademetriou L, Economou M, Stefanadis C. The implication of obesity on total antioxidant capacity in apparently healthy men and women: the ATTICA study. *Nutr Metab Cardiovasc Dis* 2007; **17**: 590-597 [PMID: 16901682 DOI: 10.1016/j.numecd.2006.05.007]

76 **Savini I**, Catani MV, Evangelista D, Gasperi V, Avigliano L. Obesity-associated oxidative stress: strategies finalized to improve redox state. *Int J Mol Sci* 2013; **14**: 10497-10538 [PMID: 23698776 DOI: 10.3390/ijms140510497]

77 **Manna P**, Jain SK. Obesity, Oxidative Stress, Adipose Tissue Dysfunction, and the Associated Health Risks: Causes and Therapeutic Strategies. *Metab Syndr Relat Disord* 2015; **13**: 423-444 [PMID: 26569333 DOI: 10.1089/met.2015.0095]

78 **Marseglia L**, Manti S, D'Angelo G, Nicotera A, Parisi E, Di Rosa G, Gitto E, Arrigo T. Oxidative stress in obesity: a critical component in human diseases. *Int J Mol Sci* 2014; **16**: 378-400 [PMID: 25548896 DOI: 10.3390/ijms16010378]

79 **Aloo SO**, Ofosu FK, Kim NH, Kilonzi SM, Oh DH. Insights on Dietary Polyphenols as Agents against Metabolic Disorders: Obesity as a Target Disease. *Antioxidants (Basel)* 2023; **12** [PMID: 36829976 DOI: 10.3390/antiox12020416]

80 **Zamani-Garmsiri F**, Emamgholipour S, Rahmani Fard S, Ghasempour G, Jahangard Ahvazi R, Meshkani R. Polyphenols: Potential anti-inflammatory agents for treatment of metabolic disorders. *Phytother Res* 2022; **36**: 415-432 [PMID: 34825416 DOI: 10.1002/ptr.7329]

81 **Badshah H**, Ullah I, Kim SE, Kim TH, Lee HY, Kim MO. Anthocyanins attenuate body weight gain *via* modulating neuropeptide Y and GABAB1 receptor in rats hypothalamus. *Neuropeptides* 2013; **47**: 347-353 [PMID: 23830691 DOI: 10.1016/j.npep.2013.06.001]

82 **Buchholz T**, Melzig MF. Polyphenolic Compounds as Pancreatic Lipase Inhibitors. *Planta Med* 2015; **81**: 771-783 [PMID: 26132857 DOI: 10.1055/s-0035-1546173]

83 **Marrelli M**, Russo C, Statti G, Argentieri MP, Meleleo D, Mallamaci R, Avato P, Conforti F. Phytochemical and biological characterization of dry outer scales extract from Tropea red onion (Allium cepa L. var. Tropea)-A promising inhibitor of pancreatic lipase. *Phytomedicine Plus* 2022; **2**: 100235 [DOI: 10.1016/j.phyplu.2022.100235]

84 **Kim NH**, Jegal J, Kim YN, Heo JD, Rho JR, Yang MH, Jeong EJ. Chokeberry Extract and Its Active Polyphenols Suppress Adipogenesis in 3T3-L1 Adipocytes and Modulates Fat Accumulation and Insulin Resistance in Diet-Induced Obese Mice. *Nutrients* 2018; **10** [PMID: 30424495 DOI: 10.3390/nu10111734]

85 **Rocha A**, Bolin AP, Cardoso CA, Otton R. Green tea extract activates AMPK and ameliorates white adipose tissue metabolic dysfunction induced by obesity. *Eur J Nutr* 2016; **55**: 2231-2244 [PMID: 26361764 DOI: 10.1007/s00394-015-1033-8]

86 **Lee MS**, Shin Y, Jung S, Kim Y. Effects of epigallocatechin-3-gallate on thermogenesis and mitochondrial biogenesis in brown adipose tissues of diet-induced obese mice. *Food Nutr Res* 2017; **61**: 1325307 [PMID: 28659734 DOI: 10.1080/16546628.2017.1325307]

87 **Valli V**, Heilmann K, Danesi F, Bordoni A, Gerhäuser C. Modulation of Adipocyte Differentiation and Proadipogenic Gene Expression by Sulforaphane, Genistein, and Docosahexaenoic Acid as a First Step to Counteract Obesity. *Oxid Med Cell Longev* 2018; **2018**: 1617202 [PMID: 29576843 DOI: 10.1155/2018/1617202]

88 **Carpéné C**, Pejenaute H, Del Moral R, Boulet N, Hijona E, Andrade F, Villanueva-Millán MJ, Aguirre L, Arbones-Mainar JM. The Dietary Antioxidant Piceatannol Inhibits Adipogenesis of Human Adipose Mesenchymal Stem Cells and Limits Glucose Transport and Lipogenic Activities in Adipocytes. *Int J Mol Sci* 2018; **19** [PMID: 30018277 DOI: 10.3390/ijms19072081]

89 **Matijašić M**, Meštrović T, Paljetak HČ, Perić M, Barešić A, Verbanac D. Gut Microbiota beyond Bacteria-Mycobiome, Virome, Archaeome, and Eukaryotic Parasites in IBD. *Int J Mol Sci* 2020; **21** [PMID: 32290414 DOI: 10.3390/ijms21082668]

90 **Hills RD Jr**, Pontefract BA, Mishcon HR, Black CA, Sutton SC, Theberge CR. Gut Microbiome: Profound Implications for Diet and Disease. *Nutrients* 2019; **11** [PMID: 31315227 DOI: 10.3390/nu11071613]

91 **Jandhyala SM**, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Nageshwar Reddy D. Role of the normal gut microbiota. *World J Gastroenterol* 2015; **21**: 8787-8803 [PMID: 26269668 DOI: 10.3748/wjg.v21.i29.8787]

92 **Larsen N**, Vogensen FK, van den Berg FW, Nielsen DS, Andreasen AS, Pedersen BK, Al-Soud WA, Sørensen SJ, Hansen LH, Jakobsen M. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLoS One* 2010; **5**: e9085 [PMID: 20140211 DOI: 10.1371/journal.pone.0009085]

93 **Tilg H**, Moschen AR. Microbiota and diabetes: an evolving relationship. *Gut* 2014; **63**: 1513-1521 [PMID: 24833634 DOI: 10.1136/gutjnl-2014-306928]

94 **Canfora EE**, Jocken JW, Blaak EE. Short-chain fatty acids in control of body weight and insulin sensitivity. *Nat Rev Endocrinol* 2015; **11**: 577-591 [PMID: 26260141 DOI: 10.1038/nrendo.2015.128]

95 **Cani PD**, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, Burcelin R. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes* 2008; **57**: 1470-1481 [PMID: 18305141 DOI: 10.2337/db07-1403]

96 **Tanase DM**, Gosav EM, Neculae E, Costea CF, Ciocoiu M, Hurjui LL, Tarniceriu CC, Maranduca MA, Lacatusu CM, Floria M, Serban IL. Role of Gut Microbiota on Onset and Progression of Microvascular Complications of Type 2 Diabetes (T2DM). *Nutrients* 2020; **12** [PMID: 33276482 DOI: 10.3390/nu12123719]

97 **Cani PD**, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, Neyrinck AM, Fava F, Tuohy KM, Chabo C, Waget A, Delmée E, Cousin B, Sulpice T, Chamontin B, Ferrières J, Tanti JF, Gibson GR, Casteilla L, Delzenne NM, Alessi MC, Burcelin R. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 2007; **56**: 1761-1772 [PMID: 17456850 DOI: 10.2337/db06-1491]

98 **Grasset E**, Puel A, Charpentier J, Collet X, Christensen JE, Tercé F, Burcelin R. A Specific Gut Microbiota Dysbiosis of Type 2 Diabetic Mice Induces GLP-1 Resistance through an Enteric NO-Dependent and Gut-Brain Axis Mechanism. *Cell Metab* 2017; **25**: 1075-1090.e5 [PMID: 28467926 DOI: 10.1016/j.cmet.2017.04.013]

99 **Koudoufio M**, Desjardins Y, Feldman F, Spahis S, Delvin E, Levy E. Insight into Polyphenol and Gut Microbiota Crosstalk: Are Their Metabolites the Key to Understand Protective Effects against Metabolic Disorders? *Antioxidants (Basel)* 2020; **9** [PMID: 33066106 DOI: 10.3390/antiox9100982]

100 **Anhê FF**, Roy D, Pilon G, Dudonné S, Matamoros S, Varin TV, Garofalo C, Moine Q, Desjardins Y, Levy E, Marette A. A polyphenol-rich cranberry extract protects from diet-induced obesity, insulin resistance and intestinal inflammation in association with increased Akkermansia spp. population in the gut microbiota of mice. *Gut* 2015; **64**: 872-883 [PMID: 25080446 DOI: 10.1136/gutjnl-2014-307142]

101 **Chen K**, Gao Z, Ding Q, Tang C, Zhang H, Zhai T, Xie W, Jin Z, Zhao L, Liu W. Effect of natural polyphenols in Chinese herbal medicine on obesity and diabetes: Interactions among gut microbiota, metabolism, and immunity. *Front Nutr* 2022; **9**: 962720 [PMID: 36386943 DOI: 10.3389/fnut.2022.962720]

102 **Hara T**, Hirasawa A, Sun Q, Sadakane K, Itsubo C, Iga T, Adachi T, Koshimizu TA, Hashimoto T, Asakawa Y, Tsujimoto G. Novel selective ligands for free fatty acid receptors GPR120 and GPR40. *Naunyn Schmiedebergs Arch Pharmacol* 2009; **380**: 247-255 [PMID: 19471906 DOI: 10.1007/s00210-009-0425-9]

103 **Takikawa M**, Kurimoto Y, Tsuda T. Curcumin stimulates glucagon-like peptide-1 secretion in GLUTag cells *via* Ca2+/calmodulin-dependent kinase II activation. *Biochem Biophys Res Commun* 2013; **435**: 165-170 [PMID: 23660191 DOI: 10.1016/j.bbrc.2013.04.092]

104 **Kato M**, Nishikawa S, Ikehata A, Dochi K, Tani T, Takahashi T, Imaizumi A, Tsuda T. Curcumin improves glucose tolerance *via* stimulation of glucagon-like peptide-1 secretion. *Mol Nutr Food Res* 2017; **61** [PMID: 27990751 DOI: 10.1002/mnfr.201600471]

105 **Rehman K**, Ali MB, Akash MSH. Genistein enhances the secretion of glucagon-like peptide-1 (GLP-1) *via* downregulation of inflammatory responses. *Biomed Pharmacother* 2019; **112**: 108670 [PMID: 30784939 DOI: 10.1016/j.biopha.2019.108670]

106 **Fan J**, Johnson MH, Lila MA, Yousef G, de Mejia EG. Berry and Citrus Phenolic Compounds Inhibit Dipeptidyl Peptidase IV: Implications in Diabetes Management. *Evid Based Complement Alternat Med* 2013; **2013**: 479505 [PMID: 24069048 DOI: 10.1155/2013/479505]

107 **Wang Y**, Alkhalidy H, Liu D. The Emerging Role of Polyphenols in the Management of Type 2 Diabetes. *Molecules* 2021; **26** [PMID: 33572808 DOI: 10.3390/molecules26030703]

108 **Tzounis X**, Vulevic J, Kuhnle GG, George T, Leonczak J, Gibson GR, Kwik-Uribe C, Spencer JP. Flavanol monomer-induced changes to the human faecal microflora. *Br J Nutr* 2008; **99**: 782-792 [PMID: 17977475 DOI: 10.1017/S0007114507853384]

109 **Unno T**, Sakuma M, Mitsuhashi S. Effect of dietary supplementation of (-)-epigallocatechin gallate on gut microbiota and biomarkers of colonic fermentation in rats. *J Nutr Sci Vitaminol (Tokyo)* 2014; **60**: 213-219 [PMID: 25078378 DOI: 10.3177/jnsv.60.213]

110 **Prabhakar PK**, Doble M. Synergistic effect of phytochemicals in combination with hypoglycemic drugs on glucose uptake in myotubes. *Phytomedicine* 2009; **16**: 1119-1126 [PMID: 19660925 DOI: 10.1016/j.phymed.2009.05.021]

111 **Prabhakar PK**, Doble M. Interaction of phytochemicals with hypoglycemic drugs on glucose uptake in L6 myotubes. *Phytomedicine* 2011; **18**: 285-291 [PMID: 20724125 DOI: 10.1016/j.phymed.2010.06.016]

112 **Upadhyay S**, Dixit M. Role of Polyphenols and Other Phytochemicals on Molecular Signaling. *Oxid Med Cell Longev* 2015; **2015**: 504253 [PMID: 26180591 DOI: 10.1155/2015/504253]

113 **Pedersen HK**, Gudmundsdottir V, Nielsen HB, Hyotylainen T, Nielsen T, Jensen BA, Forslund K, Hildebrand F, Prifti E, Falony G, Le Chatelier E, Levenez F, Doré J, Mattila I, Plichta DR, Pöhö P, Hellgren LI, Arumugam M, Sunagawa S, Vieira-Silva S, Jørgensen T, Holm JB, Trošt K; MetaHIT Consortium, Kristiansen K, Brix S, Raes J, Wang J, Hansen T, Bork P, Brunak S, Oresic M, Ehrlich SD, Pedersen O. Human gut microbes impact host serum metabolome and insulin sensitivity. *Nature* 2016; **535**: 376-381 [PMID: 27409811 DOI: 10.1038/nature18646]

114 **Guasch-Ferré M**, Hruby A, Toledo E, Clish CB, Martínez-González MA, Salas-Salvadó J, Hu FB. Metabolomics in Prediabetes and Diabetes: A Systematic Review and Meta-analysis. *Diabetes Care* 2016; **39**: 833-846 [PMID: 27208380 DOI: 10.2337/dc15-2251]

115 **Bragg F**, Kartsonaki C, Guo Y, Holmes M, Du H, Yu C, Pei P, Yang L, Jin D, Chen Y, Schmidt D, Avery D, Lv J, Chen J, Clarke R, Hill M, Li L, Millwood I, Chen Z. Circulating Metabolites and the Development of Type 2 Diabetes in Chinese Adults. *Diabetes Care* 2022; **45**: 477-480 [PMID: 34848488 DOI: 10.2337/dc21-1415]

116 **Vanweert F**, Schrauwen P, Phielix E. Role of branched-chain amino acid metabolism in the pathogenesis of obesity and type 2 diabetes-related metabolic disturbances BCAA metabolism in type 2 diabetes. *Nutr Diabetes* 2022; **12**: 35 [PMID: 35931683 DOI: 10.1038/s41387-022-00213-3]

117 **Bloomgarden Z**. Diabetes and branched-chain amino acids: What is the link? *J Diabetes* 2018; **10**: 350-352 [PMID: 29369529 DOI: 10.1111/1753-0407.12645]

118 **White PJ**, Lapworth AL, An J, Wang L, McGarrah RW, Stevens RD, Ilkayeva O, George T, Muehlbauer MJ, Bain JR, Trimmer JK, Brosnan MJ, Rolph TP, Newgard CB. Branched-chain amino acid restriction in Zucker-fatty rats improves muscle insulin sensitivity by enhancing efficiency of fatty acid oxidation and acyl-glycine export. *Mol Metab* 2016; **5**: 538-551 [PMID: 27408778 DOI: 10.1016/j.molmet.2016.04.006]

119 **Bartova S**, Madrid-Gambin F, Fernández L, Carayol J, Meugnier E, Segrestin B, Delage P, Vionnet N, Boizot A, Laville M, Vidal H, Marco S, Hager J, Moco S. Grape polyphenols decrease circulating branched chain amino acids in overfed adults. *Front Nutr* 2022; **9**: 998044 [PMID: 36386937 DOI: 10.3389/fnut.2022.998044]

120 **Harris CS**, Cuerrier A, Lamont E, Haddad PS, Arnason JT, Bennett SA, Johns T. Investigating wild berries as a dietary approach to reducing the formation of advanced glycation endproducts: chemical correlates of in vitro antiglycation activity. *Plant Foods Hum Nutr* 2014; **69**: 71-77 [PMID: 24448675 DOI: 10.1007/s11130-014-0403-3]

121 **Ramkissoon JS**, Mahomoodally MF, Ahmed N, Subratty AH. Antioxidant and anti-glycation activities correlates with phenolic composition of tropical medicinal herbs. *Asian Pac J Trop Med* 2013; **6**: 561-569 [PMID: 23768830 DOI: 10.1016/S1995-7645(13)60097-8]

122 **Coelho OGL**, Ribeiro PVM, Alfenas RCG. Can grape polyphenols affect glycation markers? A systematic review. *Crit Rev Food Sci Nutr* 2023; **63**: 1208-1218 [PMID: 34369228 DOI: 10.1080/10408398.2021.1962796]

123 **Larrosa M**, González-Sarrías A, Yáñez-Gascón MJ, Selma MV, Azorín-Ortuño M, Toti S, Tomás-Barberán F, Dolara P, Espín JC. Anti-inflammatory properties of a pomegranate extract and its metabolite urolithin-A in a colitis rat model and the effect of colon inflammation on phenolic metabolism. *J Nutr Biochem* 2010; **21**: 717-725 [PMID: 19616930 DOI: 10.1016/j.jnutbio.2009.04.012]

124 **Li Y**, Liu Y, Liu S, Gao M, Wang W, Chen K, Huang L. Diabetic vascular diseases: molecular mechanisms and therapeutic strategies. *Signal Transduct Target Ther* 2023; **8**: 152 [PMID: 37037849 DOI: 10.1038/s41392-023-01400-z]

125 **Nesto RW**. Correlation between cardiovascular disease and diabetes mellitus: current concepts. *Am J Med* 2004; **116** Suppl 5A: 11S-22S [PMID: 15019859 DOI: 10.1016/j.amjmed.2003.10.016]

126 **Giugliano D**, Ceriello A, Paolisso G. Oxidative stress and diabetic vascular complications. *Diabetes Care* 1996; **19**: 257-267 [PMID: 8742574 DOI: 10.2337/diacare.19.3.257]

127 **Rojas A**, Figueroa H, Re L, Morales MA. Oxidative stress at the vascular wall. Mechanistic and pharmacological aspects. *Arch Med Res* 2006; **37**: 436-448 [PMID: 16624640 DOI: 10.1016/j.arcmed.2005.11.012]

128 **Gao L**, Mann GE. Vascular NAD(P)H oxidase activation in diabetes: a double-edged sword in redox signalling. *Cardiovasc Res* 2009; **82**: 9-20 [PMID: 19179352 DOI: 10.1093/cvr/cvp031]

129 **Deanfield JE**, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. *Circulation* 2007; **115**: 1285-1295 [PMID: 17353456 DOI: 10.1161/CIRCULATIONAHA.106.652859]

130 **Krüger-Genge A**, Blocki A, Franke RP, Jung F. Vascular Endothelial Cell Biology: An Update. *Int J Mol Sci* 2019; **20** [PMID: 31500313 DOI: 10.3390/ijms20184411]

131 **Boulanger CM**. Endothelium. *Arterioscler Thromb Vasc Biol* 2016; **36**: e26-e31 [PMID: 27010027 DOI: 10.1161/ATVBAHA.116.306940]

132 **Tousoulis D**, Kampoli AM, Tentolouris C, Papageorgiou N, Stefanadis C. The role of nitric oxide on endothelial function. *Curr Vasc Pharmacol* 2012; **10**: 4-18 [PMID: 22112350 DOI: 10.2174/157016112798829760]

133 **Zou MH**, Cohen R, Ullrich V. Peroxynitrite and vascular endothelial dysfunction in diabetes mellitus. *Endothelium* 2004; **11**: 89-97 [PMID: 15370068 DOI: 10.1080/10623320490482619]

134 **Szabo C**. Role of nitrosative stress in the pathogenesis of diabetic vascular dysfunction. *Br J Pharmacol* 2009; **156**: 713-727 [PMID: 19210748 DOI: 10.1111/j.1476-5381.2008.00086.x]

135 **Förstermann U**, Xia N, Li H. Roles of Vascular Oxidative Stress and Nitric Oxide in the Pathogenesis of Atherosclerosis. *Circ Res* 2017; **120**: 713-735 [PMID: 28209797 DOI: 10.1161/CIRCRESAHA.116.309326]

136 **Martins TF**, Palomino OM, Álvarez-Cilleros D, Martín MA, Ramos S, Goya L. Cocoa Flavanols Protect Human Endothelial Cells from Oxidative Stress. *Plant Foods Hum Nutr* 2020; **75**: 161-168 [PMID: 32185628 DOI: 10.1007/s11130-020-00807-1]

137 **Zhou H**, Fu B, Xu B, Mi X, Li G, Ma C, Xie J, Li J, Wang Z. Rosmarinic Acid Alleviates the Endothelial Dysfunction Induced by Hydrogen Peroxide in Rat Aortic Rings *via* Activation of AMPK. *Oxid Med Cell Longev* 2017; **2017**: 7091904 [PMID: 28883905 DOI: 10.1155/2017/7091904]

138 **Serraino I**, Dugo L, Dugo P, Mondello L, Mazzon E, Dugo G, Caputi AP, Cuzzocrea S. Protective effects of cyanidin-3-O-glucoside from blackberry extract against peroxynitrite-induced endothelial dysfunction and vascular failure. *Life Sci* 2003; **73**: 1097-1114 [PMID: 12818719 DOI: 10.1016/S0024-3205(03)00356-4]

139 **Spanier G**, Xu H, Xia N, Tobias S, Deng S, Wojnowski L, Forstermann U, Li H. Resveratrol reduces endothelial oxidative stress by

modulating the gene expression of superoxide dismutase 1 (SOD1), glutathione peroxidase 1 (GPx1) and NADPH oxidase subunit (Nox4). *J Physiol Pharmacol* 2009; **60** Suppl 4: 111-116 [PMID: 20083859]

140 **Alvarez S**, Zaobornyj T, Actis-Goretta L, Fraga CG, Boveris A. Polyphenols and red wine as peroxynitrite scavengers: a chemiluminescent assay. *Ann N Y Acad Sci* 2002; **957**: 271-273 [PMID: 12074979 DOI: 10.1111/j.1749-6632.2002.tb02923.x]

141 **Liang XX**, Wang RY, Guo YZ, Cheng Z, Lv DY, Luo MH, He A, Luo SX, Xia Y. Phosphorylation of Akt at Thr308 regulates p-eNOS Ser1177 during physiological conditions. *FEBS Open Bio* 2021; **11**: 1953-1964 [PMID: 33993653 DOI: 10.1002/2211-5463.13194]

142 **Madeira SV**, Auger C, Anselm E, Chataigneau M, Chataigneau T, Soares de Moura R, Schini-Kerth VB. eNOS activation induced by a polyphenol-rich grape skin extract in porcine coronary arteries. *J Vasc Res* 2009; **46**: 406-416 [PMID: 19155632 DOI: 10.1159/000194271]

143 **Edirisinghe I**, Burton-Freeman B, Tissa Kappagoda C. Mechanism of the endothelium-dependent relaxation evoked by a grape seed extract. *Clin Sci (Lond)* 2008; **114**: 331-337 [PMID: 17927567 DOI: 10.1042/CS20070264]

144 **Wallerath T**, Poleo D, Li H, Förstermann U. Red wine increases the expression of human endothelial nitric oxide synthase: a mechanism that may contribute to its beneficial cardiovascular effects. *J Am Coll Cardiol* 2003; **41**: 471-478 [PMID: 12575978 DOI: 10.1016/S0735-1097(02)02826-7]

145 **Wallerath T**, Li H, Gödtel-Ambrust U, Schwarz PM, Förstermann U. A blend of polyphenolic compounds explains the stimulatory effect of red wine on human endothelial NO synthase. *Nitric Oxide* 2005; **12**: 97-104 [PMID: 15740983 DOI: 10.1016/j.niox.2004.12.004]

146 **Wallerath T**, Deckert G, Ternes T, Anderson H, Li H, Witte K, Förstermann U. Resveratrol, a polyphenolic phytoalexin present in red wine, enhances expression and activity of endothelial nitric oxide synthase. *Circulation* 2002; **106**: 1652-1658 [PMID: 12270858 DOI: 10.1161/01.CIR.0000029925.18593.5C]

147 **Xia N**, Strand S, Schlufter F, Siuda D, Reifenberg G, Kleinert H, Förstermann U, Li H. Role of SIRT1 and FOXO factors in eNOS transcriptional activation by resveratrol. *Nitric Oxide* 2013; **32**: 29-35 [PMID: 23583951 DOI: 10.1016/j.niox.2013.04.001]

148 **Goldin A**, Beckman JA, Schmidt AM, Creager MA. Advanced glycation end products: sparking the development of diabetic vascular injury. *Circulation* 2006; **114**: 597-605 [PMID: 16894049 DOI: 10.1161/CIRCULATIONAHA.106.621854]

149 **Brownlee M**, Vlassara H, Cerami A. Nonenzymatic glycosylation and the pathogenesis of diabetic complications. *Ann Intern Med* 1984; **101**: 527-537 [PMID: 6383165 DOI: 10.7326/0003-4819-101-4-527]

150 **Cerami A**, Vlassara H, Brownlee M. Role of advanced glycosylation products in complications of diabetes. *Diabetes Care* 1988; **11** Suppl 1: 73-79 [PMID: 3069394]

151 **Khalid M**, Petroianu G, Adem A. Advanced Glycation End Products and Diabetes Mellitus: Mechanisms and Perspectives. *Biomolecules* 2022; **12** [PMID: 35454131 DOI: 10.3390/biom12040542]

152 **Peppa M**, Uribarri J, Vlassara H. Glucose, Advanced Glycation End Products, and Diabetes Complications: What Is New and What Works. *Clin Diabetes* 2003; **21**: 186-187 [DOI: 10.2337/diaclin.21.4.186]

153 **Twarda-Clapa A**, Olczak A, Białkowska AM, Koziołkiewicz M. Advanced Glycation End-Products (AGEs): Formation, Chemistry, Classification, Receptors, and Diseases Related to AGEs. *Cells* 2022; **11** [PMID: 35455991 DOI: 10.3390/cells11081312]

154 **Brings S**, Fleming T, Freichel M, Muckenthaler MU, Herzig S, Nawroth PP. Dicarbonyls and Advanced Glycation End-Products in the Development of Diabetic Complications and Targets for Intervention. *Int J Mol Sci* 2017; **18** [PMID: 28475116 DOI: 10.3390/ijms18050984]

155 **Uribarri J**, del Castillo MD, de la Maza MP, Filip R, Gugliucci A, Luevano-Contreras C, Macías-Cervantes MH, Markowicz Bastos DH, Medrano A, Menini T, Portero-Otin M, Rojas A, Sampaio GR, Wrobel K, Garay-Sevilla ME. Dietary advanced glycation end products and their role in health and disease. *Adv Nutr* 2015; **6**: 461-473 [PMID: 26178030 DOI: 10.3945/an.115.008433]

156 **Gautieri A**, Passini FS, Silván U, Guizar-Sicairos M, Carimati G, Volpi P, Moretti M, Schoenhuber H, Redaelli A, Berli M, Snedeker JG. Advanced glycation end-products: Mechanics of aged collagen from molecule to tissue. *Matrix Biol* 2017; **59**: 95-108 [PMID: 27616134 DOI: 10.1016/j.matbio.2016.09.001]

157 **Rojas A**, Añazco C, González I, Araya P. Extracellular matrix glycation and receptor for advanced glycation end-products activation: a missing piece in the puzzle of the association between diabetes and cancer. *Carcinogenesis* 2018; **39**: 515-521 [PMID: 29373651 DOI: 10.1093/carcin/bgy012]

158 **Rojas A**, Delgado-López F, González I, Pérez-Castro R, Romero J, Rojas I. The receptor for advanced glycation end-products: a complex signaling scenario for a promiscuous receptor. *Cell Signal* 2013; **25**: 609-614 [PMID: 23200851 DOI: 10.1016/j.cellsig.2012.11.022]

159 **Hudson BI**, Lippman ME. Targeting RAGE Signaling in Inflammatory Disease. *Annu Rev Med* 2018; **69**: 349-364 [PMID: 29106804 DOI: 10.1146/annurev-med-041316-085215]

160 **Moldogazieva NT**, Mokhosoev IM, Mel'nikova TI, Porozov YB, Terentiev AA. Oxidative Stress and Advanced Lipoxidation and Glycation End Products (ALEs and AGEs) in Aging and Age-Related Diseases. *Oxid Med Cell Longev* 2019; **2019**: 3085756 [PMID: 31485289 DOI: 10.1155/2019/3085756]

161 **Wautier MP**, Chappey O, Corda S, Stern DM, Schmidt AM, Wautier JL. Activation of NADPH oxidase by AGE links oxidant stress to altered gene expression *via* RAGE. *Am J Physiol Endocrinol Metab* 2001; **280**: E685-E694 [PMID: 11287350 DOI: 10.1152/ajpendo.2001.280.5.E685]

162 **Yeh WJ**, Hsia SM, Lee WH, Wu CH. Polyphenols with antiglycation activity and mechanisms of action: A review of recent findings. *J Food Drug Anal* 2017; **25**: 84-92 [PMID: 28911546 DOI: 10.1016/j.jfda.2016.10.017]

163 **Khanam A**, Ahmad S, Husain A, Rehman S, Farooqui A, Yusuf MA. Glycation and Antioxidants: Hand in the Glove of Antiglycation and Natural Antioxidants. *Curr Protein Pept Sci* 2020; **21**: 899-915 [PMID: 32039678 DOI: 10.2174/1389203721666200210103304]

164 **González I**, Morales MA, Rojas A. Polyphenols and AGEs/RAGE axis. Trends and challenges. *Food Res Int* 2020; **129**: 108843 [PMID: 32036875 DOI: 10.1016/j.foodres.2019.108843]

165 **Sun C**, Zhao C, Guven EC, Paoli P, Simal-Gandara J, Ramkumar KM, Wang S, Buleu F, Pah A, Turi V, Damian G, Dragan S, Tomas M, Khan W, Wang M, Delmas D, Portillo MP, Dar P, Chen L, Xiao J. Dietary polyphenols as antidiabetic agents: Advances and opportunities. *Food Frontiers* 2020; **1**: 18-44 [DOI: 10.1002/fft2.15]

166 **Gugliucci A**, Bastos DH, Schulze J, Souza MF. Caffeic and chlorogenic acids in Ilex paraguariensis extracts are the main inhibitors of AGE generation by methylglyoxal in model proteins. *Fitoterapia* 2009; **80**: 339-344 [PMID: 19409454 DOI: 10.1016/j.fitote.2009.04.007]

167 **Genaro-Mattos TC**, Maurício ÂQ, Rettori D, Alonso A, Hermes-Lima M. Antioxidant Activity of Caffeic Acid against Iron-Induced Free Radical Generation--A Chemical Approach. *PLoS One* 2015; **10**: e0129963 [PMID: 26098639 DOI: 10.1371/journal.pone.0129963]

168 **Bhuiyan MN**, Mitsuhashi S, Sigetomi K, Ubukata M. Quercetin inhibits advanced glycation end product formation *via* chelating metal ions, trapping methylglyoxal, and trapping reactive oxygen species. *Biosci Biotechnol Biochem* 2017; **81**: 882-890 [PMID: 28388357 DOI: 10.1080/09168451.2017.1282805]

169 **Wang W**, Yagiz Y, Buran TJ, Nunes CDN, Gu L. Phytochemicals from berries and grapes inhibited the formation of advanced glycation end-products by scavenging reactive carbonyls. *Food Res Int* 2011; **44**: 2666-2673 [DOI: 10.1016/j.foodres.2011.05.022]

170 **Thornalley PJ**. The glyoxalase system: new developments towards functional characterization of a metabolic pathway fundamental to biological life. *Biochem J* 1990; **269**: 1-11 [PMID: 2198020 DOI: 10.1042/bj2690001]

171 **Frandsen J**, Narayanasamy P. Flavonoid Enhances the Glyoxalase Pathway in Cerebellar Neurons to Retain Cellular Functions. *Sci Rep* 2017; **7**: 5126 [PMID: 28698611 DOI: 10.1038/s41598-017-05287-z]

172 **Buttari B**, Profumo E, Facchiano F, Ozturk EI, Segoni L, Saso L, Riganò R. Resveratrol prevents dendritic cell maturation in response to advanced glycation end products. *Oxid Med Cell Longev* 2013; **2013**: 574029 [PMID: 23936610 DOI: 10.1155/2013/574029]

173 **Kanlaya R**, Thongboonkerd V. Molecular Mechanisms of Epigallocatechin-3-Gallate for Prevention of Chronic Kidney Disease and Renal Fibrosis: Preclinical Evidence. *Curr Dev Nutr* 2019; **3**: nzz101 [PMID: 31555758 DOI: 10.1093/cdn/nzz101]

174 **Dong L**, Li Y, Chen Q, Liu Y, Wu Z, Pan D, Yan N, Liu L. Cereal polyphenols inhibition mechanisms on advanced glycation end products and regulation on type 2 diabetes. *Crit Rev Food Sci Nutr* 2023; 1-19 [PMID: 37222572 DOI: 10.1080/10408398.2023.2213768]

175 **Jonas RA**, Crabtree TR, Jennings RS, Marques H, Katz RJ, Chang HJ, Stuijfzand WJ, van Rosendael AR, Choi JH, Doh JH, Her AY, Koo BK, Nam CW, Park HB, Shin SH, Cole J, Gimelli A, Khan MA, Lu B, Gao Y, Nabi F, Nakazato R, Schoepf UJ, Driessen RS, Bom MJ, Thompson RC, Jang JJ, Ridner M, Rowan C, Avelar E, Généreux P, Knaapen P, de Waard GA, Pontone G, Andreini D, Al-Mallah MH, Guglielmo M, Bax JJ, Earls JP, Min JK, Choi AD, Villines TC. Diabetes, Atherosclerosis, and Stenosis by AI. *Diabetes Care* 2023; **46**: 416-424 [PMID: 36577120 DOI: 10.2337/dc21-1663]

176 **Yuan T**, Yang T, Chen H, Fu D, Hu Y, Wang J, Yuan Q, Yu H, Xu W, Xie X. New insights into oxidative stress and inflammation during diabetes mellitus-accelerated atherosclerosis. *Redox Biol* 2019; **20**: 247-260 [PMID: 30384259 DOI: 10.1016/j.redox.2018.09.025]

177 **Poznyak A**, Grechko AV, Poggio P, Myasoedova VA, Alfieri V, Orekhov AN. The Diabetes Mellitus-Atherosclerosis Connection: The Role of Lipid and Glucose Metabolism and Chronic Inflammation. *Int J Mol Sci* 2020; **21** [PMID: 32155866 DOI: 10.3390/ijms21051835]

178 **Qiu Q**, Zhang F, Zhu W, Wu J, Liang M. Copper in Diabetes Mellitus: a Meta-Analysis and Systematic Review of Plasma and Serum Studies. *Biol Trace Elem Res* 2017; **177**: 53-63 [PMID: 27785738 DOI: 10.1007/s12011-016-0877-y]

179 **Banerjee J**, Mishra N, Damle G, Dhas Y. Beyond LDL-c: The importance of serum oxidized LDL in predicting risk for type 2 diabetes in the middle-aged Asian Indians. *Diabetes Metab Syndr* 2019; **13**: 206-213 [PMID: 30641698 DOI: 10.1016/j.dsx.2018.08.036]

180 **Shah MS**, Brownlee M. Molecular and Cellular Mechanisms of Cardiovascular Disorders in Diabetes. *Circ Res* 2016; **118**: 1808-1829 [PMID: 27230643 DOI: 10.1161/CIRCRESAHA.116.306923]

181 **Islam MA**, Amin MN, Siddiqui SA, Hossain MP, Sultana F, Kabir MR. Trans fatty acids and lipid profile: A serious risk factor to cardiovascular disease, cancer and diabetes. *Diabetes Metab Syndr* 2019; **13**: 1643-1647 [PMID: 31336535 DOI: 10.1016/j.dsx.2019.03.033]

182 **Heinloth A**, Heermeier K, Raff U, Wanner C, Galle J. Stimulation of NADPH oxidase by oxidized low-density lipoprotein induces proliferation of human vascular endothelial cells. *J Am Soc Nephrol* 2000; **11**: 1819-1825 [PMID: 11004212 DOI: 10.1681/ASN.V11101819]

183 **Wang Y**, Wang GZ, Rabinovitch PS, Tabas I. Macrophage mitochondrial oxidative stress promotes atherosclerosis and nuclear factor-κB-mediated inflammation in macrophages. *Circ Res* 2014; **114**: 421-433 [PMID: 24297735 DOI: 10.1161/CIRCRESAHA.114.302153]

184 **Feldman F**, Koudoufio M, Desjardins Y, Spahis S, Delvin E, Levy E. Efficacy of Polyphenols in the Management of Dyslipidemia: A Focus on Clinical Studies. *Nutrients* 2021; **13** [PMID: 33669729 DOI: 10.3390/nu13020672]

185 **Cayatte AJ**, Rupin A, Oliver-Krasinski J, Maitland K, Sansilvestri-Morel P, Boussard MF, Wierzbicki M, Verbeuren TJ, Cohen RA. S17834, a new inhibitor of cell adhesion and atherosclerosis that targets nadph oxidase. *Arterioscler Thromb Vasc Biol* 2001; **21**: 1577-1584 [PMID: 11597929 DOI: 10.1161/hq1001.096723]

186 **Zang M**, Xu S, Maitland-Toolan KA, Zuccollo A, Hou X, Jiang B, Wierzbicki M, Verbeuren TJ, Cohen RA. Polyphenols stimulate AMP-activated protein kinase, lower lipids, and inhibit accelerated atherosclerosis in diabetic LDL receptor-deficient mice. *Diabetes* 2006; **55**: 2180-2191 [PMID: 16873680 DOI: 10.2337/db05-1188]

187 **Frémont L**, Belguendouz L, Delpal S. Antioxidant activity of resveratrol and alcohol-free wine polyphenols related to LDL oxidation and polyunsaturated fatty acids. *Life Sci* 1999; **64**: 2511-2521 [PMID: 10403511 DOI: 10.1016/S0024-3205(99)00209-X]

188 **Aviram M**, Fuhrman B. Wine flavonoids protect against LDL oxidation and atherosclerosis. *Ann N Y Acad Sci* 2002; **957**: 146-161 [PMID: 12074969 DOI: 10.1111/j.1749-6632.2002.tb02913.x]

189 **Berrougui H**, Grenier G, Loued S, Drouin G, Khalil A. A new insight into resveratrol as an atheroprotective compound: inhibition of lipid peroxidation and enhancement of cholesterol efflux. *Atherosclerosis* 2009; **207**: 420-427 [PMID: 19552907 DOI: 10.1016/j.atherosclerosis.2009.05.017]

190 **Regnström J**, Nilsson J, Tornvall P, Landou C, Hamsten A. Susceptibility to low-density lipoprotein oxidation and coronary atherosclerosis in man. *Lancet* 1992; **339**: 1183-1186 [PMID: 1349935 DOI: 10.1016/0140-6736(92)91129-V]

191 **Heinecke JW**. Oxidants and antioxidants in the pathogenesis of atherosclerosis: implications for the oxidized low density lipoprotein hypothesis. *Atherosclerosis* 1998; **141**: 1-15 [PMID: 9863534 DOI: 10.1016/S0021-9150(98)00173-7]

192 **Li J**, Zhong Z, Yuan J, Chen X, Huang Z, Wu Z. Resveratrol improves endothelial dysfunction and attenuates atherogenesis in apolipoprotein E-deficient mice. *J Nutr Biochem* 2019; **67**: 63-71 [PMID: 30856465 DOI: 10.1016/j.jnutbio.2019.01.022]

193 **Yang H**, Yang T, Heng C, Zhou Y, Jiang Z, Qian X, Du L, Mao S, Yin X, Lu Q. Quercetin improves nonalcoholic fatty liver by ameliorating inflammation, oxidative stress, and lipid metabolism in db/db mice. *Phytother Res* 2019; **33**: 3140-3152 [PMID: 31452288 DOI: 10.1002/ptr.6486]

194 **Hosseini A**, Razavi BM, Banach M, Hosseinzadeh H. Quercetin and metabolic syndrome: A review. *Phytother Res* 2021; **35**: 5352-5364 [PMID: 34101925 DOI: 10.1002/ptr.7144]

195 **García-Martínez BI**, Ruiz-Ramos M, Pedraza-Chaverri J, Santiago-Osorio E, Mendoza-Núñez VM. Effect of Resveratrol on Markers of Oxidative Stress and Sirtuin 1 in Elderly Adults with Type 2 Diabetes. *Int J Mol Sci* 2023; **24** [PMID: 37108584 DOI: 10.3390/ijms24087422]

196 **Bapir M**, Untracht GR, Cooke D, McVey JH, Skene SS, Campagnolo P, Whyte MB, Dikaios N, Rodriguez-Mateos A, Sampson DD, Sampson DM, Heiss C. Cocoa flavanol consumption improves lower extremity endothelial function in healthy individuals and people with type 2 diabetes. *Food Funct* 2022; **13**: 10439-10448 [PMID: 36164983 DOI: 10.1039/d2fo02017c]

197 **García-Martínez BI**, Ruiz-Ramos M, Pedraza-Chaverri J, Santiago-Osorio E, Mendoza-Núñez VM. Hypoglycemic Effect of Resveratrol: A Systematic Review and Meta-Analysis. *Antioxidants (Basel)* 2021; **10** [PMID: 33430470 DOI: 10.3390/antiox10010069]

198 **Sirvent P**, Chavanelle V, Otero YF, Bargetto M, Le Joubioux F, Boisseau N, Maugard T, Cazaubiel M, Pereira B, Guigas B, Hadjadj S, Peltier SL, Marette A, Bard JM. TOTUM-63, a plant-based polyphenol-rich extract, improves glycaemic control in subjects with prediabetes or early

stage newly-diagnosed type 2 diabetes in a randomized, double-blind, placebo-controlled trial. *Diabetes Obes Metab* 2022; **24**: 2331-2340 [PMID: 35837981 DOI: 10.1111/dom.14817]

199 **Gu W**, Geng J, Zhao H, Li X, Song G. Effects of Resveratrol on Metabolic Indicators in Patients with Type 2 Diabetes: A Systematic Review and Meta-Analysis. *Int J Clin Pract* 2022; **2022**: 9734738 [PMID: 35685602 DOI: 10.1155/2022/9734738]

200 **Delpino FM**, Figueiredo LM, Nunes BP. Effects of melatonin supplementation on diabetes: A systematic review and meta-analysis of randomized clinical trials. *Clin Nutr* 2021; **40**: 4595-4605 [PMID: 34229264 DOI: 10.1016/j.clnu.2021.06.007]

201 **Mahjabeen W**, Khan DA, Mirza SA. Role of resveratrol supplementation in regulation of glucose hemostasis, inflammation and oxidative stress in patients with diabetes mellitus type 2: A randomized, placebo-controlled trial. *Complement Ther Med* 2022; **66**: 102819 [PMID: 35240291 DOI: 10.1016/j.ctim.2022.102819]

202 **Grabež M**, Škrbić R, Stojiljković MP, Vučić V, Rudić Grujić V, Jakovljević V, Djuric DM, Suručić R, Šavikin K, Bigović D, Vasiljević N. A prospective, randomized, double-blind, placebo-controlled trial of polyphenols on the outcomes of inflammatory factors and oxidative stress in patients with type 2 diabetes mellitus. *Rev Cardiovasc Med* 2022; **23**: 57 [PMID: 35229548 DOI: 10.31083/j.rcm2302057]

203 **Gómez-Martínez S**, Díaz-Prieto LE, Vicente Castro I, Jurado C, Iturmendi N, Martín-Ridaura MC, Calle N, Dueñas M, Picón MJ, Marcos A, Nova E. Moringa oleifera Leaf Supplementation as a Glycemic Control Strategy in Subjects with Prediabetes. *Nutrients* 2021; **14** [PMID: 35010932 DOI: 10.3390/nu14010057]

204 **Delpino FM**, Figueiredo LM. Resveratrol supplementation and type 2 diabetes: a systematic review and meta-analysis. *Crit Rev Food Sci Nutr* 2022; **62**: 4465-4480 [PMID: 33480264 DOI: 10.1080/10408398.2021.1875980]

205 **Nyambuya TM**, Nkambule BB, Mazibuko-Mbeje SE, Mxinwa V, Mokgalaboni K, Orlando P, Silvestri S, Louw J, Tiano L, Dludla PV. A Meta-Analysis of the Impact of Resveratrol Supplementation on Markers of Renal Function and Blood Pressure in Type 2 Diabetic Patients on Hypoglycemic Therapy. *Molecules* 2020; **25** [PMID: 33266114 DOI: 10.3390/molecules25235645]

206 **Davis DW**, Tallent R, Navalta JW, Salazar A, Lyons TJ, Basu A. Effects of Acute Cocoa Supplementation on Postprandial Apolipoproteins, Lipoprotein Subclasses, and Inflammatory Biomarkers in Adults with Type 2 Diabetes after a High-Fat Meal. *Nutrients* 2020; **12** [PMID: 32605005 DOI: 10.3390/nu12071902]

207 **Raimundo AF**, Félix F, Andrade R, García-Conesa MT, González-Sarrías A, Gilsa-Lopes J, do Ó D, Raimundo A, Ribeiro R, Rodriguez-Mateos A, Santos CN, Schär M, Silva A, Cruz I, Wang B, Pinto P, Menezes R. Combined effect of interventions with pure or enriched mixtures of (poly)phenols and anti-diabetic medication in type 2 diabetes management: a meta-analysis of randomized controlled human trials. *Eur J Nutr* 2020; **59**: 1329-1343 [PMID: 32052147 DOI: 10.1007/s00394-020-02189-1]

208 **Hoseini A**, Namazi G, Farrokhian A, Reiner Ž, Aghadavod E, Bahmani F, Asemi Z. The effects of resveratrol on metabolic status in patients with type 2 diabetes mellitus and coronary heart disease. *Food Funct* 2019; **10**: 6042-6051 [PMID: 31486447 DOI: 10.1039/c9fo01075k]

209 **Huang J**, Qin S, Huang L, Tang Y, Ren H, Hu H. Efficacy and safety of Rhizoma curcumea longae with respect to improving the glucose metabolism of patients at risk for cardiovascular disease: a meta-analysis of randomised controlled trials. *J Hum Nutr Diet* 2019; **32**: 591-606 [PMID: 30983042 DOI: 10.1111/jhn.12648]

210 **Adibian M**, Hodaei H, Nikpayam O, Sohrab G, Hekmatdoost A, Hedayati M. The effects of curcumin supplementation on high-sensitivity C-reactive protein, serum adiponectin, and lipid profile in patients with type 2 diabetes: A randomized, double-blind, placebo-controlled trial. *Phytother Res* 2019; **33**: 1374-1383 [PMID: 30864188 DOI: 10.1002/ptr.6328]

211 **Schell J**, Betts NM, Lyons TJ, Basu A. Raspberries Improve Postprandial Glucose and Acute and Chronic Inflammation in Adults with Type 2 Diabetes. *Ann Nutr Metab* 2019; **74**: 165-174 [PMID: 30763939 DOI: 10.1159/000497226]

212 **Mollace V**, Scicchitano M, Paone S, Casale F, Calandruccio C, Gliozzi M, Musolino V, Carresi C, Maiuolo J, Nucera S, Riva A, Allegrini P, Ronchi M, Petrangolini G, Bombardelli E. Hypoglycemic and Hypolipemic Effects of a New Lecithin Formulation of Bergamot Polyphenolic Fraction: A Double Blind, Randomized, Placebo- Controlled Study. *Endocr Metab Immune Disord Drug Targets* 2019; **19**: 136-143 [PMID: 30501605 DOI: 10.2174/1871530319666181203151513]

213 **Rienks J**, Barbaresko J, Oluwagbemigun K, Schmid M, Nöthlings U. Polyphenol exposure and risk of type 2 diabetes: dose-response meta-analyses and systematic review of prospective cohort studies. *Am J Clin Nutr* 2018; **108**: 49-61 [PMID: 29931039 DOI: 10.1093/ajcn/nqy083]

214 **Seyyedebrahimi S**, Khodabandehloo H, Nasli Esfahani E, Meshkani R. The effects of resveratrol on markers of oxidative stress in patients with type 2 diabetes: a randomized, double-blind, placebo-controlled clinical trial. *Acta Diabetol* 2018; **55**: 341-353 [PMID: 29357033 DOI: 10.1007/s00592-017-1098-3]

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ORIGINAL ARTICLE

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Identification of hub genes associated with *Helicobacter pylori* infection and type 2 diabetes mellitus: A pilot bioinformatics study

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Abstract

BACKGROUND

Helicobacter pylori (*H. pylori*) infection is related to various extragastric diseases including type 2 diabetes mellitus (T2DM). However, the possible mechanisms connecting *H. pylori* infection and T2DM remain unknown.

AIM

To explore potential molecular connections between *H. pylori* infection and T2DM.

METHODS

We extracted gene expression arrays from three online datasets (GSE60427, GSE27411 and GSE115601). Differentially expressed genes (DEGs) commonly present in patients with *H. pylori* infection and T2DM were identified. Hub genes were validated using human gastric biopsy samples. Correlations between hub genes and immune cell infiltration, miRNAs, and transcription factors (TFs) were further analyzed.

RESULTS

A total of 67 DEGs were commonly presented in patients with *H. pylori* infection and T2DM. Five significantly upregulated hub genes, including *TLR4*, *ITGAM*, *C5AR1*, *FCER1G*, and *FCGR2A*, were finally identified, all of which are closely related to immune cell infiltration. The gene-miRNA analysis detected 13 miRNAs with at least two gene cross-links. TF-gene interaction networks showed that *TLR4* was coregulated by 26 TFs, the largest number of TFs among the 5 hub genes.

CONCLUSION

We identified five hub genes that may have molecular connections between *H. pylori* infection and T2DM. This study provides new insights into the pathogenesis

of *H. pylori*-induced onset of T2DM.

Key Words: *Helicobacter pylori*; Type 2 diabetes mellitus; Bioinformatics analysis; Differentially expressed genes; Hub genes

Core Tip: This bioinformatic research is the one of the first studies to identify the key genes and pathways associated with both *Helicobacter pylori* (*H. pylori*) infection and type 2 diabetes mellitus (T2DM), using integrated bioinformatics analyses. Five hub genes were identified, including *TLR4*, *C5AR1*, *ITGAM*, *FCGR2A*, *FCER1G*, and all of which were closely related to immune cell infiltration. We also verified their expression in clinical specimens. Hopefully, this study will shed some light on the pathogenesis of *H. pylori*-induced T2DM in the future. This study is of great clinical importance.

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INTRODUCTION

The infection rate of *Helicobacter pylori* (*H. pylori*) is still increasing recently and it infects almost 50% of the world' population. The prevalence rate is even higher in less developed countries[1]. It not only affects gastric disease but also affects extragastric diseases such as non-alcoholic fatty liver disease[2], cardiovascular disease[3], autoimmune disease [4], and endocrine disorders, such as diabetes[5]. In recent years, the prevalence rate of type 2 diabetes mellitus (T2DM) and its complications have also increased significantly[6]. The consequences of poor glycemic control in the long and short term can be significant on social and economic levels[7,8]. Patients with T2DM are more susceptible to *H. pylori* infection, according to our previous meta-analysis[9,10]. There is a significant decrease in the eradication rate of *H. pylori* infection in T2DM patients with *H. pylori* infection compared to T2DM patients without infection[11]. Additionally, *H. pylori*-infected T2DM patients have worse glycemic control capability[12]. All these clinical studies strongly suggest that there is an association between *H. pylori* infection and T2DM.

However, the detailed mechanisms underlying *H. pylori* infection and T2DM remain unclear. According to previous studies, both innate and adaptive immune reactions may be activated in the mucosa of the stomach as a result of *H. pylori* infection[13]. This local inflammation in the stomach may spread systematically as a result of proinflammatory cytokines released by the stomach[14]. Chronic low-grade inflammation, which is a feature of *H. pylori*-associated T2DM, would be more likely to develop as a result[15]. Our previous mechanistic study suggested that *H. pylori* infection induces hepatic insulin resistance by the c-Jun/miR-203/SOCS3 signaling pathway[16]. The gut microbiota may also play a role in the immune and metabolic homeostasis of the host, and the infection of *H. pylori* not only disrupts the balance of commensal bacterial species in the gastric mucosa but also causes alterations in the microbial composition of the human gut[17]. However, these hypotheses have not been formally confirmed and validated.

This study aimed to investigate the potential molecular connections between *H. pylori* infection and T2DM. We identified differentially expressed genes (DEGs) by analyzing gene expression datasets through comprehensive bioinformatics analysis. DEGs were screened by combining the results from GEO datasets. Protein-protein interaction (PPI) construction, Gene Ontology (GO) term analysis, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed to identify the hub genes linked to the two diseases. A miRNA-hub gene network and transcription factor (TF)-gene mRNA interaction network were also constructed. We sought to provide new insights into the pathogenesis of *H. pylori*-induced onset of T2DM.

MATERIALS AND METHODS

Data sources

The NCBI-GEO database is a publicly available database containing gene expression datasets[18,19]. Three datasets were retrieved from the GEO database (https://www.ncbi.nlm.nih.gov/geo/), including two gene expression profiles related to *H. pylori* (GSE60427 and GSE27411) and one dataset related to T2DM (GSE115601). Detailed information on the microarray datasets is provided in Supplementary Table 1. Gene expression profiles were set accordingly, including: (1) Tissue samples collected from diseased and normal gastric tissues; and (2) datasets with more than three samples.

Identification of DEGs

The NCBI-GEO2R interactive tool was utilized to analyze and compare data under similar experimental conditions from two or more sample groups to identify genes significantly differentially expressed for both diseases (https://www.ncbi.

nlm.nih.gov/geo/query/acc.cgi?)[20]. Genes that satisfied the criteria of log fold change > 0.4 with adjusted *P* value less than 0.05 were identified as DEGs. Genes presenting upregulation or downregulation in both *H. pylori* and T2DM were selected using the Venn diagram web tool (http://bioinfogp.cnb.csic.es/tools/venny/).

Functional enrichment analysis of DEGs

DAVID (Database for Annotation, Visualization, and Integrated Discovery), as an online tool, was used to predict the functions of hub genes based on GO enrichment analysis and KEGG pathway analysis (https://david.ncifcrf.gov/)[21] at three levels: Biological process (BP), molecular function (MF), and cellular component (CC). Bubble maps were used for representing BP, MF, CC, and KEGG pathways, using R package of ggPlot2. A statistically significant *P* value was defined as *P* value less than 0.05.

Construction of PPI network and identification of hub genes

A public online database, named STRING (https://string-db.org/), can be used to search for and predict PPIs. This inclusive resource facilitates the investigation of direct physical associations between proteins, as well as the detection of indirect functional connections unveiled through correlation analyses[22]. When common DEGs between different groups were identified, they were uploaded to STRING's official website (https://cn.string-db.org/) and the interactions between DEGs and STRING database proteins were then assigned (with a minimum needed interaction score of 0.40). We followed the method of Liu *et al*[23], in which PPI interaction networks were visualized using Cytoscape (Version 3.6.1). Cytoscape is from National Institute of General Medical Sciences, United States. We used CytoHubba (Version 0.1) to identify hub genes using a maximal clique centrality algorithm.

Evaluation of infiltrated immune cells

To explore the association between infiltrating immune cells and *H. pylori* infection, data on proportions of the 22 immune cell types were obtained using the "cell-type identification by estimating relative subsets of RNA transcripts" (CIBERSORT) algorithm (https://cibersort.stanford.edu/). As a result, only samples with a *P* value of < 0.05 were included in the immune cell infiltration matrix. Boxplots and violin plots were utilized to visualize the proportions of infiltrated immune cells in each sample and each group. The correlation between expression of the five hub genes and the abundance of six immune cell subsets [B cells, CD4+ T cells, CD8+ T cells, macrophages, dendritic cells (DCs), and neutrophils] was analyzed in the gene module of TIMER (http://timer.cistrome.org/)[24].

MiRNAs prediction and gene–miRNA interaction network construction

In order to predict their targeted miRNAs, hub genes were selected and analyzed using the miRWalk database (http://mirwalk.umm.uni-heidelberg.de/). The filter setting with a score of > 0.90 was implemented. The target gene binding region was the 3'-UTR, and the intersection with other databases was set to miRDB. Further data processing was carried out by Cytoscape.

TF-gene interaction network

The Network Analyst database (https://www.networkanalyst.ca/) was applied to identify human TFs of the related hub genes[25]. The database includes all three data sources named JASPAR, ENCODE and ChIP Enrichment Analysis. ChIP Enrichment Analysis was used to identify target TFs of hub genes in our current study. Moreover, the Cytoscape tool was used to visualize the TF-gene interaction network among TFs and hub genes.

Singlegene gene set enrichment analysis

Gene set enrichment analysis (GSEA) of each hub gene was performed using the "clusterProfiler" R package to identify regulatory pathways and biological functions associated with each hub gene. An adjusted $P < 0.05$ was used to indicate significant thresholds for GSEA.

Hub genes validated in clinical specimens

The results of our bioinformatics-based analysis were further verified by RT-qPCR assays. Gastric antrum tissues from patients and controls were collected (control: $n = 30$; T2DM: $n = 30$; *H. pylori*: $n = 30$; T2DM + *H. pylori*: $n = 30$).

H. pylori infection was diagnosed by the 13C-urea breath test (Headway Bio-Sci Co., Ltd, Shenzhen, China) according to the manufacturer's instructions. A delta over baseline of > 4% indicates a positive *H. pylori* infection status. Patients with T2DM were diagnosed based on one of the following American Diabetes Association diagnostic criteria: fasting blood glucose level ≥ 7.0 mmol/L, 2-hour postload glucose level ≥ 11.1 mmol/L during an oral glucose tolerance test, glycated hemoglobin level ≥ 6.5%, or a random plasma glucose level ≥ 11.1 mmol/L in a patient with classic symptoms of hyperglycemia or hyperglycemic crisis. This study was approved by the ethics committee of the First Affiliated Hospital of Nanjing Medical University (2021-SRFA-034). Total RNA was extracted from each tissue sample using TRIzol (Invitrogen, F10488, Waltham, MA, United States), following the manufacturer's instructions. The kit, EasyScript All-in-One First-Strand cDNA Synthesis SuperMix for RT-qPCR Kit (TransGen Biotech, Beijing, China), was utilized for reverse transcription, with incubations performed at a tempertature 42°C for 15 min and then at 85°C for 15 s. Subsequently, StarLighter SYBR Green RT-qPCR Mix (Universal) (Forever Star, Beijing, China) kit was utilized for RT-qPCR analysis, with an ABI 7500 system (Applied Biosystems, United States). The primers used are listed in Supplementary Table 2. The reaction conditions were as follows: Predenaturation (95°C for 5 min), 40 cycles of denaturation (94°C for 20 s), annealing and extension (60°C for 34 s). β-actin was served as an internal control for RT-qPCR. The $2^{-\Delta\Delta Ct}$ method was utilized to determine relative the expression levels of genes. Statistical analysis was performed using GraphPad Prism (Version 9.0,

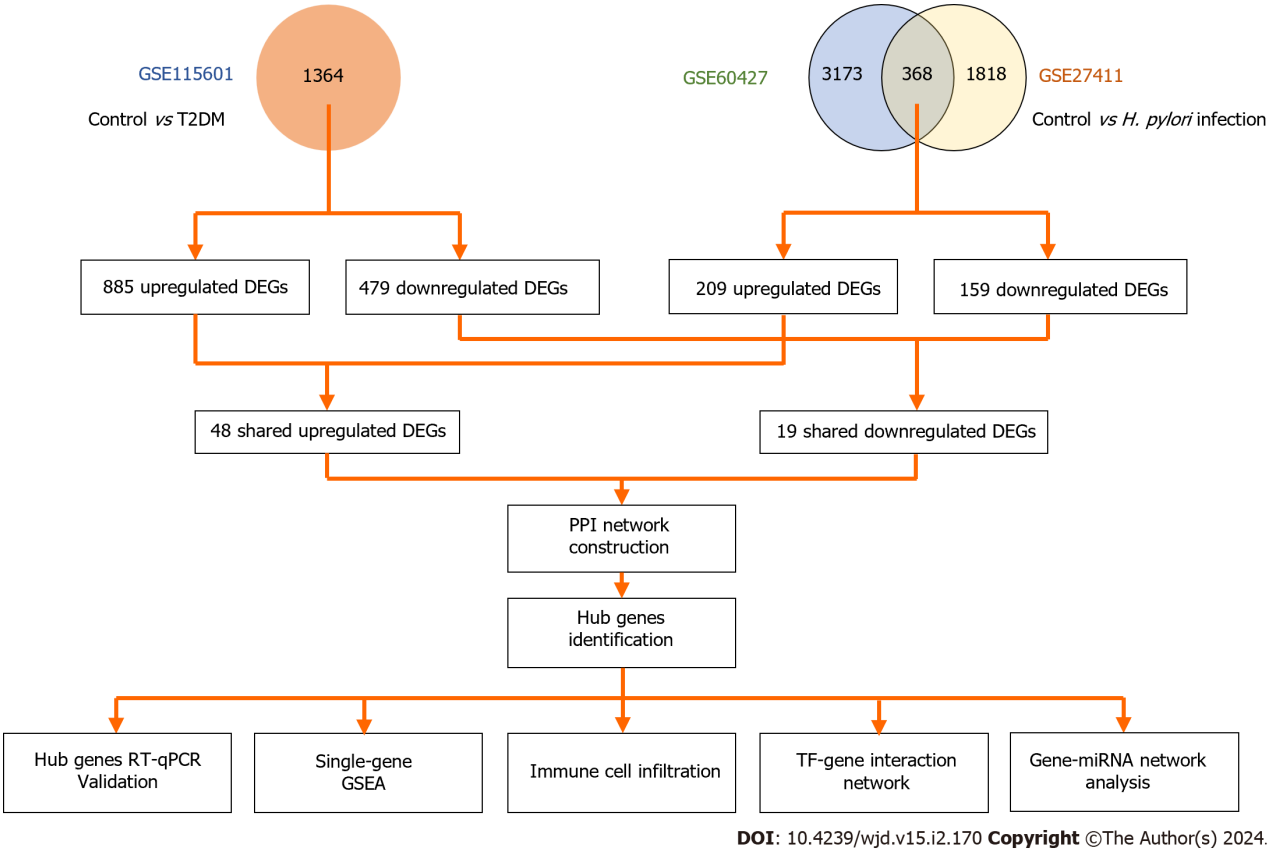

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Figure 1 Overall workflow of the study. T2DM: Type 2 diabetes mellitus; *H. pylori*: *Helicobacter pylori*; DEGs: Differentially expressed genes; TF: Transcriptional factor; PPI: Protein-protein interaction; GSEA: Gene set enrichment analysis.

Boston, MA, United States). Expression differences of hub genes were compared using one-way ANOVA in four groups (control, *H. pylori* infection, T2DM, and T2DM with *H. pylori* infection), and pairwise comparisons within the two groups were performed using Student's *t* test. Statistically significant was defined as $P < 0.05$.

RESULTS

Identification of DEGs

Figure 1 illustrated the overall study design. In brief, a total of 3541, 2186 and 1364 DEGs were identified from the GSE60427, GSE217411 and GSE115601 datasets, respectively. In the GEO datasets, volcano plots (Figure 2A-C) and heatmaps (Supplementary Figure 1) were used to illustrate the dysregulated genes (including upregulated and downregulated). Among these datasets, 67 common DEGs were extracted, including 48 upregulated and 19 downregulated genes (Supplementary Table 3; Figure 2D).

Functional annotation of DEGs

After DEGs were selected, GO and KEGG pathway enrichment analyses were performed to explore the biological functions of these genes involving three functional categories: BP, MF, and CC. Major BP terms associated with DEGs included regulation of the immune effector process, neutrophil activation and neutrophil mediated immunity (Figure 3A). Major CC terms associated with these DEGs included the secretory granule membrane, blood microparticle, and tertiary granule (Figure 3B). Finally, MF-associated GO terms were mainly associated with sulfur compound binding, heparin binding, glycosaminoglycan binding, *etc.* (Figure 3C). According to KEGG pathway analysis results, the DEGs were mainly enriched for pathways related to complement and coagulation cascades, *Staphylococcus aureus* infection, and neutrophil extracellular trap formation (Figure 3D).

PPI network construction and hub gene selection

The PPI network of DEGs obtained from STRING was subjected to the MCODE plugin of Cytoscape to analyze significant modules. A total of 38 nodes and 84 edges were mapped in the PPI network (Figure 4A). From these modules, the top functional cluster of modules was selected based on the cutoff criteria of node > 3 and score > 3 (Figure 4B).

Then, the key genes with degree connectivity were ranked by the CytoHubba plugin of Cytoscape. Finally, five intersecting genes (*TLR4*, *ITGAM*, *C5AR1*, *FCER1G* and *FCGR2A*) with the highest degree were considered hub genes for

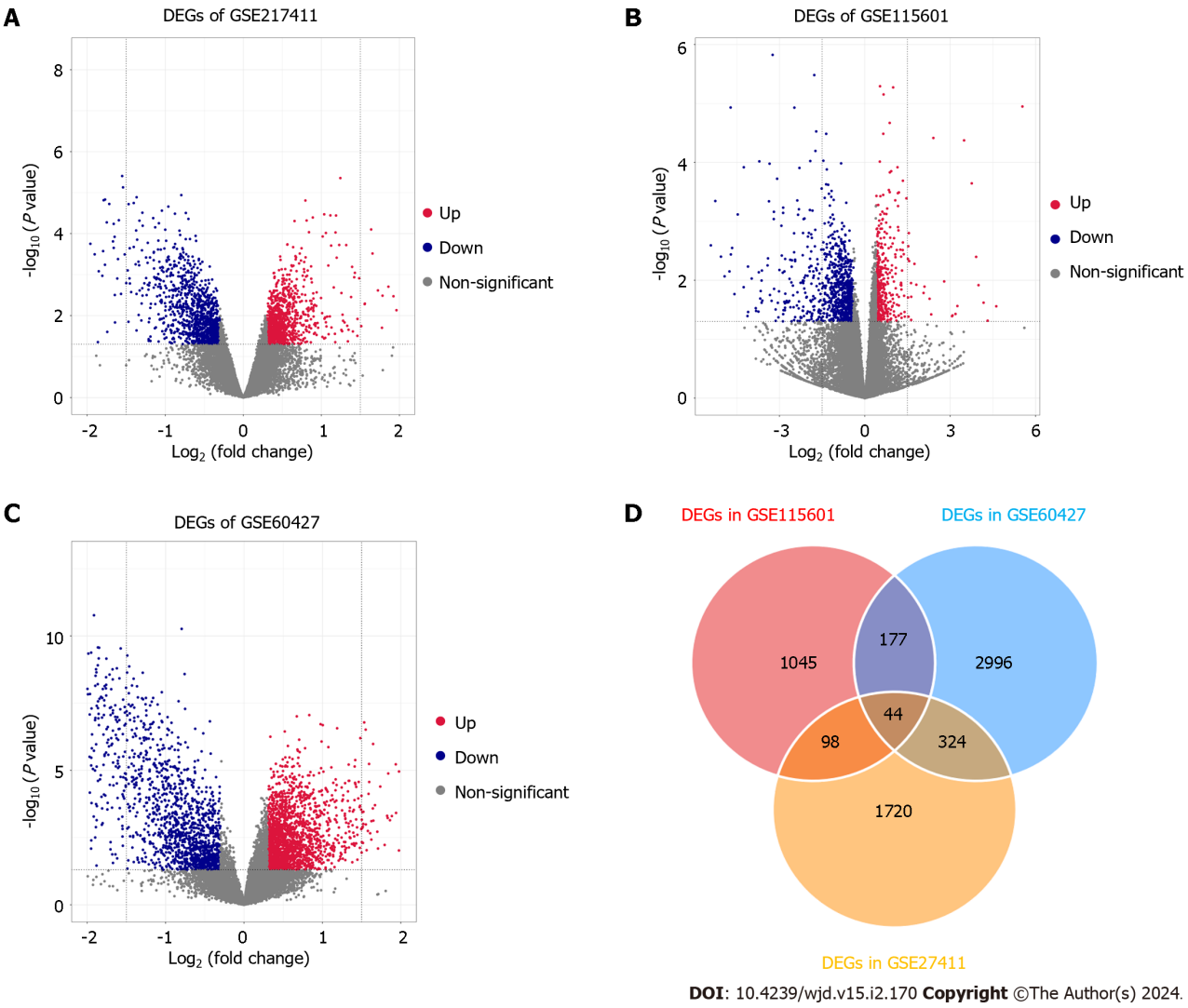

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Figure 2 The expression levels of differentially expressed genes in three datasets. A-C: The volcano plot distribution of differentially expressed genes (DEGs) of GSE60427 (A), GSE27411 (B) and GSE115601 (C). The blue dots indicate the screened downregulated DEGs, red dots indicate the screened upregulated DEGs, and the grey dots indicate genes with no significant differences; D: The Venn diagram of DEGs based on the three datasets. DEGs: Differentially expressed genes.

further analyses (Figure 4C and D).

Validation of hub genes in human gastric tissues

Expression levels of the five hub genes in the three datasets are shown in Supplementary Figure 2; and were significantly upregulated in patients with either *H. pylori* infection or T2DM alone compared to negative controls. Human gastric tissues from four groups were collected (control group, *H. pylori* infection alone group, T2DM alone group and T2DM with *H. pylori* infection group). All included patients underwent upper gastrointestinal endoscopy and were pathologically diagnosed with chronic superficial gastritis without acute inflammation or atrophy according to the Sydney System[26]. The baseline characteristics of the groups are shown in Supplementary Table 4. Through RT-qPCR analysis, we found that *TLR4*, *ITGAM*, *C5AR1*, *FCER1G* and *FCGR2*A were expressed at significantly higher levels in the T2DM with *H. pylori* infection group ($P < 0.05$) than in the T2DM group or the *H. pylori* infection group alone (Figure 4E).

Immune infiltration analysis

Using the CIBERSORT algorithm, we explored differences in immune infiltration between *H. pylori*-infected versus normal gastric tissues. Compared with normal tissues, *H. pylori*-infected gastric tissues generally contained a higher proportion of regulatory T cells, activated NK cells, eosinophils and neutrophils, whereas the proportions of plasma cells, activated mast cells and M2 macrophages were lower in *H. pylori*-infected gastric tissues (Figure 5A and B).

The results obtained using TIMER showed that *TLR4* and *ITGAM* expression correlated positively with CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and DCs. *C5AR1*, *FCER1G* and *FCGR2A* expression was significantly associated with infiltration of B cells, CD8+ T cells, macrophages, neutrophils, and DCs, among which their mRNA expression levels all correlated negatively with B cells (Figure 5C).

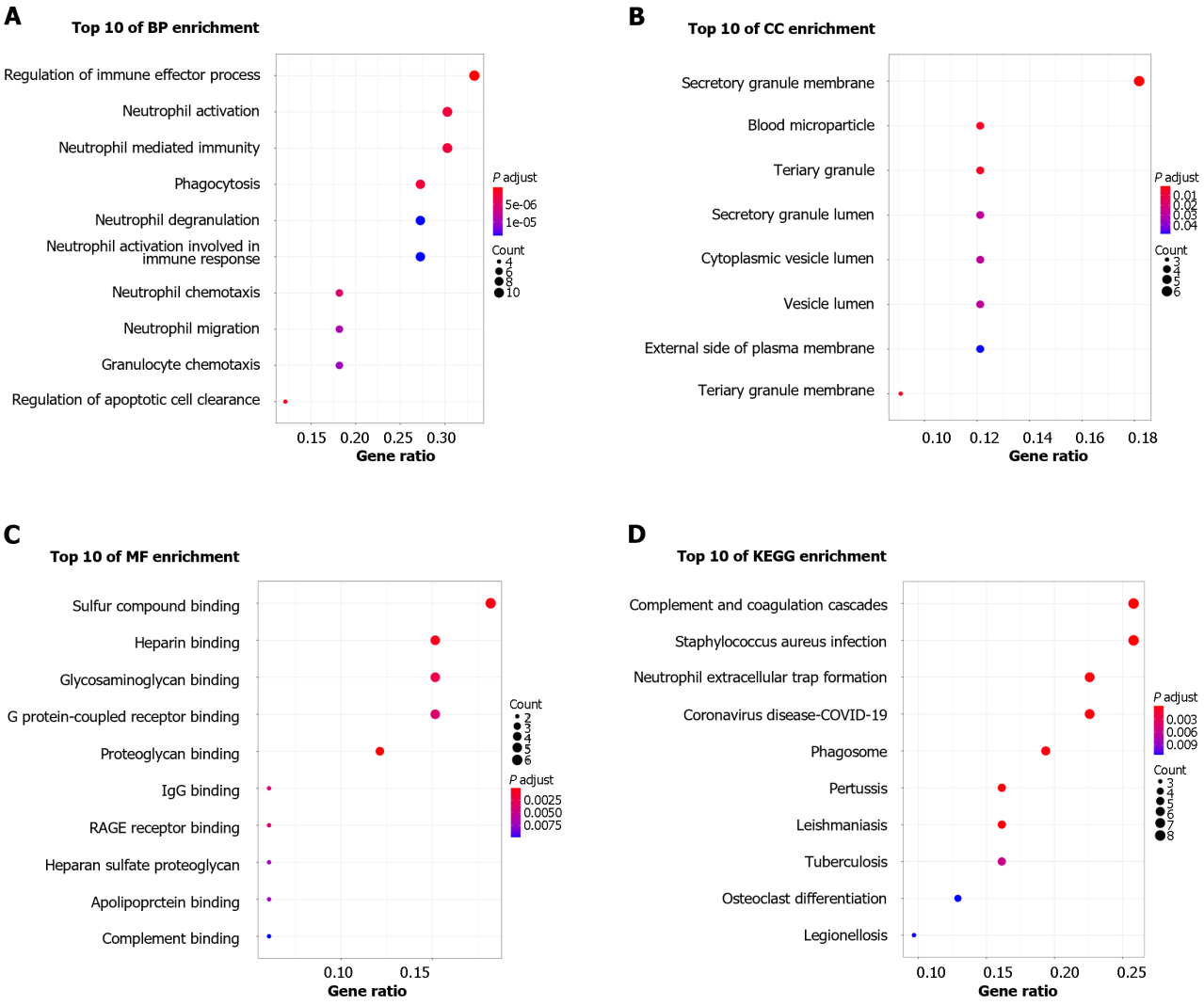

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Figure 3 Functional enrichment analysis of common differentially expressed genes. A: Biological process analysis of differentially expressed genes (DEGs); B: Cellular component analysis of DEGs; C: Molecular function analysis of DEGs; D: Kyoto Encyclopedia of Genes and Genomes pathway analysis of DEGs. BP: Biological process; CC: Cellular component; MF: Molecular function; KEGG: Kyoto Encyclopedia of Genes and Genomes.

Prediction of further miRNA and analysis of gene-miRNA network

A total of 225 miRNAs was predicted after we uploading the 5 identified hub genes to the miRWalk database. The gene–miRNA interaction network is shown in Figure 6A. We detected 13 miRNAs (miR-6848-5p, miR-6796-5p, miR-6740-5p, miR-8060, miR-6730-5p, miR-5698, miR-12119, miR-6881-5p, miR-6846-5p, miR-7703, miR-6728-5p, miR-7107-5p and miR-1914-3p) associated with at least two gene cross-links, as shown in Supplementary Table 5.

TF-gene interaction network

The top ranked TFs were SPI1, MECOM, GATA2, TP63, SALL4, GATA1, MITF, RUNX1 and FLI1 (Figure 6B). Based on the results, we found that *TLR4* was coregulated by 26 TFs, the highest among the identified hub genes.

Functional analysis of hub genes by single-gene GSEA

We performed GSEA on *TLR4*, *ITGAM*, *C5AR1*, *FCER1G* and *FCGR2A* to explore the role of these genes in the course of *H. pylori* infection and T2DM and found the top 10 significant items (Figure 7). According to GSEA results, it suggested that all these five genes play a direct or indirect role in the pathogenesis of *H. pylori* infection and T2DM. For example, *FCG2A* is involved in the signaling pathway of "type 1 diabetes mellitus" and the "insulin signaling pathway", *C5AR1* and *FCER1G* are involved in the signaling pathway of "type 1 diabetes mellitus", and *ITGAM* is involved in the signaling pathway of "glycosaminoglycan biosynthesis chondroitin sulfate".

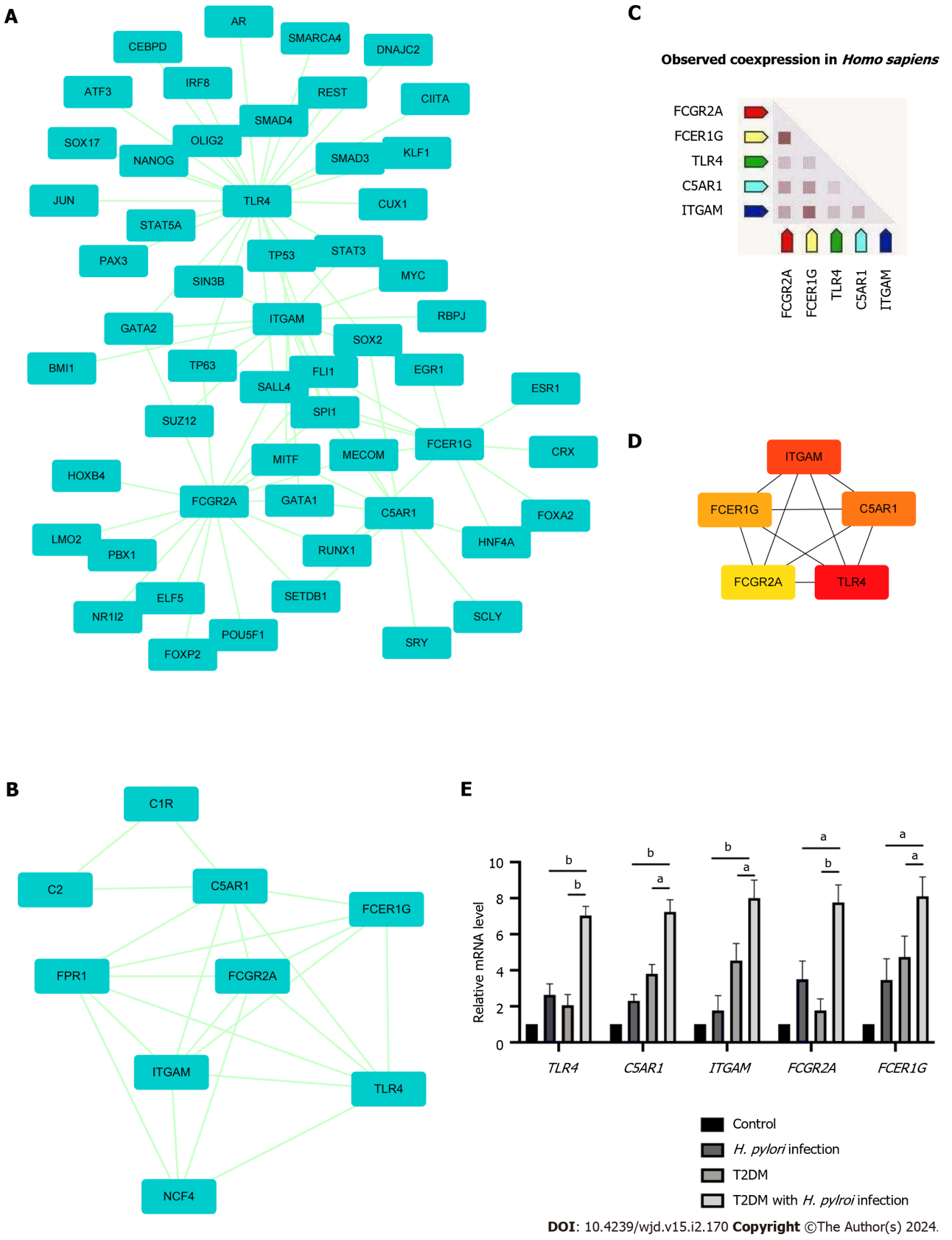

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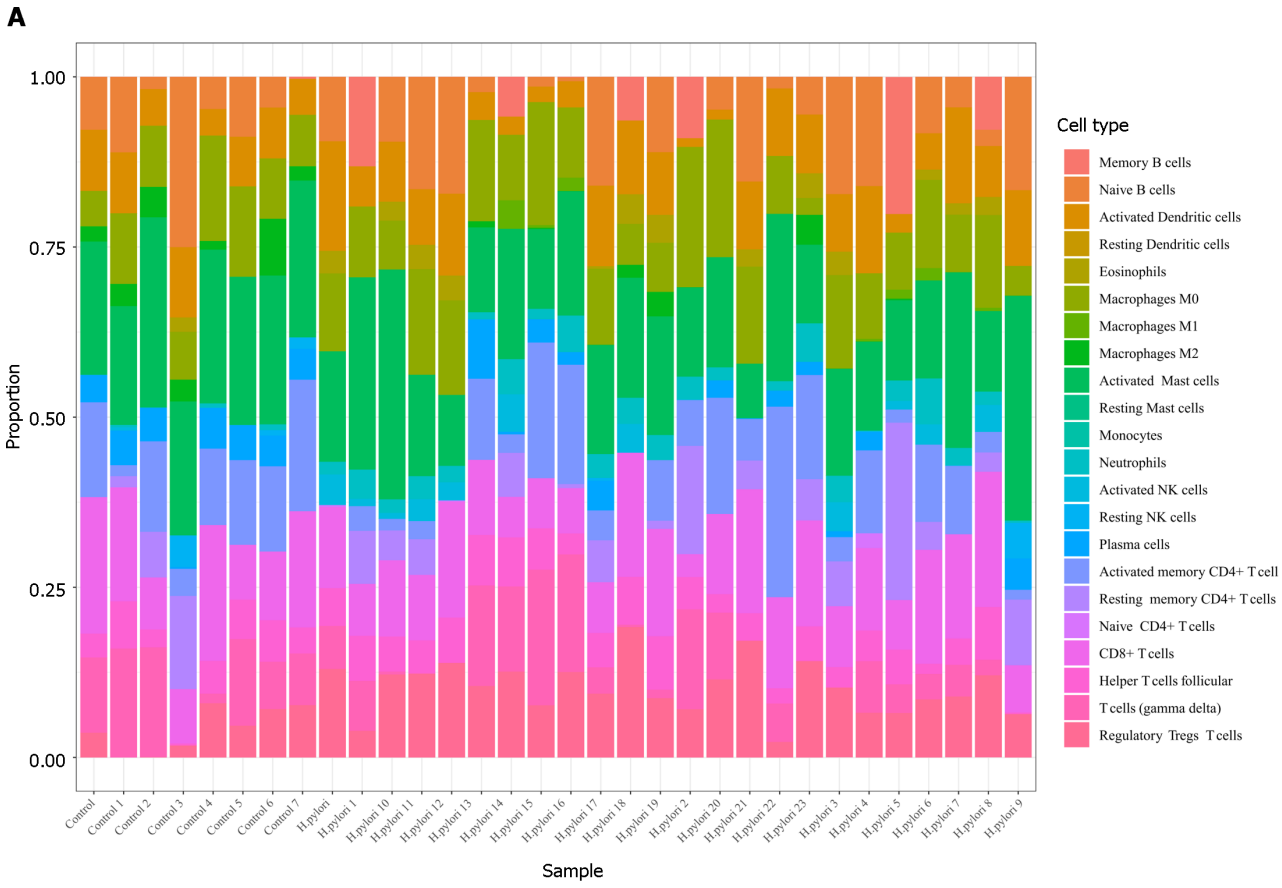
Figure 4 Protein-protein interaction network showing interactions between common genes and identification of differentially expressed genes from this network. A: The protein-protein interaction (PPI) network of differentially expressed genes was constructed by Cytoscape software. The criteria of the PPI network were as follows: Confidence score ≥ 0.4 and a maximum number of interactions ≤ 5; B: The top module of the PPI network. MCODE score ≥ 3, 9 nodes and 21 edges; C: Construction of the PPI network among the 5 hub genes; D: Coexpression analysis of the 5 hub genes using STRING; E: The expression of 5 hub genes in clinical specimens by RT-qPCR analysis. $^{a}P < 0.05$; $^{b}P < 0.01$). T2DM: Type 2 diabetes mellitus; *H. pylori*: *Helicobacter pylori*.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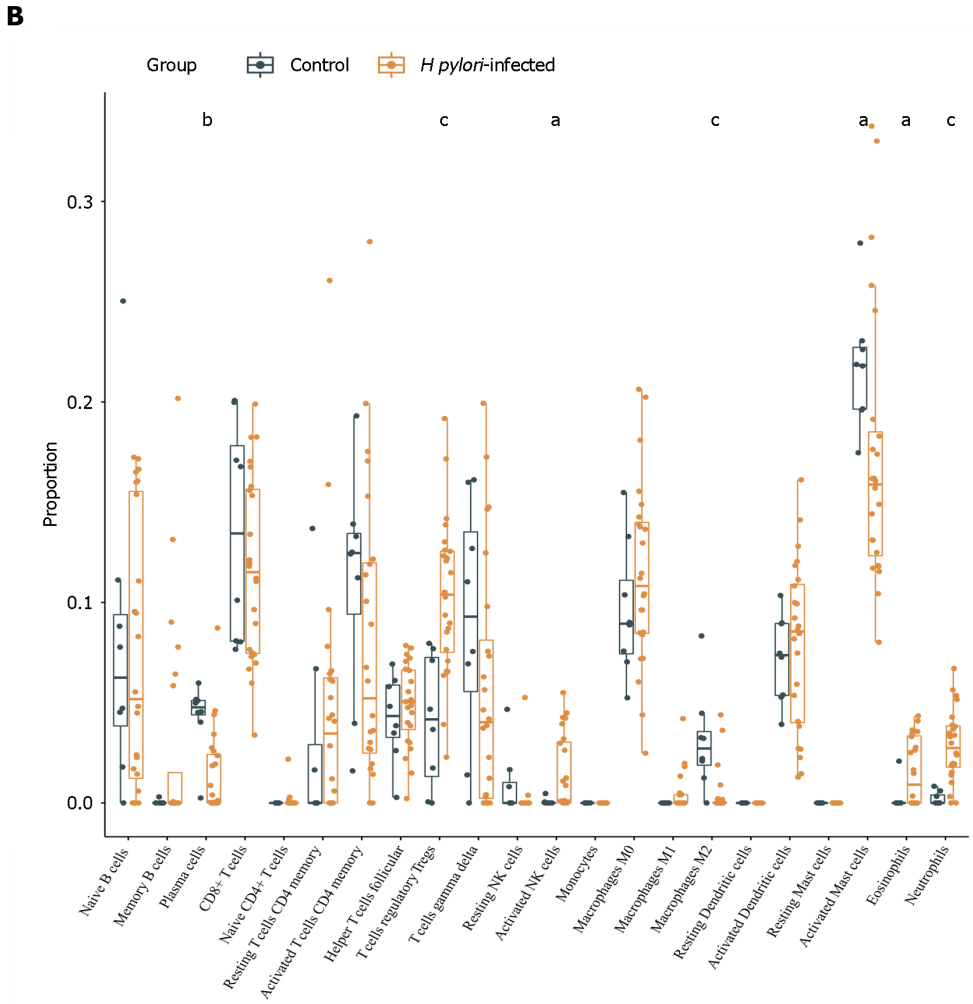
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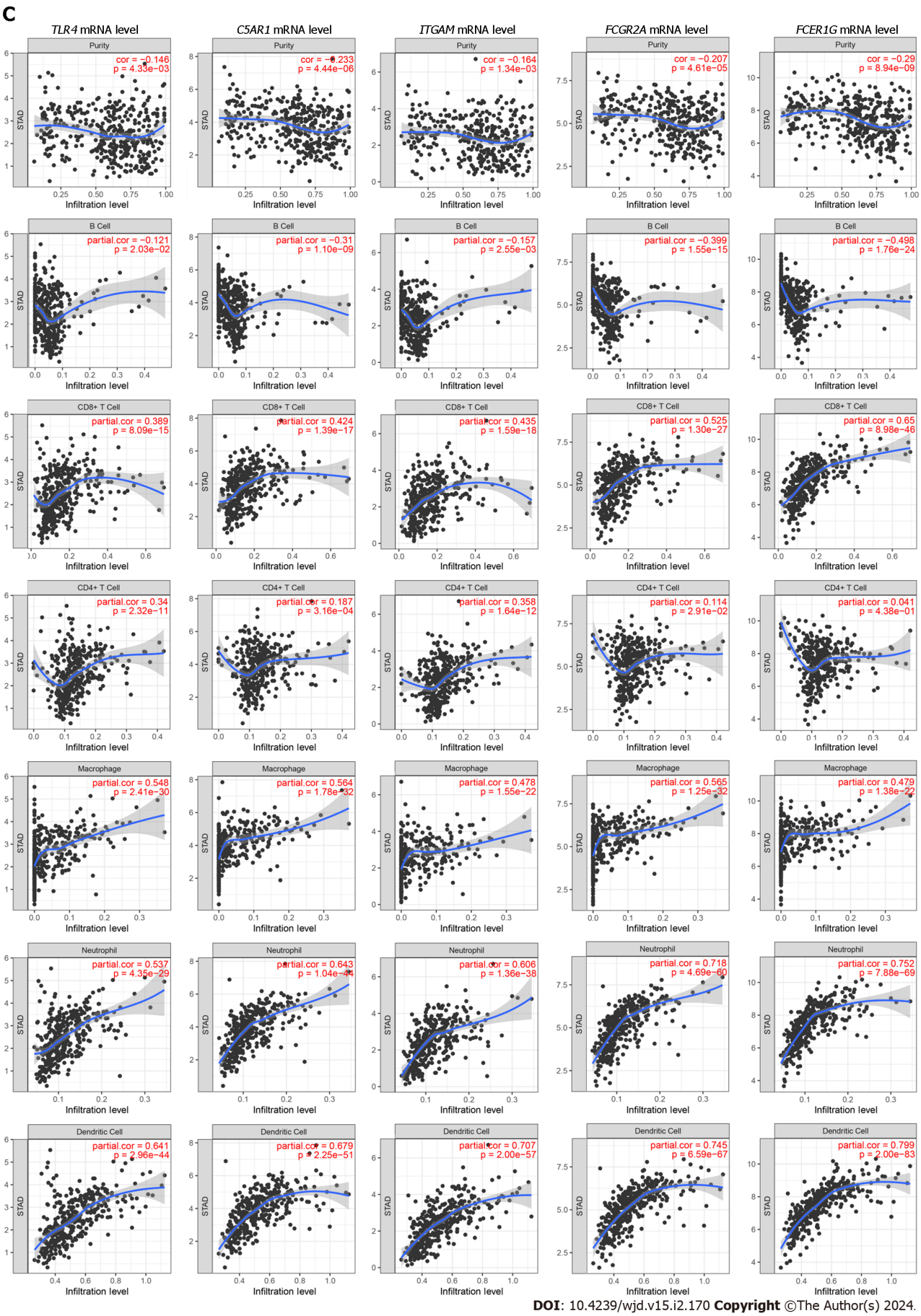

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Figure 5 The relationship between hub genes and immune infiltration. A and B The differences in immune infiltration between *Helicobacter pylori* (*H.*

pylori)-infected gastric tissues and normal gastric tissues; C: Correlation analysis between hub gene expression and immune cell infiltration levels in *H. pylori* infection. *H. pylori*: *Helicobacter pylori*. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$.

DISCUSSION

Approximately 50% of the world's population is infected with *H. pylori*, and the infection rate is even higher in patients with T2DM. Infected patients with T2DM have worse blood glucose control abilities, with great social and economic burdens[7,8]. However, the detailed mechanism of the interaction between T2DM and *H. pylori* infection remains unknown. Therefore, it is necessary to increase our understanding of the underlying mechanisms leading to the risk of *H. pylori* infection and T2DM to develop effective treatment approaches.

In this study, we investigated the biological functions, expression levels, and correlations with immune infiltrates of common genes with significantly altered expression in both *H. pylori*-infected individuals and T2DM patients through integrated bioinformatics analyses. Our results showed that expression of 67 overlapping genes was altered in gastric samples from both *H. pylori*-infected individuals and T2DM patients. Among these genes, 48 were upregulated and 19 downregulated. Five hub genes were further identified through PPI analysis. However, regardless of the statistical probability, the causality between a candidate genotype and the phenotype of the host remains uncertain[27]. To further identify the relationship between genotype (the 5 hub genes) and phenotype (*H. pylori*-associated T2DM), rigorous validation of mechanisms at the molecular, cellular, tissue, and whole-organism levels is needed.

Chronic low-grade inflammation has been definitively shown to correspond with obesity[28] and diabetes[29]. However, whether obesity and diabetes drive the inflammation or vice versa remains to be elucidated. Gut microbiota play a critical role in the development of the host immune system, making it an important immune organ[30]. Disturbance of the gut microbiota promotes inflammation within the lining of the intestines[31]. The dysbiosis of the gut results in bacterial infiltration, allowing microbes to contact the epithelium and causing inflammation[32]. Toll-like receptors (TLR) play a key role in host recognition of microbes[33]. *TLR4* has been implicated in recognition of bacterial lipopolysaccharides, a key element of the cell walls of gram-negative bacteria. This triggers the expression of proinflammatory cytokines and chemokines, including tumor necrosis factor-alpha[34]. This inflammatory response is strongly linked to insulin resistance, and both *TLR4* and its coreceptor CD14 are needed to induce insulin resistance in mice[35]. It is believed that *TLR4*, one of the TLR family members, possesses the potential to trigger nuclear factor-κB when confronted with short-chain fatty acids. Consequently, this leads to subsequent stimulation of the immune system[36]. Therefore, the inflammation caused by *TLR4* serves a crucial function in the development of T2DM related to *H. pylori*. The study conducted by Devaraj *et al*[37] exhibited a notable rise in the level of *TLR4* expression among individuals diagnosed with type 1 diabetes. This finding implies that *TLR4* actively participates in the inflammatory state associated with diabetes. Moreover, knockout of *TLR4* alleviated inflammation in rats with diabetes and *TLR4* antagonists attenuated atherogenesis in mice with diabetes[38]. Based on our results, we speculated that *TLR4* participates in the pathogenesis of H. pylori-associated T2DM *via* the TLR signaling pathway.

Other hub genes, *ITGAM*[39], *C5AR1*[40], *FCER1G*[41] and *FCGR2A*[42], are also reported to be associated with diabetes. *ITGAM*, a monocyte/macrophage marker, is upregulated in T2DM patients[39]. *FCER1G* was identified as a significant gene related to diabetic kidney disease. Gene Expression Omnibus validation using additional datasets showed that FCER1G is upregulated in diabetic glomerular lesions compared with normal tissues. This report also revealed that abnormal upregulation of *FCER1G* is related to diabetic glomerular lesions[41].

Clinical variability between individuals infected with any pathogen is enormous, ranging from silent to lethal. One of the main reasons is immunity differs among individuals[43]. Tumor-infltrating immune cells function together to defend the body against invading factors, such as bacterial infection. Therefore, they can be used as important predictors for diagnosis and treatment of diseases[44]. Based on KEGG pathway and immune cell infiltration analyses, we found that *H. pylori* infection is associated with multiple immune cell changes, especially NK cells and regulatory T cells. Through single-gene GSEA, we found that high expression of the hub genes *TLR4*, *FCGR2A*, and *FCER1G* was associated with NK cell-mediated cytotoxicity in diabetes, which suggests that *H. pylori* infection might change hub gene expression and downstream NK cells to induce T2DM. Further analysis suggested that these 5 hub genes all correlated with B cells, CD8+ T cells, macrophages, neutrophils, and DCs. It has been shown that isolated NK cells from T2DM subjects show defects in the NK cell-activating receptors NKG2D and NKp46, in association with functional defects in NK degranulation capacity [45]. Restrepo *et al*[46] demonstrated that chronic hyperglycaemia is significantly associated with defects in complement receptors and Fcγ receptors on isolated monocytes, resulting in phagocytosis impairment. An *in vitro* study using macrophages derived from mouse bone marrow and treated with high glucose showed reduced antibacterial activity and phagocytosis for the treated macrophages[47]. In the same study, reduced phagocytosis was shown in peritoneal macrophages from mice with T2DM. This might be related to the reduced glycolytic capacity and reserve of macrophages following long-term sensitization to high levels of glucose. Reactive oxygen species production was reportedly reduced in isolated neutrophils from T2DM tuberculosis patients following phorbol 12-myristate 13-acetate stimulation, and this defect in reactive oxygen species production was associated with increased levels of resistin in T2DM patient serum[48]. In a comparable study, Perner *et al*[49] documented the inhibition of superoxide production in neutrophils isolated from healthy individuals when subjected to a high-glucose environment. This hindrance was observed to be a consequence of the suppression of glucose-6-phosphate dehydrogenase, which disrupted the generation of nicotinamide adenine dinucleotide phosphate. Thus, we speculate that these 5 hub genes are involved in *H. pylori*-associated T2DM through immune infiltration. We will validate their relationship through experiments in the future.

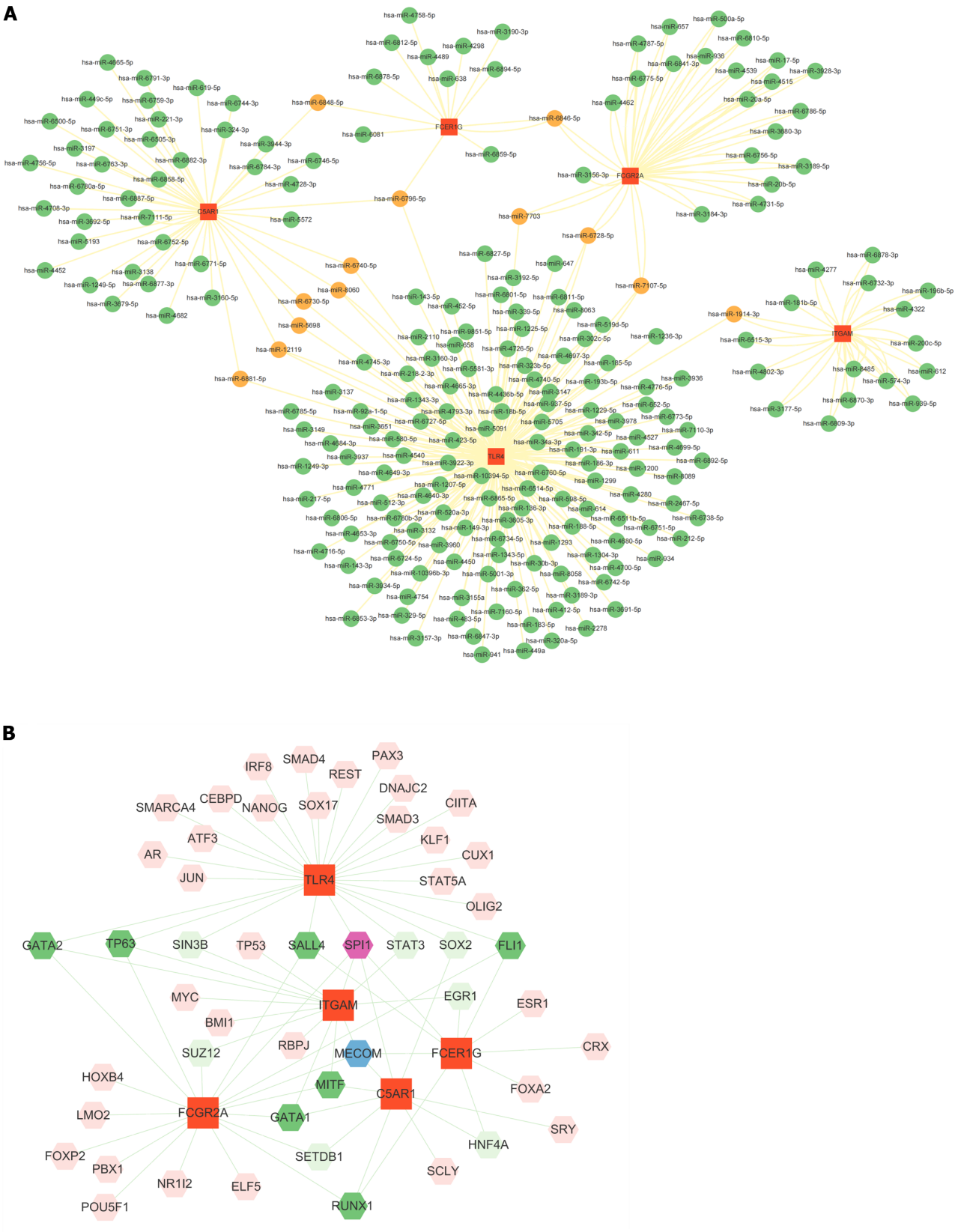

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Figure 6 The interaction of hub genes with miRNA/transcriptional factors. A: Interaction network between the hub genes and their targeted miRNAs. Hub genes are presented in red squares, whereas miRNAs are shown in green circles. Orange circles represent miRNAs targeting two or more genes simultaneously; B: Construction of the transcriptional factor-gene interaction network from Cytoscape.

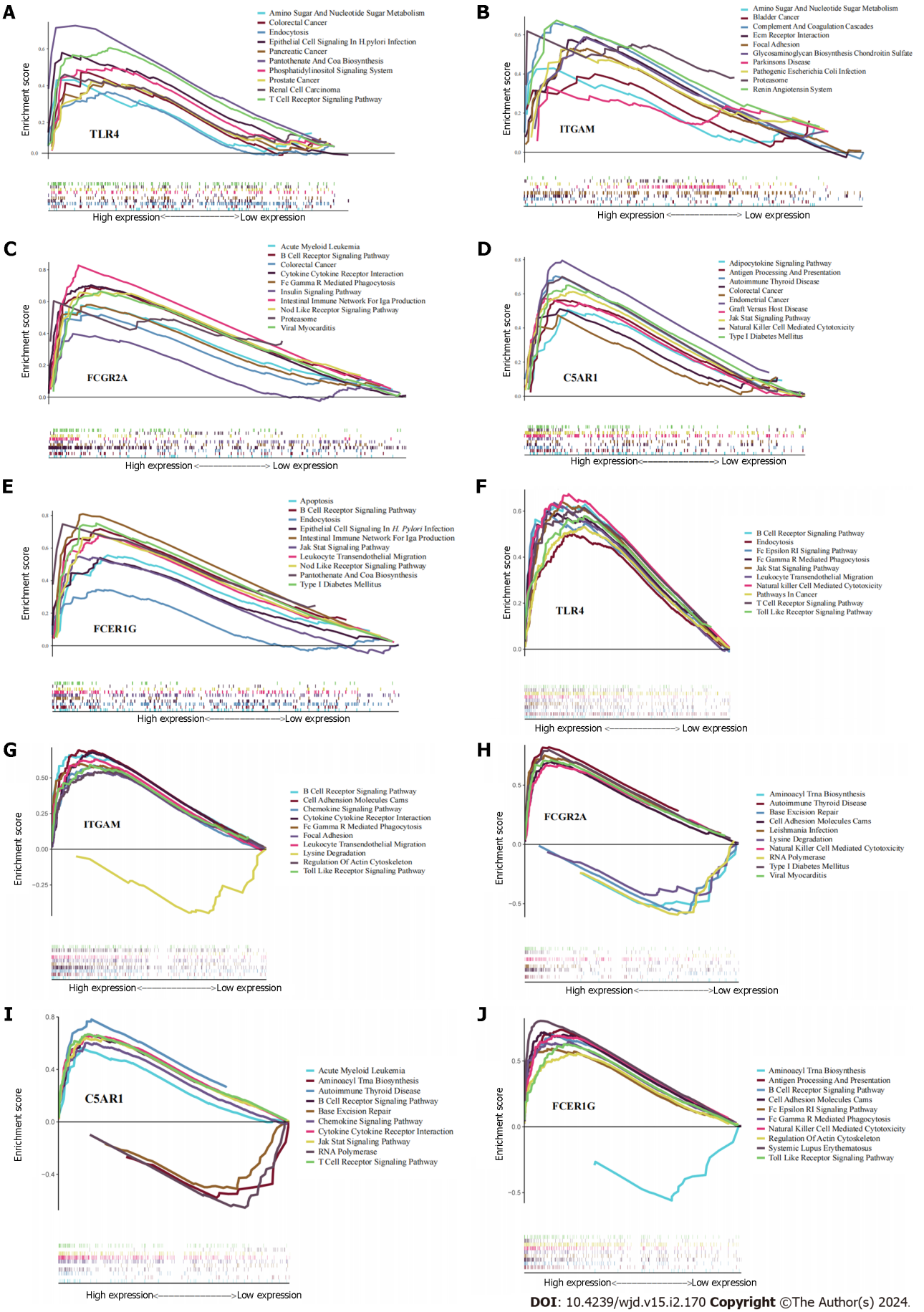

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Figure 7 Results of single-gene gene set enrichment analysis. A-E: *Helicobacter pylori* infection; F-J: Type 2 diabetes mellitus.

This study provides some new insights into the pathogenesis of *H. pylori*-associated T2DM. However, several limitations should be mentioned. First of all, this study had a relatively small sample size and a larger sample size would be necessary for further investigations. Secondly, hub genes were identified using bioinformatics analysis and validated by a small clinical sample. Validation including RNA-seq from a larger clinical cohort is needed. It is necessary to investigate the potential underlying mechanisms involved in these findings in future large-scale prospective studies. Thirdly, despite statistical probability, the causality between a candidate genotype and the phenotype of the host is uncertain[27]. To identify the relationship between genotype (the 5 hub genes) and phenotype (*H. pylori*-associated T2DM), rigorous validation of mechanisms at the molecular, cellular, tissue, and whole-organism levels is needed.

CONCLUSION

We report 67 common DEGs and five hub genes (*TLR4*, *ITGAM*, *C5AR1*, *FCER1G* and *FCGR2A*) in *H. pylori* infection and T2DM. We validated expression of the five hub genes by RT-qPCR. All hub genes were significantly upregulated in T2DM patients with *H. pylori* infection compared with noninfected T2DM patients. Immune infiltration analysis showed that *H. pylori*-infected gastric tissues generally contained a higher proportion of regulatory T cells, activated NK cells, eosinophils and neutrophils. Our gene-miRNA analysis detected 13 miRNAs with at least two gene cross-links, and TF-gene interaction networks showed that *TLR4* to be coregulated by 26 TFs, the largest number of TFs among the 5 hub genes. This study provides a new idea for elucidating the pathogenesis of H. pylori-associated T2DM at the genetic level.

ARTICLE HIGHLIGHTS

Research background

This prevalence rate of *Helicobacter pylori* (*H. pylori*) is high, especially in less developed countries. Its infection related to not only gastric diseases but also extragastric diseases such as type 2 diabetes mellitus (T2DM). However, the underlying mechanisms connecting *H. pylori* infection and T2DM remains unclear.

Research motivation

The potential molecular connections between *H. pylori* infection and T2DM are needed to be identified, in order to further elucidate the pathogenesis and the new treatment strategy of *H. pylori*-infected T2DM.

Research objectives

We aimed to explore the potential molecular connections between *H. pylori* infection and T2DM using bioinformatics analysis. In the future research, we will investigating these identified genes and downstream signaling pathway to further understand their relationship.

Research methods

Differentially expressed genes from three datasets commonly present in patients with *H. pylori* infection and T2DM were identified. Hub genes were validated by RT-qPCR using human gastric biopsy samples. Correlations between hub genes and immune cell infiltration, miRNAs, and transcription factors were further analyzed.

Research results

This is the first study to identify the key genes and pathways associated with *H. pylori* infection and T2DM using integrated bioinformatics analysis. We identified five hub genes, all of which were closely related to immune cell infiltration.

Research conclusions

We were the first to find out that the 5 hub genes identified are playing important roles in the pathogenesis of *H. pylori*-infected T2DM.

Research perspectives

It is necessary to investigate the potential underlying mechanisms involved in these findings in future large-scale prospective studies.

FOOTNOTES

Co-corresponding authors: Guo-Xin Zhang and Xiao-Ying Zhou.

Author contributions: Zhou XY and Zhang GX concepted and designed the research study; Chen H and Zhou X developed methodology; Chen H acquired the data; Zhou XY analyzed and interpretated the data; Chen H wrote the first version of the manuscript; Zhang GX and Zhou XY revised the manuscript; all authors were involved in the critical review of the results and have contributed to, read, and

approved the final manuscript. Zhou XY and Zhang GX contributed equally to this work as co-corresponding authors. The reasons for designating Zhou XY and Zhang GX as co-corresponding authors are threefold. First, the research was performed as a collaborative effort, and the designation of co-corresponding authorship accurately reflects the distribution of responsibilities and burdens associated with the time and effort required to complete the study and the resultant paper. This also ensures effective communication and management of post-submission matters, ultimately enhancing the paper's quality and reliability. Second, the overall research team encompassed authors with a variety of expertise and skills from different fields, and the designation of co-corresponding authors best reflects this diversity. This also promotes the most comprehensive and in-depth examination of the research topic, ultimately enriching readers' understanding by offering various expert perspectives. Third, Zhou XY and Zhang GX contributed to almost the same funding on this research. The choice of these researchers as co-corresponding authors acknowledges and respects this equal contribution, while recognizing the spirit of teamwork and collaboration of this study. In summary, we believe that designating Zhou XY and Zhang GX as co-corresponding authors of is fitting for our manuscript as it accurately reflects our team's collaborative spirit, equal contributions, and diversity.

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Institutional review board statement: The original data in this study were retrieved from the public GEO database with an open license for data use. This study was approved by the ethic committee of the First Affiliated Hospital of Nanjing Medical University (Approval No. 2022-SR-406).

Informed consent statement: All study participants or their legal guardian provided informed written consent about personal and medical data collection prior to study enrollment.

Conflict-of-interest statement: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Data sharing statement: The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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REFERENCES

1 **Butt J**, Epplein M. How do global trends in Helicobacter pylori prevalence inform prevention planning? *Lancet Gastroenterol Hepatol* 2023; **8**: 498-499 [PMID: 37086740 DOI: 10.1016/S2468-1253(23)00101-2]
2 **Alvarez CS**, Florio AA, Butt J, Rivera-Andrade A, Kroker-Lobos MF, Waterboer T, Camargo MC, Freedman ND, Graubard BI, Lazo M, Guallar E, Groopman JD, Ramírez-Zea M, McGlynn KA. Associations between Helicobacter pylori with nonalcoholic fatty liver disease and other metabolic conditions in Guatemala. *Helicobacter* 2020; **25**: e12756 [PMID: 33006810 DOI: 10.1111/hel.12756]
3 **Zhang P**, He Q, Song D, Wang Y, Liu X, Ding G, Xing W. Association of Helicobacter pylori Infection With Carotid Atherosclerosis in a Northern Chinese Population: A Cross-Sectional Study. *Front Cardiovasc Med* 2021; **8**: 795795 [PMID: 35174222 DOI: 10.3389/fcvm.2021.795795]
4 **Wang L**, Cao ZM, Zhang LL, Dai XC, Liu ZJ, Zeng YX, Li XY, Wu QJ, Lv WL. Helicobacter Pylori and Autoimmune Diseases: Involving Multiple Systems. *Front Immunol* 2022; **13**: 833424 [PMID: 35222423 DOI: 10.3389/fimmu.2022.833424]
5 **Mansori K**, Dehghanbanadaki H, Naderpour S, Rashti R, Moghaddam AB, Moradi Y. A systematic review and meta-analysis of the prevalence of Helicobacter pylori in patients with diabetes. *Diabetes Metab Syndr* 2020; **14**: 601-607 [PMID: 32417710 DOI: 10.1016/j.dsx.2020.05.009]
6 **Zhang K**, Ma Y, Luo Y, Song Y, Xiong G, Sun X, Kan C. Metabolic diseases and healthy aging: identifying environmental and behavioral risk factors and promoting public health. *Front Public Health* 2023; **11**: 1253506 [PMID: 37900047 DOI: 10.3389/fpubh.2023.1253506]
7 **Pan Y**, Zhong S, Zhou K, Tian Z, Chen F, Liu Z, Geng Z, Li S, Huang R, Wang H, Zou W, Hu J. Association between Diabetes Complications and the Triglyceride-Glucose Index in Hospitalized Patients with Type 2 Diabetes. *J Diabetes Res* 2021; **2021**: 8757996 [PMID: 34671683 DOI: 10.1155/2021/8757996]
8 **Wang M**, He Y, He Q, Di F, Zou K, Wang W, Sun X. Comparison of clinical characteristics and disease burden between early- and late-onset type 2 diabetes patients: a population-based cohort study. *BMC Public Health* 2023; **23**: 2411 [PMID: 38049796 DOI: 10.1186/s12889-023-17280-5]
9 **Zhou X**, Zhang C, Wu J, Zhang G. Association between Helicobacter pylori infection and diabetes mellitus: a meta-analysis of observational studies. *Diabetes Res Clin Pract* 2013; **99**: 200-208 [PMID: 23395214 DOI: 10.1016/j.diabres.2012.11.012]
10 **Bener A**, Ağan AF, Al-Hamaq AOAA, Barisik CC, Öztürk M, Ömer A. Prevalence of Helicobacter pylori Infection among Type 2 Diabetes

Mellitus. *Adv Biomed Res* 2020; **9**: 27 [PMID: 33072639 DOI: 10.4103/abr.abr_248_19]
11 **Yao CC**, Kuo CM, Hsu CN, Yang SC, Wu CK, Tai WC, Liang CM, Wu KL, Huang CF, Bi KW, Lee CH, Chuah SK. First-line Helicobacter pylori eradication rates are significantly lower in patients with than those without type 2 diabetes mellitus. *Infect Drug Resist* 2019; **12**: 1425-1431 [PMID: 31239721 DOI: 10.2147/IDR.S194584]
12 **Song X**, Cai C, Jin Q, Chen X, Yu C. The efficacy of Helicobacter pylori eradication in diabetics and its effect on glycemic control: A systematic review and meta-analysis. *Helicobacter* 2021; **26**: e12781 [PMID: 33465265 DOI: 10.1111/hel.12781]
13 **Thai TD**, Chuenchom C, Donsa W, Faksri K, Sripa B, Edwards SW, Salao K. Helicobacter pylori extract induces purified neutrophils to produce reactive oxygen species only in the presence of plasma. *Biomed Rep* 2023; **19**: 89 [PMID: 37901879 DOI: 10.3892/br.2023.1671]
14 **Han L**, Shu X, Wang J. Helicobacter pylori-Mediated Oxidative Stress and Gastric Diseases: A Review. *Front Microbiol* 2022; **13**: 811258 [PMID: 35211104 DOI: 10.3389/fmicb.2022.811258]
15 **Wu YY**, Hsieh CT, Tsay GJ, Kao JT, Chiu YM, Shieh DC, Lee YJ. Recruitment of CCR6(+) Foxp3(+) regulatory gastric infiltrating lymphocytes in Helicobacter pylori gastritis. *Helicobacter* 2019; **24**: e12550 [PMID: 30412323 DOI: 10.1111/hel.12550]
16 **Zhou X**, Liu W, Gu M, Zhou H, Zhang G. Helicobacter pylori infection causes hepatic insulin resistance by the c-Jun/miR-203/SOCS3 signaling pathway. *J Gastroenterol* 2015; **50**: 1027-1040 [PMID: 25689935 DOI: 10.1007/s00535-015-1051-6]
17 **Martin-Nuñez GM**, Cornejo-Pareja I, Clemente-Postigo M, Tinahones FJ. Gut Microbiota: The Missing Link Between Helicobacter pylori Infection and Metabolic Disorders? *Front Endocrinol (Lausanne)* 2021; **12**: 639856 [PMID: 34220702 DOI: 10.3389/fendo.2021.639856]
18 **Pfeifer SP**. From next-generation resequencing reads to a high-quality variant data set. *Heredity (Edinb)* 2017; **118**: 111-124 [PMID: 27759079 DOI: 10.1038/hdy.2016.102]
19 **Davis S**, Meltzer PS. GEOquery: a bridge between the Gene Expression Omnibus (GEO) and BioConductor. *Bioinformatics* 2007; **23**: 1846-1847 [PMID: 17496320 DOI: 10.1093/bioinformatics/btm254]
20 **Sufyan M**, Ali Ashfaq U, Ahmad S, Noor F, Hamzah Saleem M, Farhan Aslam M, El-Serehy HA, Aslam S. Identifying key genes and screening therapeutic agents associated with diabetes mellitus and HCV-related hepatocellular carcinoma by bioinformatics analysis. *Saudi J Biol Sci* 2021; **28**: 5518-5525 [PMID: 34588861 DOI: 10.1016/j.sjbs.2021.07.068]
21 **Huang DW**, Sherman BT, Tan Q, Collins JR, Alvord WG, Roayaei J, Stephens R, Baseler MW, Lane HC, Lempicki RA. The DAVID Gene Functional Classification Tool: a novel biological module-centric algorithm to functionally analyze large gene lists. *Genome Biol* 2007; **8**: R183 [PMID: 17784955 DOI: 10.1186/gb-2007-8-9-r183]
22 **Rosandić M**, Paar V, Gluncić M, Basar I, Pavin N. Key-string algorithm--novel approach to computational analysis of repetitive sequences in human centromeric DNA. *Croat Med J* 2003; **44**: 386-406 [PMID: 12950141]
23 **Liu S**, Ren W, Yu J, Li C, Tang S. Identification of Hub Genes Associated with Diabetes Mellitus and Tuberculosis Using Bioinformatic Analysis. *Int J Gen Med* 2021; **14**: 4061-4072 [PMID: 34354368 DOI: 10.2147/IJGM.S318071]
24 **Wang Y**, Zhao M, Zhang Y. Identification of fibronectin 1 (FN1) and complement component 3 (C3) as immune infiltration-related biomarkers for diabetic nephropathy using integrated bioinformatic analysis. *Bioengineered* 2021; **12**: 5386-5401 [PMID: 34424825 DOI: 10.1080/21655979.2021.1960766]
25 **Stolte M**, Meining A. The updated Sydney system: classification and grading of gastritis as the basis of diagnosis and treatment. *Can J Gastroenterol* 2001; **15**: 591-598 [PMID: 11573102 DOI: 10.1155/2001/367832]
26 **Cheng KP**, Yang YJ, Hung HC, Lin CH, Wu CT, Hung MH, Sheu BS, Ou HY. Helicobacter pylori eradication improves glycemic control in type 2 diabetes patients with asymptomatic active Helicobacter pylori infection. *J Diabetes Investig* 2019; **10**: 1092-1101 [PMID: 30556347 DOI: 10.1111/jdi.12991]
27 **Casanova JL**, Su HC; COVID Human Genetic Effort. A Global Effort to Define the Human Genetics of Protective Immunity to SARS-CoV-2 Infection. *Cell* 2020; **181**: 1194-1199 [PMID: 32405102 DOI: 10.1016/j.cell.2020.05.016]
28 **She Y**, Mangat R, Tsai S, Proctor SD, Richard C. The Interplay of Obesity, Dyslipidemia and Immune Dysfunction: A Brief Overview on Pathophysiology, Animal Models, and Nutritional Modulation. *Front Nutr* 2022; **9**: 840209 [PMID: 35252310 DOI: 10.3389/fnut.2022.840209]
29 **Koh GY**, Rowling MJ, Pritchard SK. Possible role of type 1 and type 2 taste receptors on obesity-induced inflammation. *Nutr Rev* 2022; **80**: 1919-1926 [PMID: 35150265 DOI: 10.1093/nutrit/nuac007]
30 **Bonde A**, Daly S, Kirsten J, Kondapaneni S, Mellnick V, Menias CO, Katabathina VS. Human Gut Microbiota-associated Gastrointestinal Malignancies: A Comprehensive Review. *Radiographics* 2021; **41**: 1103-1122 [PMID: 33989072 DOI: 10.1148/rg.2021200168]
31 **Lock JY**, Caboni M, Strandwitz P, Morrissette M, DiBona K, Joughin BA, Lewis K, Carrier RL. An *in vitro* intestinal model captures immunomodulatory properties of the microbiota in inflammation. *Gut Microbes* 2022; **14**: 2039002 [PMID: 35316142 DOI: 10.1080/19490976.2022.2039002]
32 **Bui TI**, Gill AL, Mooney RA, Gill SR. Modulation of Gut Microbiota Metabolism in Obesity-Related Type 2 Diabetes Reduces Osteomyelitis Severity. *Microbiol Spectr* 2022; **10**: e0017022 [PMID: 35315698 DOI: 10.1128/spectrum.00170-22]
33 **Rong Z**, Huang Y, Cai H, Chen M, Wang H, Liu G, Zhang Z, Wu J. Gut Microbiota Disorders Promote Inflammation and Aggravate Spinal Cord Injury Through the TLR4/MyD88 Signaling Pathway. *Front Nutr* 2021; **8**: 702659 [PMID: 34589510 DOI: 10.3389/fnut.2021.702659]
34 **Noori MS**, Courreges MC, Bergmeier SC, McCall KD, Goetz DJ. Modulation of LPS-induced inflammatory cytokine production by a novel glycogen synthase kinase-3 inhibitor. *Eur J Pharmacol* 2020; **883**: 173340 [PMID: 32634441 DOI: 10.1016/j.ejphar.2020.173340]
35 **Lu Z**, Zhang X, Li Y, Lopes-Virella MF, Huang Y. TLR4 antagonist attenuates atherogenesis in LDL receptor-deficient mice with diet-induced type 2 diabetes. *Immunobiology* 2015; **220**: 1246-1254 [PMID: 26162692 DOI: 10.1016/j.imbio.2015.06.016]
36 **Yuan Y**, Lu L, Bo N, Chaoyue Y, Haiyang Y. Allicin Ameliorates Intestinal Barrier Damage *via* Microbiota-Regulated Short-Chain Fatty Acids-TLR4/MyD88/NF-κB Cascade Response in Acrylamide-Induced Rats. *J Agric Food Chem* 2021; **69**: 12837-12852 [PMID: 34694121 DOI: 10.1021/acs.jafc.1c05014]
37 **Devaraj S**, Tobias P, Jialal I. Knockout of toll-like receptor-4 attenuates the pro-inflammatory state of diabetes. *Cytokine* 2011; **55**: 441-445 [PMID: 21498084 DOI: 10.1016/j.cyto.2011.03.023]
38 **Ekuni D**, Yoneda T, Endo Y, Kasuyama K, Irie K, Mizutani S, Azuma T, Tomofuji T, Morita M. Occlusal disharmony accelerates the initiation of atherosclerosis in apoE knockout rats. *Lipids Health Dis* 2014; **13**: 144 [PMID: 25189624 DOI: 10.1186/1476-511X-13-144]
39 **Westerbacka J**, Cornér A, Kolak M, Makkonen J, Turpeinen U, Hamsten A, Fisher RM, Yki-Järvinen H. Insulin regulation of MCP-1 in human adipose tissue of obese and lean women. *Am J Physiol Endocrinol Metab* 2008; **294**: E841-E845 [PMID: 18270300 DOI: 10.1152/ajpendo.00653.2006]

40 **Li L**, Wei T, Liu S, Wang C, Zhao M, Feng Y, Ma L, Lu Y, Fu P, Liu J. Complement C5 activation promotes type 2 diabetic kidney disease *via* activating STAT3 pathway and disrupting the gut-kidney axis. *J Cell Mol Med* 2021; **25**: 960-974 [PMID: 33280239 DOI: 10.1111/jcmm.16157]

41 **Liu S**, Wang C, Yang H, Zhu T, Jiang H, Chen J. Weighted gene co-expression network analysis identifies FCER1G as a key gene associated with diabetic kidney disease. *Ann Transl Med* 2020; **8**: 1427 [PMID: 33313172 DOI: 10.21037/atm-20-1087]

42 **Mehrbod P**, Eybpoosh S, Farahmand B, Fotouhi F, Khanzadeh Alishahi M. Association of the host genetic factors, hypercholesterolemia and diabetes with mild influenza in an Iranian population. *Virol J* 2021; **18**: 64 [PMID: 33766078 DOI: 10.1186/s12985-021-01486-3]

43 **Casanova JL**. Human genetic basis of interindividual variability in the course of infection. *Proc Natl Acad Sci U S A* 2015; **112**: E7118-E7127 [PMID: 26621739 DOI: 10.1073/pnas.1521644112]

44 **Qi Z**, Yan F, Chen D, Xing W, Li Q, Zeng W, Bi B, Xie J. Identification of prognostic biomarkers and correlations with immune infiltrates among cGAS-STING in hepatocellular carcinoma. *Biosci Rep* 2020; **40** [PMID: 33006365 DOI: 10.1042/BSR20202603]

45 **Gajovic N**, Jurisevic M, Pantic J, Radosavljevic G, Arsenijevic N, Lukic ML, Jovanovic I. Attenuation of NK cells facilitates mammary tumor growth in streptozotocin-induced diabetes in mice. *Endocr Relat Cancer* 2018; **25**: 493-507 [PMID: 29459428 DOI: 10.1530/ERC-17-0529]

46 **Restrepo BI**, Twahirwa M, Rahbar MH, Schlesinger LS. Phagocytosis *via* complement or Fc-gamma receptors is compromised in monocytes from type 2 diabetes patients with chronic hyperglycemia. *PLoS One* 2014; **9**: e92977 [PMID: 24671137 DOI: 10.1371/journal.pone.0092977]

47 **Srinontong P**, Wandee J, Aengwanich W. Paraquat modulates immunological function in bone marrow-derived macrophages. *Acta Vet Hung* 2022 [PMID: 35262507 DOI: 10.1556/004.2022.00003]

48 **Chao WC**, Yen CL, Wu YH, Chen SY, Hsieh CY, Chang TC, Ou HY, Shieh CC. Increased resistin may suppress reactive oxygen species production and inflammasome activation in type 2 diabetic patients with pulmonary tuberculosis infection. *Microbes Infect* 2015; **17**: 195-204 [PMID: 25528597 DOI: 10.1016/j.micinf.2014.11.009]

49 **Perner A**, Nielsen SE, Rask-Madsen J. High glucose impairs superoxide production from isolated blood neutrophils. *Intensive Care Med* 2003; **29**: 642-645 [PMID: 12552364 DOI: 10.1007/s00134-002-1628-4]

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ORIGINAL ARTICLE

Case Control Study

Experience of humanistic nursing in hemodialysis nursing for patients with diabetic kidney disease

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Abstract

BACKGROUND

Diabetic kidney disease (DKD) is a prevalent complication of diabetes that often requires hemodialysis for treatment. In the field of nursing, there is a growing recognition of the importance of humanistic care, which focuses on the holistic needs of patients, including their emotional, psychological, and social well-being. However, the application of humanistic nursing in the context of hemodialysis for DKD patients remains relatively unexplored.

AIM

To explore the experience of humanistic nursing in hemodialysis nursing for DKD patients.

METHODS

Ninety-six DKD patients treated with hemodialysis from March 2020 to June 2022 were included in the study and divided into the control cluster (48 cases) and the study cluster (48 cases) according to different nursing methods; the control cluster was given routine nursing and the study cluster was given humanized nursing. The variances of negative emotion mark, blood glucose, renal function, the incidence of complications, life mark and nursing satisfaction before and after nur-sing were contrasted between the two clusters.

RESULTS

No significant difference in negative emotion markers between the two clusters were observed before nursing ($P > 0.05$), and the negative emotion markers of the

two clusters decreased after nursing. The Hamilton Anxiety Rating Scale and Hamilton Depression Rating Scale markers were lower in the study cluster than the control cluster. The healing rate of patients in the study cluster was significantly higher than the control cluster (97.92% *vs* 85.42%, $P < 0.05$). Blood glucose parameters were not significantly different between the groups prior to nursing ($P > 0.05$). However, after nursing, blood urea nitrogen and serum creatinine (SCr) levels in the study cluster were lower than those in the control cluster ($P < 0.05$). The incidence rate of complications was significantly lower in the study group compared to the control cluster (6.25% *vs* 20.83%, $P < 0.05$). There was no significant difference in the life markers between the two clusters before nursing. While the life markers increased after nursing for both groups, the 36-item health scale markers in the study cluster were higher than those within the control cluster ($P < 0.05$). Finally, the nursing satisfaction rate was 93.75% in the study cluster, compared to 75% in the control cluster ($P < 0.05$).

CONCLUSION

In hemodialysis for DKD patients, the implementation of humanistic nursing achieved ideal results, effectively reducing patients' psychological negative emotion markers so that they can actively cooperate with the diagnosis and nursing, facilitate the control of blood glucose and the maintenance of residual renal function, reduce the occurrence of complications, and finally enhance the life quality and nursing satisfaction of patients. It is worthy of being widely popularized and applied.

Key Words: Diabetic kidney disease; Hemodialysis; Humanistic nursing; Incidence of complication; Effect

Core Tip: The study aimed to explore the experience of humanistic nursing in hemodialysis for patients with diabetic kidney disease (DKD). The results showed that humanistic nursing effectively reduced patients' negative emotions, improved healing, controlled blood glucose levels, and maintained renal function. It also reduced the incidence of complications and enhanced patients' life quality and nursing satisfaction. These findings highlight the importance of humanistic nursing in improving the care and well-being of DKD patients undergoing hemodialysis. The implementation of humanistic nursing should be widely promoted and applied in clinical practice.

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INTRODUCTION

Diabetic kidney disease (DKD) is a renal disease that occurs as a result of diabetic injury. Its incidence is increasing each year due to the rising prevalence of diabetes[1]. Hemodialysis is the primary treatment for DKD, as it effectively eliminates harmful substances from the bloodstream, thus improving the patients' quality of life. However, it is important to note that DKD patients undergoing hemodialysis face unique challenges compared to other dialysis patients. Firstly, the underlying cause of their kidney disease is different, stemming from diabetic kidney injury. Secondly, the close association between DKD and the increasing prevalence of diabetes often leads to additional complications and comorbidities. Lastly, DKD patients may encounter specific challenges such as disease and program awareness, adhering to treatment regimens, and a higher likelihood of developing complications during hemodialysis[2]. These distinctions highlight the need for tailored interventions and care approaches for this particular patient population.

Hemodialysis is a treatment method for kidney disease caused by diabetes complications. Over time, uncontrolled diabetes can damage the blood vessels and filters in the kidneys, impairing their function. Hemodialysis involves the use of a machine called a dialyzer to filter the blood, removing waste products and excess fluids that damaged kidneys can no longer effectively eliminate. This treatment helps maintain fluid and electrolyte balance, control blood pressure, and remove accumulated waste products. It is important to note that hemodialysis is not a cure for DKD, but rather a supportive therapy to manage symptoms and maintain overall health. Other treatments, such as medication, lifestyle modifications, and kidney transplantation, may also be considered as part of the DKD management plan.

However, hemodialysis is an invasive procedure that can be time-consuming, which presents specific challenges and requirements. Additionally, many patients lack awareness about their disease and the hemodialysis program, leading to low compliance with diagnosis and care. This can further contribute to complications during hemodialysis and poor patient prognosis[3,4]. In response to these challenges, nursing measures are often employed to enhance patient care. One such approach is humanistic nursing, which has emerged because of evolving nursing models. Humanistic nursing focuses on treating patients holistically, considering their physical, emotional, and psychological needs. By incorporating this approach, healthcare professionals aim to provide individualized and compassionate care, fostering a positive patient

experience during hemodialysis. To investigate the application of humanistic nursing in hemodialysis nursing for DKD patients, this paper aims to share our experiences and findings.

MATERIALS AND METHODS

Study subjects

Ninety-six DKD patients who received hemodialysis from March 2020 to June 2022 were included in the study and divided into the control cluster (48 cases) and the study cluster (48 cases) according to different nursing methods. Among them, there were 25 males and 23 females within the control cluster, aged 38-77 years, with an average of (56.43 ± 6.84) years. In the study cluster, there were 26 males and 22 females, aged 39-78 years, with an average of (57.05 ± 7.11) years. Inclusion criteria were as follows: (1) DKD; (2) Receiving hemodialysis for the first time; and (3) Informed consent. Exclusion criteria were as follows: (1) Mental, consciousness and communication disorders; (2) Incomplete follow-up data; (3) Renal parenchymal injury caused by other causes; and (4) Combined with other serious diabetic complications such as myocardial infarction, cirrhosis of the liver and kidney failure. To implement random allocation, we used a random number generator or statistical software to randomly assign patients to different groups.

Setting and participants

Control cluster: Routine nursing: (1) Inform relevant precautions, closely observe all vital signs of patients, and timely report to the doctor once abnormalities are found; (2) Give specific guidance in diet, life and exercise; (3) Regularly test blood glucose to prevent the occurrence of hypoglycemia; (4) Do an excellent job in daily ward environment nursing; (5) Prevent and symptomatically treat complications, including maintaining skin cleanliness, oral care, puncture site care, *etc.*; and (6) Vascular access and catheter care, puncture with rope ladder method and satisfactory buckle method during puncture, and continuously replace the puncture site to ensure that the fistula can be evenly stressed; operate in strict accordance with asepsis during puncture, catheterization process, upper and bottom dialysis machines, replace the catheter heparin cap after the end of dialysis and clean and disinfect the catheter, and inform patients to keep the skin around the catheter dry.

Study clusters: Humanized nursing was implemented on the basis of the control cluster, and all nursing staff were trained before implementation to enhance their own perception of humanized nursing, and patient-centered nursing was always achieved in nursing. The measures included: (1) Health education: Active communication with patients, health education could be conducted in the form of health knowledge lectures, one-on-one education, and WeChat video push, including methods, processes, precautions, and effectiveness, and the advantages of humanized nursing were informed to patients, and similar cases of successful nursing were listed to enhance patient confidence; (2) Psychological counseling: Communication with patients with mild tone, understanding their genuine emotions, and guiding patients to actively name their concerns, and targeted counseling was given for negative emotional causes, including cognitive therapy, attention transfer method, music relaxation therapy, and emotional catharsis method; (3) Fresh green plants were placed indoors to relax the patient's mood; (4) Diet and exercise individualized nursing: The patient's condition, weight and self-metabolism were analyzed, the most appropriate daily food intake and exercise were calculated for the patient, and personalized guidance was made. Diet instructs patients to eat more foods containing calcium, protein-rich and fresh vegetables, low-sugar fruits, *etc.*; and (5) Obtain social support: Communicate with patients' families, often stand at the patient's point of view to understand their behavior and enhance the participation of family members in patient care, and strive to create a harmonious, warm, and relaxed family environment at home.

Variables

Negative emotion comparative: The Hamilton Anxiety Rating Scale (HAMA) and Hamilton Depression Rating Scale (HAMD) were used to assess the negative emotion[5]. HAMA is a standardized assessment tool used by healthcare professionals to quantify the severity of anxiety symptoms in individuals. It measures the presence and intensity of various anxiety-related symptoms, such as tension, apprehension, and insomnia. On the other hand, HAMD is a widely used instrument that evaluates the severity of depressive symptoms. It assesses factors like mood, guilt, sleep disturbances, and suicidal tendencies. Both scales provide valuable insights into the level of anxiety and depression experienced by individuals, aiding in diagnosis, treatment planning, and monitoring of progress over time.

Comparative of compliance rate: The compliance of patients is often judged based on the following criteria: (1) Complete compliance: The patient follows the doctor's advice throughout and actively cooperates with nursing staff; (2) Partial compliance: The patient may not fully cooperate but can complete nursing activities under the guidance of nursing staff; and (3) Non-compliance: The patient completely refuses to cooperate with nursing staff. Patient compliance is determined by observing and evaluating their interaction with the healthcare team and their adherence to treatment and nursing plans. However, it is important to consider individual differences, patient education level, and social support when assessing patient compliance[6].

Comparative blood glucose parameters: A total of 4 mL of peripheral venous blood was drawn from the patients in a fasting state or 2 h after a meal, and the supernatant was collected after centrifugation[7].

Comparative renal function indexes: A total of 4 mL of peripheral venous blood was drawn from the patients under a fasting state, and the supernatant was collected after centrifugation to detect blood urea nitrogen (BUN) and serum

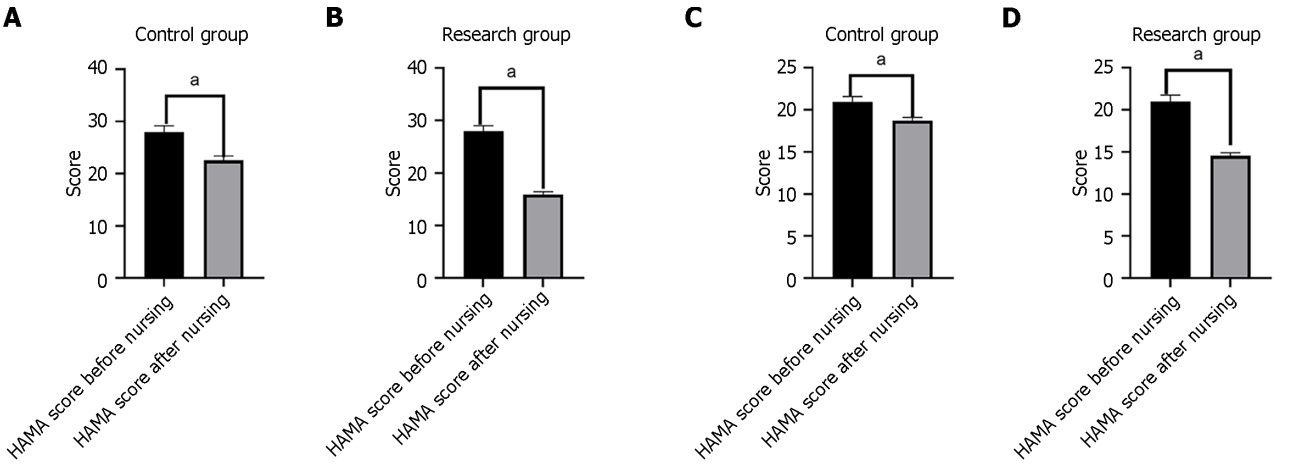

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Figure 1 Comparative of negative emotions. A: Before treatment, Hamilton Anxiety Rating Scale (HAMA) scores of the two groups were compared; B: After treatment, HAMA scores of the two groups were compared; C: Before treatment, Hamilton Depression Rating Scale (HAMD) scores of the two groups were compared; D: After treatment, HAMD scores of the two groups were compared. $^{a}P < 0.05$, the difference between groups with statistical significance. HAMA: Hamilton Anxiety Rating Scale; HAMD: Hamilton Depression Rating Scale.

creatinine (SCr) by automatic biochemical analyzer[8].

Comparison of complications: Common complications in hemodialysis include infection, catheter dysfunction, hypoglycemia, hypotension, and heart failure. Intradialytic hypotension (IDH) is common after dialysis. IDH, which can be caused by aggressive ultrafiltration due to weight gain during dialysis, can lead to myocardial stunning and cardiac arrhythmias, which are associated with an increased risk of death[9]. Obviously, kidney failure has a major impact on heart function. Studies have shown that more than half of deaths in patients with end-stage renal disease (ESRD) are due to cardiovascular disease, with arrhythmias and cardiac arrest accounting for 38 percent of deaths[10]. The incidence of ESRD has nearly doubled in the past 20 years. Infection is the second leading cause of death in this patient population, and vascular access-associated infection is the most common identifiable source of infection in hemodialysis patients[11]. The quality of vascular access is the most important factor that determines dialysis treatment efficacy. Vascular lumen stenosis can lead to increased risk of thrombosis, catheter dysfunction and adverse effects on blood flow[12]. Case studies have shown that glucose is transferred from the dialysate into the blood during dialysis and reactive hypoglycemia occurs after the end of dialysis. Persistent hypoglycemia can lead to permanent brain damage[13]. Therefore, monitoring the above complications is of great significance for evaluating the efficacy of dialysis. All complications that occurred during hemodialysis were recorded for both clusters[14].

Quality-of-life comparison: Patient quality-of-life was assessed using the 36-item health scale (SF-36) with a total possible score of 100 points[15].

Comparison of nursing satisfaction rate: The self-generated satisfaction questionnaire was used to evaluate satisfaction with the nursing care received, out of a total of 100 points. The score ranges were as follows: Satisfaction: ≥ 80 points, essential satisfaction: 60-79 points, dissatisfaction: < 60 points; satisfaction rate = (satisfaction + essential satisfaction)/total × 100%[16].

Statistical methods

Measurement data were expressed as (mean ± SD), and *t*-tests were used. Enumeration data were expressed as *n* (%), and a χ^2 test was used. $P < 0.05$ was considered statistically significant and data were analyzed in GraphPad Prism 8 software.

RESULTS

Negative emotion contrast

There was no observable variance in negative emotion marks between the two clusters before nursing care ($P > 0.05$). After nursing, the negative emotion marks of the two clusters decreased, and the HAMA and HAMD marks of the study cluster were lower than those of the control cluster ($P < 0.05$) (Figure 1).

Comparison of compliance rates

The compliance rate of patients in the control cluster was 85.42%, whereas it increased to 97.92% in the study cluster ($P < 0.05$) (Table 1).

Table 1 Comparative of compliance rate

Cluster	Cases	Full compliance	Basic compliance	Non-compliance	Compliance rate
Control cluster	48	16	25	7	41 (85.42)
Study cluster	48	30	17	1	47 (97.92)
χ^2	/	/	/	/	4.909
P value	/	/	/	/	0.027

Data are *n* or *n* (%).

Table 2 Comparative of complication rate

Cluster	Cases	Infection	Catheter dysfunction	Hypotension	Hypoglycemia	Heart failure	Occurrence
Control cluster	48	2	3	2	2	1	10 (20.83)
Study cluster	48	1	1	1	1	0	3 (6.25)
χ^2	/	/	/	/	/	/	4.360
P value	/	/	/	/	/	/	0.037

Data are *n* or *n* (%).

Table 3 Comparative of life

Cluster	Time	Social functioning	Physical function	Role function	Affective function	Cognitive function
Control cluster	Pre-nursing	51.67 ± 4.47	56.27 ± 3.57	50.14 ± 4.27	62.11 ± 4.27	61.21 ± 4.72
	Post-care[a]	58.57 ± 4.28	62.52 ± 3.74	58.16 ± 3.88	68.65 ± 5.08	69.66 ± 5.17
Study cluster	Pre-nursing	51.59 ± 4.07	56.31 ± 4.07	50.09 ± 4.13	62.09 ± 4.31	61.26 ± 4.86
	Post care[a,b]	65.25 ± 5.63	67.23 ± 4.24	67.15 ± 4.25	77.15 ± 5.52	78.65 ± 5.06

[a]$P < 0.05$, intra-cluster comparative.
[b]$P < 0.05$, inter-cluster comparative.
Data are points summarized as mean ± SD.

Comparison of blood glucose indicators

There was no significant variance in blood glucose indices between the two clusters before nursing ($P > 0.05$). After nursing, blood glucose indices decreased in both clusters, and fasting plasma glucose (FPG), 2-hour plasma glucose (2hPG) and hemoglobin A1c (HbA1c) levels in the study cluster were lower than those within the control cluster ($P < 0.05$) (Figure 2).

Comparison of renal function indicators

There was no significant variance in renal function indices between the two clusters before nursing ($P > 0.05$). After nursing, the renal function indices decreased for both clusters, and BUN and SCr levels in the study cluster were lower than those within the control cluster ($P < 0.05$) (Figure 3).

Comparison of complications

The complication rate of the control cluster was 20.83%, compared to 6.25% in the study ($P < 0.05$) (Table 2). Complications included infection, catheter dysfunction, hypotension, hypoglycemia, and heart failure.

Quality of life comparison

There were no significant differences in the quality-of-life marks between the two clusters before nursing ($P > 0.05$). The quality-of-life marks increased for both groups after nursing, but the SF-36 marks were higher in the study cluster than the control cluster ($P < 0.05$) (Table 3). The questionnaire covers eight domains: Physical functioning, role limitations due to physical health, bodily pain, general health perceptions, vitality, social functioning, role limitations due to emotional problems, and mental health.

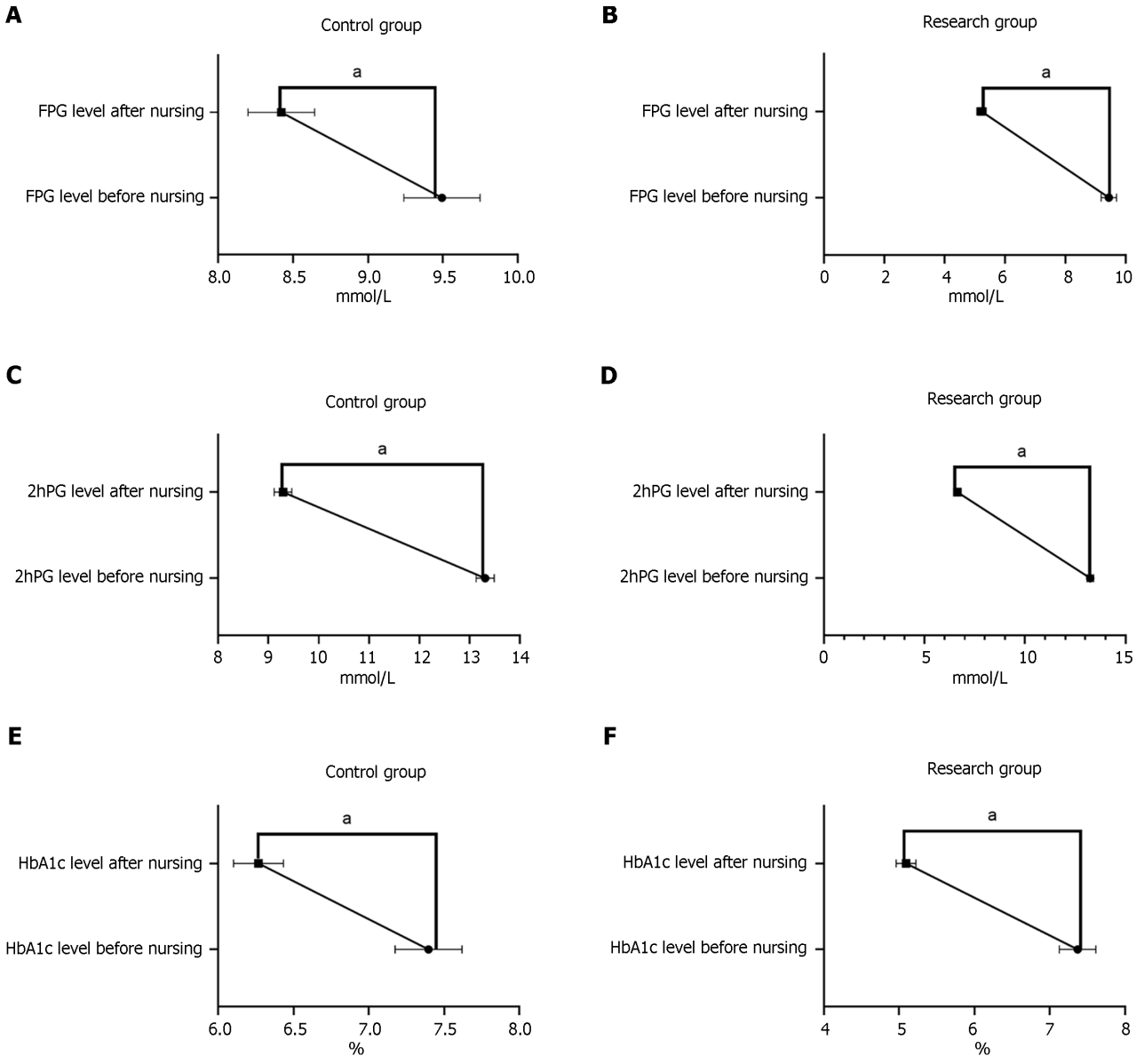

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Figure 2 Comparative of blood glucose indicators. A: Comparison of fasting plasma glucose (FPG) level before and after treatment in control group; B: Comparison of FPG levels before and after treatment in the study group; C: Comparison of 2-h plasma glucose (2hPG) level in control group before and after treatment; D: Comparison of 2hPG levels before and after treatment in the study group; E: Comparison of hemoglobin A1c (HbA1c) level before and after treatment in control group; F: Comparison of HbA1c levels before and after treatment in the study group. $^{a}P < 0.05$, the difference between groups with statistical significance. FPG: Fasting plasma glucose; HbA1c: Hemoglobin A1c; 2Hpg: 2-h plasma glucose.

Comparison of nursing satisfaction rate

The nursing satisfaction rate was higher in the study cluster compared to the control cluster (93.75% *vs* 75%; $P < 0.05$) (Figure 4).

DISCUSSION

Compared to conventional hemodialysis patients with nephropathy, hemodialysis patients with DKD have a higher risk of discomfort and complications, resulting in a lower degree of nursing cooperation. Therefore, it is very important to strength the care of hemodialysis patients with DKD in the hemodialysis room[17,18]. In routine nursing, attention is focused on healing and attention to the patient's psychology is neglected. This contributes to passivity and singularity, which leads to the high negative emotions of patients during healing and will hinder healing progress in serious cases. Overall, there is not a positive effect from nursing care; therefore, the exploration of a more effective nursing method is necessary[19].

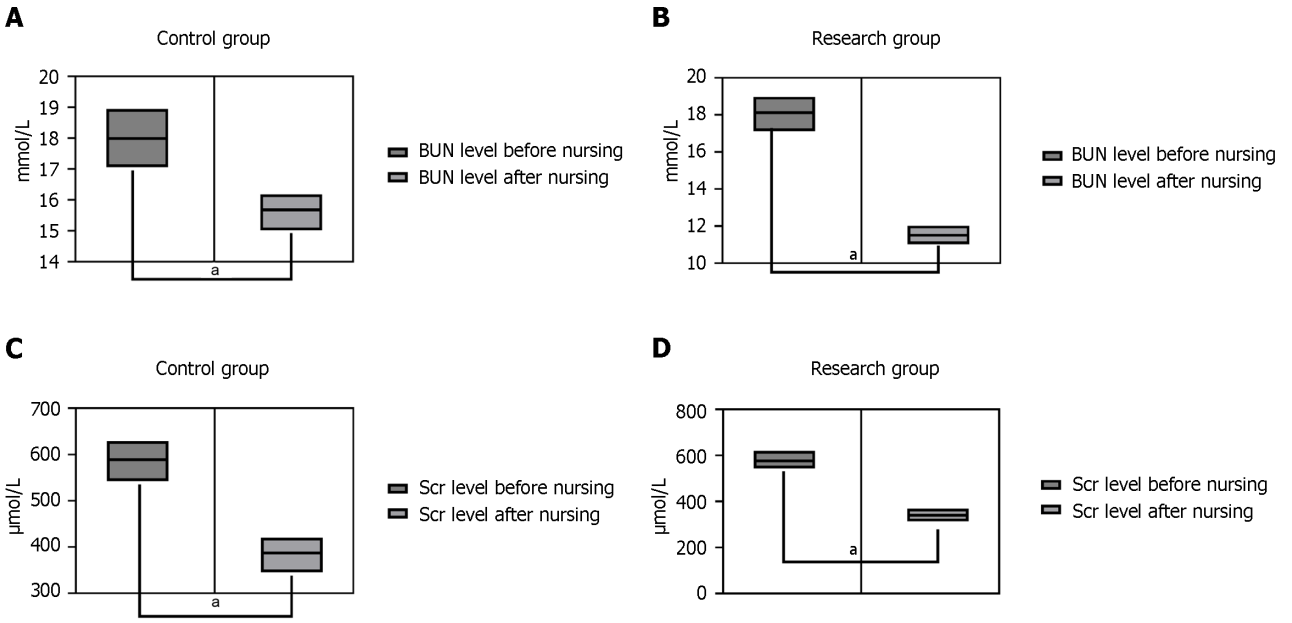

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Figure 3 Comparative of renal function indicators. A: Comparison of blood urea nitrogen (BUN) levels before and after treatment in control group; B: Comparison of BUN levels before and after treatment in the study group; C: Comparison of serum creatinine (SCr) level before and after treatment in control group; D: Comparison of SCr levels before and after treatment in the study group. [a]*P* < 0.05, the difference between groups with statistical significance.

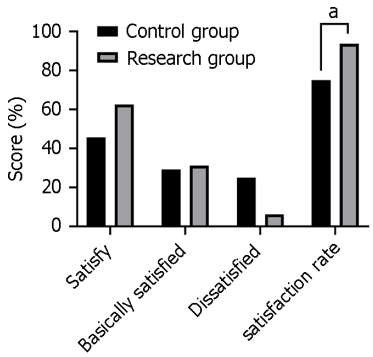

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Figure 4 Comparative of nursing satisfaction rate. [a]*P* < 0.05, the difference between groups with statistical significance.

With the continuous change of nursing model, humanistic nursing appears and is widely used in various fields. Humanistic nursing can be traced back to human nursing at the earliest. It requires nursing staff to have the cognition of human science, to implement humanistic nursing for patients, in order to make the patient' psychology in a satisfactory and comfortable state to actively cooperate with nursing staff to complete the whole nursing, to achieve the best nursing [20]. With the continuous enhancement of humanistic nursing, its core refers to respecting the patient's life value, personality dignity, privacy and face in nursing, without making them feel embarrassed and uncomfortable, which will greatly reduce the psychological discomfort caused by physical discomfort, so as to enhance the patient's negative emotions[21,22]. Humanized nursing attaches importance to the psychological needs of patients, through effective communication to obtain the real ideas of patients' thoughts, and through scientific and appropriate methods to guide them, in order to help patients establish the best rehabilitation state. Relevant studies have found that meeting the psychological needs of patients can enhance their own defense against stress response, facilitate the reconstruction of psychological balance, and is important for the enhancement of healing and body immunity[23].

Reasonable control of blood glucose is of great meaning for the enhancement of renal function in patients with DKD, and the purpose of nursing implementation is to help patients achieve the expected healing goals, so the detection of blood glucose and renal function indicators in patients can reflect the effect of nursing implementation to a certain extent. Among them, HbA1c has been officially included in the diagnostic criteria of diabetes, FPG and 2hPG are also the main diagnostic criteria, and they are used for the diagnosis of blood glucose levels in diabetic patients[24]. Diabetic nephropathy is a chronic kidney disease. Studies have shown that inflammation and depression have a two-way connection between people with chronic disease[25]. In chronic kidney disease, the increase in anxiety susceptibility may be associated with the inflammatory process of the toxin, the increase of oxidative stress, brain microvascular damage

and the participation of the renin-angiotensin system[26]. So we can speculate that after nursing interventions, the patient's anxiety and depression were relieved. This affects the function of inflammation and kidney function, which affects the level of blood creatinine.

SCr and BUN are the parameters usually used to estimate renal function of which SCr is a small molecule substance metabolized by muscle and BUN is a nitrogen-containing compound in plasma, and the excretion of both into the body is completed by glomerular filtration. Glomerular filtration is also decreased when kidney function is compromised, causing BUN and SCr to be incompletely eliminated from the body, thus resulting in increased serum levels of both[27]. This study found that the implementation of humanistic nursing can better control the blood glucose level of patients with DKD facilitating the maintenance of residual renal function. The study found that after the implementation of humanistic nursing, the healing, life and nursing satisfaction of patients were remarkably enhanced, and the complications and negative emotions were remarkably reduced, which suggests that humanistic nursing has a very high application value in hemodialysis.

CONCLUSION

In hemodialysis for patients with DKD, the implementation of humanistic nursing has achieved ideal results, which can effectively reduce the psychological negative emotion mark of patients, so that they can actively cooperate with the diagnosis and nursing, to facilitate the control of blood glucose and the enhancement of residual renal function, to reduce the occurrence of complications, and finally to enhance the life quality and nursing satisfaction of patients. It is worthy of being widely popularized and applied. However, the main limitations of our study are the small number of studied patients and the too short follow-up time. Our intention is to continue to study these aspects in future research with a more wide number of DKD patients.

ARTICLE HIGHLIGHTS

Research background

Diabetic nephropathy is one of the common complications of diabetes, and as the disease progresses, patients may need to receive hemodialysis treatment. In the process of receiving hemodialysis, patients need long-term treatment, and the condition is changeable, so quality nursing services are needed to improve the treatment effect and the quality of life of patients. Humanistic nursing is a kind of patient-centered nursing concept, emphasizing respect for patients' lives and personalities, paying attention to patients' emotions and needs, and providing patients with comprehensive and personalized nursing services.

Research motivation

The traditional hemodialysis nursing model often only focuses on the physiological needs of patients, ignoring the psychological and social needs of patients, resulting in patients are prone to anxiety, depression and other adverse emotions in the treatment process, affecting the treatment effect. Therefore, the purpose of this study is to explore the application effect of anthropogenic nursing in hemodialysis nursing of diabetic nephropathy patients, to provide patients with more comprehensive and personalized nursing services.

Research objectives

The first is to discuss the application effect of humanistic nursing in hemodialysis nursing of diabetic nephropathy patients; the second is to evaluate the impact of humanistic nursing on patients' quality of life, mental health and satisfaction.

Research methods

A total of 96 diabetic kidney disease patients receiving hemodialysis treatment from March 2020 to June 2022 were selected as the study objects. The control group was given routine nursing, while the research group was given humanized nursing on this basis, including the following aspects: Nurses and patients fully communicate and exchange, understand the patient's condition, family background, psychological state, *etc*., to provide personalized nursing services for patients; psychological support: Nurses provide psychological counseling and support to patients to help them relieve bad emotions and enhance treatment confidence; health education: Nurses provide comprehensive health education to patients, including diet guidance, exercise guidance, medication guidance, *etc*., to improve patients' self-management ability; social support: Nurses provide family and social support to help patients deal with difficulties and problems in life. The control group received routine care. At the end of the trial, all patients were assessed for quality of life, mental health and satisfaction.

Research results

By comparing data from the experimental and control groups, we found that patients in the experimental group were better than those in the control group in terms of quality of life, mental health and satisfaction. After receiving humanistic care, anxiety, depression and other bad emotions were effectively alleviated, the treatment effect was improved, and the

quality of life was improved. At the same time, the satisfaction of the experimental group was also higher than that of the control group.

Research conclusions

Humanistic nursing has a remarkable effect on hemodialysis nursing of diabetic nephropathy patients. Through humanistic nursing, patients' mental health and quality of life have been effectively improved, the treatment effect has been improved, and the satisfaction of patients has also been improved. Therefore, we suggest to promote and apply humanistic nursing concept in hemodialysis nursing of diabetic nephropathy patients.

Research perspectives

From the perspective of patient needs, understand the physiological, psychological and social needs of patients. From the perspective of nursing practice, this paper explores the application method and effect of humanistic nursing concept in hemodialysis nursing practice. From the perspective of nursing staff, this paper discusses the requirements of humanistic nursing on the quality and working style of nursing staff, and the experience and feeling of nursing staff in the process of implementing humanistic nursing.

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FOOTNOTES

Author contributions: Chai XY performed experiments and wrote the manuscript; Bao XY designed the study and revised the manuscript; Dai Y was involved in analytical tools; Dai XX and Zhang Y collected the human material; Zhang Y served as scientific advisor; Yang YL is the guarantor.

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REFERENCES

1 **Siegel KR**, Ali MK, Zhou X, Ng BP, Jawanda S, Proia K, Zhang X, Gregg EW, Albright AL, Zhang P. Cost-effectiveness of Interventions to Manage Diabetes: Has the Evidence Changed Since 2008? *Diabetes Care* 2020; **43**: 1557-1592 [PMID: 33534729 DOI: 10.2337/dci20-0017]

2 **Varghese RT**, Jialal I, Doerr C. Diabetic Nephropathy (Nursing). 2023 Jul 24. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan- [PMID: 33760450]

3 **Scarton L**, Hebert LE, Goins RT, Umans JG, Jiang L, Comiford A, Chen S, White A, Ritter T, Manson SM. Diabetes and health-related quality of life among American Indians: the role of psychosocial factors. *Qual Life Res* 2021; **30**: 2497-2507 [PMID: 33837892 DOI: 10.1007/s11136-021-02830-4]

4 **Brown EA**, Zhao J, McCullough K, Fuller DS, Figueiredo AE, Bieber B, Finkelstein FO, Shen J, Kanjanabuch T, Kawanishi H, Pisoni RL,

Perl J; PDOPPS Patient Support Working Group. Burden of Kidney Disease, Health-Related Quality of Life, and Employment Among Patients Receiving Peritoneal Dialysis and In-Center Hemodialysis: Findings From the DOPPS Program. *Am J Kidney Dis* 2021; **78**: 489-500.e1 [PMID: 33872688 DOI: 10.1053/j.ajkd.2021.02.327]

5 **Tamru K**, Aga F, Berhanie E, Aynalem YA, Shiferaw WS. Incidence of diabetic nephropathy in patients with type 2 diabetes mellitus at a tertiary healthcare setting in Ethiopia. *Diabetes Metab Syndr* 2020; **14**: 1077-1083 [PMID: 32650279 DOI: 10.1016/j.dsx.2020.06.028]

6 **Yu L**, Yang X, Hao J. Study on the effect of high quality nursing in patients with diabetic nephropathy. *Panminerva Med* 2021 [PMID: 34609118 DOI: 10.23736/S0031-0808.21.04465-7]

7 **Guo Y**, Song Q, Cui Y, Wang C. Clinical Effects of Primary Nursing on Diabetic Nephropathy Patients Undergoing Hemodialysis and Its Impact on the Inflammatory Responses. *Evid Based Complement Alternat Med* 2022; **2022**: 1011415 [PMID: 35983002 DOI: 10.1155/2022/1011415]

8 **Guo F**, Lin YL, Raji M, Leonard B, Chou LN, Kuo YF. Processes and outcomes of diabetes mellitus care by different types of team primary care models. *PLoS One* 2020; **15**: e0241516 [PMID: 33152002 DOI: 10.1371/journal.pone.0241516]

9 **Morfin JA**, Fluck RJ, Weinhandl ED, Kansal S, McCullough PA, Komenda P. Intensive Hemodialysis and Treatment Complications and Tolerability. *Am J Kidney Dis* 2016; **68**: S43-S50 [PMID: 27772642 DOI: 10.1053/j.ajkd.2016.05.021]

10 **Ahmadmehrabi S**, Tang WHW. Hemodialysis-induced cardiovascular disease. *Semin Dial* 2018; **31**: 258-267 [PMID: 29624739 DOI: 10.1111/sdi.12694]

11 **Gupta V**, Yassin MH. Infection and hemodialysis access: an updated review. *Infect Disord Drug Targets* 2013; **13**: 196-205 [PMID: 24001331 DOI: 10.2174/1871526511313030008]

12 **Masud A**, Costanzo EJ, Zuckerman R, Asif A. The Complications of Vascular Access in Hemodialysis. *Semin Thromb Hemost* 2018; **44**: 57-59 [PMID: 28898900 DOI: 10.1055/s-0037-1606180]

13 **Rigg GA**, Bercu BA. Hypoglycemia--a complication of hemodialysis. *N Engl J Med* 1967; **277**: 1139-1140 [PMID: 6055002 DOI: 10.1056/NEJM196711232772109]

14 **El Berri H**, Gedik FG, Belkhadir J, Catton H, Hammerich A, Oweis A, Slama S. Tackling diabetes: how nurses can make the difference. *East Mediterr Health J* 2020; **26**: 1318-1319 [PMID: 33226097 DOI: 10.26719/2020.26.11.1318]

15 **Liao K**, Lin KC, Chiou SJ. Self-efficacy remains a vital factor in reducing the risk of dialysis in type 2 diabetes care. *Medicine (Baltimore)* 2021; **100**: e26644 [PMID: 34260563 DOI: 10.1097/MD.0000000000026644]

16 **Chen HC**, Lai MJ, Wu WC, Lee CY, Lin HJ, Lin CC, Chang CT, Wang CCN, Chou CY. Association of diabetes, education level, and care dependency with use of temporary vascular access in patients with chronic kidney disease. *Semin Dial* 2021; **34**: 130-136 [PMID: 33103809 DOI: 10.1111/sdi.12930]

17 **Li L**, Chen H, Peng C, Yang L. Analysis on Value of Continuous Nursing Based on WeChat in Improving Healthy Quality of Life and Self-Management Behavior of Patients with Diabetic Nephropathy. *Evid Based Complement Alternat Med* 2022; **2022**: 5131830 [PMID: 36185090 DOI: 10.1155/2022/5131830]

18 **Beetham KS**, Krishnasamy R, Stanton T, Sacre JW, Douglas B, Isbel NM, Coombes JS, Howden EJ. Effect of a 3-Year Lifestyle Intervention in Patients with Chronic Kidney Disease: A Randomized Clinical Trial. *J Am Soc Nephrol* 2022; **33**: 431-441 [PMID: 34893535 DOI: 10.1681/ASN.2021050668]

19 **Blanchfield D**, O'Connor L. A participatory action research study to inform combined type 2 diabetes and chronic kidney disease care provided in the context of advanced practice nursing. *J Adv Nurs* 2022; **78**: 3427-3443 [PMID: 35855655 DOI: 10.1111/jan.15362]

20 **Mott AK**. Diabetes Mellitus Self-Management to Decrease the Risk for Chronic Kidney Disease. *Nephrol Nurs J* 2021; **48**: 65-63 [PMID: 33683845]

21 **Watanabe H**, Anezaki H, Kazawa K, Tamaki Y, Hashimoto H, Moriyama M. Long-term effectiveness of a disease management program to prevent diabetic nephropathy: a propensity score matching analysis using administrative data in Japan. *BMC Endocr Disord* 2022; **22**: 135 [PMID: 35596152 DOI: 10.1186/s12902-022-01040-4]

22 **Lucena AF**, Magro CZ, Proença MCDC, Pires AUB, Moraes VM, Aliti GB. Validation of the nursing interventions and activities for patients on hemodialytic therapy. *Rev Gaucha Enferm* 2018; **38**: e66789 [PMID: 29538608 DOI: 10.1590/1983-1447.2017.03.66789]

23 **Pérez-Alba A**, Catalán Navarrete S, Renau Ortells E, García Peris B, Agustina Trilles A, Cerrillo García V, Calvo Gordo C. Nursing program to support home hemodialysis. Experience of a center. *Nefrologia (Engl Ed)* 2021; **41**: 360-362 [PMID: 36166254 DOI: 10.1016/j.nefroe.2021.06.002]

24 **Yuan L**, Yuan H, Feng Q, Zhao J. Effect of continuous nursing on quality of life of hemodialysis patients: A protocol for systematic review and meta-analysis. *Medicine (Baltimore)* 2021; **100**: e24942 [PMID: 33761650 DOI: 10.1097/MD.0000000000024942]

25 **Katon WJ**. Epidemiology and treatment of depression in patients with chronic medical illness. *Dialogues Clin Neurosci* 2011; **13**: 7-23 [PMID: 21485743 DOI: 10.31887/DCNS.2011.13.1/wkaton]

26 **Huang CW**, Wee PH, Low LL, Koong YLA, Htay H, Fan Q, Foo WYM, Seng JJB. Prevalence and risk factors for elevated anxiety symptoms and anxiety disorders in chronic kidney disease: A systematic review and meta-analysis. *Gen Hosp Psychiatry* 2021; **69**: 27-40 [PMID: 33516963 DOI: 10.1016/j.genhosppsych.2020.12.003]

27 **Acharya DK**, Nilmanat K, Boonyasopun U. Textual Organization of Hemodialysis Nursing Practice: An Institutional Ethnography. *J Nepal Health Res Counc* 2022; **20**: 180-185 [PMID: 35945873 DOI: 10.33314/jnhrc.v20i01.4023]

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Case Control Study

Analysis of the influencing factors and clinical related characteristics of pulmonary tuberculosis in patients with type 2 diabetes mellitus

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Abstract

BACKGROUND

In China, the prevalence of type 2 diabetes mellitus (T2DM) among diabetic patients is estimated to be between 90%-95%. Additionally, China is among the 22 countries burdened by a high number of tuberculosis cases, with approximately 4.5 million individuals affected by active tuberculosis. Notably, T2DM poses a significant risk factor for the development of tuberculosis, as evidenced by the increased incidence of T2DM coexisting with pulmonary tuberculosis (T2DM-PTB), which has risen from 19.3% to 24.1%. It is evident that these two diseases are intricately interconnected and mutually reinforcing in nature.

AIM

To elucidate the clinical features of individuals diagnosed with both T2DM and tuberculosis (T2DM-PTB), as well as to investigate the potential risk factors associated with active tuberculosis in patients with T2DM.

METHODS

T2DM-PTB patients who visited our hospital between January 2020 and January 2023 were selected as the observation group, Simple DM patients presenting to our hospital in the same period were the control group, Controls and case groups were matched 1:2 according to the principle of the same sex, age difference (± 3) years and disease duration difference (± 5) years, patients were investigated for general demographic characteristics, diabetes-related characteristics, body immune status, lifestyle and behavioral habits, univariate and multivariate analysis of the data using conditional logistic regression, calculate the odds ratio (OR) values and 95%CI of OR values.

RESULTS
A total of 315 study subjects were included in this study, including 105 subjects in the observation group and 210 subjects in the control group. Comparison of the results of both anthropometric and biochemical measures showed that the constitution index, systolic blood pressure, diastolic blood pressure and lymphocyte count were significantly lower in the case group, while fasting blood glucose and high-density lipoprotein cholesterol levels were significantly higher than those in the control group. The results of univariate analysis showed that poor glucose control, hypoproteinemia, lymphopenia, TB contact history, high infection, smoking and alcohol consumption were positively associated with PTB in T2DM patients; married, history of hypertension, treatment of oral hypoglycemic drugs plus insulin, overweight, obesity and regular exercise were negatively associated with PTB in T2DM patients. Results of multivariate stepwise regression analysis found lymphopenia (OR = 17.75, 95%CI: 3.40-92.74), smoking (OR = 12.25, 95%CI: 2.53-59.37), history of TB contact (OR = 6.56, 95%CI: 1.23-35.03) and poor glycemic control (OR = 3.37, 95%CI: 1.11-10.25) was associated with an increased risk of developing PTB in patients with T2DM, While being overweight (OR = 0.23, 95%CI: 0.08-0.72) and obesity (OR = 0.11, 95%CI: 0.02-0.72) was associated with a reduced risk of developing PTB in patients with T2DM.

CONCLUSION
T2DM-PTB patients are prone to worse glycemic control, higher infection frequency, and a higher proportion of people smoking, drinking alcohol, and lack of exercise. Lymphopenia, smoking, history of TB exposure, poor glycemic control were independent risk factors for T2DM-PTB, and overweight and obesity were associated with reduced risk of concurrent PTB in patients with T2DM.

Key Words: Type 2 diabetes; Pulmonary tuberculosis; Blood sugar; Infection; Risk factors

Core Tip: Diabetes mellitus is a metabolic disorder resulting from a combination of genetic factors, environmental influences, and lifestyle choices, which lead to impairments in insulin secretion or function. In recent times, there has been a significant increase in the incidence of diabetes accompanied by hyperglycemia as its primary manifestation. By conducting case-control studies within hospital settings, we aim to examine the distinctive features of patients with type 2 diabetes mellitus and pulmonary tuberculosis and investigate the potential risk factors associated with the development of tuberculosis in this specific population.

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder caused by genetic factors, environment and lifestyle caused by defects in insulin secretion or function. In recent years, the prevalence of DM, the main manifestation of hyperglycemia, has risen sharply, reaching 9.7%[1]. In China, type 2 DM (T2DM) accounts for 90%-95% of in all diabetic patients[2]. Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium tuberculosis* (*M. tuberculosis*)[3], most of latent tuberculosis infected people have no obvious clinical symptoms. Patients with latent TB, when body resistance decreases or cell-mediated allergy increases, develop active TB, with pulmonary tuberculosis (PTB) being the most common[4]. China is one of the 22 countries with high TB burden with about 4.5 million active TB patients[5]. As many as 130000 TB patients die each year, more than twice the total number of other infectious diseases in China[5]. T2DM is one of the risks of developing TB, and the incidence of T2DM with PTB (T2DM-PTB) increased from 19.3% to 24.1%. These two diseases are closely related and mutually promote[6]. On the one hand, due to the high tissue sugar content, metabolic disorder and decreased immune function, *M. tuberculosis* increases the production of resistant strains, and affects the prognosis of T2DM-PTB patients; on the other hand, TB will aggravate the glucose metabolism disorder of T2DM patients, increase the incidence of ketoacidosis, and present a dangerous prognosis[7]. T2DM-PTB faces new challenges in the world public health field due to its severity, treatment difficulties, and poor prognosis.

China is in a period of rapid growth in the incidence of DM, and the burden of tuberculosis is serious. DM combined with tuberculosis has become a major public health problem and the rising prevalence of DM is a potential threat to TB control. Based on this, World Health Organization recommends a collaborative framework for clinical management and control of DM with TB. Therefore, this study conducted a hospital-based case-control study to observe the characteristics of T2DM-PTB patients and explore the risk factors for pulmonary TB in T2DM, providing a scientific basis for the prevention and control of T2DM-PTB.

MATERIALS AND METHODS

General information

In this study, cases (observation group) of selected tuberculosis patients with T2DM from January 2020 to January 2023 were randomly selected in our hospital, and compared with diabetic patients without tuberculosis in the same period (control group).

Selection of the cases

Inclusion criteria for the observation group: (1) DM diagnosis earlier than PTB; (2) active tuberculosis (never received anti-tuberculosis chemotherapy or received chemotherapy for 1 month); (3) lived locally for more than 1 year, age > 18 years old; and (4) was aware of the study and signed informed consent.

Exclusion criteria for observation group: (1) Recovered PTB patients, disseminated PTB, tuberculosis pleurisy, and other extrapulmonary tuberculosis; (2) has other endocrine diseases, such as hyperthyroidism, systemic lupus erythematosus, rheumatoid arthritis; (3) has diseases that can affect immune function such as acquired immunodeficiency syndrome (AIDS), malignant tumor, chronic hepatitis, cirrhosis, primary kidney disease, renal failure, blood disease, renal transplantation, gastrectomy; or (4) study subjects have used hormones and immunosuppressants within 4 months.

Selection of control group

The control group was patients with T2DM in our hospital at the same time. Two controls were matched for each case by the principle of equal gender, age difference (± 3) years and disease duration difference (± 5) years.

Inclusion criteria for the control group: (1) DM patients aged > 18 years who had lived locally for more than 1 year; and (2) were aware of the study and signed informed consent.

Exclusion criteria for the control group: (1) Now have other endocrine diseases, such as hyperthyroidism, systemic lupus erythematosus, rheumatoid arthritis, *etc.*; (2) now has diseases that can affect the immune function, such as AIDS, malignant tumor, chronic hepatitis and cirrhosis, chemical, primary renal disease, renal failure, hematological disorders, post-renal transplantation, gastrectomy, *etc.*; or (3) patients with pulmonary infection, or patients with tuberculosis lesions or suspicious lesions after chest X-ray examination.

Diagnostic criteria

Diagnostic criteria for T2DM[8]: Patients presented with typical T2DM, abnormal glucose test (random glucose 11.1 mmol/L or fasting glucose 7.0 mmol/L; or oral glucose tolerance test 2h glucose 11.1 mmol/L).

Diagnostic criteria for PTB[9]: (1) Clinical symptoms such as cough, expectoration and fever, typical PTB findings combined with chest X-ray and chest computed tomography; (2) tuberculin skin test (PPD) reaction 10 mm; (3) positive TB antibody or γ -interferon release test; (4) positive mycobacterium smear culture; and (5) histopathology consistent with positive tuberculous change and acid fast staining.

Sample size calculation

The sample size was calculated according to the sample size of the paired case-control study (number of cases: number of controls = 1: r):

$$n = /[Z_{\alpha}\sqrt{(1+1/\mathrm{r})\bar{p}(1-\bar{p})} + Z_{\beta}\sqrt{p_1(1-p_1)/\mathrm{r} + p_0(1-p_0)}]^2(p_1-p_0)^2$$

$$p_1p_0p_0 = \mathrm{OR}/[1 + (\mathrm{OR}-1)]$$

$$\bar{p}p_1p_0 = (+\mathrm{r})/(1 + \mathrm{r})$$

$p_1p_0Z_{\alpha}$ and Z_{β} are the exposure rate of a major risk factor in the observation group and control group, respectively. OR represents the odds ratio of the risk factor, the standard normal cut-off for the type I error probability α and the standard normal cut-off for the type II error probability β. The literature shows that the glucose control level of T2DM patients is closely related to the occurrence of PTB[10], poor glucose control can increase the risk of PTB, its OR value is about 3[11], the incidence of poor glucose control in T2DM patients in China is about 60%[12], namely = 0.60, this study took α = 0.05 (bilateral), β = 0.10, r = 2. The observation group should be more than 81 cases and more than 162 cases in the control group. A total of 105 patients in the observation group and 210 patients in the control group were included in this study, which met the study requirements.

Questionnaire survey

Using uniform questionnaire and inquiry, the questionnaire mainly included: general demographic characteristics: age, gender, marital status, educational level, work and monthly income, DM related characteristics: family history of DM, course of disease, diet control and blood glucose monitoring; body immune status: whether the subjects had upper respiratory tract infections (such as cold, sinusitis, tonsillitis, otitis media, *etc.*), bronchitis or pneumonia, skin infections (lip herpes, genital herpes, warts, furuncle or abscess) in the last year. The questionnaire was adapted from the immune system assessment questionnaire developed by the Chronic Immunodeficiency Center of the University Medical Center in Freiburg[13]; Lifestyle and behavioral habits: smoking, alcohol consumption, physical exercise, sleep status, tuberculosis exposure history, per capita living area, dust exposure history and contact personnel, *etc.*

Physical examination

Measurement of height and weight: After calibrating the instrument, the patient is required to take off his shoes and coat according to the standard method. During the measurement, feet are tight, back straight and eyes straight forward.

Blood pressure measurement: Blood pressure is measured by desktop mercury column sphygmomanometer. The respondent needs to rest for at least 5 min in a quiet state, and can be measured after the mood is stable. During the measurement, the respondent was exposed to his right arm, the arm was placed flat on the table and heart, and the feet were placed flat on the ground to relax. Select the appropriate cuff, record the systolic blood pressure and diastolic blood pressure in the first and fifth tone of KorotKoff, measuring three times, at least one minute between each two times, and averaging the three readings.

Laboratory examination

Fasting blood was drawn and sent to the clinical laboratory, Timely centrifugation, Isolate the serum, Hitachi 7180 was used to determine fasting blood glucose, total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), albumin; separated plasma, A SYSMEX XE-2100 hematology for hemoglobin, lymphocyte count, *etc*. Glycated hemoglobin was determined by Alometric.

Quality control

Before the formal investigation, the pre-investigation should be conducted in a certain group of people to find out the problems and deficiencies in time, adjust the design of unreasonable items, and improve the content of the questionnaire. All the investigators have received systematic and unified professional training, and can participate in the project research only after passing the examination. Inform the research subjects before the investigation, and sign the informed consent form. Investigators in strict accordance with the requirements of the training inquiry and physical examination, not induced questions, truthfully fill in the questionnaire, to ensure the authenticity and reliability of the data, and in the survey by personnel audit questionnaire, unified number and corresponding records, the unqualified questionnaire check or remove as far as possible. Data entry was conducted by uniformly trained researchers. The double data entry method was adopted, and the data was checked after entry. When the two questionnaires were inconsistent, the original data were checked, and errors were found and re-entered.

Statistical analysis

Data were entered and statistically analyzed using SPSS 20.0 software. If the quantitative data conforms to the normal distribution, the mean standard deviation; the non-normal data adopts the median and the interquartile spacing; the qualitative data is represented by the frequency (percentage). For quantitative data satisfying the normal distribution, non-parametric test for non-parametric data; and test for comparison between groups. Univariate analysis screened the potential risk factors for concurrent PTB in DM patients, multivariate conditional Logistic regression analysis (fitting by Cox model in survival analysis) screened the independent influencing factors of concurrent PTB, using the stepwise method, and 0.05 and 0.10 were used as the significance level of introduced and excluded variables, respectively. The strength of the association of the various influencing factors with PTB was measured by the odds ratio (OR) and its 95%CI. Before the analysis, all the variables were checked for collinearity. The test level shall be taken as $\alpha = 0.05$.

RESULTS

General demographic characteristics

A total of 105 eligible T2DM-PTB patients and 210 patients with T2DM alone were collected during the study period. The age range of the observation group was 26-86 years, and the mean age was 55.3 ± 11.5 years; the control group was 26-83 years, 55.7 ± 11.4 years, gender, marital status, educational level, occupational and worker's insurance ($P > 0.05$), as shown in Table 1.

DM-related characteristics

The mean duration of DM in the observation group was 6.8 ± 5.7 years, 7.4 ± 5.6 years, with no statistical difference ($P > 0.05$); the proportion of history of hypertension was lower than the control group ($P < 0.05$), but no significant difference in family history of DM ($P > 0.05$); the incidence of poor glucose control in the observation group was 77.9%, significantly higher than 56.5% ($P < 0.001$). In both groups, drugs were the main way to control blood glucose, accounting for 45.7% and 43.8%, respectively, but the proportion of the observation group using oral medicine and insulin was significantly lower than that of the control group (10.5% *vs* 24.8%, $P < 0.05$). There were significant differences between the two groups, and the proportion was 43.8%, while the control group was 43.3%, statistically different ($P < 0.05$); there was no significant difference in the family history of DM and regular blood glucose monitoring ($P > 0.05$; Table 2).

Test results of laboratory indicators

Systolic BP, diastolic blood pressure, body weight, body mass index (BMI), and lymphocyte count were lower than the control group, while fasting glucose and HDL were higher than the control group ($P < 0.05$). Serum cholesterol and triglyceride levels may differ between the two groups ($P = 0.052$), but not in height, glycated hemoglobin, hemoglobin, albumin and LDL ($P > 0.05$; Table 3).

Table 1 General demographic characteristics of the observed and control groups, *n* (%)

Characteristics	Observation group (*n* = 105)	Control group (*n* = 210)	χ^2/t	*P* value
Age (yr)	55.3 ± 11.5	55.7 ± 11.4	-0.254	0.800
Gender			0.009	0.926
Male	80 (76.2)	159 (75.7)		
Female	35 (23.4)	51 (24.3)		
marital status			7.307	0.007
Unwed/divorced/widowed	14 (13.3)	27 (12.9)		
Married	91 (86.7)	183 (87.1)		
Educational level			0.335	0.163
Illiteracy	13 (12.4)	32 (15.2)		
Primary school	18 (17.1)	32 (15.2)		
Middle school	62 (59.1)	126 (60.0)		
College degree or above	12 (11.4)	20 (9.5)		
Occupation			6.491	0.261
Housework and unemployed	34 (32.4)	65 (30.9)		
Peasant	22 (21.0)	65 (30.9)		
Office worker	13 (12.4)	27 (12.9)		
Self-employed worker	12 (11.4)	23 (11.0)		
Worker	14 (13.3)	21 (10.0)		
Other	10 (9.5)	9 (4.3)		
Worker's insurance			2.293	0.130
Yes	45 (42.9)	109 (51.9)		
No	60 (57.1)	101 (48.1)		

Nutritional status

In terms of nutritional status, clear differences between the rates of overweight, obesity, hypoproteinemia and lymphopenia, as detailed in Table 4. The incidence of overweight and obesity was 29.5% and 8.6%, respectively, significantly lower than the control group 47.0% and 17.5%, and the incidence of hypoproteinemia and lymphopenia was 24.2% and 74.2%, respectively, both higher than 11.4% and 45.1% in the control group, while neither group was statistically different between the incidence of hyperlipidemia and anemia, shown in Table 4.

Immunological status of the body

In the case of the observation group, upper respiratory tract infection was 69.5% in the past year, which was significantly higher than 47.1% of the control group. Both the observation and control groups were lower in the incidence of bronchitis or pneumonia, gastrointestinal infection, and skin infection, and there was no significant difference between the two groups. There were significant differences between high infection in the two groups, with the proportion of high infection in 70.5% in the observation group and 54.3% in the control group. The difference was statistically significant ($P < 0.05$). However, there was no statistical difference in the incidence of herpes between the two groups, as detailed in Table 5. The rates of pharyngeal, tonsillectomy, nasal polypectomy, and appendectomy were low in both groups (< 2%, not listed).

Lifestyle and behavior habits

The comparison of lifestyle and behavioral habits between the two groups are shown in Table 6. The proportion of participants with a history of TB contact was significantly higher than the control group (21.0% *vs* 6.7%), while the kitchen was less well-ventilated than the control group (61.9% *vs* 81.2%). More than 70% of the patients in both groups were able to do indoor ventilation regularly, but the proportion of people in the observation group without ventilation habits was higher than that in the control group. There were no statistical differences in dust contact history, migrant work history, contact situation and per capita living area. A comparison of the distribution of smoking between the two groups found that the proportion of current smokers in the observation group was the highest, 49.5%, while the proportion of non-smokers in the control group was 50.0%. The proportion of current smokers in the observation group was significantly higher than that in the control group ($P < 0.05$).39% and 18% of the observation and control groups were current drinkers, and 2.9% and 10.0% were previous drinkers. The proportion of patients in the observation group was 40%, significantly

Table 2 Comparison between the observed and control groups in diabetes-related characteristics, *n* (%)

Characteristics	Observation group (*n* = 105)	Control group (*n* = 210)	χ^2/t	*P* value
Disease course (yr)	6.8 ± 5.7	7.4 ± 5.6	-0.863	0.389
Family history of diabetes			1.585	0.208
Yes	31 (29.5)	77 (36.7)		
No	74 (71.5)	133 (63.3)		
History of hypertension			4.123	0.042
Yes	34 (32.4)	93 (44.3)		
No	71 (67.6)	117 (55.7)		
Poor glycemic control			12.281	< 0.001
Yes	74 (77.9)	100 (56.5)		
No	31 (22.1)	110 (43.5)		
Regular blood glucose monitoring			0.135	0.713
Yes	25 (23.8)	54 (25.7)		
No	80 (76.2)	156 (74.3)		
Diabetes treatment modality			10.285	0.016
Unregular treatment	24 (22.9)	36 (17.1)		
Oral hypoglycaemic agent	48 (45.7)	92 (43.8)		
Insulin	22 (20.9)	30 (14.3)		
Oral antidiabetic drugs + insulin	11 (10.5)	52 (24.8)		
Diet control attitude			7.141	0.028
Think it's very important	29 (27.6)	53 (25.3)		
Think it is generally important	30 (28.6)	91 (43.3)		
Think it's not important	46 (43.8)	66 (31.4)		

lower than 61% in the control group. According to the sleep status analysis, the proportion of people with difficulty falling asleep and habitual snoring was higher than that of the control group, while the drug sleep assistance and sleep duration was not significantly different between the two groups.

Univariate analysis of the risk of patients with T2DM-PTB

Using univariate condition logistic, regression analysis, 12 factors at α = 0.05, including marital status, history of hypertension, poor blood glucose control, DM treatment, BMI grouping, hypoproteinemia, lymphopenia, TB exposure, high infection, smoking, drinking, and regular exercise. The relative hazards of each contributing factor are shown in Table 7. The analysis found that poor glycemic control, hypoproteinemia, lymphopenia, history of TB exposure, high infection, smoking, and alcohol consumption were the risk factors for T2DM-PTB. In this study, non-smokers, former smokers, and passive smokers were combined as current non-smokers, With it as a reference group, univariate logistic regression analysis found that T2DM patients had more than five times higher risk of smoking PTB than non-smokers (OR = 5.12, 95%CI: 2.61-10.7; $P < 0.001$); Combining non-drinkers and previous drinkers as current non-drinkers, With it as a reference group, The univariate results showed that the risk of PTB in T2DM patients was 3.39 times higher than that in current non-drinkers (OR = 3.39, 95%CI: 1.88-6.12; $P < 0.001$). Regarding nutritional status, hypoproteinemia (OR = 2.48, 95%CI: 1.21-5.09) and lymphopenia (OR = 3.91, 95%CI: 2.12-7.21) were both risk factors for T2DM-PTB; while overweight (OR = 0.38, 95%CI: 0.22-0.66; $P = 0.001$) and obesity (OR = 0.32, 95%CI: 0.14-0.70; $P = 0.005$) than T2DM with normal weight, and patients had a lower risk of concurrent PTB. Unmarried (OR = 0.29, 95%CI: 0.12-0.73), history of hypertension (OR = 0.59, 95%CI: 0.36-0.98), oral medication and insulin (OR = 0.31, 95%CI: 0.13-0.73), and regular exercise (OR = 0.34, 95%CI: 0.20-0.58) were protective factors for T2DM-PTB.

Multivariate logistic regression analysis of the risk of patients with T2DM-PTB

Before the multivariate condition logistic regression analysis, the univariate analysis of OR is significant, marital status, high blood pressure, poor blood glucose control, DM treatment, BMI group, low proteinemia, lymphopenia, TB exposure history high infection, smoking, drinking, regular exercise 12 factors for collinear diagnosis. The results show that the tolerance is between 0.746 and 0.924, and the variance expansion factors are less than 10, and there is no collinearity problem. The possible risk factors selected by the results of univariate analysis were used as independent variables, and

Table 3 Test results of observation and control groups

Items	Observation group (*n* = 105)	Control group (*n* = 210)	χ^2/t	*P* value
Height (cm)	169.52 ± 8.81	168.68 ± 7.54	0.881	0.379
Weight (kg)	66.53 ± 13.62	71.75 ± 12.08	-3.249	0.001
Body mass index (kg/m^2)	23.00 ± 3.30	25.18 ± 3.40	-5.377	< 0.001
Systolic blood pressure (mmHg)	129.00 ± 20.00	134.00 ± 24.00	-2.544	0.011
Diastolic blood pressure (mmHg)	80.00 ± 10.50	83.00 ± 14.00	-2.504	0.012
Hemoglobin A1c (%)	8.85 ± 2.15	8.90 ± 2.80	-0.873	0.382
Fasting blood glucose (mmol/L)	10.54 ± 3.43	7.83 ± 2.81	-4.517	< 0.001
Hemoglobin (g/L)	136.50 ± 27.00	138.00 ± 21.00	-0.295	0.768
Albumin (g/L)	40.20 ± 8.40	39.68 ± 5.80	-0.247	0.805
Lymphocyte count (10^9/L)	1.65 ± 0.78	2.08 ± 0.94	-4.168	< 0.001
Triglyceride (mmol/L)	1.28 ± 0.31	1.47 ± 0.36	-1.940	0.052
Cholesterol (mmol/L)	4.68 ± 1.13	4.39 ± 1.62	-1.870	0.061
High-density lipoprotein (mmol/L)	1.20 ± 0.47	1.10 ± 0.39	-2.032	0.042
Low-density lipoprotein (mmol/L)	2.85 ± 1.08	2.70 ± 1.30	-0.713	0.476

Table 4 Comparison of nutritional status between the observed and control groups, *n* (%)

Items	Observation group (*n* = 105)	Control group (*n* = 210)	χ^2/t	*P* value
BMI divide into groups			19.702	< 0.001
Normal	65 (61.9)	71 (35.5)		
Overload	31 (29.5)	94 (47.0)		
Fat	9 (8.6)	35 (17.5)		
Anemia	18 (18.4)	28 (15.2)	0.465	0.495
Hypoproteinemia	24 (24.2)	20 (11.4)	7.702	0.006
Lymphocytopenia	72 (74.2)	83 (45.1)	21.773	< 0.001
Hyperlipidemic and hyperlipidemia	46 (50.0)	98 (58.7)	1.812	0.178
Simple hypertriglyceridemia	13 (14.1)	38 (22.8)	2.790	0.095
Simple hypercholesterolemia	8 (8.7)	9 (5.4)	1.057	0.304
Combined hyperlipidemia familial	3 (3.3)	7 (4.2)	0.138	0.710
Simple low HDL cholesterolemia	22 (24.7)	44 (26.7)	0.114	0.736

BMI: Body mass index; HDL: High-density lipoprotein.

the conditional logistic regression analysis was performed with PTB as the dependent variable (the control group was 0), and 0.05 and 0.10 as the significance level of the introduced and excluded variables. The assignment method for each variable is shown in Table 8.

The statistically significant 12 factors were analyzed by multivariate Logistic regression, which showed that five factors entered the model, as detailed in Table 9. Among them, lymphopenia, poor glycemic control, history of TB exposure and smoking were risk factors for T2DM-PTB, while overweight and obesity were protective factors. Patients with T2DM-PTB with OR lymphopenia (17.75, P = 0.001) and smoking (OR = 12.25, P = 0.002) had more than ten times the risk of TB. Being overweight (OR = 0.23, P = 0.011) and obesity (OR = 0.11, P = 0.021) reduced the risk of PTB in T2DM by 77% and 89%, respectively.

Table 5 Comparison of body immune status between the observed and control groups, *n* (%)

Items	Observation group (*n* = 105)	Control group (*n* = 210)	χ^2/t	*P* value
Upper respiratory tract infection	73 (69.5)	99 (47.1)	14.145	< 0.001
Bronchitis or pneumonia	5 (4.8)	12 (5.7)	0.124	0.724
Gastrointestinal infection	9 (8.6)	20 (9.5)	0.076	0.783
Skin infection	3 (2.9)	9 (4.3)	0.390	0.532
High infection	74 (70.5)	114 (54.3)	7.626	0.006
Bleb	16 (15.2)	26 (12.4)	0.495	0.482

Table 6 Comparison of lifestyle and behavioral habits between the observed and control groups, *n* (%)

Items	Observation group (*n* = 105)	Control group (*n* = 210)	χ^2/t	*P* value
History of tuberculosis contact	22 (21.0)	14 (6.7)	14.113	< 0.001
History of dust contact	7 (6.7)	15 (7.1)	0.024	0.876
Smoke			29.017	< 0.001
Current smoker	52 (49.5)	48 (22.8)		
Non-smoker	33 (31.4)	105 (50.0)		
A former smoker	13 (12.4)	18 (8.6)		
Passive smoker	7 (6.7)	39 (18.6)		
Drink			18.924	< 0.001
Current drinker	41 (39.0)	38 (18.1)		
No drinkers	61 (58.1)	151 (71.9)		
A former drinker	3 (2.9)	21 (10.0)		
Whether you exercise regularly			16.625	< 0.001
Yes	42 (40.0)	128 (61.0)		
No	63 (60.0)	82 (39.0)		

DISCUSSION

This paper compared the characteristics of the two groups, and the results showed that there were obvious differences in marriage, education, low protein, *etc*. Our previous study found that the proportion of unmarried (unmarried, divorced, widowed) in T2DM-PTB patients was significantly higher. Part of this is that a person has a great relationship between their physical and mental health. The ability to care for each other and support each other is very important for maintaining mental and physical stability between couples[14]. However, the older unmarried, divorced, widowed, often have loneliness, anxiety, anger, sadness and other bad psychological state. This bad mood and mood, it is likely to affect the body's metabolic function and immunity, thus making the body more susceptible to *M. tuberculosis* infection. Moreover, it is also possible that it is related to the poor family income of PTB patients, with some impact on their marriage.

T2DM-PTB is a common chronic wasting disease, patients often lead to nutritional deficiency, and then lead to body injury, and then lead to disease recurrence, affecting the prognosis[15,16]. The incidence of T2DM-PTB is as high as 45%-78.3%[17]. The occurrence of T2DM-PTB is associated with multiple causes[18]. T2DM has high blood sugar levels in the body, but due to the lack of insulin, the body cannot convert blood sugar into energy, and can only use protein, fat and other metabolites as energy sources, resulting in malnutrition[19]. T2DM is a serious risk of human health disease. In addition, because the body is in a state of consumption for a long time, PTB will also cause the body's catabolism abnormalities, thus reducing the body's protein and fat reserves, resulting in the body's malnutrition. The T2DM-PTB group had higher hypoproteinemia than the T2DM group. It is possible that protein deficiency reduces the cellular immune function of the body, which further improves the body's sensitivity to infection, leading to the infection aggravating[20]. Another reason may be that in the chronic process from *M. tuberculosis* infection to TB, the metabolism of the patient will accelerate and the production of interleukin-6 and tumor necrosis factor-α may lead to fever, liver synthesis of acute phase reaction protein and inhibit the production of serum albumin[21,22]. PTB is a chronic wasting disease that is prone to anemia. At the same time, *M. tuberculosis* proliferation in the patient's body tissue can cause a large amount of nutrients to be consumed, including hematopoietic substances, and eventually lead to anemia in the patient. About 16%-94% of PTB patients will develop anemia[23]. Our previous study found that there was no significant

Table 7 Results of the univariate conditional logistic regression analysis

Variable	Odds ratio	95%CI	*P* value
Married	0.29	0.12-0.73	0.009
Poor glycemic control	2.36	1.32-4.22	0.004
Diabetes treatment modality			
Unregular treatment	1.00	-	-
Oral hypoglycaemic agent	0.76	0.41-1.44	0.401
Insulin	0.98	0.45-2.14	0.960
Oral antidiabetic drugs + insulin	0.31	0.13-0.73	0.007
History of hypertension	0.59	0.36-0.98	0.040
Body mass index divide into groups			
Normal	1.00	-	-
Overload	0.38	0.22-0.66	0.001
Fat	0.32	0.14-0.70	0.005
Hypoproteinemia	2.48	1.21-5.09	0.013
Lymphocytopenia	3.91	2.12-7.21	< 0.001
History of tuberculosis contact	4.49	1.97-10.23	< 0.001
High infection	1.95	1.21-3.15	0.006
Smoke	5.12	2.61-10.7	< 0.001
Drink	3.39	1.88-6.12	< 0.001
Regular exercise	0.34	0.20-0.58	< 0.001

Table 8 Assignment statement in the multivariate logistic regression analysis of the risk in type 2 diabetes mellitus with pulmonary tuberculosis patients

Items	Assignment method
Marital status	Unmarried, divorced and widowed = 1, married = 2
Hypertension	No = 0, Yes = 1
Poor glycemic control	No = 0, Yes = 1
Diabetes treatment methods	Oral medication = 1, insulin = 2, oral medication and insulin simultaneously = 3, no regular treatment = 4
BMI grouping	BMI < 24 = 1, BMI: 24.0-27.9 = 2, BMI ≥ 28 = 3
Hypoproteinemia	No = 0, Yes = 1
Lymphocytopenia	No = 0, Yes = 1
History of tuberculosis contact	No = 0, Yes = 1
High infection	No = 0, Yes = 1
Smoke	No = 0, Yes = 1
Drink	No = 0, Yes = 1
Regular exercise	No = 0, Yes =1

BMI: Body mass index.

relationship between T2DM-PTB and the incidence of diabetic TB, and the incidence of T2DM-PTB will gradually increase with the development of the disease.

Our multifactorial study found that in patients with T2MD, having lymphopenia, smoking, a history of TB, and failure to control their blood sugar increase the risk of TB. In the execution of cellular immunity, lymphocytes are the most important effector cells, which can not only reflect the immune status of the human body, but also be used as a new index

Table 9 Multivariate conditional logistic regression analysis of risk factors for type 2 diabetes mellitus with pulmonary tuberculosis

Variable	β	SE	Wald	*P* value	Odds ratio (95%CI)
BMI divide into groups					
Normal	-	-	-	-	1.00
Overload	-1.457	0.573	6.475	0.011	0.23 (0.08-0.72)
Fat	-2.182	0.943	5.357	0.021	0.11 (0.02-0.72)
Poor glycemic control	1.215	0.568	4.581	0.032	3.37 (1.11-10.25)
Lymphocytopenia	2.876	0.844	11.622	0.001	17.75 (3.40-92.74)
History of tuberculosis contact	1.882	0.854	4.849	0.028	6.56 (1.23-35.03)
Smoke	2.506	0.805	9.686	0.002	12.25 (2.53-59.37)

BMI: Body mass index.

to evaluate the protein reserve of the human body internal organs. By measurement, it can also indirectly evaluate the nutritional status of the human body. The previous study of our group found that T2DM was accompanied by lymphocyte decline, and the incidence of PTB was 10 times higher than that of T2DM. The decrease in lymphocyte number indicates a weakening of cellular immune function as an anti-tuberculosis immune mechanism, which leads to the development of TB[24]. After extensive research, it has been proved that balanced nutrition and proper body weight can ensure the normal metabolic activities and immune function of the body. Malnourished people often reduce the total number of T lymphocytes, the function of the decline, but also can make the mechanical barrier of the body is damaged, mucosal resistance decreased, resulting in immune dysfunction, which is easy to cause a variety of infection[25,26].

A large number of studies have shown that smoking can improve the risk of TB. Preliminary meta-analysis showed that the risk of TB in smokers is twice that of non-smokers[27]. The previous study of our research group found that the risk of TB in diabetic patients is 10 times that of non-smokers. This suggests that among diabetics, smoking causes a much greater risk of TB than the average patient. Smoking can damage airway epithelial cilia, inhibit lung phagocyment by macrophages, reduce the removal of lung, and increase the risk of lung infection. There are also reports that nicotine in cigarettes directly damage macrophages, killing *M. tuberculosis*[28]. Smoking for a long time leads to a decrease in the expression of surface proteins associated with antigen presentation in lung macrophages. After the pathogen enters the body, some of them cannot be presented to the immune system, leading to a decrease in the killing of pathogenic bacteria [29]. Our study showed that smoking and alcoholism is a risk factor for TB, while smoking and alcohol abuse is also a risk factor for TB in African population[30]. Smoking and passive smoking are closely linked to drinking, this is because drinking has a special social environment, drinking and smoking and passive smoking often coexist, thus improving the risk of infection. Previous studies have shown that the risk of active pulmonary TB with over 40 g of daily drinking significantly increases[31], and the univariate analysis of our research group also indicates that alcohol consumption is an important cause of T2DM-PTB. Some scholars believe that excessive drinking will cause TB, and alcohol will cause direct toxicity to the body's immune system, making the body more susceptible to TB[32,33]. Animal experiments have shown that chronic and acute alcohol intake can directly damage macrophages and cellular immunity, leading to the development of PTB[34].

BMI is a comprehensive index of long-term lack of energy. Previous studies[35,36] found that BMI (BMI < 18.5 kg/m^2) is closely related to TB incidence, but the proportion of people with BMI < 18.5 kg/m^2 is low (3.5%), and the statistical significance is unclear, so further expansion of the sample is needed.

Previous studies have shown that the risk of developing TB gradually increases with increasing age, and men are more likely to develop TB[37,38]. Since age is proportional to sex, its relationship with T2DM-PTB cannot be evaluated.

This project intends to use a 1:2 ratio case-control design to obtain more valuable data with a smaller sample size, especially in a small number of diabetic patients with active TB. Using conditional logistic regression analysis, the deficiency of increased required sample content due to stratification avoided previous univariate stratification analysis. In addition, this study uses a matching case-control study, which enables the matching factors to reach a balance between the case group and the control group. In the comparative analysis, the influence of these factors can be excluded, so it has a high accuracy in the estimation of the model. Combining a 1:2 ratio of case-control trials with conditional Logistic regression can improve the detection efficiency of clinical trials and ensure the quality of the trials with a smaller sample size.

However, this paper also has some shortcomings. Since the samples in this study were collected from hospitals, there are certain limitations in the selection of samples, so it is inevitable that selective bias will occur.

CONCLUSION

In conclusion, T2DM-PTB patients are prone to worse glycemic control, higher infection frequency, smoking, drinking and lack of exercise; lymphocytopenia, smoking, exposure to TB history, and poor glycemic control are independent risk

factors for T2DM-PTB, overweight and obesity, T2DM, and decreased risk of concurrent PTB.

ARTICLE HIGHLIGHTS

Research background

The characteristics of patients with type 2 diabetes (T2DM) were clarified, and the risk factors of active tuberculosis (TB) in T2DM were explored to provide scientific basis for the prevention and control of the disease.

Research motivation

In China, T2DM accounts for 90%-95% of all diabetic patients, and China is one of the 22 countries with high TB burden, with about 4.5 million active TB patients; T2DM is one of the risks of developing TB, and the incidence of T2DM with TB (T2DM-PTB) increases from 19.3% to 24.1%. These two diseases are closely related and mutually reinforcing.

Research objectives

Clarify the characteristics of patients with T2DM complicated with TB, and explore the risk factors of active tuberculosis in T2DM patients, so as to provide a scientific basis for the prevention and control of diseases.

Research methods

T2DM-PTB patients in our hospital were selected as the observation group, and simple T2DM patients in our hospital at the same time were selected as the control group. The general demographic characteristics, diabetes-related characteristics, body immune status, lifestyle and behavioral habits were investigated, and the data were analyzed by conditional logistic regression.

Research results

The results found that the physical index, systolic blood pressure, diastolic blood pressure and lymphocyte count were significantly lower than the control group, while fasting blood glucose and high-density lipoprotein cholesterol levels were significantly higher than the control group, poor glucose control, hypoproteinemia, lymphopenia, TB exposure history, high infection, smoking, alcohol consumption were positively associated with PTB in T2DM; married, history of hypertension, treatment of oral hypoglycemic agents + insulin, overweight, obesity and regular exercise were negatively associated with concurrent PTB in patients with T2DM.

Research conclusions

Patients with T2DM-PTB are prone to worse glycemic control, higher infection frequency, and a higher proportion of people smoking, alcohol consumption, and lack of exercise. Lymphopenia, smoking, history of TB exposure, and poor glycemic control were independent risk factors for T2DM-PTB, and overweight and obesity were associated with a decreased risk of concurrent PTB in patients with T2DM.

Research perspectives

The empirical and comparative perspectives.

FOOTNOTES

Co-first authors: Han Shi and Yuan Yuan.

Author contributions: Shi H and Yuan Y designed the research; and Shi H and Yuan Y contributed equally to this work as co-first authors equally to this work; Li X, Yuan Y, Li YF, Fan L, Yang XM and Shi H contributed new reagents/analytic tools; Yuan Y, Li X, Li YF, Fan L, Yang YM and Shi H analyzed the data; Shi H and Yuan Y wrote the paper; and all authors were involved in the critical review of the results and have contributed to, read, and approved the final manuscript.

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REFERENCES

1 **Refardt J**. Diagnosis and differential diagnosis of diabetes insipidus: Update. *Best Pract Res Clin Endocrinol Metab* 2020; **34**: 101398 [PMID: 32387127 DOI: 10.1016/j.beem.2020.101398]
2 **Hua KF**, Zhang MY, Zhang Y, Ren BJ, Wu YH. Characteristics of OGTT and Correlation Between the Insulin to C-Peptide Molar Ratio, HOMA-IR, and Insulin Antibodies in T2DM Patients. *Diabetes Metab Syndr Obes* 2022; **15**: 2417-2425 [PMID: 35971523 DOI: 10.2147/DMSO.S373475]
3 **Natarajan A**, Beena PM, Devnikar AV, Mali S. A systemic review on tuberculosis. *Indian J Tuberc* 2020; **67**: 295-311 [PMID: 32825856 DOI: 10.1016/j.ijtb.2020.02.005]
4 **Fei S**, Feng X, Luo J, Guo L, Pan Q. Obesity and Coronavirus Disease 2019. *J Transl Int Med* 2022; **10**: 207-218 [PMID: 36776236 DOI: 10.2478/jtim-2022-0020]
5 **Cook JA**. Associations between use of crack cocaine and HIV-1 disease progression: research findings and implications for mother-to-infant transmission. *Life Sci* 2011; **88**: 931-939 [PMID: 21219914 DOI: 10.1016/j.lfs.2011.01.003]
6 **Ugarte-Gil C**, Curisinche M, Herrera-Flores E, Hernandez H, Rios J. Situation of the tuberculosis-diabetes comorbidity in adults in Peru: 2016-2018. *Rev Peru Med Exp Salud Publica* 2021; **38**: 254-260 [PMID: 34468572 DOI: 10.17843/rpmesp.2021.382.6764]
7 **Armstrong LR**, Kammerer JS, Haddad MB. Diabetes mellitus among adults with tuberculosis in the USA, 2010-2017. *BMJ Open Diabetes Res Care* 2020; **8** [PMID: 32641300 DOI: 10.1136/bmjdrc-2020-001275]
8 **Ru N**, Zou WB, Wu H, Hu LH, Li XB, Liu GF, Li ZS, Liao Z; Chronic Pancreatitis Group of Chinese Medical Doctor Association. Chinese guidelines for the diagnosis and treatment of pancreatic exocrine insufficiency (2018 edition). *J Dig Dis* 2019; **20**: 567-571 [PMID: 31006979 DOI: 10.1111/1751-2980.12753]
9 **Bureau of Disease Prevention and Control**, Ministry of Health, Department of Medical Administration, Chinese Center for Disease Control and Prevention. Technical Specifications for Tuberculosis Prevention and Control in China, 2020 edition. Beijing: Peking Union Medical College Press, 2020
10 **van 't Riet E**, Dekker JM, Sun Q, Nijpels G, Hu FB, van Dam RM. Role of adiposity and lifestyle in the relationship between family history of diabetes and 20-year incidence of type 2 diabetes in U.S. women. *Diabetes Care* 2010; **33**: 763-767 [PMID: 20067970 DOI: 10.2337/dc09-1586]
11 **Leung CC**, Lam TH, Chan WM, Yew WW, Ho KS, Leung GM, Law WS, Tam CM, Chan CK, Chang KC. Diabetic control and risk of tuberculosis: a cohort study. *Am J Epidemiol* 2008; **167**: 1486-1494 [PMID: 18400769 DOI: 10.1093/aje/kwn075]
12 **Wang L**, Peng W, Zhao Z, Zhang M, Shi Z, Song Z, Zhang X, Li C, Huang Z, Sun X, Wang L, Zhou M, Wu J, Wang Y. Prevalence and Treatment of Diabetes in China, 2013-2018. *JAMA* 2021; **326**: 2498-2506 [PMID: 34962526 DOI: 10.1001/jama.2021.22208]
13 **Hope AA**, Hsieh SJ, Petti A, Hurtado-Sbordoni M, Verghese J, Gong MN. Assessing the Usefulness and Validity of Frailty Markers in Critically Ill Adults. *Ann Am Thorac Soc* 2017; **14**: 952-959 [PMID: 28358584 DOI: 10.1513/AnnalsATS.201607-538OC]
14 **Gao J**, Lu Y, Gokulnath P, Vulugundam G, Li G, Li J, Xiao J. Benefits of Physical Activity on Cardiometabolic Diseases in Obese Children and Adolescents. *J Transl Int Med* 2022; **10**: 236-245 [PMID: 36776239 DOI: 10.2478/jtim-2022-0041]
15 **Traub J**, Reiss L, Aliwa B, Stadlbauer V. Malnutrition in Patients with Liver Cirrhosis. *Nutrients* 2021; **13** [PMID: 33562292 DOI: 10.3390/nu13020540]
16 **Taylor R**. Type 2 diabetes: etiology and reversibility. *Diabetes Care* 2013; **36**: 1047-1055 [PMID: 23520370 DOI: 10.2337/dc12-1805]
17 **Santoro A**, Kahn BB. Adipocyte Regulation of Insulin Sensitivity and the Risk of Type 2 Diabetes. *N Engl J Med* 2023; **388**: 2071-2085 [PMID: 37256977 DOI: 10.1056/NEJMra2216691]
18 **Kichloo A**, Shaka H, El-Amir Z, Wani F, Singh J, Velazquez GR, Edigin E, Dahiya D. In-patient outcomes of patients with diabetic ketoacidosis and concurrent protein energy malnutrition: A national database study from 2016 to 2017. *Postgrad Med* 2021; **133**: 854-859 [PMID: 33858299 DOI: 10.1080/00325481.2021.1916231]
19 **Fu L**, Ramos-Roman MA, Deng Y. Metabolic Adaptation in Lactation: Insulin-dependent and -independent Glycemic Control. *J Transl Int Med* 2022; **10**: 191-196 [PMID: 36776235 DOI: 10.2478/jtim-2022-0036]
20 **Pike J**, Chandra RK. Effect of vitamin and trace element supplementation on immune indices in healthy elderly. *Int J Vitam Nutr Res* 1995; **65**: 117-121 [PMID: 7591530]
21 **Hullalli R**, Gudadinni, Motappa R. WITHDRAWN: Prevalence of Diabetes Mellitus among Newly Detected Sputum Positive Pulmonary Tuberculosis Patients and Associated Risk Factors: A Cross-sectional Study. *Curr Diabetes Rev* 2023 [PMID: 37138479 DOI: 10.2174/1573399819666230501195227]
22 **Karyadi E**, Schultink W, Nelwan RH, Gross R, Amin Z, Dolmans WM, van der Meer JW, Hautvast JG, West CE. Poor micronutrient status of active pulmonary tuberculosis patients in Indonesia. *J Nutr* 2000; **130**: 2953-2958 [PMID: 11110853 DOI: 10.1093/jn/130.12.2953]
23 **Lee SW**, Kang YA, Yoon YS, Um SW, Lee SM, Yoo CG, Kim YW, Han SK, Shim YS, Yim JJ. The prevalence and evolution of anemia associated with tuberculosis. *J Korean Med Sci* 2006; **21**: 1028-1032 [PMID: 17179681 DOI: 10.3346/jkms.2006.21.6.1028]
24 **Pavan Kumar N**, Nair D, Banurekha VV, Dolla C, Kumaran P, Sridhar R, Babu S. Type 2 diabetes mellitus coincident with pulmonary or latent tuberculosis results in modulation of adipocytokines. *Cytokine* 2016; **79**: 74-81 [PMID: 26771473 DOI: 10.1016/j.cyto.2015.12.026]

25 **Black RE**, Morris SS, Bryce J. Where and why are 10 million children dying every year? *Lancet* 2003; **361**: 2226-2234 [PMID: 12842379 DOI: 10.1016/S0140-6736(03)13779-8]

26 **Nobs SP**, Zmora N, Elinav E. Nutrition Regulates Innate Immunity in Health and Disease. *Annu Rev Nutr* 2020; **40**: 189-219 [PMID: 32520640 DOI: 10.1146/annurev-nutr-120919-094440]

27 **Lin HH**, Ezzati M, Murray M. Tobacco smoke, indoor air pollution and tuberculosis: a systematic review and meta-analysis. *PLoS Med* 2007; **4**: e20 [PMID: 17227135 DOI: 10.1371/journal.pmed.0040020]

28 **Qiu F**, Liang CL, Liu H, Zeng YQ, Hou S, Huang S, Lai X, Dai Z. Impacts of cigarette smoking on immune responsiveness: Up and down or upside down? *Oncotarget* 2017; **8**: 268-284 [PMID: 27902485 DOI: 10.18632/oncotarget.13613]

29 **Bothamley GH**. Smoking and tuberculosis: a chance or causal association? *Thorax* 2005; **60**: 527-528 [PMID: 15994256 DOI: 10.1136/thx.2004.036012]

30 **Tekkel M**, Rahu M, Loit HM, Baburin A. Risk factors for pulmonary tuberculosis in Estonia. *Int J Tuberc Lung Dis* 2002; **6**: 887-894 [PMID: 12365575]

31 **Lönnroth K**, Williams BG, Stadlin S, Jaramillo E, Dye C. Alcohol use as a risk factor for tuberculosis - a systematic review. *BMC Public Health* 2008; **8**: 289 [PMID: 18702821 DOI: 10.1186/1471-2458-8-289]

32 **Szabo G**. Alcohol's contribution to compromised immunity. *Alcohol Health Res World* 1997; **21**: 30-41 [PMID: 15706761]

33 **Greenberg S**, Xie J, Kolls J, Nelson S, Didier P, Mason C. Ethanol suppresses Mycobacteria tuberculosis-induced mRNA for nitric oxide synthase in alveolar macrophages, in vivo. *Alcohol Clin Exp Res* 1995; **19**: 394-401 [PMID: 7542849 DOI: 10.1111/j.1530-0277.1995.tb01521.x]

34 **Mellencamp MA**. Effects of ethanol consumption on susceptibility to pulmonary and gastrointestinal factors. *Alcohol Clin Exp Res* 1996; **20**: 192A-195A [PMID: 8947263 DOI: 10.1111/j.1530-0277.1996.tb01774.x]

35 **Lönnroth K**, Williams BG, Cegielski P, Dye C. A consistent log-linear relationship between tuberculosis incidence and body mass index. *Int J Epidemiol* 2010; **39**: 149-155 [PMID: 19820104 DOI: 10.1093/ije/dyp308]

36 **Lönnroth K**, Jaramillo E, Williams BG, Dye C, Raviglione M. Drivers of tuberculosis epidemics: the role of risk factors and social determinants. *Soc Sci Med* 2009; **68**: 2240-2246 [PMID: 19394122 DOI: 10.1016/j.socscimed.2009.03.041]

37 **Buskin SE**, Gale JL, Weiss NS, Nolan CM. Tuberculosis risk factors in adults in King County, Washington, 1988 through 1990. *Am J Public Health* 1994; **84**: 1750-1756 [PMID: 7977912 DOI: 10.2105/ajph.84.11.1750]

38 **Hussain H**, Akhtar S, Nanan D. Prevalence of and risk factors associated with Mycobacterium tuberculosis infection in prisoners, North West Frontier Province, Pakistan. *Int J Epidemiol* 2003; **32**: 794-799 [PMID: 14559752 DOI: 10.1093/ije/dyg247]

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ORIGINAL ARTICLE

Retrospective Study

Vitamin D, selenium, and antidiabetic drugs in the treatment of type 2 diabetes mellitus with Hashimoto's thyroiditis

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Abstract

BACKGROUND

Diabetes and thyroiditis are closely related. They occur in combination and cause significant damage to the body. There is no clear treatment for type-2 diabetes mellitus (T2DM) with Hashimoto's thyroiditis (HT). While single symptomatic drug treatment of the two diseases is less effective, combined drug treatment may improve efficacy.

AIM

To investigate the effect of a combination of vitamin D, selenium, and hypoglycemic agents in T2DM with HT.

METHODS

This retrospective study included 150 patients with T2DM and HT treated at The Central Hospital of Shaoyang from March 2020 to February 2023. Fifty patients were assigned to the control group, test group A, and test group B according to different treatment methods. The control group received low-iodine diet guidance and hypoglycemic drug treatment. Test group A received the control treatment plus vitamin D treatment. Test group B received the group A treatment plus selenium. Blood levels of markers of thyroid function [free T3 (FT3), thyroid stimulating hormone (TSH), free T4 (FT4)], autoantibodies [thyroid peroxidase antibody (TPOAB) and thyroid globulin antibody (TGAB)], blood lipid index [low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), triacylglycerol (TG)], blood glucose index [fasting blood glucose (FBG), and hemoglobin A1c (HbA1c)] were measured pre-treatment and 3 and 6 months after treatment. The relationships between serum 25-hydroxyvitamin D3 [25 (OH) D3] level and each of these indices were analyzed.

RESULTS
The levels of 25 (OH) D_3, FT3, FT4, and LDL-C increased in the order of the control group, test group A, and test group B (all $P < 0.05$). The TPOAB, TGAB, TC, TG, FBG, HbA1c, and TSH levels increased in the order of test groups B, A, and the control group (all $P < 0.05$). All the above indices were compared after 3 and 6 months of treatment. Pre-treatment, there was no divergence in serum 25 (OH) D_3 level, thyroid function-related indexes, autoantibodies level, blood glucose, and blood lipid index between the control group, test groups A and B (all $P > 0.05$). The 25 (OH) D_3 levels in test groups A and B were negatively correlated with FT4 and TGAB (all $P < 0.05$).

CONCLUSION
The combination drug treatment for T2DM with HT significantly improved thyroid function, autoantibody, and blood glucose and lipid levels.

Key Words: Type-2 diabetes mellitus; Hashimoto's thyroiditis; Vitamin D; Selenium agent; Hypoglycemic drugs; Curative effect

Core Tip: Selenium yeast and active vitamin D can reduce thyroid-related antibodies in type-2 diabetes mellitus (T2DM) and Hashimoto's thyroiditis (HT) and improve thyroid function. Hypoglycemia drugs can lower blood sugar levels in patients and promote blood sugar stability. While most patients with T2DM and HT are currently treated with a single symptomatic drug, the effects are unsatisfactory. In this study, the combination of vitamin D and selenium yeast added to hypoglycemic agents to treat T2DM patients with HT showed a remarkable therapeutic effect.

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INTRODUCTION

Diabetes is a chronic metabolic disease characterized by chronic hyperglycemia caused by a relative lack of insulin in the body. Its incidence increases annually, and approximately 90% of cases involve type-2 diabetes mellitus (T2DM)[1]. Thyroid disease is mainly characterized by dysfunction in thyroid hormone secretion. Hashimoto's thyroiditis (HT) is a typical autoimmune disease that has also shown an increasing incidence in recent years[2]. The main manifestations of HT are elevated levels of thyroid autoantibodies and goiter, which often lead to hypothyroidism with disease progression. Diabetes and thyroiditis are closely related and often occur in combination. Foreign reports show that thyroid dysfunction has a higher prevalence in the diabetic population, at 12.5%-51.6%, which is two to three times that of other populations[3,4]. The study found that the incidence of HT in T2DM patients was significantly higher than in the general population[5]. The pathogenesis of T2DM and HT is believed to mainly involve insulin resistance, immune factors, infection, oxidative stress, genetics, leptin, molecular cytology, and other related factors; however, there is no clear consensus on the pathogenesis of T2DM with HT. Western medicine generally adopts symptomatic treatments for these two diseases, including hypoglycemic medications, improved thyroid function, and treatment of complications.

Iodine, selenium, and vitamin D are essential for thyroid hormone production in the human body. Deficiencies can cause changes in thyroid structure and function[6]. HT is often accompanied by vitamin D deficiency. In foreign literature, vitamin D deficiency in patients with HT was as high as 60.6% and was even lower in female patients[7]. Vitamin D levels are negatively correlated with thyroid-stimulating hormone levels. Patients with HT with insufficient or deficient vitamin D levels are more likely to have subclinical and clinical hypothyroidism than HT patients with normal vitamin D levels[8]. However, some studies have conflicting results regarding the effect of vitamin D on the incidence of HT[9,10]. Related literature reports that the occurrence of T2DM is relevant to changes in serum 25-hydroxyvitamin D3 [25 (OH) D3] levels. Supplementation with vitamin D increases serum 25 (OH) D_3 levels[11]. Selenium supplementation can upregulate activated regulatory T cells' horizons and partially reduce thyroid autoantibodies' horizons[12,13]. Yu *et al* [14] explored the effect of the combined treatment of thyroxine and selenium on HT, and the results suggested that the combination of the two drugs was significantly better than thyroxine alone in preventing HT progression. However, few studies have reported the efficacy of combined treatments with vitamin D, selenium yeast, and hypoglycemic drugs in patients with T2DM and HT. This study explored the therapeutic effects of vitamin D, selenium, and vitamin D combined with selenium in patients with T2DM and HT. The study further examined 25 (OH) D_3 indicators associated with combined T2DM and HT.

MATERIALS AND METHODS

Object

This retrospective study included 150 patients with T2DM and HT treated at The Central Hospital of Shaoyang between March 2020 and February 2023. According to the different treatment methods, the patients were split into test groups A and B and a control group, with 50 cases per group. The inclusion criteria were: Meeting the diagnostic criteria for T2DM [15] and in a stable condition; combined with HT and meeting the HT diagnostic criteria[16]: (1) Swollen and tough thyroid isthmus, (2) positivity for serum thyroid globulin antibody (TGAB) and thyroid peroxidase antibody (TPOAB); (3) thyroid ultrasound showing diffuse enlargement and hypoechoic thyroid gland; (4) thyroid fine needle puncture findings consistent with cytological changes of thyroiditis; and (5) thyroid function showing normal range of free T4 (FT4) and thyroid stimulating hormone (TSH) levels (< 10 Uiu/L), which was not treated after the initial diagnosis. Among them, (1), (2), (3) and (5) are necessary. If the case is atypical, (4) is required for diagnosis. No neurological diseases at study completion. The exclusion criteria were: (1) Type-1 diabetes mellitus; (2) severe infectious diseases and other autoimmune diseases; (3) heart, liver, kidney, and other serious diseases or malignant tumors; (4) pregnancy; (5) use of immunosuppressants, immune checkpoint inhibitors, or glucocorticoid drugs and a recent history of drugs affecting thyroid function; (6) history of thyroid trauma or surgical treatment combined with parathyroid dysfunction; and (7) chronic inflammation caused by other factors.

The control group received low-iodine diet guidance and hypoglycemic drug treatment. That is, saxagliptin tablets (Bristol-Myers Squibb Company, national drug approval number J20110029) were administered orally once daily (5 mg daily).

Test group A was administered oral vitamin D (Qingdao Double Whale Pharmaceutical Co., LTD., Sinopod H20113033, 4000 u/d) + hypoglycemic drug treatment in addition to the control group treatment[11].

Test group B was administered vitamin D + selenium yeast + hypoglycemic drug treatment. That is, based on test group A, oral selenium yeast (Mudanjiang Lingtai Pharmaceutical Co., LTD., Sinomedmedicine approval number: H10940161, 100 μg/time, 2 times/day). Treatment was discontinued in cases of adverse reactions, including cardiopulmonary events, allergies, or elevated blood calcium levels. All patients in each group were treated for 6 months [11,14].

Observation index

(1) General information: Sex, age, and body mass index (BMI) were collected and recorded; (2) laboratory indicators: After 8 h of overnight fasting, the subjects were sent to the central laboratory for a venous blood sample the following morning. The samples were immediately stored at 4 °C. An automatic chemiluminescence analyzer (I2000SR, Abbott, United States) was used to detect serum 25 (OH) D_3, thyroid function [TSH, free T3 (FT3), FT4], autoantibody (TGAB, TPOAB); automatic biochemical apparatus (Beckman Coulter, AU5800 model) determination of blood lipid index [low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), triacylglycerol (TG)], blood glucose index [fasting blood glucose (FBG), hemoglobin A1c (HbA1c)]. These indicators were measured in all patients pre-treatment and after 3 and 6 months of treatment; and (3) the correlations between serum 25 (OH) D_3 levels and each index in test groups A and B were analyzed.

Statistical analysis

IBM SPSS Statistics for Windows, version 26.0, was used to analyze the project data. Counting variables are expressed as n, (%) and compared by χ^2 test. Continuous variables are reported as mean ± SD. One-way analysis of variance (ANOVA) was used to compare the three groups. If differences were observed, a pound-for-pair comparison was performed. Pearson's correlation analysis was used to analyze the relationships between serum 25 (OH) D_3 levels and each index. The test level of statistical analysis was $\alpha = 0.05$.

RESULTS

Comparison of general data among the three groups of patients

Comparisons of general data, such as sex, age, and BMI among the three groups ($P > 0.05$), are shown in Table 1.

Changes in serum 25 (OH) D_3 levels in the three groups before and after 3 and 6 months of treatment

Pre-treatment, the 25 (OH) D3 levels in the control group and test groups A and B were 15.15 ± 3.64, 15.62 ± 3.75, and 14.85 ± 4.17 mg/L, respectively ($P > 0.05$ for the comparison between the three groups). After 3 months of treatment, the 25 (OH) D3 levels in test groups A and B were 19.24 ± 4.14 and 22.88 ± 4.60 mg/L, respectively, which were higher than that in the control group (16.18 ± 3.09 mg/L). Compared with test group A, the levels in test group B were higher ($P < 0.05$). After 6 months of treatment, the levels of 25 (OH) D3 in test groups A and B were 24.87 ± 4.75 and 29.31 ± 5.17 mg/L, respectively, both of which were higher than that of the control group (16.19 ± 3.14 mg/L). Compared with that in test group A, the level in test group B was higher ($P < 0.05$) (Figure 1).

Changes in thyroid function in the three groups before and after 3 and 6 months of treatment

After 3 and 6 months of treatment, The TSH levels of test groups A and B were lower than those of the control group. Compared with those in test group A, the values in group B were lower ($P < 0.05$). The FT3 and FT4 levels in test groups A and B were higher than those in the control group; compared with those in test group A, the levels in group B were

Table 1 Comparison of general data among the three patient groups

Group	Sex (male/female)	Age (yr)	BMI (kg/m²)
Control group (n = 50)	23/27	53.78 ± 7.49	22.88 ± 2.31
Test group A (n = 50)	26/24	52.76 ± 7.88	23.07 ± 2.17
Test group B (n = 50)	22/28	52.52 ± 8.13	22.98 ± 2.30
χ^2/F value	1.361	0.364	0.085
P value	0.715	0.695	0.918

BMI: Body mass index.

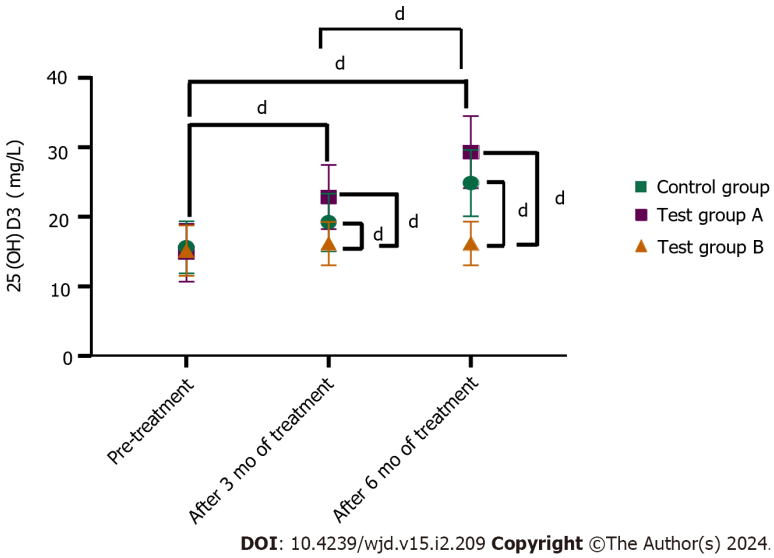

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Figure 1 Comparison of serum 25-hydroxyvitamin D3 levels in the three groups pre-treatment and 3 and 6 months after treatment. ^{d}P < 0.0001. 25 (OH) D_3: 25-hydroxyvitamin D_3.

higher (P < 0.05) (Table 2).

Changes in autoantibody levels in the three groups before and after 3 and 6 months of treatment

Pre-treatment, the TPOAB levels of the control group, test groups A and B were 365.23 ± 87.26, 364.74 ± 86.78, and 365.76 ± 85.99 pmol/L, respectively (P > 0.05 for the comparison between all three groups). After 3 months of treatment, the TPOAB levels in test groups A and B were 78.26 ± 48.23 and 270.34 ± 46.25 pmol/L, respectively, both of which were lower than that of the control group (347.26 ± 79.56 pmol/L). Compared with that in test group A, the level in group B was lower (P < 0.05). After 6 months of treatment, the TPOAB levels in test groups A and B were 233.15 ± 41.26 and 201.23 ± 38.17 pmol/L, respectively, both of which were lower than that of the control group (318.23 ± 74.23) pmol/L. Compared with that in test group A, the level in group B was lower (P < 0.05) (Figure 2A).

Pre-treatment, the TGAB levels in the control group, test group A, and test group B were 138.29 ± 16.43, 139.22 ± 16.47, and 138.56 ± 16.73 U/mL, respectively (P > 0.05 for the comparison of all three groups). After 3 months of treatment, the TGAB levels in test groups A and B were 119.34 ± 12.05 and 117.23 ± 11.34 U/mL, respectively, both of which were lower than that of the control group (124.56 ± 15.03) U/mL. Compared with that in test group A, the level in group B was lower (P < 0.05). After 6 months of treatment, the TGAB levels in test groups A and B were 93.15 ± 11.23 and 89.37 ± 10.42 U/mL, respectively, both of which were lower than that of the control group (123.64 ± 14.34) U/mL. It was lower in test group B than in group A (P < 0.05) (Figure 2B).

Changes in blood glucose index and blood lipid index in the three groups before and after 3 and 6 months of treatment

After 3 and 6 months of treatment, the TC, TG FBG, and HbA1c levels in test groups A and B were lower than those in the control group, while these levels were lower in test group B than in group A (all P < 0.05). The LDL-C levels in test groups A and B were higher than in the control group. Compared with the test group A, the levels of group B were higher (all P < 0.05) (Tables 3 and 4).

Table 2 Comparison of thyroid function pre-treatment and after 3 and 6 months of treatment in the three groups

Group	TSH (mU/L)			FT3 (pmol/L)			FT4 (pmol/L)		
	Pre-treatment	After 3 months of treatment	After 6 months of treatment	Pre-treatment	After 3 months of treatment	After 6 months of treatment	Pre-treatment	After 3 months of treatment	After 6 months of treatment
Control group (n = 50)	14.50 ± 2.30	13.29 ± 2.34	12.89 ± 2.18	2.39 ± 0.77	2.57 ± 0.83	2.65 ± 0.82	7.35 ± 1.35	8.27 ± 1.45	8.96 ± 2.05
Test group A (n = 50)	14.49 ± 2.23	8.37 ± 2.33[a]	4.98 ± 1.45[a]	2.43 ± 0.80	3.98 ± 0.86[a]	5.21 ± 1.26[a]	7.28 ± 1.38	11.27 ± 2.16[a]	15.51 ± 2.40[a]
Test group B (n = 50)	14.70 ± 2.34	8.54 ± 2.41[a,b]	4.05 ± 1.27[a,b]	2.48 ± 0.81	4.05 ± 0.90[a,b]	5.47 ± 1.34[a,b]	7.26 ± 1.26	11.87 ± 2.27[a,b]	16.91 ± 2.73[a,b]
F value	0.133	70.399	416.857	0.162	47.220	89.340	0.063	52.264	155.329
P value	0.875	< 0.001	< 0.001	0.850	< 0.001	< 0.001	0.939	< 0.001	< 0.001

[a]P < 0.05 *vs* group pre-treatment.
[b]P < 0.05 *vs* 3 months after treatment.
TSH: Thyroid-stimulating hormone; FT3: Free T3; FT4: Free T4

Table 3 Comparison of blood glucose index and blood lipid index pre-treatment, 3 months after treatment, and 6 months after treatment in 3 groups

Group	TC (mU/L)			TG (pmol/L)		
	Pre-treatment	After 3 months of treatment	After 6 months of treatment	Pre-treatment	After 3 months of treatment	After 6 months of treatment
Control group (n = 50)	5.13 ± 0.86	4.46 ± 0.81	4.16 ± 0.77	3.21 ± 1.02	2.97 ± 0.91	2.93 ± 0.91
Test group A (n = 50)	4.98 ± 0.89	3.76 ± 0.75[a]	2.76 ± 0.68[a]	3.17 ± 0.96	2.43 ± 0.71[a]	1.98 ± 0.65[a]
Test group B (n = 50)	4.96 ± 0.92	3.07 ± 0.68[a,b]	2.40 ± 0.69[a,b]	3.16 ± 1.05	2.08 ± 0.64[a,b]	1.83 ± 0.60[a,b]
F value	0.546	43.665	84.262	0.033	17.243	32.575
P value	0.581	< 0.001	< 0.001	0.967	< 0.001	< 0.001

[a]P < 0.05 *vs* group pre-treatment.
[b]P < 0.05 *vs* 3 months after treatment.
TC: Total cholesterol; TG: Triacylglycerol.

Correlations between serum 25 (OH) D_3 level and each index in test group A

Test group A of serum 25 (OH) D_3 levels and negatively correlated with FT4, TGAB level (P < 0.05). The other indices were not significantly correlated (P > 0.05) (Table 5).

Correlations between serum 25 (OH) D_3 level and each index in test group B

Serum 25 (OH) D_3 levels in test group B were negatively correlated with FT4 and TGAB levels (P < 0.05). The other indices were not significantly correlated (P > 0.05) (Table 6).

DISCUSSION

The onset of HT is insidious and difficult to detect. Its early clinical symptoms are not obvious. By the time the patient is diagnosed, there are already symptoms of hypothyroidism present. Early clinical symptoms are not obvious, and symptoms of hypothyroidism already exist when the condition is detected and diagnosed. The reduced secretion of thyroid hormones damages the physiological function and affects the normal life of patients[17,18]. Diabetes is a common endocrine disease in clinical settings. Diabetes combined with HT causes significant damage to the body. HT treatment

Table 4 Comparison of blood glucose index and blood lipid index pre-treatment, 3 months after treatment, and 6 months after treatment in 3 groups

Group	LDL-C (pmol/L)			FBG(pmol/L)			HbA1c(%)		
	Pre-treatment	After 3 months of treatment	After 6 months of treatment	Pre-treatment	After 3 months of treatment	After 6 months of treatment	Pre-treatment	After 3 months of treatment	After 6 months of treatment
Control group (n = 50)	1.31 ± 0.43	1.43 ± 0.44	1.48 ± 0.45	13.52 ± 3.35	13.46 ± 3.33	13.37 ± 3.28	9.16 ± 1.55	8.90 ± 1.35	7.65 ± 1.26
Test group A (n = 50)	1.34 ± 0.43	1.76 ± 0.46[a]	2.18 ± 0.50[a]	13.69 ± 3.76	12.34 ± 3.82[a]	12.98 ± 2.98[a]	8.98 ± 1.58	8.55 ± 1.30	7.09 ± 1.15[a,b]
Test group B (n = 50)	1.33 ± 0.40	1.87 ± 0.48[a]	2.41 ± 0.58[a,b]	13.60 ± 3.80	9.64 ± 1.45[a]	7.30 ± 1.48[a,b]	9.35 ± 1.52	8.20 ± 1.2[a]	6.45 ± 1.10[a,b]
F value	0.067	11.717	44.330	0.031	20.859	49.495	0.712	3.603	13.130
P value	0.935	< 0.001	< 0.001	0.970	< 0.001	< 0.001	0.492	0.030	< 0.001

[a]P < 0.05 *vs* group pre-treatment.
[b]P < 0.05 *vs* 3 months after treatment.
LDL-C: Low-density lipoprotein cholesterol; FBG: Fasting blood glucose; HbA1c: Hemoglobin A1c.

Table 5 Correlations between serum 25-hydroxyvitamin D3 level and various indexes in test group A

Index	25 (OH) D_3	
	r	*P* value
TSH	0.008	0.866
FT3	-0.027	0.853
FT4	-0.326	0.021
TPOAB	-0.017	0.905
TGAB	-0.322	0.021
TC	-0.041	0.776
TG	0.021	0.143
LDL-C	0.177	0.218
FBG	0.111	0.444
HbA1c	0.035	0.810

TSH: Thyroid-stimulating hormone; FT3: Free T3: FT4: Free T4; TPOAB: Thyroid peroxidase antibodies; TGAB: Thyroglobulin antibodies; TC: Total cholesterol; TG: Triacylglycerol; LDL-C: Low-density lipoprotein cholesterol; FBG: Fasting blood glucose; HbA1c: Hemoglobin a1c; 25 (OH) D_3: 25-hydroxyvitamin D3.

mainly involves selenium, glucocorticoids, and a limited intake of iodine. Diabetes treatment is primarily targeted at aspects related to its pathogenesis[19]. The effects of single symptomatic treatments for the combination of these two diseases are unsatisfactory. However, combined treatments can improve treatment efficacy and patients' quality of life.

A large number of studies have confirmed that HT is closely related to trace elements, such as iodine and selenium[20-23]. Selenium is mainly present in the human body as selenium protein that participates in the synthesis and metabolism of thyroid hormone and can also be used as an antioxidant to reduce inflammation in HT patients[24]. However, the influence of selenium on the occurrence and development of HT is still controversial. Early studies have shown that selenium is ineffective in treating HT, and other studies have shown that selenium supplementation cannot enhance the immune function of healthy people[25]. However, in recent years, more and more studies have found that selenium supplementation can reduce the serum autoantibody TPOAB level of HT patients, and other studies have found that selenium can not only reduce the serum TPOAB level of patients but also reduce the serum TGAB level of patients[13,26,27]. Selenium mainly regulates the natural immune response through methionine sulfoxide reductase, and low selenium status can increase the incidence of thyroid diseases[28]. Wu *et al*'s epidemiological study in China also confirmed that

Table 6 Correlation between serum 25-hydroxyvitamin D3 level and various indexes in test group B

Index	25 (OH) D_3	
	r	*P* value
TSH	-0.205	0.866
FT3	-0.069	0.633
FT4	-0.291	0.040
TPOAB	0.107	0.459
TGAB	-0.457	0.001
TC	0.003	0.985
TG	0.148	0.306
LDL-C	-0.025	0.861
FBG	0.079	0.587
HbA1c	0.230	0.108

TSH: Thyroid-stimulating hormone; FT3: Free T3: FT4: Free T4; TPOAB: Thyroid peroxidase antibodies; TGAB: Thyroglobulin antibodies; TC: Total cholesterol; TG: Triacylglycerol; LDL-C: Low-density lipoprotein-cholesterol; FBG: Fasting blood glucose; HbA1c: Hemoglobin a1c; 25 (OH) D_3: 25-hydroxyvitamin D3.

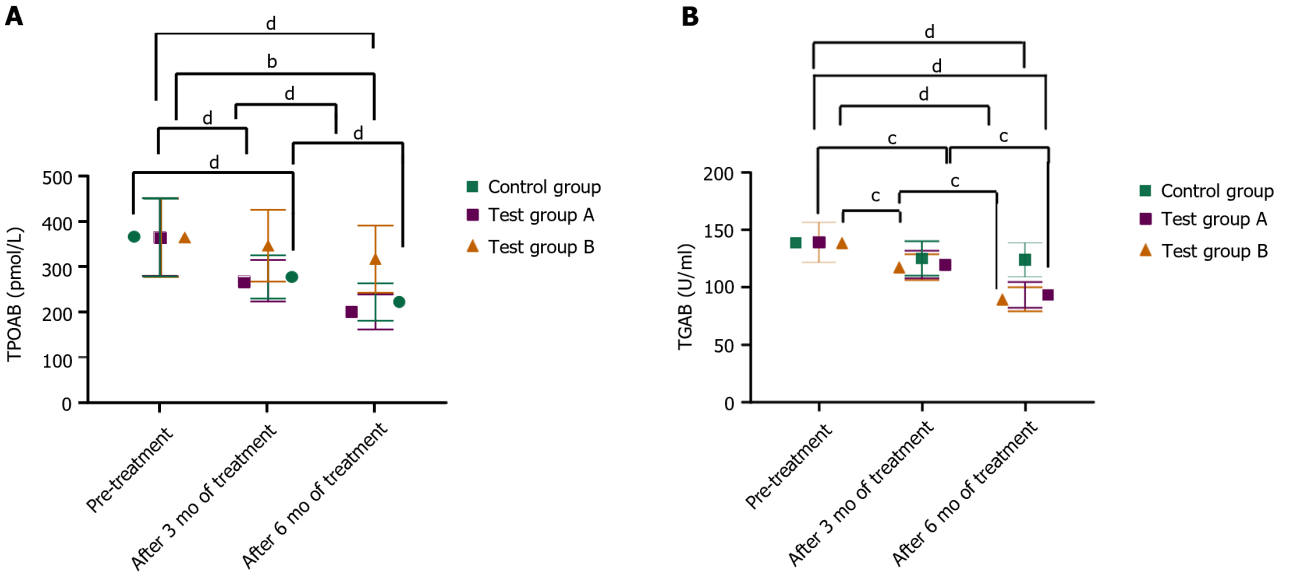

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Figure 2 Comparison of autoantibody levels in the three groups pre-treatment and 3 and 6 months after treatment. A: Changes in thyroid peroxidase antibody level; B: Changes in thyroid globulin antibody level. $^{b}P < 0.01$; $^{c}P < 0.001$; $^{d}P < 0.0001$. TPOAB: Thyroid peroxidase antibody; TGAB: Thyroid globulin antibody.

low selenium status was related to the increased risk of HT, and increasing the intake of trace element selenium could reduce the incidence of HT[29]. According to the available evidence, selenium supplementation appears to be associated with the downregulation of thyroid antibody titers and improvements in mood or general health[30].

However, whether there is a relationship between HT and vitamin D remains controversial. Recently, a review has shown that vitamin D deficiency is related to the pathophysiological process of HT, hypothyroidism, and thyroid autoimmunity to a certain extent[19]. A randomized controlled trial further confirmed the benefit of vitamin D supplementation in HT remission. 120 Newly diagnosed HT patients were randomly divided into two groups: Group 1 (intervention group) and group 2 (control group). Group 1 patients received 60000 IU of vitamin D3 per week and 500 mg of calcium tablets daily for 8 wk. Patients in group 2 were only supplemented with 500 mg calcium tablets daily for 8 wk, and the follow-up results after 3 months showed that compared with patients in group 2 (-16.6%), the TPOAB level in patients in group 1 was significantly decreased (-46.73%) ($P = 0.028$)[31]. In this study, after 3 and 6 months of treatment, the improvement of 25 (OH) D_3 level, thyroid function index level, and autoantibody in trial group A and trial group B were

better than those in the control group, and trial group B was better than trial group A ($P < 0.05$), indicating that the combined treatment of vitamin D, selenium and hypoglycemic drugs in T2DM patients with HT was more effective. It can be seen that supplementation of vitamin D and selenium yeast can increase the content of 25 (OH) D_3 in the body, improving thyroid function and the level of autoantibodies in patients.

In a 2010 study, Muscogiuri *et al*[32] found that patients with vitamin D < 20 ng/mL had a higher incidence of autoimmune thyroiditis than those with vitamin D > 20 ng/mL and found a linear correlation between vitamin D_3 and TPOAB. A large sample data by Choi *et al*[33] also showed that in the general population, the incidence of positive TPOAB was 10.1%, and in female patients, the level of vitamin D3 in TPOAB-positive people was lower than that in negative people. Studies have shown that polymorphisms of vitamin D receptors, such as BsmI and TaqI, play an important role in autoimmune thyroiditis[34]. Our study found that serum 25 (OH) D_3 in groups A and B before treatment was negatively correlated with FT4 and TGAB ($P < 0.05$). That is, the lower the level of vitamin D_3, the higher the risk of hypothyroidism. However, there are few studies on the relationship between 25 (OH) D_3 and thyroid function. Since the thyroid antibodies in our study mainly include TPOAB and TGAB and thyroid function TSH, FT3, and FT4, we cannot rule out whether there is a linear correlation between vitamin D_3 and other antibodies that cause hypothyroidism. More research on vitamin D_3 and thyroid function is needed.

Saxagliptin is a commonly used clinical drug in the treatment of T2DM. It mainly inhibits the physiological activity of the DPP-4 enzyme, promotes the improvement of glucagon-like peptide-1 level, fully stimulates islet cells, and rationally increases the release of long-acting insulin, thereby reducing the blood glucose level of patients and achieving the effect of promoting the stability of blood glucose level[35-37]. Wang *et al*[38] randomly divided 25 obese subjects with impaired fasting glucose or impaired glucose tolerance with an average age of 45 years into 4 groups: Life intervention group, saxagliptin 2.5 mg group, saxagliptin 5 mg group, metformin 1500 mg group. Relevant parameters were measured at baseline, 4 wk, 12 wk, and 24 wk. The final study showed that the saxagliptin 5 mg group reduced subjects' FBG and HbA1c and significantly reduced blood glucose levels 2 h after meals after 24 wk of intervention. As we all know, dyslipidemia in T2DM patients is mainly manifested by increased levels of TC, TG, and LDL-C and decreased levels of LDL-C. Angellotti *et al*[39] found that vitamin D supplementation could significantly reduce serum TG levels in patients who did not take cholesterol-lowering drugs. Combined with the results of this study, it was found that the three groups of patients were treated with saxagliptin, but after 3 and 6 months of treatment, the levels of blood glucose indexes and lipid indexes of test group A and B were better than those of the control group, and test group B was better than test group A ($P < 0.05$). These results indicate that the combination of vitamin D, selenium, and hypoglycemic agents has a more significant effect on T2DM patients with HT. The reason may be that selenium yeast has an obvious inhibitory effect on thyroglobulin. After taking selenium yeast, the levels of the two antibodies can be reduced, which is conducive to improving hypothyroidism caused by HT. Studies have shown that selenoproteins also affect insulin secretion and its biosynthesis. Selenium exists in glutathione peroxidase, protects pancreatic β cells, prevents them from being oxidized, maintains the normal function of beta cells, promotes glucose metabolism, and plays a hypoglycemic role[24]. Appropriate selenium supplementation in T2DM patients can help the islets recover some functions and improve the condition of diabetes. Vitamin D in T2DM patients can effectively improve insulin resistance, promote insulin secretion, regulate blood sugar and lipid metabolism, and inhibit inflammation and oxidative stress. Tahrani *et al*[40] found that female T2DM patients with vitamin D deficiency had a higher HbA1c level, and after vitamin D supplementation, the Hba1c level was lower than before. Al-shahwan *et al*[41] supplemented 45 T2DM patients with 2000 IU of vitamin D per day, and the results showed that the level of vitamin D in T2DM patients increased and the degree of insulin resistance decreased significantly.

There are still some shortcomings in this study, such as single-center, retrospective, and sample size limitations, which may impact the results. The follow-up study will expand the region and sample for exploration to provide more comprehensive research support.

CONCLUSION

The combination of vitamin D, selenium, and oral hypoglycemic drugs in treating patients with T2DM and HT has a significant clinical effect, effectively improving thyroid function, autoantibodies, blood glucose, and blood lipid levels. The elevated 25 (OH) D_3, FT4, and TGAB levels were reduced.

ARTICLE HIGHLIGHTS

Research background

The pathogeneses of type-2 diabetes mellitus (T2DM) and Hashimoto's thyroiditis (HT) mainly involve insulin resistance, immune factors, infection, genetics, leptin, oxidative stress, molecular cytology, and other related fields; however, there is currently no clear consensus on the pathogenesis of the co-occurrence of these conditions. Symptomatic treatment for these two diseases, including hypoglycemic drugs and improvement in function, is generally performed clinically. Selenium yeast and active vitamin D can reduce thyroid-related antibody levels in T2DM and HT and improve thyroid function. Hypoglycemia drugs can reduce blood sugar levels in patients and promote blood sugar stability.

Research motivation

T2DM combined with HT may cause significant damage to the body. Currently, vitamin D amaryl, and selenium yeast are used in combination and applied to research in patients with T2DM combined HT rarely reported.

Research objectives

This article explored the therapeutic effect of vitamin D + selenium + hypoglycemic agents in patients with T2DM and HT and explored the serum 25-hydroxyvitamin D3 [25 (OH) D3] level and relations with related indicators.

Research methods

The control group was administered low-iodine diet guidance and hypoglycemic drug treatment. Test group A was additionally administered vitamin D treatment, while test group B was administered selenium yeast treatment in addition to the treatment in test group A. All three groups were treated for 6 months.

Research results

The improvement ranges of 25 (OH) D_3 level, thyroid function index level, autoantibody, blood glucose, and blood lipid levels in test groups A and B were better than those in the control group, and the improvement of test group B was better.

Research conclusions

The combination of vitamin D, selenium, and oral hypoglycemic agents in the treatment of patients with T2DM and HT had a significant clinical effect and effectively improved thyroid function and autoantibody and blood glucose and blood lipid levels, increased 25 (OH) D_3 levels, and decreased free T4 and thyroid globulin antibody levels in these patients.

Research perspectives

The combination of vitamin D, selenium, and oral hypoglycemic agents for treating patients with T2DM and HT has obvious therapeutic effects and is worthy of clinical application.

FOOTNOTES

Author contributions: Feng F and Zhou B jointly researched, wrote, and revised the paper; Zhou CL, Huang P, Wang G, and Yao K participated in the analysis and provided clinical advice.

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REFERENCES

1 **Wang L**, Gao P, Zhang M, Huang Z, Zhang D, Deng Q, Li Y, Zhao Z, Qin X, Jin D, Zhou M, Tang X, Hu Y, Wang L. Prevalence and Ethnic Pattern of Diabetes and Prediabetes in China in 2013. *JAMA* 2017; **317**: 2515-2523 [PMID: 28655017 DOI: 10.1001/jama.2017.7596]

2 **Ihnatowicz P**, Drywień M, Wątor P, Wojsiat J. The importance of nutritional factors and dietary management of Hashimoto's thyroiditis. *Ann Agric Environ Med* 2020; **27**: 184-193 [PMID: 32588591 DOI: 10.26444/aaem/112331]

3 **Jayanthi R**, Srinivasan AR. Biochemical isthmus [nexus] between type 2 diabetes mellitus and thyroid status-an update. *Diabetes Metab Syndr* 2019; **13**: 1173-1177 [PMID: 31336461 DOI: 10.1016/j.dsx.2019.01.037]

4 **Biondi B**, Kahaly GJ, Robertson RP. Thyroid Dysfunction and Diabetes Mellitus: Two Closely Associated Disorders. *Endocr Rev* 2019; **40**: 789-824 [PMID: 30649221 DOI: 10.1210/er.2018-00163]

5 **Han M**, Wu H, Yang W, Chen J. Analysis of risk factors for the development of type 2 diabetes mellitus complicated with Hashimoto's thyroiditis. *BMC Endocr Disord* 2022; **22**: 173 [PMID: 35804367 DOI: 10.1186/s12902-022-01092-6]

6 **Dahiya V**, Vasudeva N, Sharma S, Kumar A. Role of Dietary Supplements in Thyroid Diseases. *Endocr Metab Immune Disord Drug Targets* 2022; **22**: 985-996 [PMID: 35440339 DOI: 10.2174/1871530322666220419125131]

7 **Cvek M**, Kaličanin D, Barić A, Vuletić M, Gunjača I, Torlak Lovrić V, Škrabić V, Punda A, Boraska Perica V. Vitamin D and Hashimoto's Thyroiditis: Observations from CROHT Biobank. *Nutrients* 2021; **13** [PMID: 34444953 DOI: 10.3390/nu13082793]

8 **Caramaschi P**, Dalla Gassa A, Ruzzenente O, Volpe A, Ravagnani V, Tinazzi I, Barausse G, Bambara LM, Biasi D. Vitamin D and autoimmune rheumatic diseases. *Clin Rheumatol* 2011; **30**: 443-444 [PMID: 21243385 DOI: 10.1007/s10067-011-1683-8]

9 **Maciejewski A**, Kowalczyk MJ, Herman W, Czyżyk A, Kowalska M, Żaba R, Łącka K. Vitamin D Receptor Gene Polymorphisms and Autoimmune Thyroiditis: Are They Associated with Disease Occurrence and Its Features? *Biomed Res Int* 2019; **2019**: 8197580 [PMID: 31531369 DOI: 10.1155/2019/8197580]

10 **Khozam SA**, Sumaili AM, Alflan MA, Shawabkeh RAS. Association Between Vitamin D Deficiency and Autoimmune Thyroid Disorder: A Systematic Review. *Cureus* 2022; **14**: e25869 [PMID: 35836431 DOI: 10.7759/cureus.25869]

11 **Hu Z**, Chen J, Sun X, Wang L, Wang A. Efficacy of vitamin D supplementation on glycemic control in type 2 diabetes patients: A meta-analysis of interventional studies. *Medicine (Baltimore)* 2019; **98**: e14970 [PMID: 30946322 DOI: 10.1097/MD.0000000000014970]

12 **Jiang H**, Chen X, Qian X, Shao S. Effects of vitamin D treatment on thyroid function and autoimmunity markers in patients with Hashimoto's thyroiditis-A meta-analysis of randomized controlled trials. *J Clin Pharm Ther* 2022; **47**: 767-775 [PMID: 34981556 DOI: 10.1111/jcpt.13605]

13 **Hu Y**, Feng W, Chen H, Shi H, Jiang L, Zheng X, Liu X, Zhang W, Ge Y, Liu Y, Cui D. Effect of selenium on thyroid autoimmunity and regulatory T cells in patients with Hashimoto's thyroiditis: A prospective randomized-controlled trial. *Clin Transl Sci* 2021; **14**: 1390-1402 [PMID: 33650299 DOI: 10.1111/cts.12993]

14 **Yu L**, Zhou L, Xu E, Bi Y, Hu X, Pei X, Jin G. Levothyroxine monotherapy versus levothyroxine and selenium combination therapy in chronic lymphocytic thyroiditis. *J Endocrinol Invest* 2017; **40**: 1243-1250 [PMID: 28534148 DOI: 10.1007/s40618-017-0693-z]

15 **American Diabetes Association**. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2018. *Diabetes Care* 2018; **41**: S13-S27 [PMID: 29222373 DOI: 10.2337/dc18-S002]

16 **Caturegli P**, De Remigis A, Rose NR. Hashimoto thyroiditis: clinical and diagnostic criteria. *Autoimmun Rev* 2014; **13**: 391-397 [PMID: 24434360 DOI: 10.1016/j.autrev.2014.01.007]

17 **Klecha AJ**, Barreiro Arcos ML, Frick L, Genaro AM, Cremaschi G. Immune-endocrine interactions in autoimmune thyroid diseases. *Neuroimmunomodulation* 2008; **15**: 68-75 [PMID: 18667802 DOI: 10.1159/000135626]

18 **Štefanić M**, Tokić S, Suver Stević M, Glavaš-Obrovac L. Association of increased eomesodermin, BCL6, and granzyme B expression with major clinical manifestations of Hashimoto's thyroiditis - an observational study. *Immunol Invest* 2018; **47**: 279-292 [PMID: 29319368 DOI: 10.1080/08820139.2018.1423571]

19 **Liontiris MI**, Mazokopakis EE. A concise review of Hashimoto thyroiditis (HT) and the importance of iodine, selenium, vitamin D and gluten on the autoimmunity and dietary management of HT patients.Points that need more investigation. *Hell J Nucl Med* 2017; **20**: 51-56 [PMID: 28315909 DOI: 10.1967/s002449910507]

20 **Vasiliu I**, Ciobanu-Apostol DG, Armasu I, Bredetean O, Serban IL, Preda C. Protective role of selenium on thyroid morphology in iodine-induced autoimmune thyroiditis in Wistar rats. *Exp Ther Med* 2020; **20**: 3425-3437 [PMID: 32905063 DOI: 10.3892/etm.2020.9029]

21 **Duntas LH**. The Role of Iodine and Selenium in Autoimmune Thyroiditis. *Horm Metab Res* 2015; **47**: 721-726 [PMID: 26361258 DOI: 10.1055/s-0035-1559631]

22 **Duntas LH**. Selenium and the thyroid: a close-knit connection. *J Clin Endocrinol Metab* 2010; **95**: 5180-5188 [PMID: 20810577 DOI: 10.1210/jc.2010-0191]

23 **Iddah MA**, Macharia BN. Autoimmune thyroid disorders. *ISRN Endocrinol* 2013; **2013**: 509764 [PMID: 23878745 DOI: 10.1155/2013/509764]

24 **Mojadadi A**, Au A, Salah W, Witting P, Ahmad G. Role for Selenium in Metabolic Homeostasis and Human Reproduction. *Nutrients* 2021; **13** [PMID: 34579133 DOI: 10.3390/nu13093256]

25 **Santos JAR**, Rama TA, da Silva DJL, Fernandes RJ, Zacca R. Supply of Antioxidants vs. Recruit Firefighters' Cellular Immune Status: A Randomized Double-Blinded Placebo-Controlled Parallel-Group Trial. *Life (Basel)* 2022; **12** [PMID: 35743844 DOI: 10.3390/life12060813]

26 **Negro R**, Attanasio R, Grimaldi F, Marcocci C, Guglielmi R, Papini E. A 2016 Italian Survey about the Clinical Use of Selenium in Thyroid Disease. *Eur Thyroid J* 2016; **5**: 164-170 [PMID: 27843806 DOI: 10.1159/000447667]

27 **Wang W**, Mao J, Zhao J, Lu J, Yan L, Du J, Lu Z, Wang H, Xu M, Bai X, Zhu L, Fan C, Zhang H, Shan Z, Teng W. Decreased Thyroid Peroxidase Antibody Titer in Response to Selenium Supplementation in Autoimmune Thyroiditis and the Influence of a Selenoprotein P Gene Polymorphism: A Prospective, Multicenter Study in China. *Thyroid* 2018; **28**: 1674-1681 [PMID: 30398407 DOI: 10.1089/thy.2017.0230]

28 **Brigelius-Flohé R**, Flohé L. Selenium and redox signaling. *Arch Biochem Biophys* 2017; **617**: 48-59 [PMID: 27495740 DOI: 10.1016/j.abb.2016.08.003]

29 **Wu Q**, Rayman MP, Lv H, Schomburg L, Cui B, Gao C, Chen P, Zhuang G, Zhang Z, Peng X, Li H, Zhao Y, He X, Zeng G, Qin F, Hou P, Shi B. Low Population Selenium Status Is Associated With Increased Prevalence of Thyroid Disease. *J Clin Endocrinol Metab* 2015; **100**: 4037-4047 [PMID: 26305620 DOI: 10.1210/jc.2015-2222]

30 **Esposito D**, Rotondi M, Accardo G, Vallone G, Conzo G, Docimo G, Selvaggi F, Cappelli C, Chiovato L, Giugliano D, Pasquali D. Influence of short-term selenium supplementation on the natural course of Hashimoto's thyroiditis: clinical results of a blinded placebo-controlled randomized prospective trial. *J Endocrinol Invest* 2017; **40**: 83-89 [PMID: 27572248 DOI: 10.1007/s40618-016-0535-4]

31 **Chaudhary S**, Dutta D, Kumar M, Saha S, Mondal SA, Kumar A, Mukhopadhyay S. Vitamin D supplementation reduces thyroid peroxidase antibody levels in patients with autoimmune thyroid disease: An open-labeled randomized controlled trial. *Indian J Endocrinol Metab* 2016; **20**: 391-398 [PMID: 27186560 DOI: 10.4103/2230-8210.179997]

32 **Muscogiuri G**, Mari D, Prolo S, Fatti LM, Cantone MC, Garagnani P, Arosio B, Di Somma C, Vitale G. 25 Hydroxyvitamin D Deficiency and Its Relationship to Autoimmune Thyroid Disease in the Elderly. *Int J Environ Res Public Health* 2016; **13** [PMID: 27571093 DOI: 10.3390/ijerph13090850]

33 **Choi YM**, Kim WG, Kim TY, Bae SJ, Kim HK, Jang EK, Jeon MJ, Han JM, Lee SH, Baek JH, Shong YK, Kim WB. Low levels of serum vitamin D3 are associated with autoimmune thyroid disease in pre-menopausal women. *Thyroid* 2014; **24**: 655-661 [PMID: 24320141 DOI: 10.1089/thy.2013.0460]

34 **Wang S**, Wu Y, Zuo Z, Zhao Y, Wang K. The effect of vitamin D supplementation on thyroid autoantibody levels in the treatment of autoimmune thyroiditis: a systematic review and a meta-analysis. *Endocrine* 2018; **59**: 499-505 [PMID: 29388046 DOI: 10.1007/s12020-018-1532-5]

35 **Subrahmanyan NA**, Koshy RM, Jacob K, Pappachan JM. Efficacy and Cardiovascular Safety of DPP-4 Inhibitors. *Curr Drug Saf* 2021; **16**: 154-164 [PMID: 32819262 DOI: 10.2174/1574886315999200819150544]

36 **Deacon CF**, Lebovitz HE. Comparative review of dipeptidyl peptidase-4 inhibitors and sulphonylureas. *Diabetes Obes Metab* 2016; **18**: 333-347 [PMID: 26597596 DOI: 10.1111/dom.12610]

37 **Li JJ**, Zhang P, Fan B, Guo XL, Zheng ZS. The efficacy of saxagliptin in T2DM patients with non-alcoholic fatty liver disease: preliminary data. *Rev Assoc Med Bras (1992)* 2019; **65**: 33-37 [PMID: 30758417 DOI: 10.1590/1806-9282.65.1.33]

38 **Wang Z**, Xu D, Huang L, Zhang T, Wang J, Chen Q, Kong L, Zhou X. Effects of saxagliptin on glucose homeostasis and body composition of obese patients with newly diagnosed pre-diabetes. *Diabetes Res Clin Pract* 2017; **130**: 77-85 [PMID: 28575729 DOI: 10.1016/j.diabres.2017.05.012]

39 **Angellotti E**, D'Alessio D, Dawson-Hughes B, Chu Y, Nelson J, Hu P, Cohen RM, Pittas AG. Effect of vitamin D supplementation on cardiovascular risk in type 2 diabetes. *Clin Nutr* 2019; **38**: 2449-2453 [PMID: 30352748 DOI: 10.1016/j.clnu.2018.10.003]

40 **Tahrani AA**, Ball A, Shepherd L, Rahim A, Jones AF, Bates A. The prevalence of vitamin D abnormalities in South Asians with type 2 diabetes mellitus in the UK. *Int J Clin Pract* 2010; **64**: 351-355 [PMID: 19863680 DOI: 10.1111/j.1742-1241.2009.02221.x]

41 **Al-Shahwan MA**, Al-Othman AM, Al-Daghri NM, Sabico SB. Effects of 12-month, 2000IU/day vitamin D supplementation on treatment naïve and vitamin D deficient Saudi type 2 diabetic patients. *Saudi Med J* 2015; **36**: 1432-1438 [PMID: 26620985 DOI: 10.15537/smj.2015.12.12923]

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ORIGINAL ARTICLE

Retrospective Study

Effect of viral hepatitis on type 2 diabetes: A Mendelian randomization study

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Abstract

BACKGROUND

The effects of viral hepatitis (VH) on type 2 diabetes (T2D) remain controversial.

AIM

To analyze the causal correlation between different types of VH and T2D using Mendelian randomization (MR).

METHODS

Single nucleotide polymorphisms of VH, chronic hepatitis B (CHB), chronic hepatitis C (CHC) and T2D were obtained from the BioBank Japan Project, European Bioinformatics Institute, and FinnGen. Inverse variance weighted, MR-Egger, and weighted median were used to test exposure-outcome associations. The MR-Egger intercept analysis and Cochran's *Q* test were used to assess horizontal pleiotropy and heterogeneity, respectively. Leave-one-out sensitivity analysis was used to evaluate the robustness of the MR analysis results.

RESULTS

The MR analysis showed no significant causal relationship between VH and T2D in Europeans [odds ratio (OR) = 1.028; 95% confidence interval (CI): 0.995-1.062, P = 0.101]. There was a negative causal association between CHB and T2D among East Asians (OR = 0.949; 95%CI: 0.931-0.968, P < 0.001), while there was no

significant causal association between CHC and T2D among East Asians (OR = 1.018; 95%CI: 0.959-1.081, P = 0.551). Intercept analysis and Cochran's Q test showed no horizontal pleiotropy or heterogeneity (P > 0.05). Sensitivity analysis showed that the results were robust.

CONCLUSION

Among East Asians, CHB is associated with a reduced T2D risk, but this association is limited by HBV load and cirrhosis. Although VH among Europeans and CHC among East Asians are not associated with the risk of T2D, focusing on blood glucose in patients with CHC is still relevant for the early detection of T2D induced by CHC-mediated pathways of hepatic steatosis, liver fibrosis, and cirrhosis.

Key Words: Viral hepatitis; Chronic hepatitis B; Chronic hepatitis C; Type 2 diabetes; Mendelian randomization

Core Tip: The effects of hepatitis B and C on type 2 diabetes (T2D) remain controversial. The study aims to analyze the causal relationship of T2D with chronic hepatitis B (CHB) and chronic hepatitis C (CHC) by Mendelian randomization (MR). This MR analysis showed that in East Asians, CHC was not associated with T2D risk, whereas CHB was associated with a reduced risk of T2D. Although this MR analysis did not find a causal relationship between CHC and T2D, focusing on blood glucose in patients with CHC is still relevant, which helps early detect T2D induced by CHC-mediated other hepatic lesions.

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INTRODUCTION

Type 2 diabetes (T2D) is a chronic metabolic disease characterized by relative insulin deficiency and abnormally elevated blood glucose[1]. An epidemiological study has shown that as the prevalence of diabetes increases each year, approximately 1 in 10 adults globally now have diabetes, and it is projected that by 2045, the world will have 693 million individuals with diabetes[2,3]. As an incurable disease, the hyperglycemic state in T2D increases the risk of macrovascular pathologies, such as cardiovascular disease, and microvascular pathologies, such as nephropathy, retinopathy, and peripheral neuropathy[4,5]. T2D is a serious threat to the life and health of patients, especially the thrombotic events caused by cardiovascular and cerebrovascular lesions, which are the leading causes of death in patients with T2D[2,6]. Obesity, high-fat diet, and physical inactivity are risk factors for T2D, and controlling these risk factors helps reduce the risk of developing T2D and improve its prognosis[7,8]. Therefore, controlling the related risk factors, particularly high-risk factors, is essential for the prevention and treatment of T2D. In recent years, an increasing number of studies have reported an association between hepatitis viruses and diabetes mellitus[9], and evidence suggests that chronic viral hepatitis (VH) may be a potential risk factor for T2D[10,11].

VH, an inflammatory disease of the liver caused by infection with the hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), or hepatitis E virus (HEV), is a major global health problem[12]. These viruses cause acute hepatitis, and HBV, HCV, and HDV infections progress to chronic hepatitis[13,14]. Globally, approximately 257 million individuals have been reported to be infected with HBV, and 71 million are infected with HCV[15]. Chronic hepatitis B (CHB) from HBV infection and chronic hepatitis C (CHC) from HCV infection cause persistent damage to the liver, resulting in liver fibrosis, cirrhosis, liver cancer, and even death[13,16]. Relevant studies have shown that hepatitis B cirrhosis and HCV infection increased the risk of T2D by 74% and 1058%, respectively[11,17], suggesting that CHB and CHC are potential risk factors for T2D. This effect may be related to the signaling pathway by which hepatitis viruses alter hepatic glucose homeostasis by mediating the overexpression of protein phosphatase 2A to inhibit Akt and FoxO1 dephosphorylation[18]. However, some studies have reported that HBV and HCV infections do not increase the incidence of T2D[19,20], which indirectly negates the relationships of them. Whether different categories of VH, especially CHB and CHC, are associated with the risk of T2D remains controversial, and the causal relationship between them needs to be further explored.

Mendelian randomization (MR) is a method for assessing the causal relationship between exposure and outcome variables using genetic variants[21]. Due to the randomized nature of allele classification, MR has properties similar to those of randomized controlled trials[22]. Although MR cannot be used as a substitute for randomized controlled trials, it provides additional evidence for causality analysis[23]. This MR analysis explored the causal relationship between T2D and VH, CHB, and CHC from a gene prediction perspective, with the aim of providing additional evidence for risk factor studies in diabetes.

MATERIALS AND METHODS

Study design

MR relies on three primary assumptions[24]: (1) Association assumption: Single nucleotide polymorphisms (SNPs) are strongly associated with exposure factors; (2) Independence assumption: SNPs are independent of confounding variables; and (3) Exclusivity assumption: SNPs do not act on outcome variables through pathways other than through exposure factors. The design is illustrated in Figure 1.

Data sources

Data on VH, CHB, CHC, and T2D were obtained from the BioBank Japan Project (https://biobankjp.org/en/), European Bioinformatics Institute (https://www.ebi.ac.uk), and FinnGen (www.finngen.fi/fi). All the data were sourced from publicly available databases; therefore, no additional ethical approval was required.

Selection of genetic instrument variables

First, SNPs strongly associated with exposure factors were screened in the genome-wide association studies (GWAS), according to a threshold of $P < 5 \times 10^{-6}$ to fulfill assumption 1. Second, independent SNPs were screened, according to $R^2 < 0.001$ and kb = 10000, to avoid potential bias due to linkage disequilibrium. Third, the *F*-value of each SNP was calculated, and SNPs with $F \leq 10$ were excluded. *F*-value was calculated publicly as $F = [R^2/(1 - R^2)] \times [(N - K - 1)/K]$, where $R^2 = 2 \times (1 - MAF) \times MAF \times \beta^2$. R^2: The cumulative explained variance of the selected instrument variables on exposure; MAF: The effect of minor allele frequency; β: The estimated effect of SNP; and N: Sample size of the GWAS. Finally, we referred to PhenoScanner (www.phenoscanner.medschl.cam.ac.uk) and related literature to remove SNPs potentially associated with T2D to fulfill assumption 2.

Data analysis

This study followed the STROBE-MR guidelines[25]. The "TwoSampleMR (0.5.7)" program package for R 4.3.1 was used to perform the two-sample MR analysis. Inverse variance weighting (IVW), MR-Egger, and weighted median were used as basic causality assessment methods. Among these methods, IVW was the primary analysis method[26] that achieved unbiased causal estimation without horizontal pleiotropy. MR-Egger and the weighted median are complementary methods to MR analysis, with the former providing valid causal estimation in some cases where pleiotropy exists, and the latter being less sensitive to outliers and measurement errors.

The MR results were corrected and analyzed using the MR-Pleiotropy Residual Sum and Outlier method (MR-PRESSO), and the MR analysis was re-executed after removing outlier SNPs ($P < 1$). Horizontal pleiotropy was assessed using MR-Egger's intercept analysis, and $P \geq 0.05$ suggested the absence of horizontal pleiotropy to fulfill assumption 3. Heterogeneity was assessed using Cochran's *Q* test, and $P \geq 0.05$ suggested the absence of heterogeneity. Leave-one-out sensitivity analysis was used to assess the robustness of the results and clarify individual SNP that significantly affected the pooled results.

RESULTS

GWAS data for exposure factors

The VH data were obtained from FinnGen, which included 377277 European participants (dataset number: finngen_R9_AB1_VIRAL_HEPATITIS). Data on CHB were obtained from the BioBank Japan Project, which contains information on 212453 East Asians (dataset number: bbj-a-99). Data on CHC were obtained from the BioBank Japan Project, which contains information on 212453 East Asians (dataset number: bbj-a-101). Eighty-six SNPs closely related to VH were provided by FinnGen, 8719 closely associated with CHB, and 1494 closely related to CHC were supplied by the BioBank Japan Project. Eleven SNPs for VH, 14 for CHB, and 13 for CHC were included after excluding the effects of linkage disequilibrium and confounding variables (Supplementary Table 1). Duplicated and mismatched SNPs were excluded based on the EAF values when harmonizing the allelic orientations of the exposure and outcome SNPs. Outlier SNPs were excluded from MR-PRESSO correction analysis. Finally, 11 SNPs for VH, nine for CHB, and six for CHC were included (Supplementary Table 2).

GWAS data for outcome variables

The T2D data for Europe were obtained from FinnGen, including 365950 European participants (dataset number: finngen_R9_T2D). Data on T2D for East Asia were obtained from the European Bioinformatics Institute, and it included 433540 East Asian individuals (dataset number: ebi-a- GCST010118) (Table 1).

MR analysis results of two samples

The causal effects between the exposure factors (VH, CHB, and CHC) and outcome variable (T2D) were analyzed using two-sample MR. A forest plot of the MR analysis is shown in Figure 2, and a scatter plot of the effect estimates for each SNP is shown in Figure 3. The results of the intercept analysis are shown in Supplementary Table 3. The results of the heterogeneity test are shown in Figure 4 and Supplementary Table 4. The sensitivity analysis is shown in Figure 5.

Table 1 Details of the Genome-wide association studies included in the Mendelian randomization

Year	Trait	Population	Sample size	Web source
2023	VH	European	377277	www.finngen.fi/en
2023	T2D	European	365950	www.finngen.fi/en
2019	CHB	East Asian	212453	https://biobankjp.org/en/
2019	CHC	East Asian	212453	https://biobankjp.org/en/
2020	T2D	East Asian	433540	https://www.ebi.ac.uk

VH: Viral hepatitis; CHB: Chronic hepatitis B; CHC: Chronic hepatitis C; T2D: Type 2 diabetes.

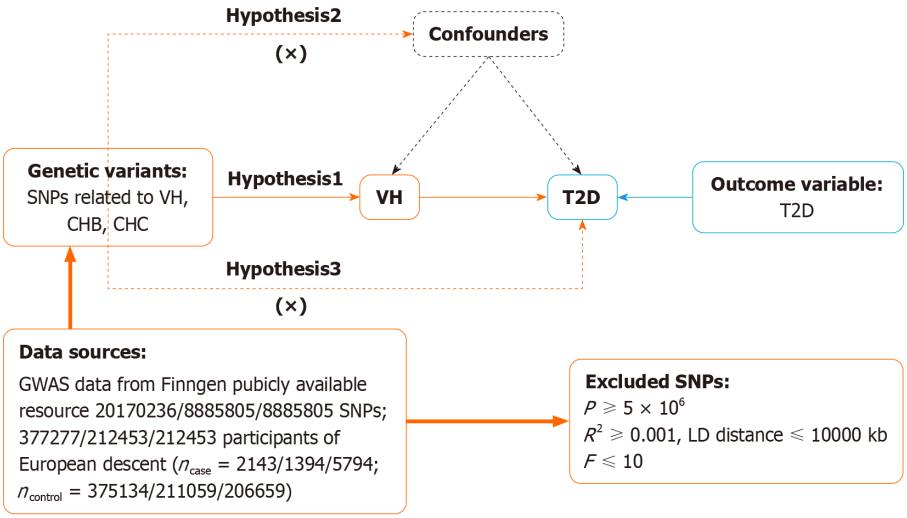

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Figure 1 Mendelian randomization design for causal analysis of viral hepatitis, chronic hepatitis B, chronic hepatitis C and type 2 diabetes. VH: Viral hepatitis; CHB: Chronic hepatitis B; CHC: Chronic hepatitis C; T2D: Type 2 diabetes; GWAS: Genome-wide association studies; SNP: Single nucleotide polymorphisms; LD: Linkage disequilibrium.

Exposure	Outcome	MR method	Forest plot	OR	95%CI	*P* value
VH	T2D	IVW		1.028	0.995-1.062	0.101
		MR-Egger		1.002	0.938-1.069	0.962
		Weighted median		1.014	0.975-1.054	0.499
CHB		IVW		0.949	0.931-0.968	< 0.001
		MR-Egger		0.940	0.901-0.981	0.026
		Weighted median		0.954	0.931-0.979	< 0.001
CHC		IVW		1.018	0.959-1.081	0.551
		MR-Egger		1.152	0.858-1.548	0.400
		Weighted median		1.009	0.933-1.091	0.821
			0.850 1.050 1.250 1.450 1.650			

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Figure 2 Forest plot of Mendelian randomization analysis on the causal relationship between viral hepatitis, chronic hepatitis B, chronic hepatitis C and type 2 diabetes. VH: Viral hepatitis; CHB: Chronic hepatitis B; CHC: Chronic hepatitis C; T2D: Type 2 diabetes; OR: Odd ratio; SNP: Single nucleotide polymorphisms; MR: Mendelian randomization; CI: Confidence interval; IVW: Inverse variance weighting.

VH: None of the three methods of analysis showed significant causal relationships between VH and T2D in Europeans: IVW [odds ratio (OR) = 1.028; 95% confidence interval (CI): 0.995-1.062; P = 0.101], MR-Egger (OR = 1.002; 95%CI: 0.938-1.069; P = 0.962), or weighted median (OR = 1.014; 95%CI: 0.975-1.054; P = 0.499). Intercept analysis showed no horizontal pleiotropy (P = 0.391). Cochran's Q test showed no heterogeneity (P = 0.119). Sensitivity analysis suggested that the

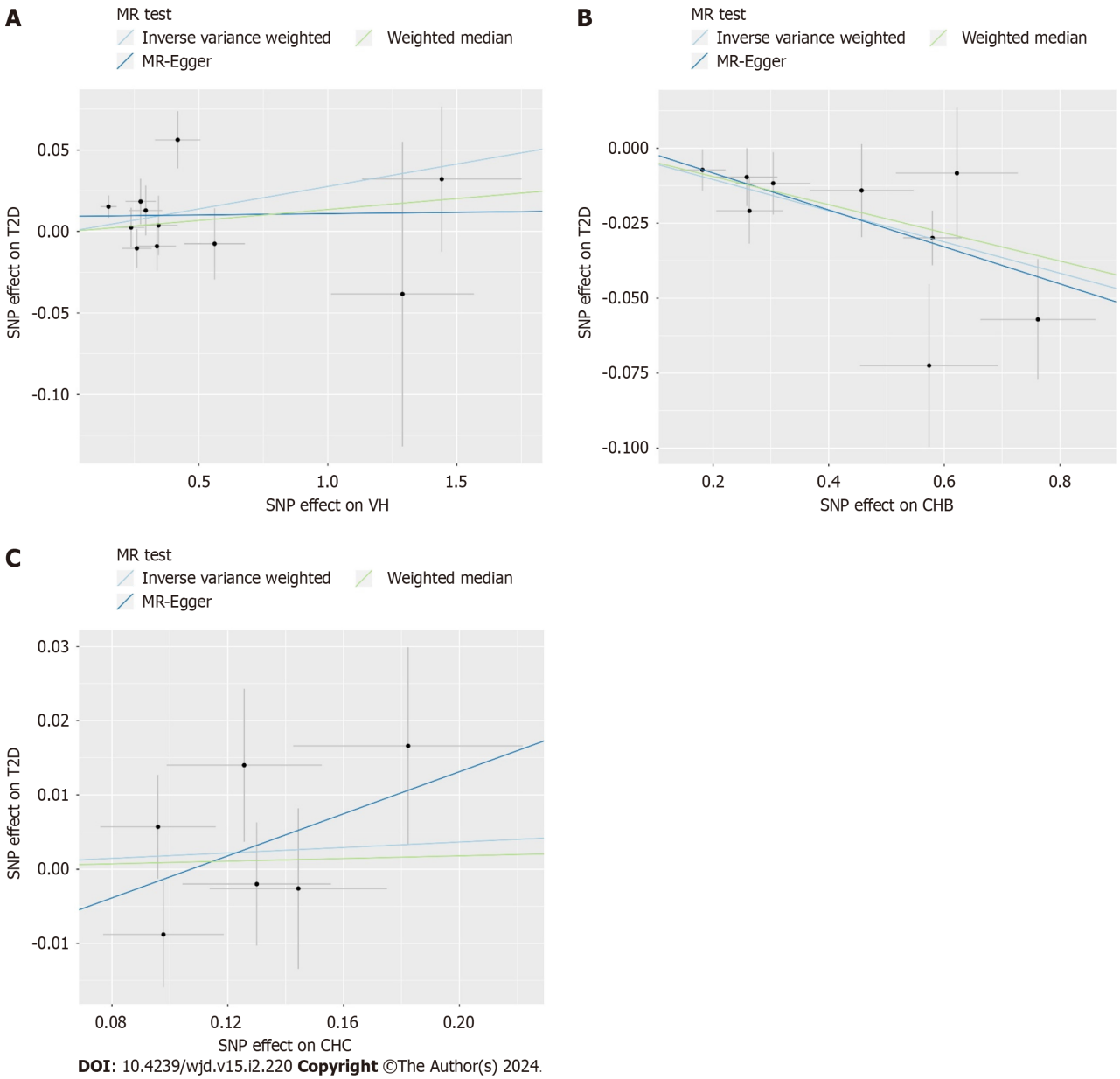

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Figure 3 Scatter plot of Mendelian randomization analysis on the causal relationship between viral hepatitis, chronic hepatitis B, chronic hepatitis C and type 2 diabetes. A: Viral hepatitis on type 2 diabetes; B: Chronic hepatitis B on type 2 diabetes; C: Chronic hepatitis C on type 2 diabetes. VH: Viral hepatitis; CHB: Chronic hepatitis B; CHC: Chronic hepatitis C; T2D: Type 2 diabetes; MR: Mendelian randomization.

results were robust.

CHB: All the three methods of analysis showed a negative causal association between CHB and T2D among East Asian individuals: IVW (OR = 0.949; 95%CI: 0.931-0.968; $P < 0.001$), MR-Egger (OR = 0.940; 95%CI: 0.901-0.981; $P = 0.026$), and weighted median (OR = 0.954; 95%CI: 0.931-0.979; $P < 0.001$). Intercept analysis showed no horizontal pleiotropy ($P = 0.640$). The Cochran's Q test revealed no heterogeneity ($P = 0.685$). Sensitivity analysis suggested that the results were robust.

CHC: None of the three analysis methods showed a significant causal relationship between CHC and T2D in East Asians: IVW (OR = 1.018; 95%CI: 0.959-1.081, $P = 0.551$), MR-Egger (OR = 1.152; 95%CI: 0.858-1.548, $P = 0.400$), and weighted median (OR = 1.009; 95%CI: 0.933-1.091, $P = 0.821$). Intercept analysis showed no horizontal pleiotropy ($P = 0.640$). The Cochran's Q test showed no heterogeneity ($P = 0.376$), and the sensitivity analysis suggested that the results were robust.

DISCUSSION

VH is an inflammatory liver disease caused by viruses, such as HAV, HBV, HCV, HDV, and HEV, and is one of the most

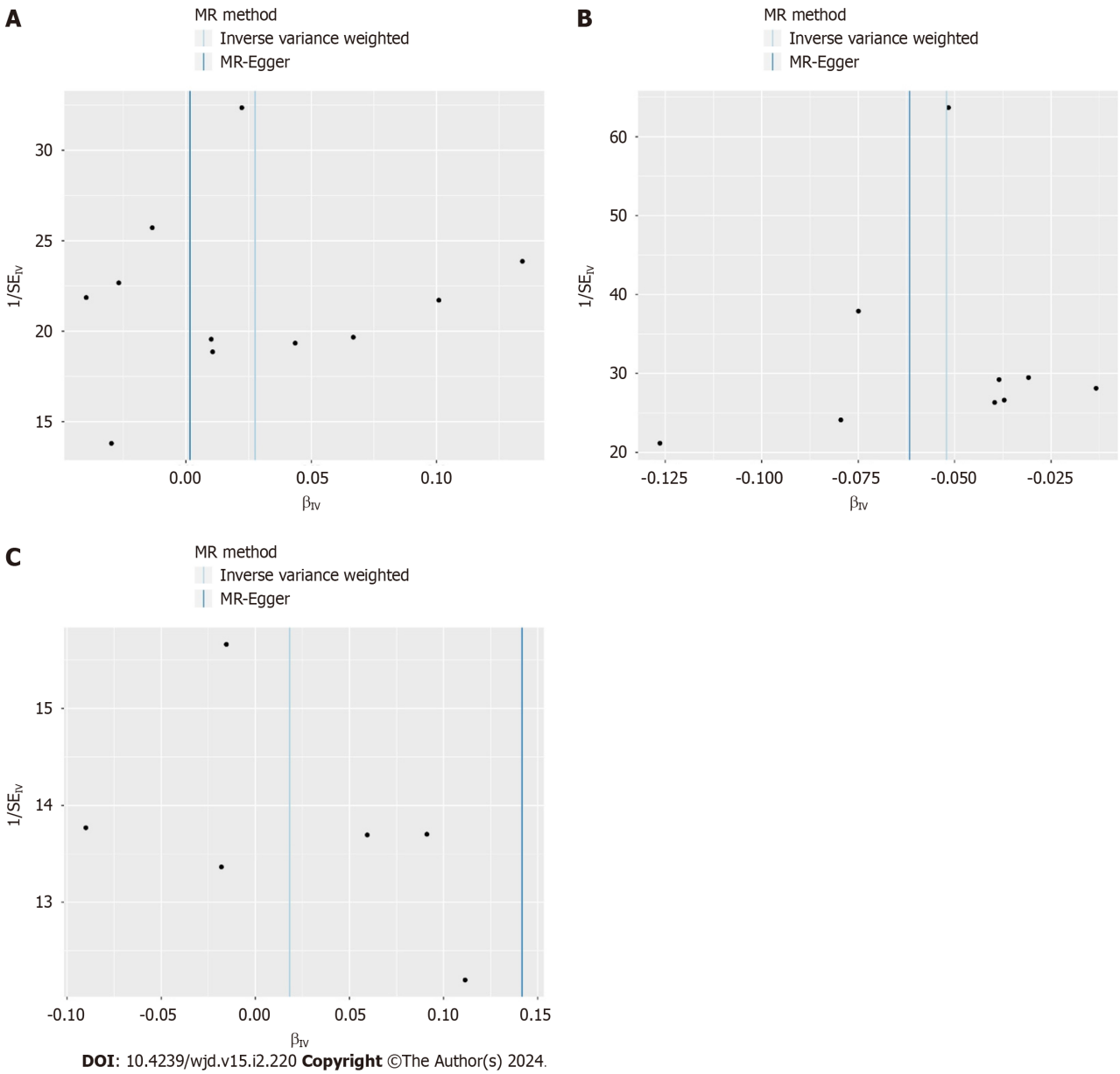

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Figure 4 Funnel plot of heterogeneity analysis on viral hepatitis, chronic hepatitis B, chronic hepatitis C and type 2 diabetes. A: Viral hepatitis on type 2 diabetes; B: Chronic hepatitis B on type 2 diabetes; C: Chronic hepatitis C on type 2 diabetes. MR: Mendelian randomization.

common liver diseases[27]. Some evidence suggests that HBV and HCV infection are associated with impaired glucose tolerance and increased incidence of diabetes mellitus[28,29], which are potential risk factors for T2D. However, other studies have found that neither HBV nor HCV infections are associated with T2D risk[20,30]. The effects of HBV and HCV infections on T2D remain controversial, and their causal relationship remains unclear. To further understand the potential impact of different types of VH on T2D, MR was used to analyze the causal relationships of VH, CHB, and CHC with T2D.

This study found no significant causal association between VH and T2D risk in the European population. Among East Asians, CHB was associated with a lower risk of T2D, whereas CHC was not associated with a risk of T2D. The Cochrane's *Q*-test and intercept analysis showed no heterogeneity or horizontal pleiotropy in these results, and the sensitivity analysis showed that the MR results were robust. As the GWAS did not include data on HAV, HDV, and HEV infections, this study did not assess the effect of these three types of VH on the risk of T2D. Additionally, the impact of CHB and CHC on T2D among Europeans is still being determined because GWAS do not contain available data on CHB and CHC in Europeans.

Our results showed that CHC was not associated with T2D risk, which is consistent with previous reports. A United States clinical trial, involving 15125 individuals found that neither the prevalence of prediabetes nor diabetes was associated with HCV infection, and the level of insulin resistance was not associated with HCV markers[20]. Additionally, an Iranian case-control study noted that CHC was not a risk factor for insulin resistance or diabetes in the Iranian population[31].

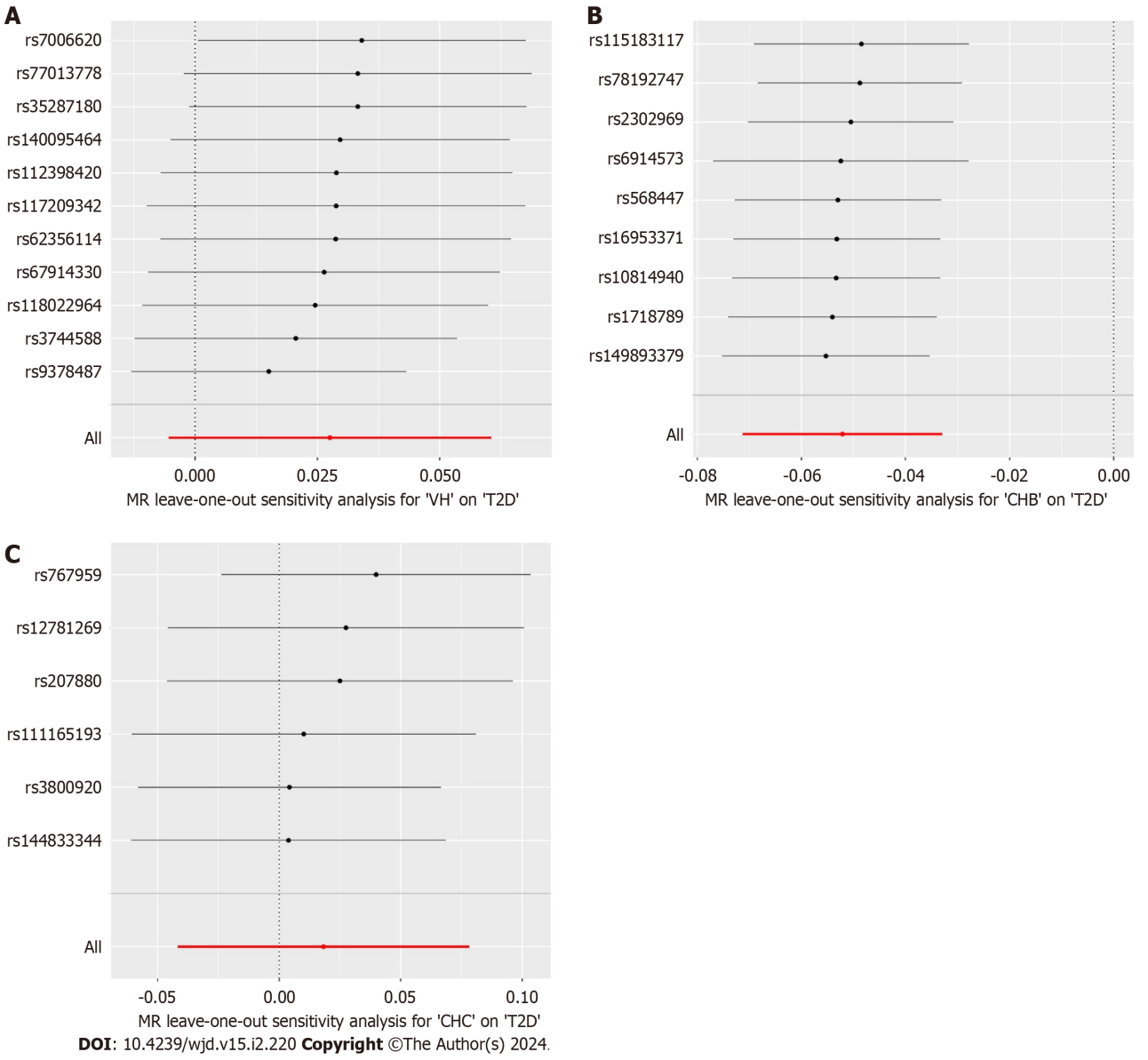

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Figure 5 The results of leave-one-out sensitivity analysis on viral hepatitis, chronic hepatitis B, chronic hepatitis C and type 2 diabetes. A: Viral hepatitis on type 2 diabetes; B: Chronic hepatitis B on type 2 diabetes; C: Chronic hepatitis C on type 2 diabetes. VH: Viral hepatitis; CHB: Chronic hepatitis B; CHC: Chronic hepatitis C; T2D: Type 2 diabetes.

However, studies supporting the notion that CHC is not associated with T2D are few, and to date, most clinical studies have pointed to a correlation between CHC and T2D. Mehta *et al*[17] suggested that CHC is a potential risk factor for T2D, and they found a higher risk of T2D in patients with CHC than that in healthy individuals. An Italian single-arm trial noted that a significant effect of HCV on glucose load developed through increased insulin resistance in the liver and muscles[32]. The impact of HCV on blood glucose levels and risk of diabetes was more pronounced in patients aged 35-49 years and in those with severe liver disease[33]. The risk for T2D in patients with CHC has been reported to increase with increasing levels of liver fibrosis[34,35]. Additionally, there is evidence that patients with CHC infected with HCV1b and HCV3 have a higher incidence of T2D[36,37], implying that the HCV genotype is an essential factor influencing the risk of T2D. These results suggest that HCV increases the risk of developing T2D by affecting insulin sensitivity, and that this association is related to the degree of liver fibrosis and HCV genotype. Therefore, HCV eradication may help reduce blood glucose levels and T2D risk. Gilad *et al*[38] and Hussein *et al*[39] found that diabetic patients coinfected with HCV who were treated with direct-acting antiviral agents (DAAs) had significant improvements in glycosylated hemoglobin levels and insulin resistance, as well as a substantial reduction in diabetes-related microvascular complications[40]. Two meta-analyses have shown that DAAs restore HCV-induced alterations in glucose homeostasis by inducing a sustained virological response, thereby reducing insulin resistance and T2D risk[41,42]. These findings indicate that anti-HCV therapy benefits patients with T2D, and provide indirect evidence that CHC is a risk factor for T2D.

Although considerable evidence points to CHC as a potential risk factor for T2D, this MR analysis, based on genetic prediction, did not reveal a causal relationship between them. The MR analysis and clinical trial results differed, possibly because of intermediate factors between CHC and T2D. Ruhl *et al*[20] stated that diabetes risk is associated with elevated alanine aminotransferase and gamma-glutamyltransferase activities rather than HCV infection status. The authors

suggested that the association between HCV infection and T2D reported in previous studies was related to elevated liver enzyme[20]. Related studies have shown that insulin resistance in individuals infected with HCV is associated with alterations in alanine transaminase, aspartate aminotransferase, and bilirubin levels[43]. Papatheodoridis *et al*[44] found that the risk of T2D in HCV-infected individuals was associated with hepatic fibrosis, cirrhosis, hepatic steatosis, and increased serum triglyceride levels.

Additionally, researchers have observed that HCV infection increases the risk of obesity and metabolic syndrome (MS) by affecting the liver. In a clinical study in Virginia, Younossi *et al*[45] found that HCV genotype three was associated with an increased risk of steatosis and fatty liver disease. A Taiwanese study has shown that HCV regulates host lipid metabolism and distribution to some extent[46]. Another Taiwanese study showed that the prevalence of MS was higher in individuals infected with HCV than that in non-infected patients (24.7% *vs* 13.2%)[47]. The effects of HCV infection on obesity and MS may be mediated through the promotion of hepatic steatosis and fibrosis. Hepatitis C core viral proteins in patients with HCV, especially in genotype 3a-infected patients, induced sterol regulatory element-binding protein 1 and peroxisome proliferator-activated receptor γ gene expression and activity, thereby increasing the transcription of genes involved in hepatic fatty acid synthesis, and ultimately promoting steatosis[48]. Hepatitis C core viral proteins, in turn, mediate oxidative stress, promote the expression of inflammatory factors, such as tumor necrosis factor-α, interleukin (IL)-6, and IL-8, and aggravate the degree of hepatic fibrosis, which exacerbates insulin resistance[49,50]. Insulin resistance plays a vital role in MS[47]. This evidence suggests that HCV may affect glucose metabolic homeostasis and increase the risk of T2D through intermediate pathways, such as hepatic steatosis, hepatic fibrosis, and cirrhosis. However, due to the assumption of the exclusivity of MR, SNPs associated with known risk factors for T2D, such as fatty liver, liver fibrosis, and cirrhosis, were excluded as confounding factors, which may be the main reason for the negative MR results.

This study suggests that CHB infection is associated with a reduced risk of T2D, which differs from the results of most clinical studies. Current studies support the notion that HBV infection is not an independent risk factor of T2D[19]. A Taiwanese study involving 1233 individuals found no significant differences in the prevalence of diabetes and glucose intolerance between asymptomatic chronic HBV-infected individuals and a non-HBV control group[30]. This is similar to the findings of another Taiwanese study that concluded that HBV itself does not confer a predisposition to diabetes[51]. Liu *et al*[52] supported this view from a serological perspective as they found that the serological status of HBV antigen (HBsAg) and hepatitis B surface antibody (HBsAb) was not associated with diabetes. Moreover, HBV infection did not increase the risk of macrovascular complications in diabetes mellitus[53].

Although most of the current literature suggests that CHB is not associated with T2D risk, some studies support CHB as a potential protective factor against T2D. A study of retired Chinese women showed that a HBsAb-positive status was associated with better metabolic status and a lower incidence of diabetes mellitus[54]. Another study found that a high HBV load is associated with a reduced risk of hepatic steatosis, a mechanism by which HBV reduces the risk of T2D[55]. This implies that a high HBV load may be an element of the reduced risk of T2D in patients with CHB rather than HBsAb positivity alone. However, most patients with CHB are treated with antiviral drugs, including tenofovir or entecavir, which reduce the HBV load in the body. As the viral load decreases, the role of HBV in regulating fat metabolism and reducing the risk of diabetes is significantly diminished, which may be the main reason why the results of this MR analysis differ from those of clinical studies.

The potential protective effects of HBV infection against obesity and MS provide indirect evidence that supports our findings. A cross-sectional study in China showed that the prevalence of MS was significantly lower in patients infected with HBV than that in non-infected patients (11.64% *vs* 12.66%)[56]. A Taiwanese clinical study included 3587 patients with HBV infection without cirrhosis and found that high HBV viral load was associated with a reduced risk of extreme obesity (OR = 0.30; 95%CI: 0.13-0.68) and centripetal obesity (OR = 0.53; 95%CI: 0.34-0.82)[57]. HBV infection may reduce the risk of hepatic steatosis by modulating lipid metabolism, which in turn reduces the risk of obesity and MS. A meta-analysis showed that the prevalence of steatosis was lower in CHB than that in the general population (OR = 0.81; 95%CI: 0.71-0.920)[58]. Another study reported that the prevalence of non-alcoholic fatty liver disease was lower in patients with HBV infection than that in non-infected patients[59]. A clinical study in Taiwan further showed that patients with positive HBsAg possessed lower hypertriglyceridemia (OR = 0.59; 95%CI: 0.52-0.66) and low-density lipoprotein-cholesterol levels (OR = 0.86; 95%CI: 0.79-0.93) than those with negative HBsAg[60]. Considering that steatosis is an essential factor that leads to the progressive impairment of glucose metabolism[61], the role of HBV in regulating hepatic lipid metabolism also contributes to the regulation of glucose metabolic homeostasis. This evidence suggests that HBV infection is associated with a lower risk of obesity and MS, and that the primary mechanism may be the modulation of hepatic fat metabolism, which corroborates our view.

Notably, the risk of T2D increases when CHB progresses to cirrhosis. A meta-analysis of 15 clinical studies showed that the incidence of diabetes was comparable between patients with non-cirrhotic CHB and those with asymptomatic HBV carriers and non-HBV[11]. In contrast, patients with hepatitis B cirrhosis had a significantly increased risk of T2D (OR = 1.99; 95%CI: 1.08-3.65)[11]. Epidemiological studies showed that only 2%-4% of patients infected with HBV each year worldwide will develop compensated cirrhosis, and only 1.5%-4% of compensated cirrhosis will further develop into decompensated cirrhosis[62]. Therefore, most patients with CHB do not have compensated or decompensated cirrhosis, which may explain why most clinical studies have not found an association between CHB and T2D. In summary, CHB is associated with a reduced risk of T2D; however, this association is limited by HBV load and cirrhosis. It weakens or disappears when patients with CHB receive antiviral therapy, and reverses when CHB progresses to cirrhosis.

Few studies have investigated the relationship between T2D and other VH, such as hepatitis A, D, and E. Lin *et al*[63] found that HAV infection was associated with an increased risk of diabetes (OR = 1.13; 95%CI: 1.08-1.18). However, HAV vaccination and successful HAV immunization were not associated with the risk of diabetes; therefore, they concluded that HAV infection was unlikely to cause diabetes[63]. Zitelli *et al*[64] found that among patients with chronic HCV

infection receiving antiviral therapy, the incidence of diabetes was 3.65 times higher in HEV-positive patients than that in HEV-negative patients, suggesting that HEV is a potential risk factor for diabetes mellitus in chronic HCV-infected individuals. In summary, there are insufficient studies elucidating the effects of hepatitis caused by HAV, HDV, and HEV infections on T2D, and this issue needs to be further explored in subsequent studies.

Our study has some limitations. First, the data on CHB and CHC were derived from East Asians; therefore, the results mainly illustrate the effect of CHB and CHC on T2D among East Asians, and it is not yet clear how they affect other races. Second, the GWAS only provided an overall dataset on VH among Europeans with availability; it did not include a dataset of different types of VH. Therefore, the results of this study can only infer that VH is not associated with T2D risk in Europeans, and cannot explain the effects of different types of VH on T2D risk among Europeans. Third, data on HAV, HDV, and HEV were unavailable in the GWAS; therefore, their effects on T2D risk were not assessed. Fourth, our data were derived from the GWAS; therefore, it was impossible to stratify the analysis for populations with different viral loads. Given these limitations, we expect future studies to improve. First, we recommend continuing to promote human genome studies worldwide, and provide more comprehensive data for MR analysis of different races. Second, we recommend conducting stratified randomized controlled trials to explore the specific effects of the different types, stages, and viral loads of VH on T2D.

CONCLUSION

This MR analysis showed that neither VH among Europeans nor CHC among East Asians were associated with T2D risk, whereas CHB was associated with reduced T2D risk among East Asians. Although VH among Europeans and CHC among East Asians are not associated with T2D risk, focusing on blood glucose in patients with CHC is still relevant for the early detection of T2D induced by CHC-mediated pathways of hepatic steatosis, liver fibrosis, and cirrhosis. Further studies are needed to explore the causal relationships and mechanisms between different types of VH and T2D.

ARTICLE HIGHLIGHTS

Research background

The causality between viral hepatitis (VH) and type 2 diabetes (T2D) remains unclear.

Research motivation

In this study, a Mendelian randomization (MR) analysis was applied to determine the causality between VH and T2D from genome-wide association study data.

Research objectives

We used a MR to identify the causality between VH, chronic hepatitis B (CHB), chronic hepatitis C (CHC) and T2D from genome-wide association study data.

Research methods

Two-sample MR was performed to obtain the causality between VH, CHB, CHC and T2D. Summary statistics from the FinnGen were used for VH, BioBank Japan Project was used for CHB and CHC, and the European Bioinformatics Institute and FinnGen were utilized for T2D.

Research results

The MR analysis showed no significant causal relationship between VH and T2D in Europeans [odds ratio (OR) = 1.028; 95% confidence interval (CI): 0.995-1.062, $P = 0.101$] as well as between CHC and T2D in East Asians (OR = 0.949; 95%CI: 0.931-0.968, $P < 0.001$), while there was a negative causal association between CHB and T2D among East Asians (OR = 0.949; 95%CI: 0.931-0.968, $P < 0.001$). These MR analysis results showed no horizontal pleiotropy or heterogeneity ($P > 0.05$), and they were robust.

Research conclusions

Among East Asians, CHB is associated with a reduced T2D risk, but this association is limited by hepatitis B virus (HBV) load and cirrhosis. Although CHC among East Asians are not associated with the risk of T2D, focusing on blood glucose in patients with CHC is still relevant for the early detection of T2D induced by CHC-mediated pathways of hepatic steatosis, liver fibrosis, and cirrhosis.

Research perspectives

Whether different categories of VH, especially CHB and CHC, are associated with the risk of T2D remains controversial. CHB is associated with a reduced T2D risk among East Asians, but this association is limited by HBV load and cirrhosis. Although VH among Europeans and CHC among East Asians are not associated with T2D risk, focusing on blood glucose in patients with CHC is still relevant for the early detection of T2D induced by CHC-mediated pathways of hepatic steatosis, liver fibrosis, and cirrhosis.

FOOTNOTES

Co-first authors: Yun-Feng Yu and Gang Hu.

Author contributions: Yu YF conceived and designed the study; Yu YF and Hu G participated in data processing and statistical analysis; Yu YF, Hu G, Yang XY, and Wu JY drafted the manuscript; Hu G, Yang XY, Wu JY, and Yu R contributed to data analysis and interpretation; Yu YF, Tong KK, Yang XY, and Yu R supervised the review of the study; and all authors seriously revised and approved the final manuscript.

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Institutional review board statement: Despite our study was an original research, this work just used genome-wide association studies statistics from public available databases for Mendelian randomization analysis, and we did not collect any new human data. As this study did not involve any human studies and/or animal experiments, the institutional review board approval was not required for our research.

Informed consent statement: Despite our study was an original research, this work just used genome-wide association studies statistics from public available databases for Mendelian randomization analysis, and we did not collect any new human data. As this study did not involve any human studies and/or animal experiments, the informed consent form was not required for our research.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: All GWAS data that support the findings of this study are openly available in the BioBank Japan Project (https://biobankjp.org/en/), European Bioinformatics Institute (https://www.ebi.ac.uk), and FinnGen (www.finngen.fi/fi).

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REFERENCES

1 **Sheng CY**, Son YH, Jang J, Park SJ. In vitro skeletal muscle models for type 2 diabetes. *Biophys Rev (Melville)* 2022; **3**: 031306 [PMID: 36124295 DOI: 10.1063/5.0096420]
2 **Majety P**, Lozada Orquera FA, Edem D, Hamdy O. Pharmacological approaches to the prevention of type 2 diabetes mellitus. *Front Endocrinol (Lausanne)* 2023; **14**: 1118848 [PMID: 36967777 DOI: 10.3389/fendo.2023.1118848]
3 **Cho NH**, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, Malanda B. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res Clin Pract* 2018; **138**: 271-281 [PMID: 29496507 DOI: 10.1016/j.diabres.2018.02.023]
4 **Zhang Y**, Zhou H. Hyper-reactive platelets and type 2 diabetes. *Zhong Nan Da Xue Xue Bao Yi Xue Ban* 2022; **47**: 374-383 [PMID: 35545331 DOI: 10.11817/j.issn.1672-7347.2022.210271]
5 **Afsar B**, Elsurer R. Increased renal resistive index in type 2 diabetes: Clinical relevance, mechanisms and future directions. *Diabetes Metab Syndr* 2017; **11**: 291-296 [PMID: 27594114 DOI: 10.1016/j.dsx.2016.08.019]
6 **Calles-Escandon J**, Garcia-Rubi E, Mirza S, Mortensen A. Type 2 diabetes: one disease, multiple cardiovascular risk factors. *Coron Artery Dis* 1999; **10**: 23-30 [PMID: 10196684]
7 **Park KS**. Prevention of type 2 diabetes mellitus from the viewpoint of genetics. *Diabetes Res Clin Pract* 2004; **66** Suppl 1: S33-S35 [PMID: 15563977 DOI: 10.1016/j.diabres.2003.11.023]
8 **Pfohl M**, Schatz H. Strategies for the prevention of type 2 diabetes. *Exp Clin Endocrinol Diabetes* 2001; **109** Suppl 2: S240-S249 [PMID: 11460574 DOI: 10.1055/s-2001-18585]
9 **Alzahrani N**. Hepatitis C virus, insulin resistance, and diabetes: A review. *Microbiol Immunol* 2022; **66**: 453-459 [PMID: 35941761 DOI: 10.1111/1348-0421.13023]
10 **Ciardullo S**, Mantovani A, Ciaccio A, Carbone M, Invernizzi P, Perseghin G. Hepatitis C virus infection and diabetes: A complex bidirectional relationship. *Diabetes Res Clin Pract* 2022; **187**: 109870 [PMID: 35398458 DOI: 10.1016/j.diabres.2022.109870]
11 **Zhang J**, Shen Y, Cai H, Liu YM, Qin G. Hepatitis B virus infection status and risk of type 2 diabetes mellitus: A meta-analysis. *Hepatol Res* 2015; **45**: 1100-1109 [PMID: 25601609 DOI: 10.1111/hepr.12481]
12 **Nagra N**, Kozarek RA, Burman BE. Therapeutic Advances in Viral Hepatitis A-E. *Adv Ther* 2022; **39**: 1524-1552 [PMID: 35220557 DOI: 10.1007/s12325-022-02070-z]

13 **Liou JW**, Mani H, Yen JH. Viral Hepatitis, Cholesterol Metabolism, and Cholesterol-Lowering Natural Compounds. *Int J Mol Sci* 2022; **23** [PMID: 35409259 DOI: 10.3390/ijms23073897]

14 **Farci P**, Niro GA, Zamboni F, Diaz G. Hepatitis D Virus and Hepatocellular Carcinoma. *Viruses* 2021; **13** [PMID: 34064419 DOI: 10.3390/v13050830]

15 **Lanini S**, Ustianowski A, Pisapia R, Zumla A, Ippolito G. Viral Hepatitis: Etiology, Epidemiology, Transmission, Diagnostics, Treatment, and Prevention. *Infect Dis Clin North Am* 2019; **33**: 1045-1062 [PMID: 31668190 DOI: 10.1016/j.idc.2019.08.004]

16 **Leoni S**, Casabianca A, Biagioni B, Serio I. Viral hepatitis: Innovations and expectations. *World J Gastroenterol* 2022; **28**: 517-531 [PMID: 35316960 DOI: 10.3748/wjg.v28.i5.517]

17 **Mehta SH**, Brancati FL, Strathdee SA, Pankow JS, Netski D, Coresh J, Szklo M, Thomas DL. Hepatitis C virus infection and incident type 2 diabetes. *Hepatology* 2003; **38**: 50-56 [PMID: 12829986 DOI: 10.1053/jhep.2003.50291]

18 **Bernsmeier C**, Calabrese D, Heim MH, Duong HT. Hepatitis C virus dysregulates glucose homeostasis by a dual mechanism involving induction of PGC1α and dephosphorylation of FoxO1. *J Viral Hepat* 2014; **21**: 9-18 [PMID: 24329853 DOI: 10.1111/jvh.12208]

19 **Shen Y**, Zhang S, Wang X, Wang Y, Zhang J, Qin G, Li W, Ding K, Zhang L, Liang F. Comparison of type 2 diabetes mellitus incidence in different phases of hepatitis B virus infection: A meta-analysis. *Liver Int* 2017; **37**: 1451-1460 [PMID: 27753241 DOI: 10.1111/liv.13275]

20 **Ruhl CE**, Menke A, Cowie CC, Everhart JE. Relationship of hepatitis C virus infection with diabetes in the U.S. population. *Hepatology* 2014; **60**: 1139-1149 [PMID: 24500979 DOI: 10.1002/hep.27047]

21 **Gao RC**, Sang N, Jia CZ, Zhang MY, Li BH, Wei M, Wu GC. Association Between Sleep Traits and Rheumatoid Arthritis: A Mendelian Randomization Study. *Front Public Health* 2022; **10**: 940161 [PMID: 35844889 DOI: 10.3389/fpubh.2022.940161]

22 **Julian TH**, Boddy S, Islam M, Kurz J, Whittaker KJ, Moll T, Harvey C, Zhang S, Snyder MP, McDermott C, Cooper-Knock J, Shaw PJ. A review of Mendelian randomization in amyotrophic lateral sclerosis. *Brain* 2022; **145**: 832-842 [PMID: 34791088 DOI: 10.1093/brain/awab420]

23 **Ference BA**, Holmes MV, Smith GD. Using Mendelian Randomization to Improve the Design of Randomized Trials. *Cold Spring Harb Perspect Med* 2021; **11** [PMID: 33431510 DOI: 10.1101/cshperspect.a040980]

24 **Davies NM**, Holmes MV, Davey Smith G. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. *BMJ* 2018; **362**: k601 [PMID: 30002074 DOI: 10.1136/bmj.k601]

25 **Burgess S**, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. *Genet Epidemiol* 2013; **37**: 658-665 [PMID: 24114802 DOI: 10.1002/gepi.21758]

26 **1000 Genomes Project Consortium**, Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, Korbel JO, Marchini JL, McCarthy S, McVean GA, Abecasis GR. A global reference for human genetic variation. *Nature* 2015; **526**: 68-74 [PMID: 26432245 DOI: 10.1038/nature15393]

27 **Bender D**, Glitscher M, Hildt E. [Viral hepatitis A to E: prevalence, pathogen characteristics, and pathogenesis]. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz* 2022; **65**: 139-148 [PMID: 34932130 DOI: 10.1007/s00103-021-03472-0]

28 **Kukla M**, Piotrowski D, Waluga M, Hartleb M. Insulin resistance and its consequences in chronic hepatitis C. *Clin Exp Hepatol* 2015; **1**: 17-29 [PMID: 28856251 DOI: 10.5114/ceh.2015.51375]

29 **Wu D**. Correlation of viral load of Hepatitis B with the gestation period and the development of diabetes mellitus. *Saudi J Biol Sci* 2019; **26**: 2022-2025 [PMID: 31889788 DOI: 10.1016/j.sjbs.2019.08.009]

30 **Huang ZS**, Huang TS, Wu TH, Chen MF, Hsu CS, Kao JH. Asymptomatic chronic hepatitis B virus infection does not increase the risk of diabetes mellitus: a ten-year observation. *J Gastroenterol Hepatol* 2010; **25**: 1420-1425 [PMID: 20659233 DOI: 10.1111/j.1440-1746.2010.06268.x]

31 **Eshraghian K**, Lankarani KB, Fattahi MR, Esmailnejad A, Peymani P. Low Prevalence of Insulin Resistance Among Iranian Patients with Chronic Hepatitis C Virus Infection: A Case-Control Study. *Curr Diabetes Rev* 2018; **14**: 446-450 [PMID: 28714382 DOI: 10.2174/1573399813666170714164446]

32 **Gualerzi A**, Bellan M, Smirne C, Tran Minh M, Rigamonti C, Burlone ME, Bonometti R, Bianco S, Re A, Favretto S, Bellomo G, Minisini R, Carnevale Schianca GP, Pirisi M. Improvement of insulin sensitivity in diabetic and non diabetic patients with chronic hepatitis C treated with direct antiviral agents. *PLoS One* 2018; **13**: e0209216 [PMID: 30571711 DOI: 10.1371/journal.pone.0209216]

33 **Wang CS**, Wang ST, Yao WJ, Chang TT, Chou P. Community-based study of hepatitis C virus infection and type 2 diabetes: an association affected by age and hepatitis severity status. *Am J Epidemiol* 2003; **158**: 1154-1160 [PMID: 14652300 DOI: 10.1093/aje/kwg259]

34 **Fabiani S**, Fallahi P, Ferrari SM, Miccoli M, Antonelli A. Hepatitis C virus infection and development of type 2 diabetes mellitus: Systematic review and meta-analysis of the literature. *Rev Endocr Metab Disord* 2018; **19**: 405-420 [PMID: 29322398 DOI: 10.1007/s11154-017-9440-1]

35 **Drazilova S**, Janicko M, Skladany L, Kristian P, Oltman M, Szantova M, Krkoska D, Mazuchova E, Piesecka L, Vahalova V, Rac M, Schreter I, Virag L, Koller T, Liptakova A, Ondrasova M, Jarcuska P. Glucose Metabolism Changes in Patients with Chronic Hepatitis C Treated with Direct Acting Antivirals. *Can J Gastroenterol Hepatol* 2018; **2018**: 6095097 [PMID: 30402450 DOI: 10.1155/2018/6095097]

36 **Memon MS**, Arain ZI, Naz F, Zaki M, Kumar S, Burney AA. Prevalence of type 2 diabetes mellitus in hepatitis C virus infected population: a Southeast Asian study. *J Diabetes Res* 2013; **2013**: 539361 [PMID: 23984431 DOI: 10.1155/2013/539361]

37 **Zhao P**, Wang JB, Jiao J. [Investigation on the incidence of diabetes in chronic hepatitis C patients and their HCV genotypes]. *Zhonghua Gan Zang Bing Za Zhi* 2006; **14**: 86-88 [PMID: 16494773]

38 **Gilad A**, Fricker ZP, Hsieh A, Thomas DD, Zahorian T, Nunes DP. Sustained Improvement in Type 2 Diabetes Mellitus is Common After Treatment of Hepatitis C Virus With Direct-acting Antiviral Therapy. *J Clin Gastroenterol* 2019; **53**: 616-620 [PMID: 30614943 DOI: 10.1097/MCG.0000000000001168]

39 **Hussein HA**, Allam AS, Moaty ASA. Evaluation of Glycated Haemoglobin (HbA1c) Level in Type 2 Diabetic Chronic HCV Non-cirrhotic Treatment-Naïve Egyptian Patients Eradicated with Sofosbuvir Plus Daclatasvir. *Curr Diabetes Rev* 2020; **16**: 165-170 [PMID: 31146663 DOI: 10.2174/1573399815666190531091128]

40 **Shiffman ML**, Gunn NT. Impact of hepatitis C virus therapy on metabolism and public health. *Liver Int* 2017; **37** Suppl 1: 13-18 [PMID: 28052632 DOI: 10.1111/liv.13282]

41 **Sacco M**, Saracco GM. The impact of direct-acting antiviral treatment on glycemic homeostasis in patients with chronic hepatitis C. *Minerva Gastroenterol (Torino)* 2021; **67**: 264-272 [PMID: 33856147 DOI: 10.23736/S2724-5985.21.02835-X]

42 **Ribaldone DG**, Sacco M, Saracco GM. The Effect of Viral Clearance Achieved by Direct-Acting Antiviral Agents on Hepatitis C Virus Positive Patients with Type 2 Diabetes Mellitus: A Word of Caution after the Initial Enthusiasm. *J Clin Med* 2020; **9** [PMID: 32092892 DOI: 10.3390/jcm9020563]

43 **Mishra PR**, Bharti A, Arora R, Mir IA, Punia VPS. Increased Insulin Resistance in Hepatitis-C Infection-Association with Altered Hepatic Function Testing. *Pathophysiology* 2022; **29**: 326-332 [PMID: 35893594 DOI: 10.3390/pathophysiology29030024]

44 **Papatheodoridis GV**, Chrysanthos N, Savvas S, Sevastianos V, Kafiri G, Petraki K, Manesis EK. Diabetes mellitus in chronic hepatitis B and C: prevalence and potential association with the extent of liver fibrosis. *J Viral Hepat* 2006; **13**: 303-310 [PMID: 16637860 DOI: 10.1111/j.1365-2893.2005.00677.x]

45 **Younossi ZM**, McCullough AJ, Ong JP, Barnes DS, Post A, Tavill A, Bringman D, Martin LM, Assmann J, Gramlich T, Mullen KD, O'Shea R, Carey WD, Ferguson R. Obesity and non-alcoholic fatty liver disease in chronic hepatitis C. *J Clin Gastroenterol* 2004; **38**: 705-709 [PMID: 15319656 DOI: 10.1097/01.mcg.0000135372.10846.2a]

46 **Tsao YC**, Chen JY, Yeh WC, Peng YS, Li WC. Association between visceral obesity and hepatitis C infection stratified by gender: a cross-sectional study in Taiwan. *BMJ Open* 2017; **7**: e017117 [PMID: 29133317 DOI: 10.1136/bmjopen-2017-017117]

47 **Huang JF**, Chuang WL, Yu ML, Yu SH, Huang CF, Huang CI, Yeh ML, Hsieh MH, Yang JF, Lin ZY, Chen SC, Dai CY, Chang WY. Hepatitis C virus infection and metabolic syndrome---a community-based study in an endemic area of Taiwan. *Kaohsiung J Med Sci* 2009; **25**: 299-305 [PMID: 19560994 DOI: 10.1016/S1607-551X(09)70520-0]

48 **Del Campo JA**, Romero-Gómez M. Steatosis and insulin resistance in hepatitis C: a way out for the virus? *World J Gastroenterol* 2009; **15**: 5014-5019 [PMID: 19859993 DOI: 10.3748/wjg.15.5014]

49 **Sheikh MY**, Choi J, Qadri I, Friedman JE, Sanyal AJ. Hepatitis C virus infection: molecular pathways to metabolic syndrome. *Hepatology* 2008; **47**: 2127-2133 [PMID: 18446789 DOI: 10.1002/hep.22269]

50 **Lecube A**, Hernández C, Genescà J, Simó R. Proinflammatory cytokines, insulin resistance, and insulin secretion in chronic hepatitis C patients: A case-control study. *Diabetes Care* 2006; **29**: 1096-1101 [PMID: 16644643 DOI: 10.2337/diacare.2951096]

51 **Huang JF**, Dai CY, Hwang SJ, Ho CK, Hsiao PJ, Hsieh MY, Lee LP, Lin ZY, Chen SC, Wang LY, Shin SJ, Chang WY, Chuang WL, Yu ML. Hepatitis C viremia increases the association with type 2 diabetes mellitus in a hepatitis B and C endemic area: an epidemiological link with virological implication. *Am J Gastroenterol* 2007; **102**: 1237-1243 [PMID: 17531012 DOI: 10.1111/j.1572-0241.2007.01181.x]

52 **Liu Y**, Jiang C, Hao Y, Xu L, Zhang W, Jin YL, Zhu T, Lam TH. Association of hepatitis B surface antigen seropositivity and hepatitis B surface antibody seropositivity with diabetes: a cross-sectional study based on two Chinese populations in Guangdong, China. *BMJ Open* 2020; **10**: e028968 [PMID: 32690726 DOI: 10.1136/bmjopen-2019-028968]

53 **Liu XY**, Zhou Y. Influence of hepatitis B virus on the prevalence of diabetes complications in patients with type 2 diabetes. *J Diabetes Investig* 2023; **14**: 429-434 [PMID: 36510700 DOI: 10.1111/jdi.13954]

54 **Li M**, Zhou H, Guan Y, Peng H, Wang S, Zhang P, Su B. Positive hepatitis B surface antibody is associated with reduced risk of diabetes mellitus in retired female Chinese workers. *J Diabetes* 2016; **8**: 158-161 [PMID: 26016384 DOI: 10.1111/1753-0407.12317]

55 **Yu MW**, Lin CL, Liu CJ, Huang YW, Hu JT, Wu WJ, Wu CF. Hepatic steatosis and development of type 2 diabetes: Impact of chronic hepatitis B and viral specific factors. *J Formos Med Assoc* 2022; **121**: 1478-1487 [PMID: 34764005 DOI: 10.1016/j.jfma.2021.10.014]

56 **Yan LB**, Liao J, Han N, Zhou LY, Wang XE, Wang YJ, Tang H. Association between Hepatitis B Virus Infection and Metabolic Syndrome in Southwest China: A Cross-sectional Study. *Sci Rep* 2020; **10**: 6738 [PMID: 32317690 DOI: 10.1038/s41598-020-62609-4]

57 **Chiang CH**, Yang HI, Jen CL, Lu SN, Wang LY, You SL, Su J, Iloeje UH, Chen CJ; REVEAL-HBV Study Group. Association between obesity, hypertriglyceridemia and low hepatitis B viral load. *Int J Obes (Lond)* 2013; **37**: 410-415 [PMID: 22531094 DOI: 10.1038/ijo.2012.63]

58 **Shi YW**, Yang RX, Fan JG. Chronic hepatitis B infection with concomitant hepatic steatosis: Current evidence and opinion. *World J Gastroenterol* 2021; **27**: 3971-3983 [PMID: 34326608 DOI: 10.3748/wjg.v27.i26.3971]

59 **Huang J**, Jing M, Wang C, Wang M, You S, Lin S, Zhu Y. The impact of hepatitis B virus infection status on the prevalence of nonalcoholic fatty liver disease: A population-based study. *J Med Virol* 2020; **92**: 1191-1197 [PMID: 31691993 DOI: 10.1002/jmv.25621]

60 **Huang CY**, Lu CW, Liu YL, Chiang CH, Lee LT, Huang KC. Relationship between chronic hepatitis B and metabolic syndrome: A structural equation modeling approach. *Obesity (Silver Spring)* 2016; **24**: 483-489 [PMID: 26719030 DOI: 10.1002/oby.21333]

61 **Kang SK**, Chung TW, Lee JY, Lee YC, Morton RE, Kim CH. The hepatitis B virus X protein inhibits secretion of apolipoprotein B by enhancing the expression of N-acetylglucosaminyltransferase III. *J Biol Chem* 2004; **279**: 28106-28112 [PMID: 15123606 DOI: 10.1074/jbc.M403176200]

62 **Zhao H**, Wang Q, Luo C, Liu L, Xie W. Recompensation of Decompensated Hepatitis B Cirrhosis: Current Status and Challenges. *Biomed Res Int* 2020; **2020**: 9609731 [PMID: 33029534 DOI: 10.1155/2020/9609731]

63 **Lin J**, Ou HY, Karnchanasorn R, Samoa R, Chuang LM, Chiu KC. Role of hepatitis A virus in diabetes mellitus. *World J Diabetes* 2021; **12**: 1928-1941 [PMID: 34888017 DOI: 10.4239/wjd.v12.i11.1928]

64 **Zitelli PMY**, Gomes-Gouvêa M, Mazo DF, Singer JDM, Oliveira CPMS, Farias AQ, Pinho JR, Tanigawa RY, Alves VAF, Carrilho FJ, Pessoa MG. Hepatitis E virus infection increases the risk of diabetes and severity of liver disease in patients with chronic hepatitis C virus infection. *Clinics (Sao Paulo)* 2021; **76**: e3270 [PMID: 34852140 DOI: 10.6061/clinics/2021/e3270]

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ORIGINAL ARTICLE

Observational Study

Serum tumor markers expression (CA199, CA242, and CEA) and its clinical implications in type 2 diabetes mellitus

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Abstract

BACKGROUND

Glucose and lipid metabolic disorder in patients with type 2 diabetes mellitus (T2DM) is associated with the levels of serum tumor markers of the digestive tract, such as cancer antigen (CA)199. Therefore, tumor markers in T2DM are important.

AIM

To evaluate the expression of serum tumor markers [CA199, CA242, and carcinoembryonic antigen (CEA)] and the clinical implications of the expression in T2DM.

METHODS

For this observational study conducted at Hefei BOE Hospital, China, we enrolled 82 patients with first-onset T2DM and 51 controls between April 2019 and December 2020. Levels of fasting blood glucose (FBG), tumor markers (CA199, CEA, and CA242), glycosylated hemoglobin (HbA1c), *etc*. were measured and group index levels were compared. Moreover, FBG and HbA1c levels were correlated with tumor marker levels. Tumor markers were tested for diagnostic accuracy in patients with > 9% HbA1c using the receiver operating curve (ROC) curve.

RESULTS

The T2DM group had high serum FBG, HbA1c, CA199, and CEA levels ($P < 0.05$). A comparative analysis of the two groups based on HbA1c levels (Group A: HbA1c ≤ 9%; Group B: HbA1c > 9%) revealed significant differences in CEA and CA199 levels ($P < 0.05$). The areas under the ROC curve for CEA and CA199 were 0.853 and 0.809, respectively. CA199, CEA, and CA242 levels positively correlated

with HbA1c (r = 0.308, 0.426, and 0.551, respectively) and FBG levels (r = 0.236, 0.231, and 0.298, respectively).

CONCLUSION

As compared to controls, serum CEA and CA199 levels were higher in patients with T2DM. HbA1c and FBG levels correlated with CA199, CEA, and CA242 levels. Patients with poorly controlled blood sugar must be screened for tumor markers.

Key Words: Type 2 diabetes mellitus; Carcinoembryonic antigen; Cancer antigen 199; Cancer antigen 242; Glycosylated hemoglobin

Core Tip: Levels of serum cancer antigen (CA)199, carcinoembryonic antigen (CEA), and CA242 demonstrated close association with glycosylated hemoglobin (HbA1c) and fasting blood glucose levels in patients with type 2 diabetes mellitus. Furthermore, CA199 and CEA levels had good predictive power for HbA1c levels. These findings suggest the need for monitoring tumor marker changes in those with poorly controlled blood sugar levels.

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INTRODUCTION

In China, an aging population and lifestyle changes have transformed diabetes from a rare disease to an epidemic over the past four decades. The global number of individuals aged ≥ 18 years affected by diabetes is projected to increase from 425 million in 2017 to 629 million in 2045, with type 2 diabetes mellitus (T2DM) accounting for > 90% of the diabetic population[1]. Long-term hyperglycemia in patients with T2DM can cause oxidative stress-, inflammation-, and vascular endothelial function-related damage. Recent studies have highlighted the association between diabetes and cancer, demonstrating that patients with T2DM are significantly more likely to develop malignant tumors than the general population[2]. Patients with tumors may experience significant changes in the blood sugar levels during therapy. Moreover, diabetes can cause levels of specific serum tumor markers to spike. Although carcinoembryonic antigen (CEA), cancer antigen (CA)199, and CA242 are used to diagnose tumors[3], the correlation between their expression levels and blood glucose levels in patients with T2DM remains unknown.

Considering these findings, the precise relationship between the levels of tumor markers (CEA, CA199, and CA242) and T2DM needs a thorough investigation. This study aimed to address the overarching question: "What is the relationship between the expression levels and clinical significance of serum tumor markers (CEA, CA199, and CA242) in patients with T2DM?" Addressing this question is crucial for enhancing early tumor screening and improving prognostic evaluation, potentially contributing to improved clinical outcomes and management strategies for patients with T2DM and comorbid cancer conditions.

MATERIALS AND METHODS

Sample size calculation

The sample size calculation for this study was based on the anticipated difference in tumor marker levels (CA199, CA242, and CEA) between patients with T2DM and the control group. Assuming a medium effect size (d = 0.5), a significance level (α) of 0.05, and a desired power of 80%, the sample size was estimated using the G*Power software. Based on these parameters, ≥ 46 participants were needed in each group. Assuming a 10% loss of data or exclusion, a minimum of 51 participants in each group was deemed necessary. Finally, 82 patients with T2DM and 51 controls were enrolled in this study.

The inclusion criteria set for this study were as follows: (1) Age ≥ 18 years; (2) patients who met the T2DM diagnostic criteria established by the guidelines for the prevention and treatment of type 2 diabetes in China (2020 Edition) formulated by the diabetes branch of the Chinese Medical Association; these included newly diagnosed patients and previously diagnosed patients with poor blood glucose control; and (3) those who or whose families provided informed consent. The exclusion criteria were as follows: (1) Patients with heart, liver, kidney and lung dysfunction, acute diabetic complications, infectious diseases, autoimmune diseases, acute and chronic inflammatory reactions, and malignant tumors; (2) patients on long-term glucocorticoid therapy, given the effect of these medications on blood sugar and lipid levels; (3) pregnant or lactating women; (4) patients with acute and chronic pancreatitis, liver cirrhosis, hepatitis, colitis,

gallstones, and obstructive jaundice, given that these conditions can cause benign elevation of serum CA199 or CEA levels; and (5) patients with incomplete clinical information or inaccurate data.

General information

We recruited 82 patients (47 men) with T2DM from BOE Technology Hospital in Hefei between April 2019 and December 2020. All patients were diagnosed with diabetes according to the 1999 World Health Organization diagnostic criteria. During the same period, 51 individuals (27 men) who underwent health examinations at our hospital's health examination center were selected as the control group. The median age was 59.5 (26–81) years in the T2DM group and 46 (27–68) years in the control group. Table 1, summarizing the general characteristics of the two groups indicates no significant inter-group differences. The exclusion criteria for the control group were as follows: (1) Individuals with type 1 diabetes, acute metabolic disorders associated with diabetes (such as ketoacidosis and hyperosmolar state), acute stroke, acute and chronic infections, thyroid disease, and cardiac insufficiency; (2) those with severe liver and kidney dysfunction; (3) those with acute and chronic hepatitis, alcoholic liver disease, cirrhosis, gallstone, pancreatitis, cholecystitis, and other digestive system diseases; (4) those with tumors; and (5) pregnant women. The study protocol was approved by the Medical Ethics Committee and participants provided written informed consent.

Methods

Upon admission, the body mass index (BMI) was calculated by measuring the patient height and weight and collecting venous blood after an overnight fast. The levels of alanine transaminase (ALT), aspartate transaminase (AST), creatinine, serum uric acid, fasting blood glucose (FBG), triglycerides, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and serum tumor markers CEA, CA199, and CA242 were determined using the Roche Cobas8000 biochemical immune assembly line and the corresponding test kits. The methods for measuring the parameters were as follows: FBG, hexokinase; ALT, IFCC; AST, colorimetric; serum uric acid, colorimetric; creatinine-enzyme; triglycerides, colorimetric; TC, enzyme colorimetric; LDL-C, selective clearance; CEA (normal value < 6.5 ng/mL) and CA199 (normal value < 35 U/mL), electrochemical luminescence; and CA242 (normal value < 20 U/mL), chemiluminescence immunoassay. Glycosylated hemoglobin (HbA1c) levels were measured using a Dongcao G8 glycated hemoglobin instrument and the corresponding detection kit. All patients underwent routine abdominal ultrasonography and chest imaging [radiograph/computed tomography (CT)]. Further examinations were performed for patients with suspected tumors including CT, magnetic resonance imaging, and gastroscopy.

Statistical analysis

Statistical analyses were performed using SPSS 19.0 software. For normally distributed quantitative data, *t*-tests were used for comparisons, and the data are presented as mean ± SD. The Mann–Whitney rank-sum test and Spearman correlation analysis were used for skewed distribution data. A binary logistic regression analysis was conducted using FBG, HbA1c, CEA, CA199, and CA242 as independent variables to assess their predictive value for the occurrence of T2DM. The receiver operating curve (ROC) analysis was performed for variables with significant differences. Statistical significance was set at $P < 0.05$.

RESULTS

Inter-group comparison of general characteristics

The results revealed no significant differences in the levels of liver and kidney function indicators, lipid metabolism-related indicators (AST, ALT, uric acid, creatinine, BMI, LDL-C, and TC), age, and sex distribution ($P > 0.05$). However, HbA1c and FBG levels were higher in the T2DM group compared to the control group ($P < 0.05$; Table 1).

Inter-group comparison of CEA, CA199, and CA242 levels

CEA and CA199 levels were significantly higher in the T2DM group than in the control group ($P < 0.001$). Although CA242 levels were also elevated in the T2DM group, the difference was statistically insignificant ($P = 0.068$; Table 2).

Logistic regression to analyze risk factors for T2DM

We investigated the association between T2DM incidence as the dependent variable and the following independent variables: FBG, HbA1C, CEA, CA199, and CA242 using a binary logistic regression analysis. T2DM occurrence was categorized as 0 (did not occur) and 1 (occurred). The results were optimized using a stepwise backward elimination method. Our findings indicated FBG [odds ratio (OR) = 43.173, 95% confidence interval (95%CI): 1.513–6.658], HbA1C (OR = 4.560, 95%CI: 1.914–10.863), CEA (OR = 1.366, 95%CI: 1.024–1.822), and CA199 (OR = 1.035, 95%CI: 1.013–1.057) as independent risk factors for the onset of T2DM, all with *P* values < 0.05 (Table 3).

Comparison of general clinical characteristics and tumor markers among patients with diabetes with varying HbA1c percentages

Based on an HbA1c threshold value of 9%, patients with diabetes were divided into two groups: Groups A (HbA1c ≤ 9%) and B (HbA1c > 9%). Age, liver and kidney function, and lipid metabolism were compared between the two groups. The results indicated no statistical differences in age, sex, disease course, liver and kidney function, or lipid metabolism-related indicators between the two groups ($P > 0.05$). However, group B had higher serum uric acid, FBG, CEA, and

Table 1 General characteristics was compared between two groups

Variables	Control group (*n* = 51)	T2DM group (*n* = 82)	χ^2/*Z*/*t* value	*P* value
Gender (male/female)	27/24	47/35	0.244	0.621
Age (yr)	57.98 ± 11.72	59.02 ± 11.58	0.503	0.616
ALT (U/L)	18.40 (13.20, 28.30)	18.75 (13.48, 30.93)	0.558	0.577
AST (U/L)	18.80 (11.80, 30.10)	16.25 (12.48, 24.80)	0.694	0.488
SUA (mmol/L)	312.51 ± 119.36	306.20 ± 102.97	0.323	0.747
Cre (mmol/L)	68.36 ± 27.54	70.16 ± 28.67	0.357	0.721
HbA1c (%)	5.30 (4.30, 6.60)	9.30 (8.18, 11.13)	9.013	0
FBG (mmol/L)	4.46 ± 0.89	10.08 ± 4.30	9.199	0
BMI (kg/m^2)	25.15 ± 4.28BMI	25.55 ± 3.40	0.602	0.548
LDL-C (mmol/L)	2.30 (1.90, 3.30)	2.34 (1.94, 3.23)	0.201	0.84
TG (mmol/L)	1.40 (1.10, 2.30)	1.81 (1.09, 2.68)	1.581	0.144
TC (mmol/L)	4.45 ± 1.39	4.46 ± 1.29	0.049	0.961

T2DM: Type 2 diabetes mellitus; ALT: Alanine transaminase; AST: Aspartate transaminase; SUA: Serum uric acid; Cre: Creatinine; HbA1c: Glycosylated hemoglobin; FBG: Fasting blood sugar; BMI: Body mass index; LDL-C: Low-density lipoprotein cholesterol; TG: Triglycerides; TC: Total cholesterol.

Table 2 Comparison of carcinoembryonic antigen, cancer antigen 199, cancer antigen 242 between two groups

Variables	Control group (*n* = 51)	T2DM group (*n* = 82)	*Z* value	*P* value
CEA	2.10 (1.40, 2.70)	2.70 (1.90, 3.65)	3.279	0.000
CA199	7.60 (4.40, 10.10)	11.30 (5.57, 22.13)	3.976	0.000
CA242	6.10 (3.10, 6.90)	6.25 (4.13, 9.20)	0.891	0.373

CA: Cancer antigen; CEA: Carcinoembryonic antigen.

Table 3 Multivariate logistic regression analysis of factors associated with the onset of type 2 diabetes mellitus

Factors	*β* value	SE	Wald	*P* value	OR	95%CI
FBG	1.155	0.378	9.330	0.002	3.173	1.513-6.658
HBA1C	1.517	0.443	11.739	0.001	4.560	1.914-10.863
CEA	0.312	0.147	4.505	0.034	1.366	1.024-1.822
CA199	0.034	0.011	9.554	0.002	1.035	1.013-1.057
CA242	0.145	0.115	1.585	0.208	1.156	0.923-1.448

FBG: Fasting blood sugar; HbA1c: Glycosylated hemoglobin; CA: Cancer antigen; CEA: Carcinoembryonic antigen; OR: Odds ratio; 95%CI: 95% confidence interval.

CA199 levels than group A ($P < 0.05$; Table 4).

ROCs of CEA and CA199

The area under the ROC curve (AUC) was calculated for both CEA and CA199 markers. For CEA, the AUC (95%CI) was identified to be 0.853 (0.774–0.933, $P < 0.001$; Figure 1). For CA199, the AUC (95%CI) was identified to be 0.809 (0.709–0.909, $P < 0.001$; Figure 1).

Correlation analysis of CEA, CA199, and CA242 levels with HbA1c, FBG, and lipid metabolism in patients with diabetes

The results demonstrated a positive correlation between serum CA199, CEA, and CA242 levels and HbA1c levels with

Table 4 Comparison of general clinical characteristics and tumor marks of different glycosylated hemoglobin % diabetes patients

Variables	Group A (HbA1c ≤ 9%) (*n* = 37)	Group B (HbA1c > 9%) (*n* = 45)	*t*/*Z* value	*P* value
Age (yr)	60.08 ± 9.58	58.16 ± 13.05	0.747	0.457
Gender (male/female)	21/16	26/19	0.009	0.926
Course of disease (yr)	6 (3.00, 10.50)	9 (4, 15.00)	1.627	0.104
BMI (kg/m^2)	26.09 ± 2.66	25.11 ± 3.88	1.349	0.181
FBG (mmol/L)	8.41 ± 2.79	11.46 ± 4.84	3.386	0.001
LDL-C (mmol/L)	2.33 (1.93, 3.02)	2.38 (1.94, 3.32)	0.680	0.496
TG (mmol/L)	2.00 (1.23, 3.01)	1.57 (0.96, 2.61)	1.142	0.254
TC (mmol/L)	4.54 ± 1.02	4.40 ± 1.48	0.494	0.623
Cre (umol/L)	71.07 ± 19.33	69.40 ± 34.72	0.261	0.795
SUA (umol/L)	333.64 ± 99.56	283.64 ± 101.27	2.242	0.028
ALT (U/L)	17.30 (12.65, 28.75)	19.90 (14.05, 31.45)	0.778	0.437
CEA (ng/mL)	1.90 (1.20, 2.60)	3.40 (2.60, 5.25)	5.488	0.000
CA199 (U/mL)	7.60 (4.15, 10.60)	21.00 (11.85, 26.85)	4.795	0.000
CA242 (U/mL)	5.90 (3.85, 7.15)	6.50 (4.80, 9.30)	1.622	0.105

HbA1c: Glycosylated hemoglobin; BMI: Body mass index; FBG: Fasting blood sugar; LDL-C: Low-density lipoprotein cholesterol; TG: Triglycerides; TC: Total cholesterol; Cre: Creatinine; SUA: Serum uric acid; ALT: Alanine transaminase; CA: Cancer antigen; CEA: Carcinoembryonic antigen.

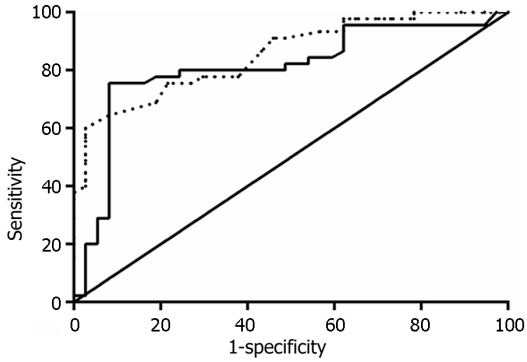

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Figure 1 The receiver operating curve for carcinoembryonic antigen and cancer antigen 199. CA: Cancer antigen; CEA: Carcinoembryonic antigen.

correlation coefficients of 0.308, 0.426, and 0.551, respectively ($P < 0.001$; Table 5) and FBG with correlation coefficients of 0.236, 0.231, and 0.298, respectively ($P < 0.05$; Table 5).

DISCUSSION

Epidemiological studies have demonstrated that the risk of certain malignancies, including hepatoma, hepatocellular carcinoma, colorectal cancer, and bladder cancer, is high in patients with T2DM[4,5]. This relationship may be attributed to long-term elevated blood glucose levels, insulin resistance, or changes in insulin-like growth factors, although the specific mechanisms remain unclear. Tumor markers, including CEA, CA199, and CA242, are mostly used for laboratory diagnosis of tumors. In patients with T2DM, chronic inflammatory lesions of beta cells in the pancreatic islets and long-term glucotoxicity and lipotoxicity can exacerbate chronic inflammation or hyperplasia of the pancreas. This process destroys normal pancreatic tissue, with subsequent replacement by adipocytes and fibrous connective tissue. Additionally the aforementioned process results in a significant release of CA199 into the bloodstream[6]. Furthermore, high blood sugar levels can affect free radical generation, increasing oxidative stress. Severe oxidative stress and high blood sugar levels may contribute to increased CEA expression[7]. Additionally, the replacement of normal pancreatic tissue by fat cells or fibrous connective tissue leads to the deposition of amyloid substances in pancreatic islet cells,

Table 5 Correlation analysis of carcinoembryonic antigen, cancer antigen 199, cancer antigen 242 levels with glycosylated hemoglobin, fasting blood sugar and lipid metabolism in diabetes patients

Variables	CEA		CA199		CA242	
	r value	*P* value	*r* value	*P* value	*r* value	*P* value
HbA1c	0.308	0.000	0.426	0.000	0.551	0.000
FBG	0.236	0.033	0.231	0.037	0.298	0.006
LDL-C	0.138	0.216	0.238	0.032	0.240	0.030
TG	0.136	0. 222	0.105	0.346	0.051	0.649
TC	0.077	0.494	0.171	0.125	0.149	0.183

HbA1c: Glycosylated hemoglobin; FBG: Fasting blood sugar; LDL-C: Low-density lipoprotein cholesterol; TG: Triglycerides; TC: Total cholesterol.

followed by tissue destruction, cell degeneration, and necrosis. Hyperglycemia further exacerbates these pathological changes, releasing glycoprotein components, including CA242, into the bloodstream[8].

This study comprehensively examined tumor markers in patients with T2DM and healthy control groups. The levels of CEA and CA199 were higher in patients with T2DM than in healthy controls, indicating that blood glucose levels may be involved in the increase of serum CEA and CA199 levels, which is consistent with the findings of Lipinski *et al*[9]. Pancreatic tissue is affected by diabetes, which is considered an important factor that leads to a false increase in serum CA199 levels. Although the CA242 levels did not significantly differ between the two groups in this study, caution is advised when drawing conclusions owing to the limited sample size. In patients with T2DM and poorly controlled blood glucose levels, a benign increase in the concentration of CA199 and CEA can occur, which does not necessarily indicate the presence of malignant tumors. The benign increase in tumor marker CA199 and CEA levels in patients with poor blood glucose control can be attributed to "glucotoxicity" damage. However, whether this increase leads to malignant tumor development cannot be determined. Therefore, patients must actively control their blood glucose levels to avoid further increases in CA199 and CEA levels[10], thereby reducing the risk of developing malignant tumors. Similarly, the slight increase in serum CA199 and CEA levels may be due to glucose metabolism disorders in patients with diabetes. Hence, increasing the cutoff value for the "normal" levels of CA199 and CEA may be necessary, for distinguishing benign digestive tract diseases from malignant digestive tract tumors in patients with diabetes.

HbA1c has a marked effect in promoting CA199 and CEA elevation, providing insight into blood sugar control during the previous 3 months in patients[11,12]. In this study, patients with diabetes were divided into two subgroups based on their HbA1c levels. CEA and CA199 levels in Group B (HbA1c > 9%) patients were significantly different from those in Group A (HbA1c ≤ 9%) patients, with positive correlations observed between serum CA199, CEA, CA242, and HbA1c levels. Notably, the positive correlation between serum CA199 and HbA1c levels in T2DM has been demonstrated previously[13]. Furthermore, we observed that LDL-C levels positively correlated with CA199 levels. Increased HbA1c levels can lead to tissue hypoxia, elevated plasma low-density lipoprotein levels, tissue collagen glycosylation, increased blood viscosity, blood stasis, abnormal anticoagulation mechanisms, and enhanced production of free radicals. Moreover, these factors can collectively cause pancreatic tissue damage, leading to elevated CA199 levels[14]. The significant relationship between increased serum CA199 and CEA and HbA1c levels in patients with T2DM underscores the diagnostic value of CA199 and CEA levels for HbA1c percentage. Hence, when clinically using CA199 and CEA to identify malignant tumors in patients with T2DM, hypoglycemic treatment should be prioritized to stabilize blood sugar levels before tumor marker detection and observation[15].

This study also observed an outstanding dependence between CEA and CA199 levels and hyperglycemia, indicating that CEA and CA199 may be related to poor blood sugar and lipid control. Previous studies have displayed that elevated CEA levels are associated with oxidative stress, which can be induced by high blood sugar levels[16]. However, increased FBG levels in patients with T2DM may contribute to upregulated CEA and CA199 expression, which could be significantly associated with a high incidence of pancreatic cancer in these patients[17]. Repetitive injury to pancreatic tissue caused by chronic glucose toxicity may be a major factor contributing to the occurrence and progression of pancreatic cancer. Active blood sugar control and early screening for pancreatic cancer could potentially reduce the risk of malignant tumors in such patients[18]. Additionally, CA199 and CEA have high diagnostic values for digestive system tumors and also demonstrate certain diagnostic values for T2DM.

Although our study highlights the association between elevated CEA, CA199, and CA242 levels and T2DM, the broad clinical implications are paramount. In a real-world setting, these tumor markers could be early indicators for potential complications in patients with T2DM. Regular monitoring of these markers could provide clinicians with actionable insights, aiding in therapeutic decisions and possibly leading to timely interventions. The correlation of these markers with metabolic indicators, such as HbA1c and FBG, further positions them as potential prognostic tools in T2DM management. As our understanding of T2DM deepens, these markers may emerge as vital tools in refining clinical strategies and bridging the gap between epidemiological data and hands-on patient care.

Some limitations of this study should be considered. A limited extrapolation of results could occur owing to all the study samples being from the same center. Furthermore, considering the relatively small sample size, a cautious interpretation of results is warranted.

CONCLUSION

Our study detected elevated serum CEA and CA199 levels in patients with T2DM. Additionally, CA199, CEA, and CA242 levels showcased significant correlations with HbA1c and FBG levels. These findings transcend mere epidemiological associations. In the clinical context, the elevated levels of the aforementioned tumor markers in patients with T2DM could indicate potential underlying pathologies or complications. Incorporating routine CA199, CEA, and CA242 assessments in patients with T2DM care might provide clinicians with valuable insights, aiding in therapeutic decisions, especially for those struggling with blood sugar management. Such proactive monitoring could lead to timely interventions, potentially mitigating complications and improving patient outcomes. As our understanding of these markers in the T2DM landscape improves, they might emerge as pivotal tools in refining patient management strategies and improving overall care.

ARTICLE HIGHLIGHTS

Research background

Glucose and lipid metabolic disorder in patients with type 2 diabetes mellitus (T2DM) is closely related to the level of serum tumor markers [such as cancer antigen (CA)199] in the digestive tract. Therefore, tumor markers of T2DM are important.

Research motivation

To assess the expression and clinical significance of serum tumor markers [CA199, CA242, and carcinoembryonic antigen (CEA)] in T2DM.

Research objectives

To study the expression of serum tumor markers (CA199, CA242, and CEA) and its clinical implications in T2DM.

Research methods

We conducted an observational study at Hefei BOE Hospital, Anhui, China, between April 2019 and December 2020 and enrolled 82 patients with first-onset T2DM and 51 controls. Levels of fasting blood glucose (FBG), tumor markers (CA199, CEA, and CA242), glycosylated hemoglobin (HbA1c), and other metabolic indicators were measured and group index levels were compared. FBG and HbA1c levels were correlated with tumor marker levels. Tumor markers were tested for diagnostic accuracy in patients with high HbA1c (> 9%) using the receiver operating curve (ROC) curve.

Research results

Compared to the control group, the T2DM group had higher serum FBG, HbA1c, CA199, and CEA levels ($P < 0.05$). A comparative analysis of the two groups based on HbA1c levels (Group A: HbA1c ≤ 9%; Group B: HbA1c > 9%) revealed significant differences in CEA and CA199 levels ($P < 0.05$). The areas under the ROC curve for CEA and CA199 were 0.853 and 0.809, respectively. Moreover, CA199, CEA, and CA242 levels were positively correlated with HbA1c ($r = 0.308$, 0.426, and 0.551, respectively) and FBG ($r = 0.236$, 0.231, and 0.298, respectively) levels.

Research conclusions

Serum CEA and CA199 levels were high in patients with T2DM. HbA1c and FBG levels correlated with CA199, CEA, and CA242 levels. Patients with poorly controlled blood sugar levels require tumor marker screening.

Research perspectives

Serum CEA and CA199 levels were higher in patients with T2DM than in controls. HbA1c and FBG levels correlated with CA199, CEA, and CA242 levels.

FOOTNOTES

Author contributions: Meng M was responsible for methodology, investigation, software, data curation, formal analysis, writing-original draft; Shi LL was responsible for conceptualization, resources, supervision, validation, writing-review, and editing.

Institutional review board statement: The study was reviewed and approved by the Hefei BOE Hospital Institutional Review Board.

Informed consent statement: All study participants provided informed written consent before participating in the study.

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REFERENCES

1 **Laakso M**. Biomarkers for type 2 diabetes. *Mol Metab* 2019; **27S**: S139-S146 [PMID: 31500825 DOI: 10.1016/j.molmet.2019.06.016]
2 **Ling S**, Zaccardi F, Issa E, Davies MJ, Khunti K, Brown K. Inequalities in cancer mortality trends in people with type 2 diabetes: 20 year population-based study in England. *Diabetologia* 2023; **66**: 657-673 [PMID: 36690836 DOI: 10.1007/s00125-022-05854-8]
3 **Chen K**, Jiao DA, Zheng S, Zhou L, Yu H, Yuan YC, Yao KY, Ma XY, Zhang Y. Diagnostic value of occult fecal blood testing for colorectal cancer screening. *World J Gastroenterol* 1997; **3**: 166-168 [PMID: 27239137 DOI: 10.3748/wjg.v3.i3.166]
4 **Pearson-Stuttard J**, Papadimitriou N, Markozannes G, Cividini S, Kakourou A, Gill D, Rizos EC, Monori G, Ward HA, Kyrgiou M, Gunter MJ, Tsilidis KK. Type 2 Diabetes and Cancer: An Umbrella Review of Observational and Mendelian Randomization Studies. *Cancer Epidemiol Biomarkers Prev* 2021; **30**: 1218-1228 [PMID: 33737302 DOI: 10.1158/1055-9965.EPI-20-1245]
5 **Scherübl H**. [Type-2-diabetes and cancer risk]. *Dtsch Med Wochenschr* 2021; **146**: 1218-1225 [PMID: 34521128 DOI: 10.1055/a-1529-4521]
6 **Zelenko Z**, Gallagher EJ, Tobin-Hess A, Belardi V, Rostoker R, Blank J, Dina Y, LeRoith D. Silencing vimentin expression decreases pulmonary metastases in a pre-diabetic mouse model of mammary tumor progression. *Oncogene* 2017; **36**: 1394-1403 [PMID: 27568979 DOI: 10.1038/onc.2016.305]
7 **Zayed AA**, Beano AM, Amer FN, Maslamani JM, Zmaili MA, Al-Khudary TH, Momani MS, Yousef AF. Serum levels of carcinoembryonic antigen in patients with type 2 diabetes. *Endocr Pract* 2016; **22**: 1310-1318 [PMID: 27482614 DOI: 10.4158/EP161221.OR]
8 **Dou H**, Sun G, Zhang L. CA242 as a biomarker for pancreatic cancer and other diseases. *Prog Mol Biol Transl Sci* 2019; **162**: 229-239 [PMID: 30905452 DOI: 10.1016/bs.pmbts.2018.12.007]
9 **Lipinski MJ**, Benedetto U, Escarcega RO, Biondi-Zoccai G, Lhermusier T, Baker NC, Torguson R, Brewer HB Jr, Waksman R. The impact of proprotein convertase subtilisin-kexin type 9 serine protease inhibitors on lipid levels and outcomes in patients with primary hypercholesterolaemia: a network meta-analysis. *Eur Heart J* 2016; **37**: 536-545 [PMID: 26578202 DOI: 10.1093/eurheartj/ehv563]
10 **Shang X**, Song C, Du X, Shao H, Xu D, Wang X. The serum levels of tumor marker CA19-9, CEA, CA72-4, and NSE in type 2 diabetes without malignancy and the relations to the metabolic control. *Saudi Med J* 2017; **38**: 204-208 [PMID: 28133696 DOI: 10.15537/smj.2017.2.15649]
11 **Linkeviciute-Ulinskiene D**, Patasius A, Zabuliene L, Stukas R, Smailyte G. Increased Risk of Site-Specific Cancer in People with Type 2 Diabetes: A National Cohort Study. *Int J Environ Res Public Health* 2019; **17** [PMID: 31905811 DOI: 10.3390/ijerph17010246]
12 **Rong F**, Dai H, Wu Y, Li J, Liu G, Chen H, Zhang X. Association between thyroid dysfunction and type 2 diabetes: a meta-analysis of prospective observational studies. *BMC Med* 2021; **19**: 257 [PMID: 34670571 DOI: 10.1186/s12916-021-02121-2]
13 **Tong W**, Gao H, Wei X, Mao D, Zhang L, Chen Q, Zhang Z, Li Y. Correlation of serum CA199 levels with glycemic control and microvascular complications in patients with type 2 diabetes mellitus. *Am J Transl Res* 2021; **13**: 3302-3308 [PMID: 34017502]
14 **Chen PC**, Lin HD. Reversible high blood CEA and CA19-9 concentrations in a diabetic patient. *Libyan J Med* 2012; **7** [PMID: 23105951 DOI: 10.3402/ljm.v7i0.19572]
15 **Ata N**, Dal K, Kucukazman M, Yeniova AÖ, Karakaya S, Unsal O, Dagdeviren M, Akın KO, Baser S, Beyan E, Ertugrul DT. The effect of glycemic control on CEA, CA 19-9, amylase and lipase levels. *Open Med (Wars)* 2015; **10**: 8-13 [PMID: 28352671 DOI: 10.1515/med-2015-0002]
16 **Hasan M**, Mohieldein A. Association between serum carcinoembryonic antigen level and oxidative stress parameters among diabetic females. *Int J Clin Exp Med* 2015; **8**: 6489-6494 [PMID: 26131277]
17 **Qin S**, Lu Y, Chen S, Hu Z, Chen H, Zhong J, Li S, Chen Z. The Relationship of Neutrophil-to-Lymphocyte Ratio or Platelet-to-Lymphocyte Ratio and Pancreatic Cancer in Patients with Type 2 Diabetes. *Clin Lab* 2019; **65** [PMID: 31307172 DOI: 10.7754/Clin.Lab.2019.181226]
18 **Donath MY**, Schumann DM, Faulenbach M, Ellingsgaard H, Perren A, Ehses JA. Islet inflammation in type 2 diabetes: from metabolic stress to therapy. *Diabetes Care* 2008; **31** Suppl 2: S161-S164 [PMID: 18227479 DOI: 10.2337/dc08-s243]

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ORIGINAL ARTICLE

Observational Study

Age-specific differences in the association between prediabetes and cardiovascular diseases in China: A national cross-sectional study

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Abstract

BACKGROUND

Cardiovascular disease (CVD) is a leading cause of morbidity and mortality worldwide, the global burden of which is rising. It is still unclear the extent to which prediabetes contributes to the risk of CVD in various age brackets among adults. To develop a focused screening plan and treatment for Chinese adults with prediabetes, it is crucial to identify variations in the connection between prediabetes and the risk of CVD based on age.

AIM

To examine the clinical features of prediabetes and identify risk factors for CVD in different age groups in China.

METHODS

The cross-sectional study involved a total of 46239 participants from June 2007 through May 2008. A thorough evaluation was conducted. Individuals with prediabetes were categorized into two groups based on age. Chinese atherosclerotic CVD risk prediction model was employed to evaluate the risk of developing CVD over 10 years. Random forest was established in both age groups. SHapley Additive exPlanation method prioritized the importance of features from the perspective of assessment contribution.

RESULTS

In total, 6948 people were diagnosed with prediabetes in this study. In prediabetes, prevalences of CVD were 5 (0.29%) in the younger group and 148 (2.85%) in the older group. Overall, 11.11% of the younger group and 29.59% of the older group were intermediate/high-risk of CVD for prediabetes without CVD based on the Prediction for ASCVD Risk in China equation in ten years. In

the younger age group, the 10-year risk of CVD was found to be more closely linked to family history of CVD rather than lifestyle, whereas in the older age group, resident status was more closely linked.

CONCLUSION
The susceptibility to CVD is age-specific in newly diagnosed prediabetes. It is necessary to develop targeted approaches for the prevention and management of CVD in adults across various age brackets.

Key Words: Age; Cardiovascular diseases; Prediabetes; Risk factors; Differences

Core Tip: Cardiovascular disease (CVD) is a leading cause of illness and death on a global scale, with its worldwide impact steadily increasing. However, it is still unclear the extent to which prediabetes contributes to the risk of CVD in various age brackets among adults. In this study, we analyzed our prediabetes data from 17 centers between June 2007 and May 2008. We found the influential features of different age brackets for the 10-year risk of CVD based on Prediction for ASCVD Risk in China. Given our findings, specific prevention strategies are needed for different age groups.

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INTRODUCTION

Cardiovascular disease (CVD) is a prominent contributor to illness and death on a global scale, with its worldwide impact steadily increasing[1]. Addressing CVD is a paramount concern for public health worldwide. The prediabetic population constitutes a substantial pool of individuals who are at risk of developing diabetes, a contributing factor for CVD. Prediabetes refers to a rise in blood sugar levels that is higher than the normal range but lower than the levels seen in clinical diabetes. Impaired fasting glucose (IFG) level or impaired glucose tolerance (IGT) is the designated term for this condition. According to previous studies, the approximate occurrence of prediabetes in China was 35.7% [95% confidence interval (95%CI): 34.2%-37.3%] in 2013 and 38.1% (95%CI: 36.4%-39.7%) in 2018[2]. Moreover, prediabetes has been linked to a higher likelihood of combined cardiovascular incidents, coronary artery disease, cerebrovascular accidents, and overall mortality[3]. During an examination of the data collected from the National Health and Nutrition Examination Surveys 2011-2014, it was found that individuals with prediabetes, as determined by ADA-fasting plasma glucose (FPG) or hemoglobin A1c (HbA1c), exhibited a significant occurrence of hypertension (36.6%), dyslipidemia (51.2%), albuminuria (7.7%), and reduced estimated glomerular filtration rate (4.6%). In total, 24.3% of the individuals were presently smoking, exhibiting a heightened projected 10-year cardiovascular event risk of around 7%[4].

It is still uncertain the extent to which prediabetes contributes to the risk of CVD in various age brackets among adults. To develop a focused glycemic screening plan and treatment for Chinese adults with prediabetes, it is crucial to identify variations in the connection between prediabetes and the risk of CVD based on age. Given that the occurrence of CVD events gradually develops, a predictive model can be utilized to estimate the likelihood for individuals without CVD.

Currently, numerous CVD risk evaluation instruments exist worldwide, with the renowned Framingham Risk Score (FRS) being the creation of Framingham Heart Research Institute. Nevertheless, these models rely on the European and American sample populations, which predominantly consist of White and Black individuals, and have a comparatively limited representation of Asians[5]. The Prediction for ASCVD Risk in China (China-PAR) CVD risk assessment model was developed in 2016 to predict the risk of atherosclerotic CVD in China. This model was specifically designed for the Chinese population and allowed for the quantitative assessment of CVD incidence risk over 10 years. The China-PAR model's development offered a significant and efficient evaluation tool for predicting CVD risk and promoting primary prevention in China[6]. The objective of this study was to forecast the likelihood of CVD in China's prediabetic population by utilizing the FRS and China-PAR models. Additionally, it aimed to analyze the disparities in CVD risk prediction between these two models and identify distinct risk factors among younger and older age groups, ultimately establishing a targeted prevention strategy.

MATERIALS AND METHODS

Study design

The study's development set was obtained from a China National Diabetes and Metabolic Disorders Survey, which was a comprehensive cross-sectional study. From June 2007 to May 2008, a large epidemiological study was conducted across

the nation. It involved 17 clinical centers located in 14 provinces and municipalities throughout the country. In the general population, individuals who were 20 years of age or older were chosen using a multistage stratified cluster sampling technique. The study design, eligibility criteria, and sampling have been previously published in great detail[7, 8].

Participants

Individuals who had resided in their present locality for more than five years were qualified to take part in the research. A total of 54240 people were chosen and asked to take part in the research, yet only 46239 grown-ups finished the questionnaire.

We included participants who were diagnosed with prediabetes using the oral glucose test (n = 7263) and excluded those who had been previously diagnosed with diabetes (n = 315). Consequently, our final analysis encompassed a total of 6948 adults, whom we subsequently categorized into two groups based on age range (as depicted in Figure 1).

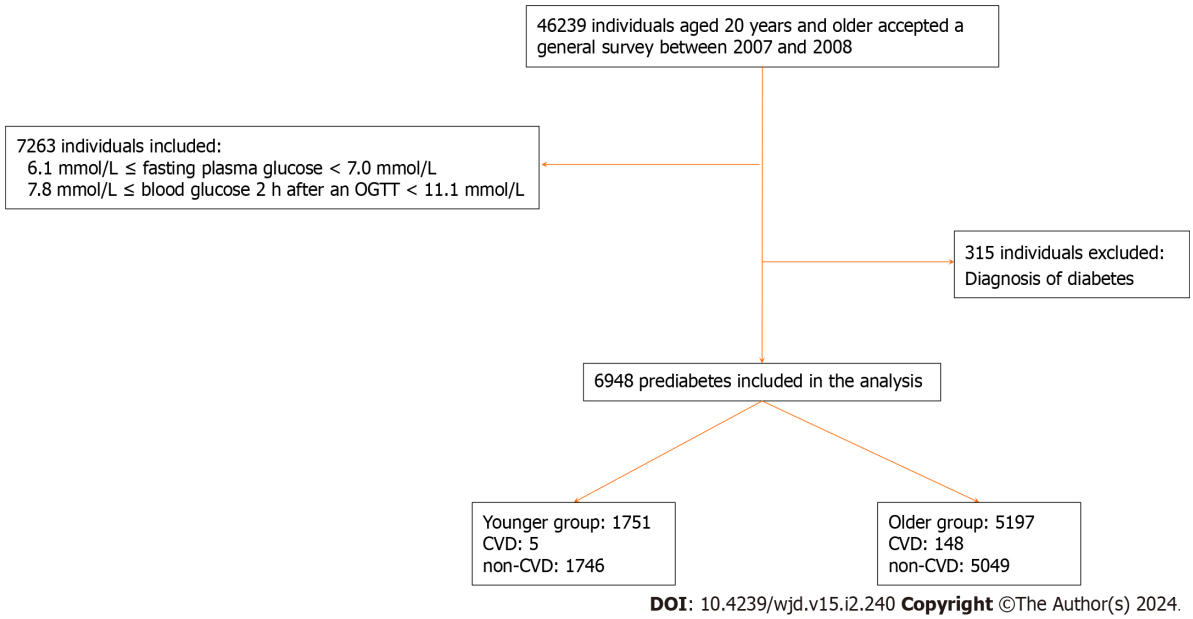

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Figure 1 Study population flow. CVD: Cardiovascular disease; OGTT: Oral glucose tolerance test.

Data collection

Trained personnel administered a typical survey to gather data on demographic traits, individual and familial medical backgrounds, and factors that pose risks to one's lifestyle[8].

Before the oral glucose tolerance test, participants were given instructions to continue with their regular physical activity and diet for a minimum of 3 d. Following a minimum of 10 hours of fasting overnight, a blood sample was obtained from a vein using a vacuum tube that contained sodium fluoride. This sample was collected to measure the glucose levels in the plasma. Individuals without any documented record of diabetes were administered a typical 75 g glucose solution, while individuals who self-reported having diabetes were provided with a steamed bun comprising roughly 80 g of intricate carbohydrates for precautionary purposes. Glucose concentrations were measured by drawing blood samples at 0, 30 min, and 120 min following the glucose or carbohydrate load[8].

Plasma glucose levels were assessed utilizing an enzymatic method involving hexokinase. Serum cholesterol and triglyceride levels were enzymatically assessed using commercially available reagents at the clinical biochemical laboratories in each province. Before starting this study, all research laboratories have successfully finished a program for standardization and certification.

Definitions

Prediabetes was diagnosed using the diagnostic criteria from the World Health Organization in 1999[9]. The plasma glucose testing results were classified into three categories: isolated IFG (fasting glucose level of ≥ 6.1 mmol/L and < 7.0 mmol/L, and PG2h level of < 7.8 mmol/L); isolated IGT (fasting glucose level of < 7.0 mmol/L, and PG2h level of ≥ 7.8 mmol/L and < 11.1 mmol/L); and undiagnosed diabetes (fasting glucose level of ≥ 7.0 mmol/L, PG2h level of ≥ 11.1 mmol/L, or both). Diabetes that had been diagnosed before was determined when the participant answered positively to the inquiry, "Has a medical professional ever informed you that you have diabetes?" The overall count of diabetes encompassed both previously diagnosed cases and those that had not been identified[8]. Prediabetes was characterized by either IFG or IGT.

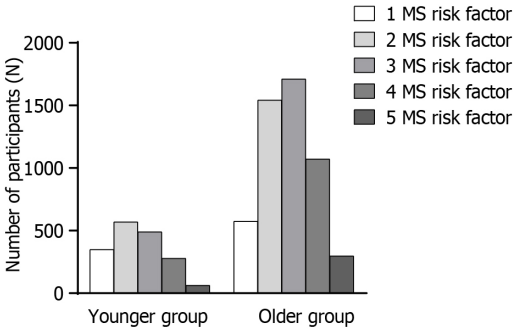

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Figure 2 Prevalence of metabolic syndrome components by age intervals. MS: Metabolic syndrome.

Assessment of the CVD risk

To assess the CVD risk, the China-PAR and FRS were employed. The China-PAR model is a tool for evaluating developed by the China-PAR Risk Assessment Research. A single expert researcher inputted the personal details and test outcomes of the volunteers *via* the online platform (http://www.cvdrisk.com.cn), which encompassed gender, age, present address (urban or rural), location (north or south), waist size, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), existing blood pressure level, usage of antihypertensive medication, presence of diabetes, smoking habits, and family history of CVD. On the China-PAR, the 10-year absolute risk percentage for CVD was categorized as low (< 5%), intermediate (5%-10%), and high risk (> 10%). The FRS scores were computed by considering six risk factors, which encompassed age, gender, TC, HDL-c, systolic blood pressure (SBP), and smoking patterns. To calculate FRS, the thresholds were set as TC < 160, 160-199, 200-239, 240-279, and ≥ 280 mg/dL; for SBP, the ranges were < 120, 120-129, 130-139, 140-159, and ≥ 160 mmHg; and for HDL-c, the values were < 40, 40-49, 50-59, and ≥ 60 mg/dL. The percentage of ten-year risk was determined by adding up the points (1 point, 6%; 2 points, 8%; 3 points, 10%; 4 points, 12%; 5 points, 16%; 6 points, 20%; 7 points, 25%; 10 points or more, greater than 30%). The percentage of absolute CVD risk over 10 years was categorized as low (less than 10%), moderate (10%-20%), and high (greater than 20%) according to classification[10].

Statistical analysis

The objective of our research was to obtain precise evaluations of the risk elements associated with CVD among various age categories in the Chinese population, specifically individuals who are 20 years old or above and have prediabetes. To ensure accuracy in a complex survey design, the estimated sample sizes were determined to align with the commonly advised criteria[11]. The calculations were adjusted to reflect the entire Chinese adult population (20 years or older) using the 2006 Chinese population data and the study's sampling method. Corrections were made for various aspects of the survey, such as oversampling of women and urban dwellers, nonresponse, highly developed economic regions, and demographic or geographic disparities between the sample and the overall population[8].

The occurrence rates of CVD were computed for the subcategories based on age factors. To investigate the correlation between the 10-year risk of CVD and demographic, lifestyle, and metabolic factors, we employ random forest (RF) analysis. SHapley Additive exPlanation (SHAP) values to provide consistent and locally accurate attribution values for each feature. This is a unified approach to explain the outcome of RF. SHAP values evaluate the importance of the output resulting from the inclusion of feature A for all combinations of features other than A. All *P* values were not adjusted for multiple testing and were considered two-tailed. The R software, version 4.3.2, was utilized for all statistical analyses. Two-tailed *P* values < 0.05 were considered significant.

RESULTS

This study involved a total of 6948 individuals who were diagnosed with prediabetes. Among this total, 1751 individuals (25.2%) were between the ages of 20 and 40, while 5197 individuals (74.8%) were above the age of 40, as shown in Table 1. In comparison to the younger participants, the older group exhibited a higher proportion of males. The older individuals with prediabetes were more likely to engage in smoking, alcohol consumption, and exercise. Additionally, they exhibited higher measurements of waist circumference (WC), PG2h, TC, HDL-c, low-density lipoprotein-cholesterol, SBP, and diastolic blood pressure (DBP). Moreover, it was observed that 5 individuals (0.29%) in the younger group and 148 individuals (2.85%) in the older group were found to have CVD.

Figure 2 and Table 2 exhibit the occurrence of metabolic syndrome components according to age intervals. According to the data presented in Table 2, prediabetes in older age exhibited a higher tendency towards central obesity and elevated blood pressure.

For prediabetes without CVD (n = 6795), the age stratification was used to compare the 10-year absolute risk grading of CVD. The findings indicated that there were statistically significant variations in the assessment outcomes of the low-, intermediate-, and high-risk categories in different age brackets on the FRS and China-PAR models (P < 0.001). However, the deductions made from the disease risk grading remained consistent. In other words, the higher the age, the higher the

Table 1 Characteristics of participants, *n* (%)

	Younger group	Older group	*P* value
Total	1751 (25.20)	5197 (74.80)	
Men	744 (42.49)	3035 (58.40)	0.015
Smoking	402 (22.96)	2204 (42.41)	0.001
Alcohol drinking	412 (23.53)	2328 (44.80)	0.009
Regular physical activity	424 (24.21)	2385 (45.89)	< 0.001
Family history of CVD	277 (15.82)	1133 (21.80)	0.173
Antihypertensive drugs	81 (4.63)	346 (6.66)	0.155
Dyslipidemia	84 (4.80)	249 (4.79)	< 0.001
Lipid-lowering drugs	13 (0.74)	87 (1.67)	0.007
BMI (kg/m^2, 95%CI)	25.0 (24.6-25.3)	24.9 (24.7-25.1)	0.688
WC (cm, 95%CI)	83.2 (82.0-84.4)	84.8 (84.2-85.3)	0.021
FPG (mmol/L, 95%CI)	5.6 (5.5-5.6)	5.5 (5.5-5.6)	0.586
PG2h (mmol/L, 95%CI)	8.2 (8.1-8.3)	8.6 (8.5-8.6)	< 0.001
TC (mmol/L, 95%CI)	4.8 (4.7-4.9)	5.0 (5.0-5.1)	< 0.001
TG (mmol/L, 95%CI)	1.9 (1.7-2.0)	1.9 (1.8-1.9)	0.985
HDL-c (mmol/L, 95%CI)	1.3 (1.2-1.3)	1.3 (1.3-1.3)	0.029
LDL-c (mmol/L, 95%CI)	2.7 (2.6-2.8)	2.9 (2.9-3.0)	< 0.001
SBP (mmHg, 95%CI)	120.0 (118.9-121.2)	131.9 (130.7-133.1)	< 0.001
DBP (mmHg, 95%CI)	79.1 (78.2-79.9)	81.2 (80.5-82.0)	< 0.001
Abnormal ECG (%)	366 (20.90)	2358 (45.37)	< 0.001
Prediabetes category			
IFG (%)	357 (20.39)	1447 (27.84)	< 0.001
IGT (%)	1394 (79.61)	3750 (72.16)	< 0.001
CVD (%)	5 (0.29)	148 (2.85)	0.003

CVD: Cardiovascular disease; BMI: Body mass index; WC: Waist circumference; HR: Heart rate; FPG: Fasting plasma glucose; PG2h: 2 h post-load plasma glucose; TC: Total cholesterol; TG: Triglycerides; HDL-c: High-density lipoprotein-cholesterol; LDL-c: Low-density lipoprotein-cholesterol; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; ECG: Electrocardiography; IFG: Impaired fasting glucose; IGT: Impaired glucose tolerance; 95%CI: 95% confidence interval.

10-year risk level of CVD (Table 3). Among the participants, a total of 5320 individuals (which accounts for 78.29% of the total) were simultaneously classified as low-, medium-, or high-risk based on both scores. The kappa test revealed a low level of agreement between the two methods (weighted κ coefficient of agreement = 0.395-0.400; $P < 0.001$) (Table 4).

Therefore, we use China-PAR to predict the 10-year risk of CVD for the Chinese. For prediabetes, intermediate/high risk of CVD ($n = 194$ in the younger group and $n = 1509$ in the older group) is more noteworthy. We utilized the RF with all the variables as input variables. The importance matrix plot for the RF method is shown in Figure 3 and revealed that the top 10 most important variables contributing to the younger group model were SBP, age, HDL-c, TC, HC, rural area, smoking, WC FPG, and TG. For the older group, the top 10 most important variables were SBP, family history of CVD, DBP, HDL-c, smoking, TG, age, WHR, FPG, and TC.

To identify the features that had the most influence, we depicted the SHAP summary plot of RF (Figure 4) for both age groups. This plot provided a visually concise figure by presenting the range and distribution of importance. It showed how high and low features' values were with SHAP values. Each dot represented the SHAP value of the feature from the individual. It was plotted horizontally and was stacked vertically to show the density of the same SHAP value. Then, each dot was colored by the value of the feature, from low (yellow) to high (purple). The higher the SHAP value of a feature, the more likely occurrence of CVD in 10 years.

Table 2 Prevalence of metabolic syndrome components according to age groups, *n* (%)

	Younger group	Older group	*P* value
Central obesity	636 (36.32)	4575 (88.03)	< 0.001
High glucose	1751 (100.00)	5197 (100.00)	-
High blood pressure	532 (30.38)	2959 (56.94)	< 0.001
High TG	708 (40.43)	2280 (43.87)	0.334
Low HDL-c	367 (20.96)	1161 (22.32)	0.500

TG: Triglycerides; HDL-c: High-density lipoprotein-cholesterol.

Table 3 Comparing the absolute 10-year risk of cardiovascular disease between the two methods across different age groups, *n* (%)

	Younger group (*n* = 1746)	Older group (*n* = 5049)	Total (*n* = 6795)	*P* value
China-PAR				
Low (< 5%)	1552 (88.89)	3540 (70.11)	5092	< 0.001
Intermediate (5%-10%)	127 (7.27)	575 (11.39)	702	< 0.001
High (> 10%)	67 (3.84)	934 (18.50)	1001	< 0.001
FRS				
Low (< 10%)	1717 (98.34)	3928 (77.80)	5645	< 0.001
Intermediate (10%-20%)	27 (1.55)	904 (17.90)	931	< 0.001
High (> 20%)	2 (0.11)	217 (4.30)	219	< 0.001

CVD: Cardiovascular disease; China-PAR: Prediction for atherosclerotic cardiovascular disease risk in China; FRS: Framingham risk score.

Table 4 Consistency analysis of the 10-year risk of cardiovascular disease absolute risk as predicted by the two models

FRS	China-PAR			Total
	Low risk	Intermediate risk	High risk	
Low risk	4923	492	230	5645
Intermediate risk	147	205	579	931
High risk	22	5	192	219
Total	5092	702	1001	6795

FRS: Framingham risk score; China-PAR: Prediction for atherosclerotic cardiovascular disease risk in China.

DISCUSSION

In the general population, fatal CVD is commonly associated with male sex, hypertension, dyslipidemia, diabetes, and smoking. Nevertheless, information is scarce concerning the presence of age-related disparities in the influence of these risk factors[12]. In China, we conducted a cross-sectional survey to examine how age and risk factors for 10-year risk of CVD interact and to determine variations in CVD risk factors among different age groups. Previous studies[13,14] support the results indicating that the younger group with hyperglycemia had a higher prevalence of CVD compared to the older group. Based on previous studies conducted locally and globally, age is a significant determinant that escalates the susceptibility to CVD[15]. Age was determined to have a significant impact on the risk of CVD after eliminating other variables that could distort the results.

Individuals with prediabetes have accompanying metabolic risk factors[16]. Metabolic syndrome is characterized by a group of metabolic risk factors, such as insulin resistance, central obesity, hyperglycemia, dyslipidemia, and high blood pressure[17]. Given that metabolic syndrome encompasses a comprehensive set of metabolic risk factors for cardiovascular events, it becomes imperative to anticipate the likelihood of CVD in these individuals. With the onset of the 21st century, CVD emerged as the primary reason for untimely death and illness globally, affecting 80% of individuals

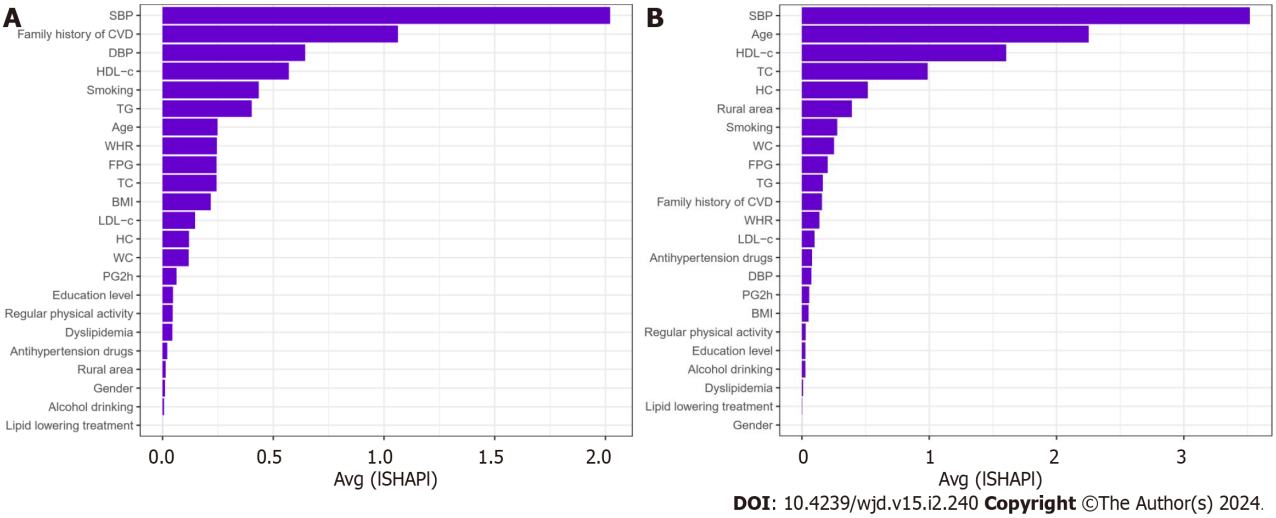

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Figure 3 Importance matrix plot of the random forest model. This importance matrix plot depicts the importance of each covariate in the final predictive model. A: Younger group; B: Older group. CVD: Cardiovascular disease; BMI: Body mass index; WC: Waist circumference; FPG: Fasting plasma glucose; PG2h: 2 h post-load plasma glucose; TC: Total cholesterol; TG: Triglycerides; HDL-c: High-density lipoprotein-cholesterol; LDL-c: Low-density lipoprotein-cholesterol; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; SHAP: SHapley Additive exPlanation.

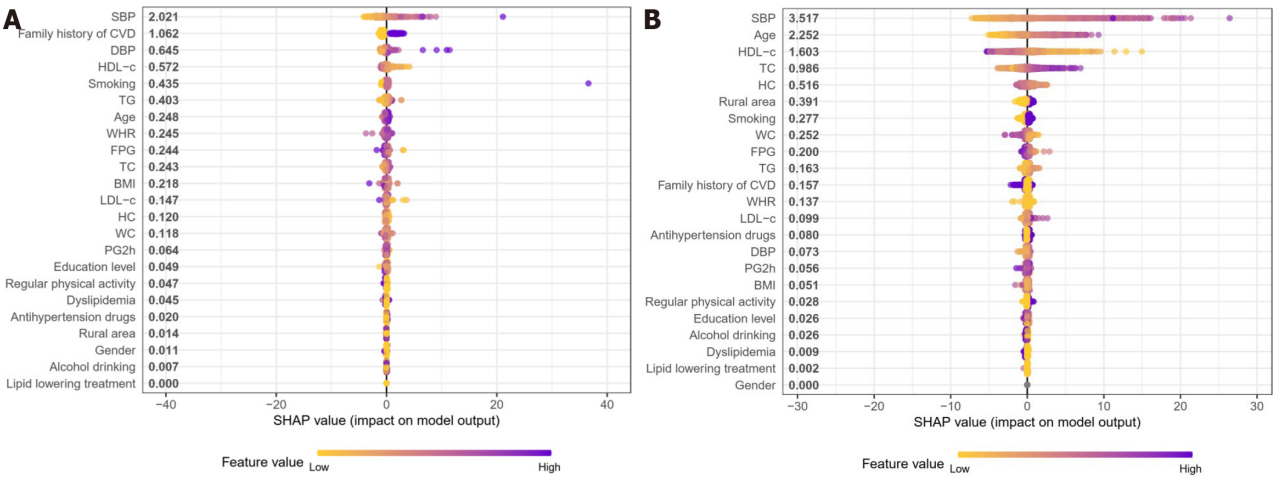

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Figure 4 SHapley Additive exPlanation summary plot of the features of the random forest model. The higher the SHapley Additive exPlanation value of a feature, the higher the probability of the 10-year risk of cardiovascular disease. A dot is created for each feature attribution value for each individual, and thus one individual is allocated one dot on the line for each feature. Dots are colored according to the values of features for the respective individual and accumulate vertically to depict density. Purple represents higher feature values, and yellow represents lower feature values. A: Younger group; B: Older group. CVD: Cardiovascular disease; BMI: Body mass index; WC: Waist circumference; FPG: Fasting plasma glucose; PG2h: 2 h post-load plasma glucose; TC: Total cholesterol; TG: Triglycerides; HDL-c: High-density lipoprotein-cholesterol; LDL-c: Low-density lipoprotein-cholesterol; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; SHAP: SHapley Additive exPlanation.

in underprivileged developing nations, following societal and economic progress. Extensive studies have been conducted since the mid-1900s to investigate the causes and risk elements, leading to the identification of various factors like tobacco use, high blood pressure, diabetes, and abnormal lipid levels as contributors to CVD[18].

Nonetheless, the correlation between prediabetes and CVD occurrences might compromise the precision of our results due to the limited number of CVD patients in our research. Hence, by utilizing the risk score, which includes the initial indications of CVD, as substitute measures, we can enhance the precision in identifying the connection between prediabetes and risk elements for CVD.

This study estimated the risk of CVD in prediabetes in the next 10 years, as shown by the China-PAR model. China-PAR incorporates the disease spectrum and prevalence of risk factors in China, including novel factors like WC and place of residence, by thoroughly considering the risk factors associated with the previous model. At the same time, the examination of the correlation between age and different risk factors was also conducted. A CVD risk prediction model suitable for the Chinese population was created, and the cut-off point of different risk stratification was proposed and verified; hence, its prediction results were more accurate. The study additionally discovered that both the FRS and China-PAR models demonstrated a positive correlation between age and the 10-year incidence risk of CVD in the prediabetes

population. This suggests that the two methods consistently predict the risk level across various age groups.

In the general populace, diabetes raises the likelihood of both microvascular and macrovascular complications as well as premature death leading to a substantial financial burden on society. While there have been limited reports on the link between prediabetes diagnosed at a later stage and the risks of CVD and mortality, numerous studies have examined the connection between prediabetes diagnosed early and the risks of CVD or mortality. The identification of prediabetes at an early stage is considered a separate contributor to the risk of CVD and is linked to a mortality rate of 15%. According to the findings of the Emerging Risk Factors Collaboration, an elevated mortality risk was observed among 820900 participants from 97 prospective studies when fasting glucose levels exceeded 5.5 mmol/L, rather than falling within the range of 3.9-5.5 mmol/L[19]. We discovered that the occurrence of prediabetes in younger patients who were recently diagnosed was strongly linked to the incidence of CVD. This implies that the prevention and treatment of CVD in the future should prioritize prediabetes, especially among the younger prediabetes population.

Regardless of age group, the timely and precise prediction of CVD risk and the subsequent adoption of preventive measures significantly improve patients' well-being and quality of life.

Implementing tactics to prevent both primary and secondary occurrences of CVD and/or its associated risk factors will alleviate the financial impact caused by this ailment. CVD risk factors can be categorized into modifiable or non-modifiable factors. Age, genetics, family history, gender, and race are among the factors involved. The risk factors that can be changed are categorized as: (1) Cardiometabolic factors, including high blood pressure, abnormal blood lipid levels, diabetes, and being overweight (which collectively make up the metabolic syndrome); and (2) lifestyle factors, such as tobacco use, lack of physical activity, poor diet, and low socio-economic status. Furthermore, there is growing evidence indicating that apart from genetic predisposition, early family-based environmental factors such as early nutrition, socioeconomic status, housing, and neighborhood play a significant role in the occurrence of CVD. Young individuals who have a familial background of CVD already possess an unfinished/unusual CVD risk profile. The authors Kataria and colleagues[20] examined the variation in plasma lipid levels and systemic blood pressure among healthy young college students who have a positive family history of CVD.

Nevertheless, in the case of elderly individuals, a family history of CVD does not pose a substantial threat to the ailment. Lifestyle and the environment in which one lives are the primary contributors to the most notable hazards. In the elderly population[21], health disparities persist among various regions and residential areas, playing a crucial role in determining overall health. Globally, it has been confirmed that there are differences in CVD mortality and levels of risk factors between urban and rural areas[22]. According to findings from a future urban-rural investigation, cardiovascular event rates were greater in rural regions compared to urban communities in middle- and low-income nations, despite urban settings having higher risk factors than rural areas[23]. Moreover, findings from a previous study conducted in Finland indicated that older individuals residing in rural regions had a higher occurrence of increased serum cholesterol levels and obesity compared to those residing in urban localities[24].

Notably, alcohol consumption has complex and sometimes paradoxical associations with CVD. In recent times, a considerable number of epidemiological studies[25] have been released concerning this subject. Experimental evidence strongly supports the advantageous impact of moderate alcohol intake, excluding instances of excessive drinking. Epidemiological data suggest that alcohol consumption protects some people against ischemic diseases to some degree. A J-shaped correlation was observed between the mean intake of alcohol and CVD, as reported in reference[26], which means for low to moderate alcohol consumption, a lower CVD risk is observed compared to abstaining and excessive drinking. Nevertheless, as most of the protective evidence of low to moderate alcohol consumption on CVD is from observational studies, it is uncertain whether this effect is a result of different forms of bias. According to a quantitative meta-analysis, individuals who consumed less than 30 g/d of alcohol and did not engage in heavy drinking episodes had the lowest risk of ischemic heart disease (relative risk = 0.64, 95%CI: 0.53, 0.71)[27]. Due to the lack of RCT, which is the gold standard, the focus in research has now shifted to new analytical methods, such as Mendelian randomization studies. However, none of these studies could truly resolve the pressing question of whether alcohol is the protective factor of CVD. Therefore, there is remaining controversy regarding the effects of moderate alcohol consumption on CVD.

In individuals with prediabetes, randomized clinical trials have demonstrated that interventions incorporating diet and physical activity can decrease the likelihood of developing diabetes. To alleviate the effects of newly diagnosed diabetes, it is imperative to enforce public health interventions. According to the latest ADA guidelines, it is recommended to annually screen individuals with prediabetes for diabetes and refer them to a lifestyle intervention aimed at promoting weight loss[28]. The authors Qiao *et al*[29] discovered that when analyzing combined data from Asian groups, 75% of individuals with prediabetes exhibited isolated IGT following glucose loading. The presence of insulin resistance increases the likelihood of developing CVD in both the general population and individuals with diabetes. Additionally, it serves as an indicator of the cardiovascular outlook for patients with CVD[30]. The findings of this research validated a correlation between age and other contributing elements, which could be significant in elucidating the variations in CVD risk factors among younger and older individuals. To prevent and manage CVD, community health centers can offer health advice to individuals across various age brackets.

This study has several strengths, including the incorporation of a vast, nationwide study sample; a thorough evaluation of their blood sugar levels, encompassing FPG and PG2h; and meticulous recognition of CVD by China-PAR.

This study has some limitations. To define prediabetes, ADA now suggests utilizing HbA1c within the range of 5.7%-6.4% (39-47 mmol/mol), according to their latest recommendation[31]. Nevertheless, in our research, we detected prediabetes by assessing FPG and PG2h. The HbA1c level was not measured, resulting in a decrease in the number of prediabetes diagnoses. Furthermore, we meticulously accounted for variables that could influence the results in the analyses, although there is a possibility of biases arising from unmeasured confounding and reverse causality. Furthermore, the present study's cross-sectional design poses challenges in determining the causal relationship between variables. Further confirmation through prospective research is needed to establish the causal relationship between the

research factors and conclusions, as the relationship is currently exploratory.

CONCLUSION

In summary, our findings suggest that prediabetes detected through FPG and PG2h might have a stronger association with CVD in younger individuals compared to older individuals. The findings of our study validated that the risk factors associated with CVD vary across age groups during the diagnosis of prediabetes. Therefore, age should be specifically considered in the care of adults with prediabetes for CVD prevention.

ARTICLE HIGHLIGHTS

Research background

Cardiovascular disease (CVD) is a leading cause of morbidity and mortality worldwide, the global burden of which is rising. It is still unclear the extent to which prediabetes contributes to the risk of CVD in various age brackets among adults.

Research motivation

To develop a focused screening plan and treatment for Chinese adults with prediabetes, it is crucial to identify variations in the connection between prediabetes and the risk of CVD based on age.

Research objectives

To examine the clinical features of prediabetes and identify risk factors for CVD in different age groups in China.

Research methods

We analyzed age-specific differences in prediabetes to identify features for the 10-year risk of CVD in a large, representative population divided by age (younger < 40 and older > 40 years old). Chinese atherosclerotic CVD risk prediction model was employed to evaluate the risk of developing CVD over 10 years. Random forest was established in both age groups. SHapley Additive exPlanation method prioritized the importance of features from the perspective of assessment contribution.

Research results

In total, 6948 people were diagnosed with prediabetes in this study. In prediabetes, prevalences of CVD were 5 (0.29%) in the younger group and 148 (2.85%) in the older group. Overall, 11.11% of the younger group and 29.59% of the older group were intermediate/high-risk of CVD for prediabetes without CVD based on the China-PAR equation in ten years. In the younger age group, the 10-year risk of CVD was found to be more closely linked to family history of CVD rather than lifestyle, whereas in the older age group, resident status was more closely linked.

Research conclusions

The susceptibility to CVD is age-specific in newly diagnosed prediabetes. It is necessary to develop targeted approaches for the prevention and management of CVD in adults across various age brackets.

Research perspectives

Identification of prediabetes may help develop strategies to prevent and control CVD in China.

FOOTNOTES

Author contributions: Xie S and Zhang B designed the research study, contributed to the discussion, and wrote, reviewed, and edited the manuscript; Yu LP reviewed the manuscript; Chen F analyzed the data; Wang Y, Deng RF, and Zhang XL provided suggestions for the revision of the manuscript; Zhang B is the guarantor of this work and, as such, has full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis; all authors approved the final version of the manuscript.

Institutional review board statement: The study program was reviewed and approved by the Clinical Research Ethics Committee of China-Japan Friendship Hospital (No. 2007-026).

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

Conflict-of-interest statement: There are no conflicts of interest to report.

Data sharing statement: The datasets generated during and/or analyzed in the current study are available from the corresponding

author upon reasonable request.

STROBE statement: The authors have read the STROBE Statement—checklist of items, and the manuscript was prepared and revised according to the STROBE Statement—checklist of items.

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REFERENCES

1 **Vogel B**, Acevedo M, Appelman Y, Bairey Merz CN, Chieffo A, Figtree GA, Guerrero M, Kunadian V, Lam CSP, Maas AHEM, Mihailidou AS, Olszanecka A, Poole JE, Saldarriaga C, Saw J, Zühlke L, Mehran R. The Lancet women and cardiovascular disease Commission: reducing the global burden by 2030. *Lancet* 2021; **397**: 2385-2438 [PMID: 34010613 DOI: 10.1016/S0140-6736(21)00684-X]

2 **Wang L**, Peng W, Zhao Z, Zhang M, Shi Z, Song Z, Zhang X, Li C, Huang Z, Sun X, Wang L, Zhou M, Wu J, Wang Y. Prevalence and Treatment of Diabetes in China, 2013-2018. *JAMA* 2021; **326**: 2498-2506 [PMID: 34962526 DOI: 10.1001/jama.2021.22208]

3 **Echouffo-Tcheugui JB**, Selvin E. Prediabetes and What It Means: The Epidemiological Evidence. *Annu Rev Public Health* 2021; **42**: 59-77 [PMID: 33355476 DOI: 10.1146/annurev-publhealth-090419-102644]

4 **Ali MK**, Bullard KM, Saydah S, Imperatore G, Gregg EW. Cardiovascular and renal burdens of prediabetes in the USA: analysis of data from serial cross-sectional surveys, 1988-2014. *Lancet Diabetes Endocrinol* 2018; **6**: 392-403 [PMID: 29500121 DOI: 10.1016/S2213-8587(18)30027-5]

5 **Ridker PM**, Cook NR. Statins: new American guidelines for prevention of cardiovascular disease. *Lancet* 2013; **382**: 1762-1765 [PMID: 24268611 DOI: 10.1016/S0140-6736(13)62388-0]

6 **Yang X**, Li J, Hu D, Chen J, Li Y, Huang J, Liu X, Liu F, Cao J, Shen C, Yu L, Lu F, Wu X, Zhao L, Gu D. Predicting the 10-Year Risks of Atherosclerotic Cardiovascular Disease in Chinese Population: The China-PAR Project (Prediction for ASCVD Risk in China). *Circulation* 2016; **134**: 1430-1440 [PMID: 27682885 DOI: 10.1161/CIRCULATIONAHA.116.022367]

7 **Yang W**, Xiao J, Yang Z, Ji L, Jia W, Weng J, Lu J, Shan Z, Liu J, Tian H, Ji Q, Zhu D, Ge J, Lin L, Chen L, Guo X, Zhao Z, Li Q, Zhou Z, Shan G, He J; China National Diabetes and Metabolic Disorders Study Investigators. Serum lipids and lipoproteins in Chinese men and women. *Circulation* 2012; **125**: 2212-2221 [PMID: 22492668 DOI: 10.1161/CIRCULATIONAHA.111.065904]

8 **Yang W**, Lu J, Weng J, Jia W, Ji L, Xiao J, Shan Z, Liu J, Tian H, Ji Q, Zhu D, Ge J, Lin L, Chen L, Guo X, Zhao Z, Li Q, Zhou Z, Shan G, He J; China National Diabetes and Metabolic Disorders Study Group. Prevalence of diabetes among men and women in China. *N Engl J Med* 2010; **362**: 1090-1101 [PMID: 20335585 DOI: 10.1056/NEJMoa0908292]

9 **Alberti KG**, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 1998; **15**: 539-553 [PMID: 9686693 DOI: 10.1002/(SICI)1096-9136(199807)15:7<539::AID-DIA668>3.0.CO;2-S]

10 **Truett J**, Cornfield J, Kannel W. A multivariate analysis of the risk of coronary heart disease in Framingham. *J Chronic Dis* 1967; **20**: 511-524 [PMID: 6028270 DOI: 10.1016/0021-9681(67)90082-3]

11 **Li L**, Wu M, Yu Z, Niu T. Nutritional Status Indices and Monoclonal Gammopathy of Undetermined Significance Risk in the Elderly Population: Findings from the National Health and Nutrition Examination Survey. *Nutrients* 2023; **15** [PMID: 37836494 DOI: 10.3390/nu15194210]

12 **Bergami M**, Scarpone M, Bugiardini R, Cenko E, Manfrini O. Sex beyond cardiovascular risk factors and clinical biomarkers of cardiovascular disease. *Rev Cardiovasc Med* 2022; **23**: 19 [PMID: 35092211 DOI: 10.31083/j.rcm2301019]

13 **Huo X**, Gao L, Guo L, Xu W, Wang W, Zhi X, Li L, Ren Y, Qi X, Sun Z, Li W, Ji Q, Ran X, Su B, Hao C, Lu J, Guo X, Zhuo H, Zhang D, Pan C, Weng J, Hu D, Yang X, Ji L. Risk of non-fatal cardiovascular diseases in early-onset versus late-onset type 2 diabetes in China: a cross-sectional study. *Lancet Diabetes Endocrinol* 2016; **4**: 115-124 [PMID: 26704379 DOI: 10.1016/S2213-8587(15)00508-2]

14 **Kim SM**, Lee G, Choi S, Kim K, Jeong SM, Son JS, Yun JM, Kim SG, Hwang SS, Park SY, Kim YY, Park SM. Association of early-onset diabetes, prediabetes and early glycaemic recovery with the risk of all-cause and cardiovascular mortality. *Diabetologia* 2020; **63**: 2305-2314 [PMID: 32820349 DOI: 10.1007/s00125-020-05252-y]

15 **Huang Y**, Cai X, Mai W, Li M, Hu Y. Association between prediabetes and risk of cardiovascular disease and all cause mortality: systematic review and meta-analysis. *BMJ* 2016; **355**: i5953 [PMID: 27881363 DOI: 10.1136/bmj.i5953]

16 **Chan JC**, Lau ES, Luk AO, Cheung KK, Kong AP, Yu LW, Choi KC, Chow FC, Ozaki R, Brown N, Yang X, Bennett PH, Ma RC, So WY. Premature mortality and comorbidities in young-onset diabetes: a 7-year prospective analysis. *Am J Med* 2014; **127**: 616-624 [PMID: 24680795 DOI: 10.1016/j.amjmed.2014.03.018]

17 **Lemieux I**, Després JP. Metabolic Syndrome: Past, Present and Future. *Nutrients* 2020; **12** [PMID: 33202550 DOI: 10.3390/nu12113501]

18 **Teo KK**, Rafiq T. Cardiovascular Risk Factors and Prevention: A Perspective From Developing Countries. *Can J Cardiol* 2021; **37**: 733-743 [PMID: 33610690 DOI: 10.1016/j.cjca.2021.02.009]

19 **Rao Kondapally Seshasai S**, Kaptoge S, Thompson A, Di Angelantonio E, Gao P, Sarwar N, Whincup PH, Mukamal KJ, Gillum RF, Holme I, Njølstad I, Fletcher A, Nilsson P, Lewington S, Collins R, Gudnason V, Thompson SG, Sattar N, Selvin E, Hu FB, Danesh J; Emerging Risk Factors Collaboration. Diabetes mellitus, fasting glucose, and risk of cause-specific death. *N Engl J Med* 2011; **364**: 829-841 [PMID: 21366474 DOI: 10.1056/NEJMoa1008862]

20 **Kataria N**, Panda A, Singh S, Patrikar S, Sampath S. Risk factors for cardiovascular disease in a healthy young population: Family matters. *Med J Armed Forces India* 2022; **78**: 405-412 [PMID: 36267508 DOI: 10.1016/j.mjafi.2020.07.002]

21 **Anderson TJ**, Saman DM, Lipsky MS, Lutfiyya MN. A cross-sectional study on health differences between rural and non-rural U.S. counties using the County Health Rankings. *BMC Health Serv Res* 2015; **15**: 441 [PMID: 26423746 DOI: 10.1186/s12913-015-1053-3]

22 **Vaughan AS**, Quick H, Pathak EB, Kramer MR, Casper M. Disparities in Temporal and Geographic Patterns of Declining Heart Disease Mortality by Race and Sex in the United States, 1973-2010. *J Am Heart Assoc* 2015; **4** [PMID: 26672077 DOI: 10.1161/JAHA.115.002567]

23 **Yusuf S**, Rangarajan S, Teo K, Islam S, Li W, Liu L, Bo J, Lou Q, Lu F, Liu T, Yu L, Zhang S, Mony P, Swaminathan S, Mohan V, Gupta R, Kumar R, Vijayakumar K, Lear S, Anand S, Wielgosz A, Diaz R, Avezum A, Lopez-Jaramillo P, Lanas F, Yusoff K, Ismail N, Iqbal R, Rahman O, Rosengren A, Yusufali A, Kelishadi R, Kruger A, Puoane T, Szuba A, Chifamba J, Oguz A, McQueen M, McKee M, Dagenais G; PURE Investigators. Cardiovascular risk and events in 17 low-, middle-, and high-income countries. *N Engl J Med* 2014; **371**: 818-827 [PMID: 25162888 DOI: 10.1056/NEJMoa1311890]

24 **Fogelholm M**, Valve R, Absetz P, Heinonen H, Uutela A, Patja K, Karisto A, Konttinen R, Mäkelä T, Nissinen A, Jallinoja P, Nummela O, Talja M. Rural-urban differences in health and health behaviour: a baseline description of a community health-promotion programme for the elderly. *Scand J Public Health* 2006; **34**: 632-640 [PMID: 17132597 DOI: 10.1080/14034940600616039]

25 **Zhao J**, Stockwell T, Roemer A, Naimi T, Chikritzhs T. Alcohol Consumption and Mortality From Coronary Heart Disease: An Updated Meta-Analysis of Cohort Studies. *J Stud Alcohol Drugs* 2017; **78**: 375-386 [PMID: 28499102 DOI: 10.15288/jsad.2017.78.375]

26 **Roerecke M**. Alcohol's Impact on the Cardiovascular System. *Nutrients* 2021; **13** [PMID: 34684419 DOI: 10.3390/nu13103419]

27 **Roerecke M**, Rehm J. Alcohol consumption, drinking patterns, and ischemic heart disease: a narrative review of meta-analyses and a systematic review and meta-analysis of the impact of heavy drinking occasions on risk for moderate drinkers. *BMC Med* 2014; **12**: 182 [PMID: 25567363 DOI: 10.1186/s12916-014-0182-6]

28 **American Diabetes Association**. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2021. *Diabetes Care* 2021; **44**: S15-S33 [PMID: 33298413 DOI: 10.2337/dc21-S002]

29 **Qiao Q**, Nakagami T, Tuomilehto J, Borch-Johnsen K, Balkau B, Iwamoto Y, Tajima N; International Diabetes Epidemiology Group; DECODA Study Group. Comparison of the fasting and the 2-h glucose criteria for diabetes in different Asian cohorts. *Diabetologia* 2000; **43**: 1470-1475 [PMID: 11151755 DOI: 10.1007/s001250051557]

30 **Hill MA**, Yang Y, Zhang L, Sun Z, Jia G, Parrish AR, Sowers JR. Insulin resistance, cardiovascular stiffening and cardiovascular disease. *Metabolism* 2021; **119**: 154766 [PMID: 33766485 DOI: 10.1016/j.metabol.2021.154766]

31 **Kester LM**, Hey H, Hannon TS. Using hemoglobin A1c for prediabetes and diabetes diagnosis in adolescents: can adult recommendations be upheld for pediatric use? *J Adolesc Health* 2012; **50**: 321-323 [PMID: 22443833 DOI: 10.1016/j.jadohealth.2012.02.009]

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ORIGINAL ARTICLE

Observational Study

Application of non-mydriatic fundus photography-assisted telemedicine in diabetic retinopathy screening

Wan Zhou, Xiao-Jing Yuan, Jie Li, Wei Wang, Hao-Qiang Zhang, Yuan-Yuan Hu, Shan-Dong Ye

Specialty type: Endocrinology and metabolism

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Peer-review model: Single blind

Peer-review report's scientific quality classification

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Abstract

BACKGROUND

Early screening and accurate staging of diabetic retinopathy (DR) can reduce blindness risk in type 2 diabetes patients. DR's complex pathogenesis involves many factors, making ophthalmologist screening alone insufficient for prevention and treatment. Often, endocrinologists are the first to see diabetic patients and thus should screen for retinopathy for early intervention.

AIM

To explore the efficacy of non-mydriatic fundus photography (NMFP)-enhanced telemedicine in assessing DR and its various stages.

METHODS

This retrospective study incorporated findings from an analysis of 93 diabetic patients, examining both NMFP-assisted telemedicine and fundus fluorescein angiography (FFA). It focused on assessing the concordance in DR detection between these two methodologies. Additionally, receiver operating characteristic (ROC) curves were generated to determine the optimal sensitivity and specificity of NMFP-assisted telemedicine, using FFA outcomes as the standard benchmark.

RESULTS

In the context of DR diagnosis and staging, the kappa coefficients for NMFP-assisted telemedicine and FFA were recorded at 0.775 and 0.689 respectively, indicating substantial intermethod agreement. Moreover, the NMFP-assisted telemedicine's predictive accuracy for positive FFA outcomes, as denoted by the

area under the ROC curve, was remarkably high at 0.955, within a confidence interval of 0.914 to 0.995 and a statistically significant *P*-value of less than 0.001. This predictive model exhibited a specificity of 100%, a sensitivity of 90.9%, and a Youden index of 0.909.

CONCLUSION
NMFP-assisted telemedicine represents a pragmatic, objective, and precise modality for fundus examination, particularly applicable in the context of endocrinology inpatient care and primary healthcare settings for diabetic patients. Its implementation in these scenarios is of paramount significance, enhancing the clinical accuracy in the diagnosis and therapeutic management of DR. This methodology not only streamlines patient evaluation but also contributes substantially to the optimization of clinical outcomes in DR management.

Key Words: Diabetes; Diabetic retinopathy; Non-mydriatic fundus photography-assisted telemedicine; Fundus fluorescein angiography

Core Tip: There is a high consistency between non-mydriatic fundus photography (NMFP)-assisted telemedicine and fundus fluorescein angiography (FFA) techniques. The area under the curve of NMFP-assisted telemedicine results for predicting a positive result from FFA was 0.955. The specificity, sensitivity, and a Youden index of NMFP-assisted telemedicine were 100%, 90.9% and 0.909, respectively. The NMFP-assisted telemedicine has a great significant value in the clinical diagnosis and treatment of diabetic retinopathy.

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INTRODUCTION

Diabetic retinopathy (DR), a leading ocular pathology causing visual impairment, demonstrates an increased incidence and rate of blindness that correlate directly with the duration of diabetes and patient age[1,2]. The current global prevalence of DR among diabetic individuals is estimated at 34.6%, with a significant 10.2% progressing to severe visual impairment[3]. In China, approximately 87% of diabetic patients are treated in primary healthcare facilities, where ophthalmological resources are critically limited, and alarmingly, around 70% of these individuals do not receive standardized ophthalmic examinations[4]. Endocrinology departments typically serve as the initial consultation point for diabetic patients. However, there is a noted emphasis on metabolic markers such as glycemia and lipidemia, while fundus complications are often underprioritized. This leads to delayed referrals to ophthalmology services, usually at advanced stages of vision loss or blindness. At this juncture, the visual impairment induced by DR is largely irreversible, even with prompt and aggressive therapeutic interventions, significantly impacting patient quality of life and imposing substantial socio-economic burdens. Early and accurate diagnosis, followed by timely therapeutic intervention, is pivotal in arresting or mitigating the progression of DR[5]. DR can be divided into non-proliferative and proliferative types based on its clinical stage. Early screening for DR has become a priority in blindness prevention. Clinically, DR is stratified into non-proliferative and proliferative phases. Therefore, the development of an efficient and straightforward screening protocol for DR, especially tailored for endocrinologists and primary care practitioners, is imperative[6,7]. Fundus fluorescein angiography (FFA) is regarded as the gold standard for DR diagnosis. However, its invasive nature makes it unsuitable for mass DR screening. This study aims to evaluate the effectiveness of non-mydriatic fundus photography (NMFP)-assisted telemedicine in assessing DR and its various stages by assessing the concordance between NMFP-assisted telemedicine and FFA in diagnosing and staging DR.

MATERIALS AND METHODS

Subjects

Clinical data from diabetic patients diagnosed at the First Affiliated Hospital of University of Science and Technology of China between June 2019 and June 2021 were subject to a retrospective analysis. The study cohort consisted of 93 individuals (42 females and 51 males), each diagnosed through NMFP-assisted telemedicine and FFA. These patients were categorized based on the International Federation of Ophthalmological Societies' 2002 guidelines for DR screening and staging: Stage I involved no evident retinopathy, only minor hemorrhages in the posterior pole; stage II featured scattered punctate hyperfluorescent spots with capillary hemangiomas; stage III mirrored stage II in symptoms; stage IV

presented with fundus or vitreous hemorrhage due to neovascularization; stage V included fundus neovascularization accompanied by fibrous proliferation; and stage VI was characterized by both neovascularization and fibrous proliferation in the fundus, along with tractional retinal detachment. Stages I to III were classified under non-proliferative DR, while stages IV to VI fell under the proliferative DR category. Cases with no abnormal fundus findings were designated as no DR (NDR). This study adhered to the World Medical Association' Declaration of Helsinki and received approval from the ethics committee of the First Affiliated Hospital of University of Science and Technology of China.

Methods

FFA: Patients initially underwent an allergy test following mydriasis. Those who exhibited no allergic reaction proceeded to receive a rapid intravenous injection of sodium fluorescein. Subsequently, their fundus was imaged using the Heidelberg confocal laser angiography system (Global Vision, Germany), a sophisticated apparatus designed for detailed fundus examination.

NMFP-assisted telemedicine: A skilled endocrinology technician, in a controlled darkroom setting, utilized the Optomed Aurora® handheld non-mydriatic fundus camera to capture detailed images of the patients' fundus. Following a brief acclimatization period of five minutes in the examination room, two high-resolution color images were taken of each fundus' posterior pole. These images focused on the macula and optic disc, covering a 45° field of view. To ensure optimal image quality, patients were given a five-minute rest period after capturing the image of one eye, followed by imaging of the other eye post-pupil recovery. Subsequently, these fundus images, along with patient data, were uploaded to a specialized diagnostic platform. Here, an ophthalmologist employed the platform's advanced software for comprehensive analysis, diagnosis, and staging of the fundus condition (Figures 1 and 2).

Observational indicators

The study rigorously assessed the alignment between NMFP-assisted telemedicine and FFA in diagnosing and staging DR. Furthermore, it scrutinized the diagnostic efficacy of NMFP-assisted telemedicine in terms of sensitivity and specificity for DR, utilizing FFA results as the standard reference.

Statistical analysis

Statistical analysis was executed utilizing SPSS 23.0 software. The Kappa (κ) test was applied to evaluate the congruence between NMFP-assisted telemedicine and FFA test results, with κ values interpreted as follows: > 0.75 denoting exceptional concordance, 0.61-0.75 suggesting significant consistency, 0.41-0.60 indicating moderate agreement, and < 0.40 reflecting limited consistency. To ascertain the diagnostic predictive efficacy of NMFP-assisted telemedicine, receiver operating characteristic (ROC) curves were employed. A P value of less than 0.05 was set as the threshold for statistical significance.

RESULTS

The consistency check between NMFP-assisted telemedicine and FFA in the screening of DR

Of 23 patients (24.7%) were diagnosed with NDR and 70 patients (75.3%) with DR using NMFP-assisted telemedicine, whereas, FFA detected NDR in 16 patients (17.2%) and DR in 77 patients (82.8%). κ test analysis suggested consistency DR screening results between NMFP-assisted telemedicine and FFA with a κ value of 0.775 ($P < 0.001$) (Table 1).

The consistency check between NMFP-assisted telemedicine and FFA in the staging and diagnosis of DR

The 70 positive cases, diagnosed using both techniques, were categorized into stages I–VI, with 52 cases showing identical staging results. Among the patients diagnosed using NMFP-assisted telemedicine, the distribution across DR stages I, II, III, IV, V, and VI was 20, 16, 17, 11, 4, and 2, respectively. In contrast, for those diagnosed using FFA, the numbers were 15, 18, 21, 10, 5, and 1, respectively. The proportion of stage I patients diagnosed using NMFP-assisted telemedicine was slightly higher than that using FFA, while the proportion of patients at DR stages II and III diagnosed using NMFP-assisted telemedicine was lower than that using FFA. The κ test analysis indicated concordance between the results of NMFP-assisted telemedicine and FFA in the diagnosis and staging of DR, with a κ value of 0.689 ($P < 0.001$) (Table 2).

Diagnostic prediction effectiveness of NMFP-assisted telemedicine by ROC curve

ROC analysis was conducted using the FFA result as the dependent variable (DR assignment = 1, NDR assignment = 0) and the NMFP-assisted telemedicine result (DR assignment = 1, NDR assignment = 0) as the independent variable. The area under the curve for NMFP-assisted telemedicine in predicting a positive result from FFA was 0.955 (0.914, 0.995; $P < 0.001$), with a specificity of 100%, sensitivity of 90.9%, and a Youden index of 0.909 (Figure 3).

Typical fundus photos in different stages

Typical fundus photos in different stages examined by NMFP-assisted telemedicine were exhibited in Figure 4.

Table 1 Kappa test analysis between non-mydriatic fundus photography assisted telemedicine and fundus fluorescein angiography in the screening of diabetic retinopathy

Fundus fluorescein angiography	Non-mydriatic fundus photography-assisted telemedicine		Total (*n*)
	NDR	DR	
NDR	16	0	16
DR	7	70	77
Total (*n*)	23	70	93

NDR: No diabetic retinopathy; DR: Diabetic retinopathy.

Table 2 Kappa test analysis between non-mydriatic fundus photography assisted telemedicine and fundus fluorescein angiography in the staging and diagnosis of diabetic retinopathy

Fundus fluorescein angiography	No-mydriatic fundus photography-assisted telemedicine						Total (*n*)
	I	II	III	IV	V	VI	
I	15	0	0	0	0	0	15
II	2	13	1	2	0	0	18
III	3	3	15	0	0	0	21
IV	0	0	1	8	1	0	10
V	0	0	0	1	2	2	5
VI	0	0	0	0	1	0	1
Total (*n*)	20	16	17	11	4	2	70

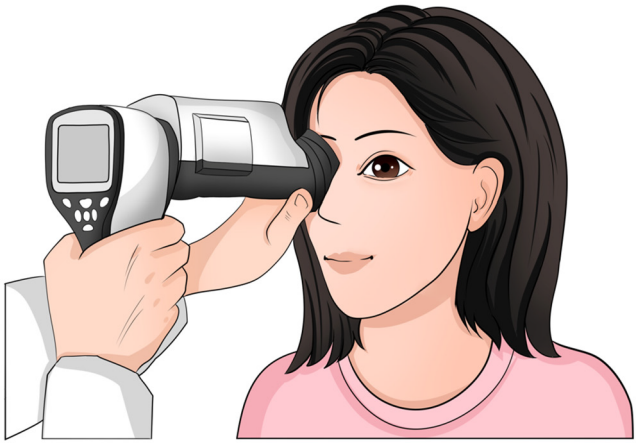

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Figure 1 Operation of the non-mydriatic portable fundus camera when performing fundus examination.

DISCUSSION

Traditional DR screening methods, including direct fundoscopy, indirect fundoscopy, and slit-lamp with preset lens methods, are known for their simplicity, speed, and patient cooperation[8,9]. However, these methods often exhibit poor accuracy and are not well-suited for mass DR screening. On the other hand, FFA, recognized as one of the most effective early DR detection methods, enables physicians to observe capillary non-perfusion patterns and disruptions in the blood-retinal barrier, providing insights into the source of macular edema-related leakage[10,11]. Consequently, FFA is regarded as the gold standard for DR diagnosis. Nevertheless, its invasive nature makes it unsuitable for pregnant women, patients with contrast allergies, or those with concurrent systemic illnesses, thereby limiting its applicability in mass DR screening.

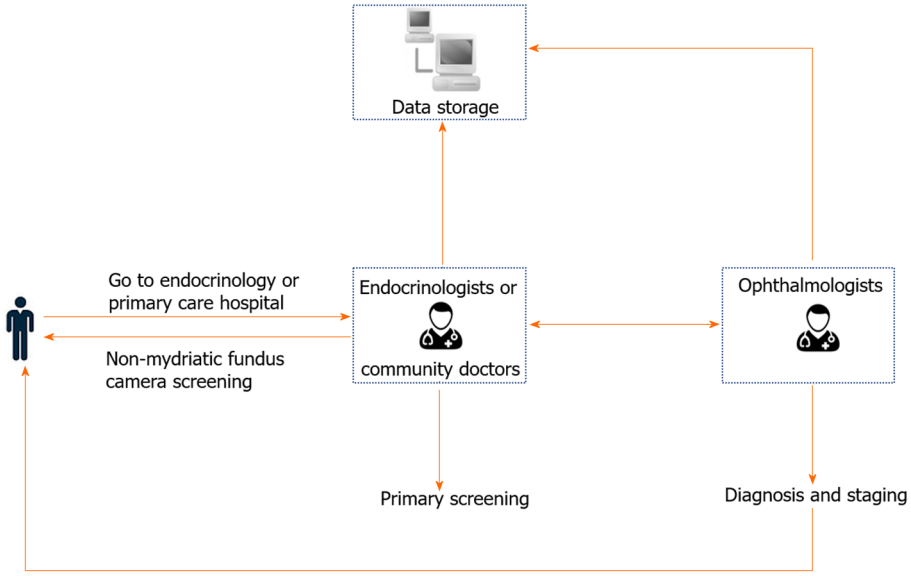

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Figure 2 Application of non-mydriatic fundus photography-assisted telemedicine.

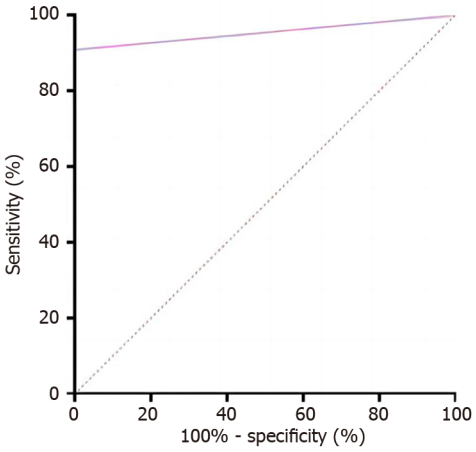

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Figure 3 The receiver operating characteristic curve identified diagnostic prediction effectiveness of non-mydriatic fundus photography-assisted telemedicine (area under the curve 0.955 with 90.9% sensitivity and 100% specificity).

NMFP, a technique that employs low-density light to capture high-resolution fundus images by enhancing camera sensitivity, has been utilized for fundus examinations since the 1980s[12]. Initially, Polaroid film and 35 mm transparencies were used, but technological advancements have significantly improved NMFP, resulting in high-quality, directly savable, and shareable images. This method offers several advantages, such as mydriasis-free operation, convenience, safety, and effectiveness. Numerous studies on DR screening have indicated that NMFP is a straightforward, objective, and cost-effective technique that enhances the efficiency of DR screening[13].

Research by Piyasena *et al*[14] demonstrated that NMFP surpasses mydriatic examination in sensitivity for detecting fundus lesions, particularly smaller ones like tiny retinal hemorrhages, microaneurysms, and neovascularization in various fundus areas[15]. Yaslam *et al*[16] also suggested that NMFP is more patient-friendly and can be widely employed for fundus examinations in both type 1 and type 2 diabetes cases. Dunn *et al*[17] showed that NMFP exhibits higher sensitivity in DR screening compared to direct fundoscopy, enhancing diagnostic accuracy for fundus pathology. In the current study, no significant difference was observed in the concordance rate between NMFP (75.3%) and FFA (82.8%), underscoring the substantial utility of NMFP in DR screening. When using FFA results as the gold standard, NMFP-assisted telemedicine demonstrated high diagnostic sensitivity and specificity in DR screening.

NMFP encompasses single-field, double-field, and seven-field photography, with most options offering a 45° field of view. Equipment models primarily include handheld, desktop, TV-type, and stereo cameras[18-21]. The advantages of the handheld NMFP employed in this study include its compact size, portability, and versatility to operate in various body positions, enabling high-definition fundus photography. Technological advancements and the widespread use of the internet have transformed DR screening into an efficient mode, with NMFP playing a pivotal role in this screening

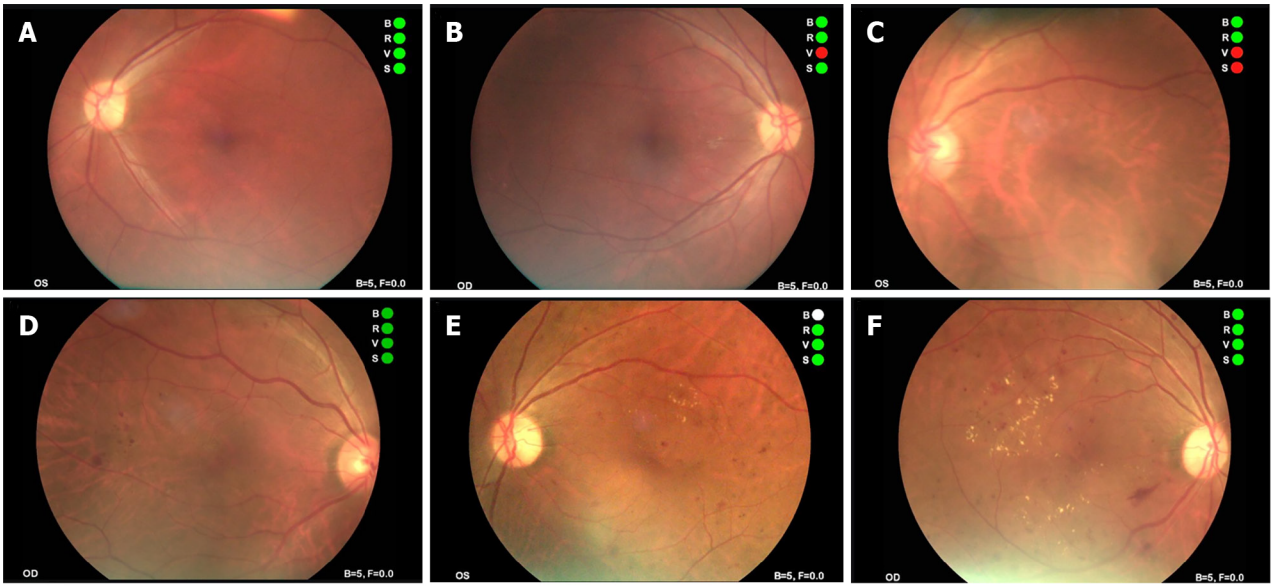

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Figure 4 Typical fundus photos in different stages of non-mydriatic fundus photography-assisted telemedicine. A and B: Photographs of normal fundus; C and D: Fundus photograph shows microhemangioma and retinal hemorrhage; E and F: Fundus photograph shows retinal hemorrhage and retinal hard exudates.

method. NMFP-assisted telemedicine relies on dedicated information transfer software tailored for remote screening of DR patients. In addition to storing and transmitting images, the software can record patients' medical history and physical examination results from each visit, streamlining data extraction, comparison, retrieval, query, and analysis for subsequent consultations. The advantages of NMFP-assisted telemedicine include: (1) Enhanced feasibility: Elderly diabetic patients with a lengthy disease course, post-cataract surgery individuals, and patients with angle-closure glaucoma often struggle with mydriasis. NMFP-assisted telemedicine significantly improves the screening feasibility for patients with small pupils[22]; (2) Patient engagement: NMFP-assisted telemedicine allows the storage of fundus images, enabling patients to visualize changes in their fundus. This facilitates timely and effective health education, empowers patients with essential knowledge about DR, and helps them comprehend disease progression. Consequently, it enhances patient compliance and shifts the focus from disease treatment to disease prevention; (3) Improved compliance: By avoiding the mydriasis procedure in fundus examination, NMFP-assisted telemedicine enhances patient compliance; (4) Accessibility: NMFP-assisted telemedicine is user-friendly, portable, and suitable for bedside data collection, making it particularly valuable for fundus screening in pediatric patients or those with limited mobility[23]; (5) Versatile use: This straightforward procedure makes fundus screening accessible to non-ophthalmologists, including endocrinologists and community physicians; (6) Telemedicine potential: Combining NMFP with telemedicine holds great promise in emphasizing the significance of ocular fundus examinations in endocrinology inpatients and primary care hospitals. It facilitates access to ophthalmic consultative services and supports clinical and epidemiologic research[16,24]. Digital fundus photography and network technology enable the acquisition of patient information, remote screening, consultation, and diagnosis, coupled with digital storage and information sharing; and (7) Cost-effectiveness: For endocrinology departments, NMFP testing is cost-effective with reusable equipment. For patients, the cost of NMFP testing is lower and within the range covered by medical insurance, making NMFP more suitable for large-scale screening in DR diagnosis. However, NMFP also has some potential risks. For instance, in telemedicine systems, patient medical information and images are transmitted over the internet, which could lead to data security and privacy issues. Moreover, remote diagnosis may lack direct face-to-face interaction with patients. Therefore, in practical applications, doctors must be vigilant in protecting patient privacy and strive to explain the diagnostic results to the patients as clearly as possible.

The presence, severity, and staging of DR are pivotal factors in the screening of DR, particularly in primary care hospitals facing limitations in medical resources. These examination results play a critical role in determining whether patients should be referred to higher-level hospitals for further consultation and prompt treatment. This approach ensures the early detection of DR, timely intervention, and facilitates the implementation of a hospital-based hierarchical diagnosis and treatment system. A real-world, multicenter, and prospective study have demonstrated the efficacy of NMFP in both DR screening and grading[25]. In our study, the results obtained from NMFP-assisted telemedicine and FFA exhibited remarkable consistency across different DR stages. Besides screening for various fundus lesions, NMFP-assisted telemedicine also allows for DR staging. The proportion of patients in stage I diagnosed using NMFP-assisted telemedicine was slightly higher than those diagnosed using FFA. In contrast, for patients in stages II and III, NMFP-assisted telemedicine had a lower proportion of diagnoses compared to FFA. NMFP-assisted telemedicine can promptly identify early signs such as hard exudates and arterial aneurysms. However, FFA excels in providing a comprehensive characterization of retinal blood flow, including all aneurysms, identification of exudates and microhemorrhages, and observation of vascular permeability alterations. Consequently, NMFP-assisted telemedicine may occasionally yield false-negative results.

Limitations

This article still has some limitations and deficiencies. Firstly, the sample size is small, and secondly, it lacks a cross-sectional comparison with other DR screening methods, which will be a direction for future research.

CONCLUSION

In summary, early screening and accurate staging of DR can reduce the risk of blindness in patients with type 2 diabetes. The pathogenesis of DR is complex, with numerous factors affecting its onset and progression. Therefore, the prevention and treatment of DR cannot rely solely on screening by ophthalmologists. Because endocrinology is often the first department that diabetic patients visit, endocrinologists need to screen diabetic patients for retinopathy detection and early intervention. NMFP-assisted telemedicine can be comprehensively used to facilitate fundus examination among endocrinology inpatients and diabetic patients in primary hospitals. In addition, the captured images and basic information from NMFP-assisted telemedicine can be archived and uploaded to a software platform to help ophthalmologists diagnose and create medical reports, thereby enabling effective prevention, control, and management of diabetes and its fundus complications. Furthermore, the collected image data can be summarized, analyzed, stored digitally, and shared for big data analysis. Diabetic patients diagnosed using NMFP-assisted telemedicine can promptly observe their fundus lesions and gain essential knowledge about DR in a timely and effective manner, thereby improving their awareness of the disease and compliance with treatment and preventing the onset and progression of DR.

ARTICLE HIGHLIGHTS

Research background

Prompt detection and precise classification of diabetic retinopathy (DR) in individuals with type 2 diabetes can lessen the likelihood of blindness. Given DR's intricate causes, relying solely on ophthalmologist examinations may not be enough for effective prevention and treatment. Since endocrinologists frequently encounter diabetic patients initially, they play a crucial role in early DR screening and intervention.

Research motivation

We endeavored to offer fresh perspectives on the screening approaches for DR.

Research objectives

This study investigates the effectiveness of telemedicine enhanced by non-mydriatic fundus photography (NMFP) in evaluating DR and its different stages.

Research methods

This study retrospectively analyzed 93 diabetic patients, comparing NMFP-assisted telemedicine with fundus fluorescein angiography (FFA) in detecting DR. It aimed to evaluate the agreement between these methods and used receiver operating characteristic (ROC) curves to assess the accuracy of NMFP against the FFA benchmark.

Research results

In diagnosing and staging DR, NMFP-assisted telemedicine and FFA showed substantial agreement with kappa coefficients of 0.775 and 0.689, respectively. NMFP's predictive accuracy for positive FFA outcomes, indicated by a ROC curve area of 0.955 (confidence interval 0.914 to 0.995) and a P-value < 0.001, was high. The model demonstrated 100% specificity, 90.9% sensitivity, and a Youden index of 0.909.

Research conclusions

This study introduces NMFP-assisted telemedicine as a practical, accurate method for examining the fundus, especially suitable for endocrinology inpatient care and primary healthcare for diabetic patients. Its use in these settings is crucial for improving the accuracy of diagnosing and treating DR. This approach simplifies patient assessments and significantly improves clinical results in DR management. The new theories proposed by this study include the importance of integrating NMFP-assisted telemedicine into endocrinology and primary healthcare for enhanced DR management. The new method proposed is the application of NMFP-assisted telemedicine itself for fundus examination in diabetic patients.

Research perspectives

Future research should focus on expanding the use of NMFP-assisted telemedicine in various healthcare settings for DR management, and conducting larger, long-term studies to evaluate its effectiveness. Additionally, exploring technological improvements and interdisciplinary collaborations can enhance the accuracy and impact of this approach in DR diagnosis and treatment.

FOOTNOTES

Co-corresponding authors: Wan Zhou and Wei Wang.

Author contributions: Yuan XJ and Li J collected and analyzed the data; Ye SD reviewed and edited the manuscript; Zhang HQ and Hu YY performed the statistical analyses and interpreted experimental results. Wang W and Zhou W contributed equally to this article, they are co-corresponding authors of this manuscript. Zhou W designed the research, analyzed the data, and wrote the manuscript; Wang W reviewed and edited the manuscript, and revised the manuscript. Zhou W is the principal corresponding author and have been responsible for the submission of the manuscript, communication during the peer review and publication processes, providing ethics committee approvals, clinical registration documents, and conflict of interest forms and statements.

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REFERENCES

1 **Chalke SD**, Kale PP. Combinational Approaches Targeting Neurodegeneration, Oxidative Stress, and Inflammation in the Treatment of Diabetic Retinopathy. *Curr Drug Targets* 2021; **22**: 1810-1824 [PMID: 33745432 DOI: 10.2174/1389450122666210319113136]
2 **Lamacchia O**, Sorrentino MR, Picca G, Paradiso M, Maiellaro P, De Cosmo S. Cardio-ankle vascular index is associated with diabetic retinopathy in younger than 70 years patients with type 2 diabetes mellitus. *Diabetes Res Clin Pract* 2019; **155**: 107793 [PMID: 31325539 DOI: 10.1016/j.diabres.2019.107793]
3 **Yau JW**, Rogers SL, Kawasaki R, Lamoureux EL, Kowalski JW, Bek T, Chen SJ, Dekker JM, Fletcher A, Grauslund J, Haffner S, Hamman RF, Ikram MK, Kayama T, Klein BE, Klein R, Krishnaiah S, Mayurasakorn K, O'Hare JP, Orchard TJ, Porta M, Rema M, Roy MS, Sharma T, Shaw J, Taylor H, Tielsch JM, Varma R, Wang JJ, Wang N, West S, Xu L, Yasuda M, Zhang X, Mitchell P, Wong TY; Meta-Analysis for Eye Disease (META-EYE) Study Group. Global prevalence and major risk factors of diabetic retinopathy. *Diabetes Care* 2012; **35**: 556-564 [PMID: 22301125 DOI: 10.2337/dc11-1909]
4 **Ha M**, Choi SY, Kim M, Na JK, Park YH. Diabetic Nephropathy in Type 2 Diabetic Retinopathy Requiring Panretinal Photocoagulation. *Korean J Ophthalmol* 2019; **33**: 46-53 [PMID: 30746911 DOI: 10.3341/kjo.2018.0034]
5 **Safi H**, Safi S, Hafezi-Moghadam A, Ahmadieh H. Early detection of diabetic retinopathy. *Surv Ophthalmol* 2018; **63**: 601-608 [PMID: 29679616 DOI: 10.1016/j.survophthal.2018.04.003]
6 **Nguyen HV**, Tan GS, Tapp RJ, Mital S, Ting DS, Wong HT, Tan CS, Laude A, Tai ES, Tan NC, Finkelstein EA, Wong TY, Lamoureux EL. Cost-effectiveness of a National Telemedicine Diabetic Retinopathy Screening Program in Singapore. *Ophthalmology* 2016; **123**: 2571-2580 [PMID: 27726962 DOI: 10.1016/j.ophtha.2016.08.021]
7 **Hu J**, Chen R, Lu Y, Dou X, Ye B, Cai Z, Pu Z, Mou L. Single-Field Non-Mydriatic Fundus Photography for Diabetic Retinopathy Screening: A Systematic Review and Meta-Analysis. *Ophthalmic Res* 2019; **62**: 61-67 [PMID: 31067550 DOI: 10.1159/000499106]
8 **Mujeeb S**, Rodrigues GR, Nayak RR, Kamath AR, Kamath SJ, Kamath G. Urine protein: Urine creatinine ratio correlation with diabetic retinopathy. *Indian J Ophthalmol* 2021; **69**: 3359-3363 [PMID: 34708805 DOI: 10.4103/ijo.IJO_1269_21]
9 **Garvican L**, Clowes J, Gillow T. Preservation of sight in diabetes: developing a national risk reduction programme. *Diabet Med* 2000; **17**: 627-634 [PMID: 11051281 DOI: 10.1046/j.1464-5491.2000.00353.x]
10 **Wang S**, Zuo Y, Wang N, Tong B. Fundus fluorescence Angiography in diagnosing diabetic retinopathy. *Pak J Med Sci* 2017; **33**: 1328-1332

[PMID: 29492053 DOI: 10.12669/pjms.336.13405]

11 **Li X**, Xie J, Zhang L, Cui Y, Zhang G, Wang J, Zhang A, Chen X, Huang T, Meng Q. Differential distribution of manifest lesions in diabetic retinopathy by fundus fluorescein angiography and fundus photography. *BMC Ophthalmol* 2020; **20**: 471 [PMID: 33261573 DOI: 10.1186/s12886-020-01740-2]

12 **Dunn HP**, Teo KZ, Smyth JW, Weerasinghe LS, Costello J, Pampapathi P, Keay L, Green T, Vukasovic M, Bruce BB, Newman NJ, Biousse V, White AJ, McCluskey P, Fraser CL. Using non-mydriatic fundus photography to detect fundus pathology in Australian metropolitan emergency departments: A prospective prevalence and diagnostic accuracy study. *Emerg Med Australas* 2021; **33**: 302-309 [PMID: 32945132 DOI: 10.1111/1742-6723.13619]

13 **Phiri R**, Keeffe JE, Harper CA, Taylor HR. Comparative study of the polaroid and digital non-mydriatic cameras in the detection of referrable diabetic retinopathy in Australia. *Diabet Med* 2006; **23**: 867-872 [PMID: 16911624 DOI: 10.1111/j.1464-5491.2006.01824.x]

14 **Piyasena MMPN**, Yip JLY, MacLeod D, Kim M, Gudlavalleti VSM. Diagnostic test accuracy of diabetic retinopathy screening by physician graders using a hand-held non-mydriatic retinal camera at a tertiary level medical clinic. *BMC Ophthalmol* 2019; **19**: 89 [PMID: 30961576 DOI: 10.1186/s12886-019-1092-3]

15 **Bedard C**, Sherry Liu S, Patterson C, Gerstein H, Griffith L. Systematic review: Can non-mydriatic cameras accurately detect diabetic retinopathy? *Diabetes Res Clin Pract* 2017; **129**: 154-159 [PMID: 28528076 DOI: 10.1016/j.diabres.2017.04.024]

16 **Yaslam M**, Al Adel F, Al-Rubeaan K, AlSalem RK, Alageel MA, Alsalhi A, AlNageeb D, Youssef AM. Non-mydriatic fundus camera screening with diagnosis by telemedicine for diabetic retinopathy patients with type 1 and type 2 diabetes: a hospital-based cross-sectional study. *Ann Saudi Med* 2019; **39**: 328-336 [PMID: 31580703 DOI: 10.5144/0256-4947.2019.328]

17 **Dunn HP**, Browning SD, Thomson D, Yates WB, McCluskey P, Keay L, White AJ, Fraser CL. Impact on patient management of non-mydriatic fundus photography compared to direct ophthalmoscopy in a regional Australian emergency department. *Emerg Med Australas* 2022; **34**: 186-193 [PMID: 34448357 DOI: 10.1111/1742-6723.13845]

18 **Bawankar P**, Shanbhag N, K SS, Dhawan B, Palsule A, Kumar D, Chandel S, Sood S. Sensitivity and specificity of automated analysis of single-field non-mydriatic fundus photographs by Bosch DR Algorithm-Comparison with mydriatic fundus photography (ETDRS) for screening in undiagnosed diabetic retinopathy. *PLoS One* 2017; **12**: e0189854 [PMID: 29281690 DOI: 10.1371/journal.pone.0189854]

19 **Neubauer AS**, Rothschuh A, Ulbig MW, Blum M. Digital fundus image grading with the non-mydriatic Visucam(PRO NM) versus the FF450(plus) camera in diabetic retinopathy. *Acta Ophthalmol* 2008; **86**: 177-182 [PMID: 17944975 DOI: 10.1111/j.1600-0420.2007.01029.x]

20 **Soto-Pedre E**, Hernaez-Ortega MC. Screening coverage for diabetic retinopathy using a three-field digital non-mydriatic fundus camera. *Prim Care Diabetes* 2008; **2**: 141-146 [PMID: 18779038 DOI: 10.1016/j.pcd.2008.04.003]

21 **Gajiwala UR**, Pachchigar S, Patel D, Mistry I, Oza Y, Kundaria D, B R S. Non-mydriatic fundus photography as an alternative to indirect ophthalmoscopy for screening of diabetic retinopathy in community settings: a comparative pilot study in rural and tribal India. *BMJ Open* 2022; **12**: e058485 [PMID: 35396308 DOI: 10.1136/bmjopen-2021-058485]

22 **Neubauer AS**, Chryssafis C, Thiel M, Priglinger S, Welge-Lüssen U, Kampik A. [Screening for diabetic retinopathy and optic disc topography with the "retinal thickness analyzer" (RTA)]. *Ophthalmologe* 2005; **102**: 251-258 [PMID: 15351898 DOI: 10.1007/s00347-004-1098-x]

23 **Le Jeune C**, Chebli F, Leon L, Anthoine E, Weber M, Péchereau A, Lebranchu P. Reliability and reproducibility of disc-foveal angle measurements by non-mydriatic fundus photography. *PLoS One* 2018; **13**: e0191007 [PMID: 29370195 DOI: 10.1371/journal.pone.0191007]

24 **Hafiz F**, Chalakkal RJ, Hong SC, Linde G, Hu R, O'Keeffe B, Boobin Y. A new approach to non-mydriatic portable fundus imaging. *Expert Rev Med Devices* 2022; **19**: 303-314 [PMID: 35473498 DOI: 10.1080/17434440.2022.2070004]

25 **Zhang Y**, Shi J, Peng Y, Zhao Z, Zheng Q, Wang Z, Liu K, Jiao S, Qiu K, Zhou Z, Yan L, Zhao D, Jiang H, Dai Y, Su B, Gu P, Su H, Wan Q, Liu J, Hu L, Ke T, Chen L, Xu F, Dong Q, Terzopoulos D, Ning G, Xu X, Ding X, Wang W. Artificial intelligence-enabled screening for diabetic retinopathy: a real-world, multicenter and prospective study. *BMJ Open Diabetes Res Care* 2020; **8** [PMID: 33087340 DOI: 10.1136/bmjdrc-2020-001596]

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ORIGINAL ARTICLE

Basic Study

Long noncoding RNA protein-disulfide isomerase-associated 3 regulated high glucose-induced podocyte apoptosis in diabetic nephropathy through targeting miR-139-3p

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Abstract

BACKGROUND

Podocyte apoptosis plays a vital role in proteinuria pathogenesis in diabetic nephropathy (DN). The regulatory relationship between long noncoding RNAs (lncRNAs) and podocyte apoptosis has recently become another research hot spot in the DN field.

AIM

To investigate whether lncRNA protein-disulfide isomerase-associated 3 (Pdia3) could regulate podocyte apoptosis through miR-139-3p and revealed the underlying mechanism.

METHODS

Using normal glucose or high glucose (HG)-cultured podocytes, the cellular functions and exact mechanisms underlying the regulatory effects of lncRNA Pdia3 on podocyte apoptosis and endoplasmic reticulum stress (ERS) were explored. LncRNA Pdia3 and miR-139-3p expression were measured through quantitative real-time polymerase chain reaction. Relative cell viability was detected through the cell counting kit-8 colorimetric assay. The podocyte apoptosis rate in each group was measured through flow cytometry. The interaction between lncRNA Pdia3 and miR-139-3p was examined through the dual luciferase reporter assay. Finally, western blotting was performed to detect the effect of lncRNA Pdia3 on podocyte apoptosis and ERS *via* miR-139-3p.

RESULTS
The expression of lncRNA Pdia3 was significantly downregulated in HG-cultured podocytes. Next, lncRNA Pdia3 was involved in HG-induced podocyte apoptosis. Furthermore, the dual luciferase reporter assay confirmed the direct interaction between lncRNA Pdia3 and miR-139-3p. LncRNA Pdia3 overexpression attenuated podocyte apoptosis and ERS through miR-139-3p in HG-cultured podocytes.

CONCLUSION
Taken together, this study demonstrated that lncRNA Pdia3 overexpression could attenuate HG-induced podocyte apoptosis and ERS by acting as a competing endogenous RNA of miR-139-3p, which might provide a potential therapeutic target for DN.

Key Words: Long noncoding RNAs; Diabetic nephropathy; Podocyte apoptosis; Endoplasmic reticulum stress; Competing endogenous RNA

Core Tip: The expression of long noncoding RNA (lncRNA) protein-disulfide isomerase-associated 3 (Pdia3) was significantly downregulated in high glucose (HG)-cultured podocytes. LncRNA Pdia3 was involved in HG-induced podocyte apoptosis. LncRNA Pdia3 overexpression attenuated HG-induced podocyte apoptosis and endoplasmic reticulum stress by acting as a competing endogenous RNA of miR-139-3p.

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INTRODUCTION

Diabetic nephropathy (DN) is one of the prominent and serious complications of diabetes mellitus. It is caused by changes in kidney structure and function, frequently resulting in end-stage renal disease and death[1]. Similar to many renal diseases[2,3], DN is characterized by progressive proteinuria, followed by a decline in glomerular filtration along with glomerulosclerosis, ultimately causing renal failure. Albuminuria results from renal glomerular filtration barrier disruption, which increases barrier permeability and protein leakage into the urine. Podocytes, also known as glomerular epithelial cells, are a crucial component of this barrier. Podocytes, as terminal differentiation cells, cannot regenerate when injured. Podocyte depletion and structural changes could destroy the glomerular filtration membrane and induce albuminuria[4]. Earlier studies have indicated that podocyte loss is among the main reasons for diabetes-induced proteinuria and the hallmark events of DN[5-7]. Podocyte apoptosis is an inciting event in DN development and correlates with DN progression.

Accumulating evidence has indicated that endoplasmic reticulum stress (ERS) plays a crucial role in DN development and progression[8], including podocyte injury[9,10]. Under normal physiological conditions, newly synthesized polypeptides translocate into the ER lumen to undergo proper folding, so that they meet the cellular quality control criteria for exit from the ER. Disrupted homeostasis, such as oxidative stress or high glucose (HG), causes an imbalance between the protein loading and folding capacity of the ER, resulting in unfolded and/or misfolded protein accumulation and ER dilatation. This process is known as ERS which consequently triggers an unfolded protein response (UPR)[11]. Numerous studies have indicated ERS-induced apoptosis as a critical mechanism mediating podocyte injury in DN[12,13]. In particular, tauroursodeoxycholic acid treatment ameliorated podocyte and glomeruli injury in diabetic mice by inhibiting ERS, thereby attenuating proteinuria and kidney histological changes[14]. Cyclin-dependent kinase 5 may play a crucial role in ERS-induced podocyte apoptosis, which was associated with podocyte apoptosis in DN[15]. However, the mechanism underlying ERS-mediated podocyte injury in DN remains largely unclear and needs further investigation.

Long noncoding RNAs (lncRNAs) belong to a class of noncoding RNAs of > 200 nucleotides in length lacking protein-coding potential. Several lncRNAs are involved in many biological processes, such as regulating transcription, translation, RNA modification, protein modification, and epigenetic modification of chromatin structures[16,17]. More importantly, lncRNAs are associated with the progression and occurrence of metabolic diseases, including diabetes and diabetic complications[18]. In particular, lncRNA TCF7 silencing attenuated HG-induced podocyte damage. Therefore, lncRNAs are a potential therapeutic target for alleviating DN development to search for novel lncRNAs and alter the expression of specific lncRNAs.

We here investigated lncRNA expression profiles and the associated competing endogenous RNA (ceRNA) network using high-throughput RNA-sequencing (RNA-seq) technologies in normal glucose (5.5 mmol/L, NG group) and HG (25 mmol/L, HG group) cultured mouse podocytes. Then, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and

Genomes (KEGG) pathway analyses were conducted to determine the function of differentially expressed lncRNAs. The present study investigated the function and underlying molecular mechanism of novel lncRNAs in ERS-podocyte apoptosis, providing the hope of developing a new and effective therapeutic strategy against DN.

MATERIALS AND METHODS

Cell culture

Conditionally immortalized mouse podocytes were cultured with Dulbecco's modified eagle medium containing 10% fetal bovine serum and antibiotics (100 U/mL of penicillin and 0.1 mg/mL of streptomycin) at 37 °C under humidified conditions of 95% air and 5% CO_2. The differentiated podocytes were used for the subsequent experiments at 80%-90% podocyte confluence. The podocytes were incubated in a medium containing 25 or 5.5 mmol/L of glucose to induce a hyperglycemic or normal condition, respectively.

RNA-Seq

TRIzol® Reagent (Life Technologies) was used to extract the total RNA from the podocytes of the NG and HG, according to the manufacturer's instructions. The RNA concentration was measured using the Qubit® RNA Assay kit in Qubit®2.0 Fluorometer (Life Technologies, CA, United States). The total RNA was purified by depleting rRNA using the Ribo-off rRNA Depletion kit (Vazyme Biotech Co., Ltd, Nanjing, China). A cDNA library was then constructed using these samples and the VAHTS™ Stranded mRNA-seq Library Prep kit for Illumina® (Vazyme Biotech Co., Ltd, Nanjing, China). Sangon Biotech (Shanghai, China) used an Illumina Novaseq6000 sequencer (Illumina Inc., San Diego, CA, United States) to sequence the libraries. Differentially expressed lncRNAs with statistical significance between the NG and HG groups were determined through *P* value/false discovery rate (FDR) filtering. A volcano plot filtering approach [|log2 (fold change)| ≥ 1.0; *q* value ≤ 0.05] was used to identify significantly and differentially expressed lncRNAs between the two groups.

Cell transfection

The cDNA fragments were cloned into the pcDNA 3.1 plasmid vector to construct lncRNA protein-disulfide isomerase-associated 3 (Pdia3) overexpressing plasmids (pcDNA3.1-lncRNA Pdia3) to overexpress ENSMUST00000153378 (lncRNA protein-disulfide isomerase-associated 3, lncRNA Pdia3 for short). The empty vector served as a control. The podocytes were transfected with small interfering RNA (siRNA) against lncRNA Pdia3 (siRNA-lncRNA Pdia3) to inhibit lncRNA Pdia3 expression. The corresponding scrambled RNA served as a negative control. Additionally, miR-139-3p mimics or inhibitors were used to increase or decrease miR-139-3p expression, respectively. The scrambled oligonucleotides (NC mimics or NC inhibitors) served as controls. The podocytes from each group were seeded into six-well plates and incubated at 37 °C for 24 h for transfection. The cells were transfected or co-transfected with the relevant plasmids using opti-MEM and Lipofectamine 2000 reagents (Invitrogen, Carlsbad, CA, United States) following the manufacturer's protocol after attaining 80% podocyte confluence.

Measurement of cell viability

Cell counting kit-8 (CCK-8) was used to measure cell viability, as described by the manufacturer. The differentiated podocytes were seeded into 96-well plates and incubated at 37 °C overnight. CCK-8 solution of 10 μL was then added to each well. The cells were incubated at 37 °C for 2 h in the dark. An automatic microplate reader was used to measure the light absorbance value of each well at 450 nm of wavelength.

Flow cytometry

The podocyte apoptosis rate in the different groups was determined through flow cytometry using an Annexin V-FITC and propidium iodide (PI) double staining kit (MultiSciences Biotechnology Corporate Limited, China) after transfection for 48 h, following the manufacturer's instructions. The cells from each group were collected and resuspended in the binding buffer to form single-cell suspensions (1×10^6 cells/mL) for staining. The podocytes were then dual stained with 10 μL annexin-V FITC and 5 μL PI at 37 °C for 5 min to avoid light exposure. Finally, flow cytometry detected the percentage of apoptotic podocytes.

Immunofluorescence

The podocytes were washed with phosphate-buffered saline (PBS) and fixed in 4% paraformaldehyde at room temperature for 30 min after transfection for 48 h. Next, the cells were permeabilized with 0.5% Triton X-100 for 5 min. The cells were blocked in 5% bovine serum albumin (BSA) for 1 h at room temperature. Subsequently, the cells were incubated with primary antibodies (podocin, 1:100, Abcam, United States; nephrin, 1:100, Abcam, United States) at 4 °C overnight. The cells were washed with PBS and incubated with fluorescence-conjugated secondary antibodies for 1 h at room temperature. The podocytes were tinted with 6-diamidino-2-phenylindole (DAPI) for 5 min and photographed under a fluorescence microscope (Olympus FV10-ASW, Tokyo, Japan).

Quantitative real-time polymerase chain reaction

Following 48 h of transfection, the total RNA was extracted and purified from podocytes using Trizol reagents according to the manufacturer's instructions. A NanoDrop spectrophotometer detected the concentration and purity of total RNA.

Subsequently, a reverse transcription kit (QIAGEN, Valencia, CA, United States) was used to reverse transcribe total RNA into cDNA. The expressions of RNA were then quantified by quantitative real-time polymerase chain reaction (qRT-PCR) using SYBR®Premix Ex Taq™ (Takara, Dalian, China). The GAPDH or U6 expressions were used as the endogenous control for lncRNA Pdia3 or miR-139-3p, respectively. The relative expression of RNA was analyzed using the $2^{-\Delta\Delta Ct}$ method. Primers were displayed as follows: LncRNA Pdia3 (forward primers 5′-ATGCGCTTCAGCTGCCTA-3′, reverse primers 5′-CGTCAGTTCCAACACATCG-3′); miR-139-3p (forward primers 5′-TCACAGAGGTTGTCCCGGC-3′, reverse primers 5′-TATGGTTGTTCACGACTCCTTCAC-3′); GAPDH (forward primers 5′-GCAAGTTCAACGGCACAG-3′, reverse primers 5′-CTCGCTCCTGGAAGATGG-3′); U6 (forward primers 5′-CTCGCTTCGGCAGCACA-3′, reverse primers 5′-AACGCTTCACGAATTTGCGT-3′).

Fluorescence in situ hybridization

Fluorescence *in situ* hybridization (FISH), which was performed using the FISH kit (Boster Biological Technology Co. Ltd, Wuhan, China) following the manufacturer's protocol, was used to analyze the subcellular localization of lncRNA Pdia3. The lncRNA Pdia3 FISH probe was designed and synthesized by Servicebio Technology (Wuhan, China). After 48 h of transfection, the podocytes were fixed in 4% paraformaldehyde for 30 min and permeabilized with 0.5% Triton X-100 for 5 min. The podocytes were incubated with the fluorescence probe at 37 °C overnight after blocking the permeabilized podocytes in 5% BSA. The podocytes were stained with DAPI for 5 min after hybridization. Finally, the images were observed under the fluorescence microscope (Olympus FV10-ASW, Japan).

Dual-luciferase reporter assay

The dual luciferase reporter assay was used to assess the direct interaction between lncRNA Pdia3 and miR-139-3p. A pmirGLO luciferase expression vector (Cosmo Bio, Tianjin, China) was used to construct the reporter plasmid. The predicted lncRNA Pdia3 3′-UTR sequence that interacts with miR-139-3p and artificially mutated sequences within the predicted target sites were synthesized and cloned into the pmirGLO luciferase vector, respectively. The wide-type (wt) or mutated (mut) luciferase reporter plasmid was then transfected with miR-139-3p mimics or NC mimics into podocytes using Lipofectamine 2000 reagents following the manufacturer's instructions. A non-related miRNA was used as NC mimics. The luciferase assay kit (Promega, Madison, WI, United States) was used to measure the luciferase activity after transfection for 48 h. The relative luciferase activity was normalized to Renilla luciferase activity.

Western blotting

After transfection for 48 h, the podocytes from each group were lysed by whole-cell lysate for 10 min on ice. The radioimmunoprecipitation assay lysis buffer was used to extract total proteins from the cultured podocytes, and a bicinchoninic acid kit was utilized to determine their concentration. The total proteins were then isolated through sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto polyvinylidene fluoride membranes. After blocking in 5% skim milk at room temperature for 1 h, the membranes were probed with primary antibodies [glucose-regulated protein 78 (GRP78), 1:1000, Abcam, United States; C/EBP homologous protein (CHOP), 1:500, Abcam, United States; caspase-12, 1:1000, Abcam, United States] overnight at 4 °C and then incubated with the secondary antibody for 1 h. The membranes were washed three times with PBST for 10 min, incubated in Western Lightning™ Chemiluminescence Reagent (PerkinElmer, United States) for 5 min, and visualized by the LabWorks™ imaging system. β-Tubulin (1:1000, Abcam, United States) was used as an internal control. ImageJ software (National Institutes of Health, Bethesda, MD, United States) was used to analyze the gray value of the target band.

Statistical analysis

Statistical Package for the Social Sciences version 20.0 was used for data analyses. All data were presented as mean ± SD. Unpaired Student's *t*-tests were used to analyze differences between the two groups. One-way analysis of variance with Student-Newman-Keuls or Dunnett's test was used to assess differences among multiple groups. The Benjamini-Hochberg method controlled the FDR using sequential modified Bonferroni correction for multiple hypothesis testing. P values of < 0.05 were considered statistically significant.

RESULTS

LncRNA Pdia3 was down-expressed in HG-cultured podocytes

Mouse podocytes were cultured under NG or HG concentrations for 48 h. RNA-seq analysis was then performed to identify differentially expressed lncRNAs between NG and HG cultured podocytes. RNA-seq revealed that 51 lncRNAs were differentially expressed between the NG and HG groups using the following criteria: P value of < 0.001, q-value of < 0.01 and $|\log2 \text{ (fold change)}| > 1$, including 20 upregulated and 31 downregulated genes (Figure 1). Among them, lncRNA Pdia3 expression was markedly lower in the HG group than in the NG group. The GO and KEGG pathway enrichment analyses were conducted to determine the biological role of lncRNAs. Bioinformatic analysis revealed an association between lncRNA Pdia3 and ERS (Supplementary material). LncRNA Pdia3 was focused on to further study its potential function and action mechanism.

LncRNA Pdia3 overexpression attenuated podocyte apoptosis and ERS in HG-cultured podocytes

First, whether or not lncRNA Pdia3 overexpression attenuated the apoptosis of HG-cultured podocytes was evaluated.

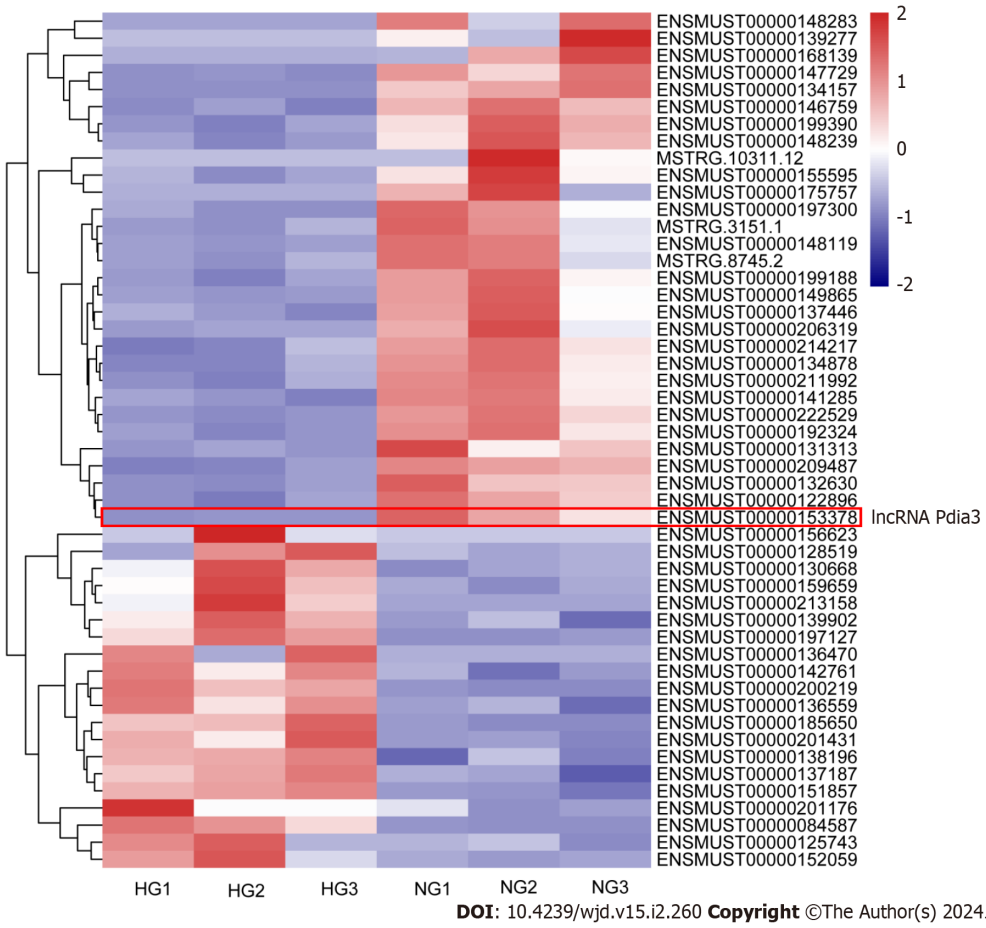


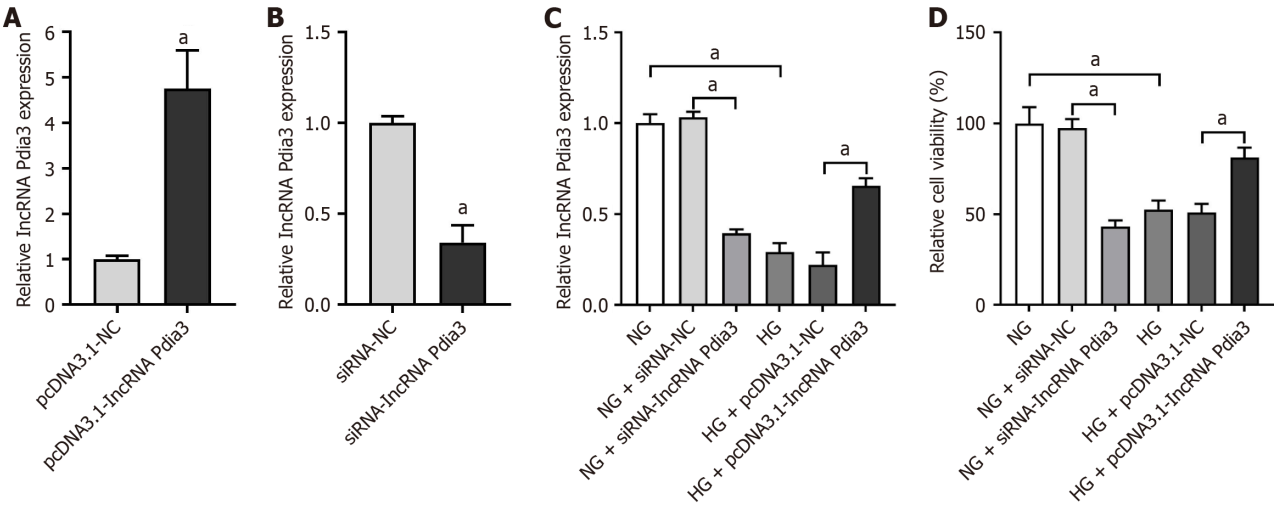
Figure 1 RNA-sequencing analysis indicated a total of 51 long noncoding RNAs that were differentially expressed between the normal glucose and high glucose groups, which included 20 upregulated and 31 downregulated long noncoding RNAs. lncRNA: Long noncoding RNA; HG: High glucose; NG: Normal glucose; Pdia3: Protein-disulfide isomerase-associated 3.

HG could reduce lncRNA Pdia3 expression according to qRT-PCR data, which was consistent with the result of RNA-seq. LncRNA Pdia3 expression significantly increased after pcDNA3.1-lncRNA Pdia3 treatment, which indicated successful transfection (Figure 2A). Following siRNA-lncRNA Pdia3 treatment, lncRNA Pdia3 expression was significantly reduced (Figure 2B), indicating the successful silencing efficiency. Compared with the NG + siRNA-NC group, qRT-PCR data indicated that the NG + siRNA-lncRNA Pdia3 group had decreased lncRNA Pdia3 expression (Figure 2C). LncRNA Pdia3 expression was significantly increased in the HG + pcDNA3.1-lncRNA Pdia3 group compared with the HG + pcDNA3.1-NC group (Figure 2C). Afterward, a CCK-8 assay revealed that siRNA-lncRNA Pdia3 transfection in NG-cultured podocytes caused a decline in cell viability. By contrast, cell viability was significantly enhanced in the HG + pcDNA3.1-lncRNA Pdia3 group compared with the HG + pcDNA3.1-NC group (Figure 2D). Furthermore, flow cytometry indicated that lncRNA Pdia3 silencing transfected by siRNA-lncRNA Pdia3 significantly increased cell apoptotic rate in NG-cultured podocytes. LncRNA Pdia3 overexpression transfected by pcDNA3.1-lncRNA Pdia3 obviously reduced cell apoptotic rate in HG-cultured podocytes (Figure 2E and F). Additionally, immunofluorescence revealed that podocin and nephrin expression were significantly decreased in the NG + siRNA-lncRNA Pdia3 group compared with the NG + siRNA-NC group. By contrast, the HG + pcDNA3.1-lncRNA Pdia3 group demonstrated greatly increased podocin and nephrin expression compared with the HG + pcDNA3.1-NC group (Figure 2G and H). These results indicated that lncRNA Pdia3 overexpression could attenuate podocyte apoptosis in HG-cultured podocytes.

We further investigated the mechanism underlying the regulatory role of lncRNA Pdia3 in podocyte apoptosis. We assessed whether lncRNA Pdia3 modulated ERS in the context of HG-induced podocyte apoptosis. Compared with the NG + siRNA-NC group, GRP78, CHOP, and caspase-12 levels significantly increased in the NG + siRNA-lncRNA Pdia3 group. After transfecting the podocytes with pcDNA3.1-lncRNA Pdia3 under the HG condition, lncRNA Pdia3 overexpression significantly reduced GRP78, CHOP, and caspase-12 levels (Figure 2I and J). These data indicated that lncRNA Pdia3 overexpression might ameliorate HG-induced ERS in podocytes.

LncRNA Pdia3 regulated podocyte apoptosis by serving as a ceRNA of miR-139-3p

Based on the aforementioned results, we investigated how lncRNA Pdia3 regulated podocyte apoptosis. The subcellular localization of lncRNA Pdia3 was assessed using FISH under the assumption of the dependence of one lncRNA's function on its subcellular distribution. As suggested by the subcellular fractionation results presented in Figure 3A, lncRNA Pdia3 was primarily expressed in the cytoplasm. Thus, we speculated that lncRNA alleviated HG-induced podocyte


A
Relative lncRNA Pdia3 expression
pcDNA3.1-NC
pcDNA3.1-lncRNA Pdia3
B
Relative lncRNA Pdia3 expression
siRNA-NC
siRNA-lncRNA Pdia3
C
Relative lncRNA Pdia3 expression
NG
NG + siRNA-NC
NG + siRNA-lncRNA Pdia3
HG
HG + pcDNA3.1-NC
HG + pcDNA3.1-lncRNA Pdia3
D
Relative cell viability (%)
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HG
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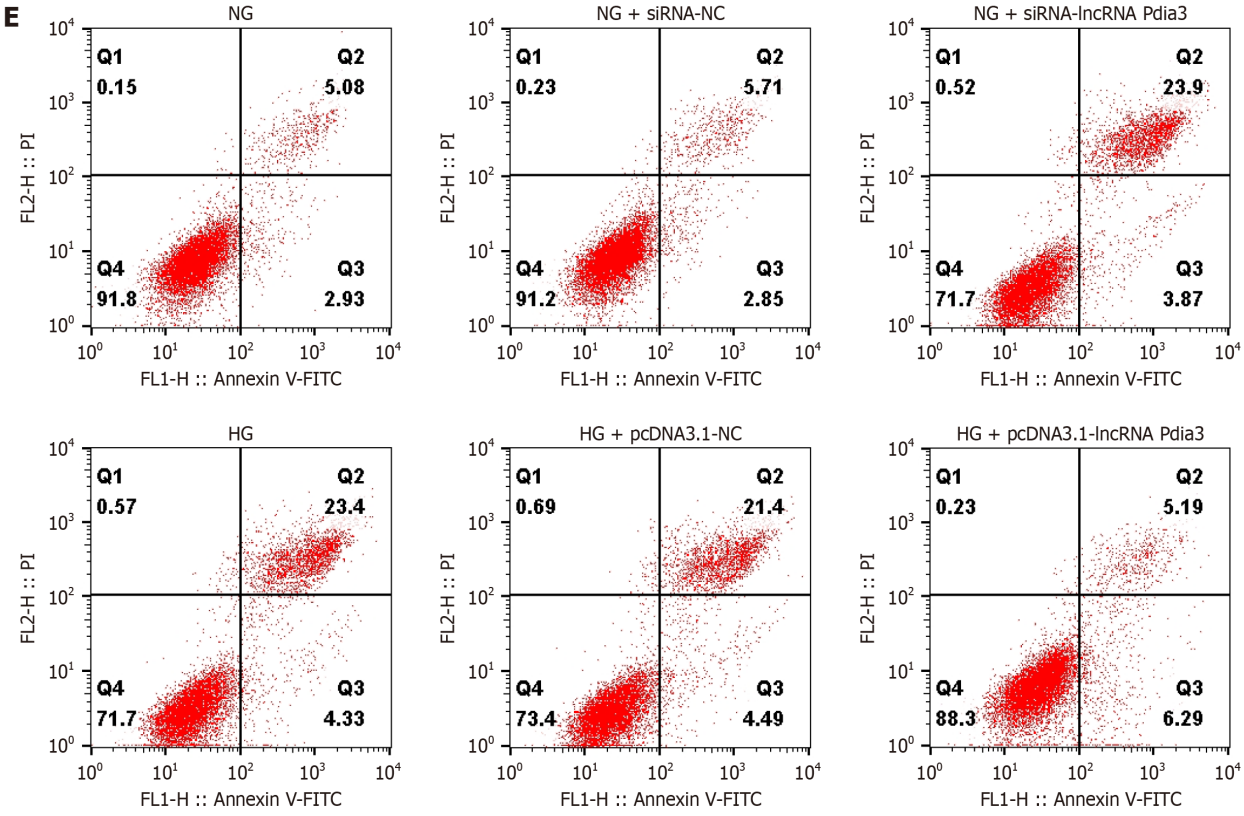
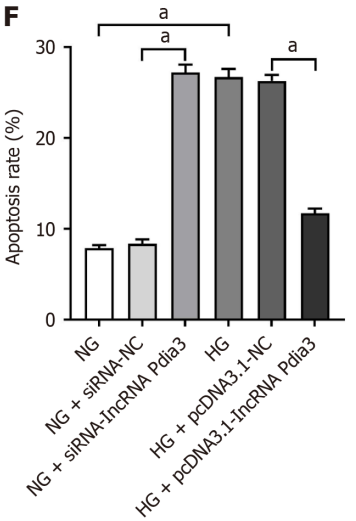
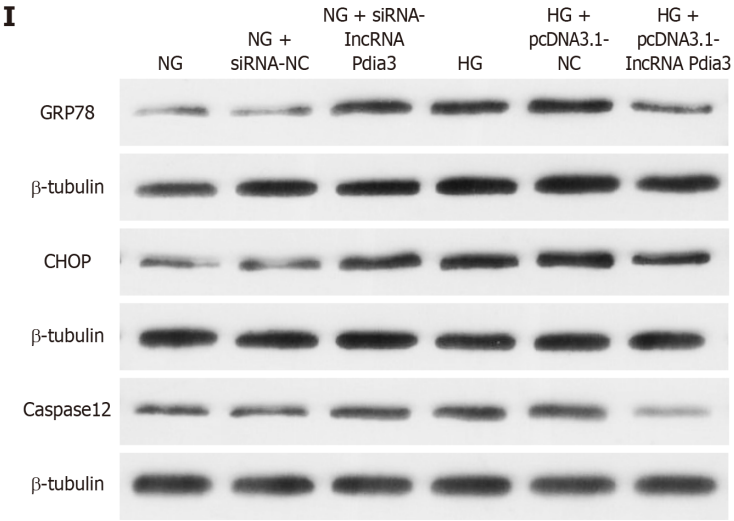

E
NG
Q1 0.15
Q2 5.08
Q4 91.8
Q3 2.93
NG + siRNA-NC
Q1 0.23
Q2 5.71
Q4 91.2
Q3 2.85
NG + siRNA-lncRNA Pdia3
Q1 0.52
Q2 23.9
Q4 71.7
Q3 3.87
HG
Q1 0.57
Q2 23.4
Q4 71.7
Q3 4.33
HG + pcDNA3.1-NC
Q1 0.69
Q2 21.4
Q4 73.4
Q3 4.49
HG + pcDNA3.1-lncRNA Pdia3
Q1 0.23
Q2 5.19
Q4 88.3
Q3 6.29
FL2-H :: PI
FL1-H :: Annexin V-FITC


F
Apoptosis rate (%)
NG
NG + siRNA-NC
NG + siRNA-lncRNA Pdia3
HG
HG + pcDNA3.1-NC
HG + pcDNA3.1-lncRNA Pdia3


I
NG
NG + siRNA-NC
NG + siRNA-lncRNA Pdia3
HG
HG + pcDNA3.1-NC
HG + pcDNA3.1-lncRNA Pdia3
GRP78
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CHOP
β-tubulin
Caspase12
β-tubulin

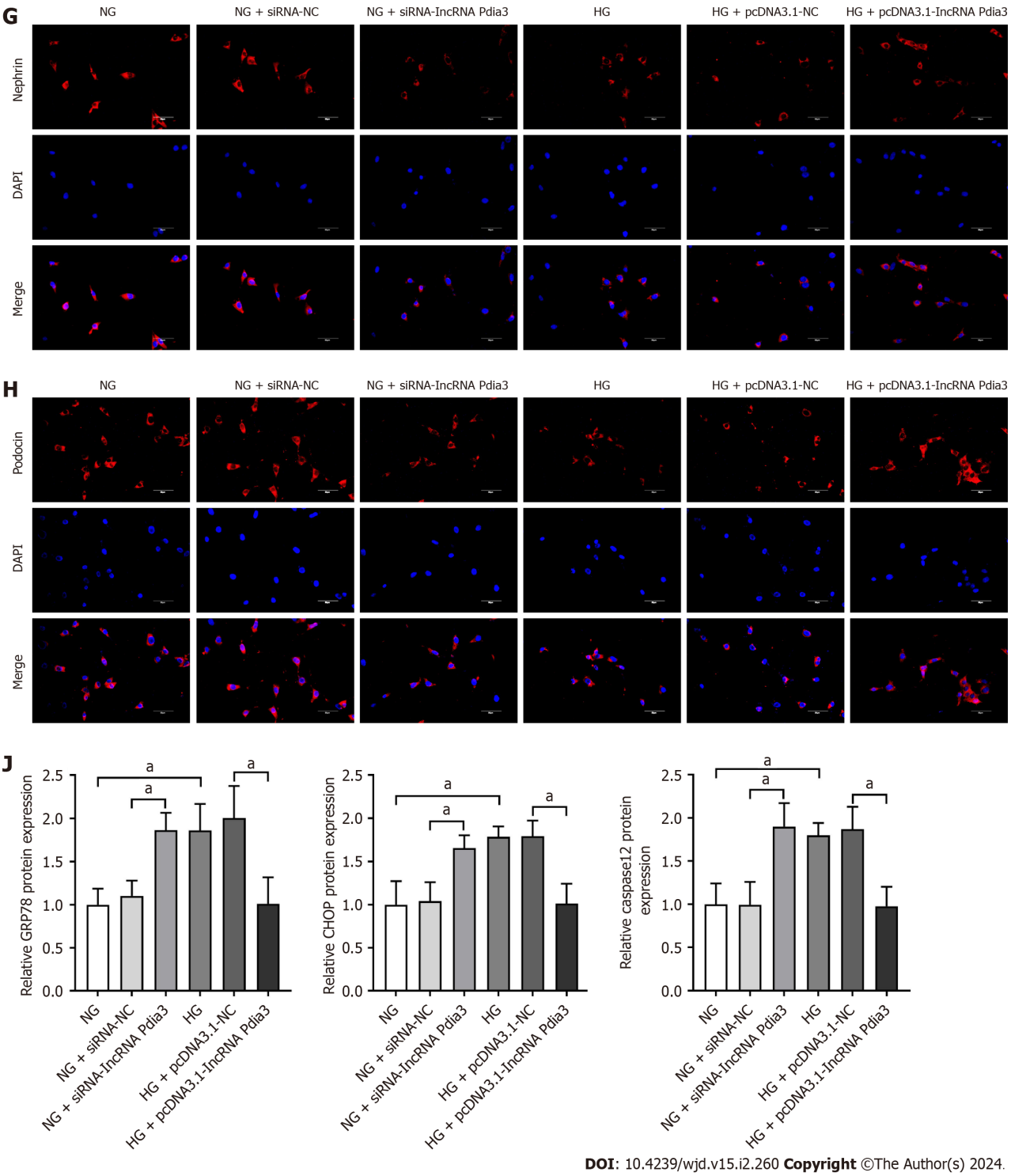

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Figure 2 The upregulation of long noncoding RNA Pdia3 attenuated podocyte apoptosis and endoplasmic reticulum stress in high glucose-cultured podocytes. The downregulation of long noncoding RNA Pdia3 aggravated podocyte apoptosis and endoplasmic reticulum stress in normal glucose-cultured podocytes. A: The transfection efficiency of pcDNA3.1-long noncoding RNA (lncRNA) Pdia3 was detected by quantitative real-time polymerase chain reaction (qRT-PCR); B: The transfection efficiency of small interfering RNA-lncRNA Pdia3 was detected by qRT-PCR; C: The expressions of lncRNA Pdia3 were measured by qRT-PCR. GAPDH served as a loading control; D: Quantitative analysis of the relative cell viability; E: Podocyte apoptosis was detected by flow cytometry; F: Quantitative analysis of the cell apoptotic rate; G and H: The expressions of nephrin and podocin in podocytes were analyzed by immunofluorescence. Scale bar = 50 μm; I: The protein levels of endoplasmic reticulum stress-related factors (glucose-regulated protein 78, C/EBP homologous protein, and caspase-12) were analyzed by western blotting. β-Tubulin was used as an internal control; J: Quantitative analysis of glucose-regulated protein 78, C/EBP homologous protein and caspase-12. The data were presented as mean ± SD. $^{a}P < 0.05$. lncRNA: Long noncoding RNA; HG: High glucose; NG: Normal glucose; siRNA: Small interfering RNA; Pdia3: Protein-disulfide isomerase-associated 3; GRP78: Glucose-regulated protein 78; CHOP: C/EBP homologous protein.

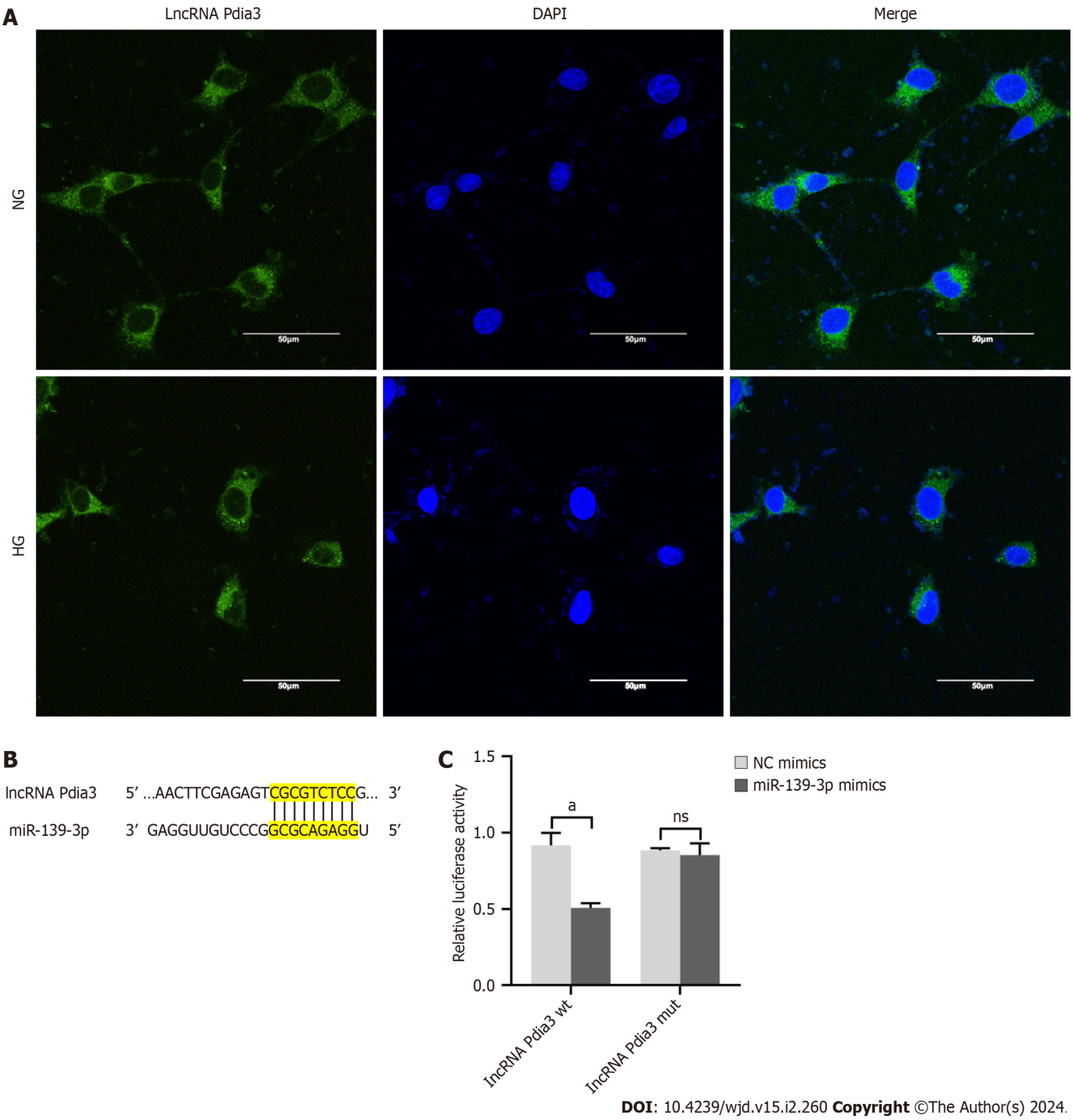

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Figure 3 The direct interaction between long noncoding RNA Pdia3 and miR-139-3p was revealed. A: The subcellular location of long noncoding RNA (lncRNA) Pdia3 was examined by fluorescence *in situ* hybridization. LncRNA Pdia3 was mainly distributed in the cytoplasm; B: The target binding site between lncRNA Pdia3 and miR-139-3p was revealed; C: Dual-luciferase reporter assay was performed to measure the luciferase activity of co-transfecting with miR-139-3p mimics or NC mimics and lncRNA Pdia3 wt or mut luciferase reporters. The data were presented as the relative ratio of firefly luciferase activity to Renilla luciferase activity. $^{a}P < 0.05$. ns: No significance. lncRNA: Long noncoding RNA; HG: High glucose; NG: Normal glucose; Pdia3: Protein-disulfide isomerase-associated 3; DAPI: 6-diamidino-2-phenylindole.

apoptosis maybe by serving as a ceRNA. Bioinformatics analysis revealed that miR-139-3p may be a possible target of lncRNA Pdia3. Figure 3B illustrated the binding sequence prediction of lncRNA Pdia3 and miR-139-3p. Moreover, the luciferase reporter assay demonstrated that lncRNA Pdia3-wt and miR-139-3p mimics co-transfection significantly decreased luciferase activity compared to lncRNA Pdia3-wt and NC mimics co-transfection. By contrast, no significant difference was observed when lncRNA Pdia3-mut was co-transfected with miR-139-3p mimics or NC mimics group (Figure 3C). Altogether, the dual luciferase reporter assay indicated that confirmed the in silico prediction of interaction between lncRNA Pdia3 and miR-139-3p.

Inhibition of miR-139-3p attenuated podocyte apoptosis and ERS in HG-cultured podocytes

Subsequently, we investigated whether miR-139-3p participated in HG-induced podocyte apoptosis. According to the qRT-PCR data, miR-139-3p expression was significantly increased in the HG group compared with that in the NG group, which was consistent with the result of RNA-seq. The qRT-PCR results revealed the high transfection efficiency of miR-

139-3p mimics (Figure 4A). After transfection with miR-139-3p inhibitors, miR-139-3p expression was significantly reduced (Figure 4B), indicating the successful silencing efficiency. The qRT-PCR data depicted that miR-139-3p expression was increased in the NG + miR-139-3p mimics group compared with the NG + NC mimics group. The miR-139-3p expression was significantly decreased by miR-139-3p inhibitors transfection in HG-cultured podocytes (Figure 4C). CCK-8 then revealed that miR-139-3p overexpression by miR-139-3p mimics significantly decreased the cell viability of the NG-cultured podocytes. Further, miR-139-3p inhibition transfected by miR-139-3p inhibitors significantly increased the cell viability of the HG-cultured podocytes (Figure 4D). Furthermore, flow cytometry indicated that miR-139-3p overexpression significantly increased the podocyte apoptosis in NG-cultured podocytes. Inhibiting miR-139-3p reduced the podocyte apoptosis in HG-cultured podocytes (Figure 4E and F). Additionalonly, immunofluorescence revealed that miR-139-3p overexpression significantly decreased podocin and nephrin expression in NG-cultured podocytes. By contrast, miR-139-3p inhibition greatly increased podocin and nephrin expression in HG-cultured podocytes (Figure 4G and H). These aforementioned results revealed the potential involvement of miR-139-3p in HG-induced podocyte apoptosis.

Furthermore, whether or not miR-139-3p regulated HG-induced ERS was determined. MiR-139-3p overexpression significantly increased GRP78, CHOP, and caspase-12 levels in NG-cultured podocytes. After transfecting the podocytes with miR-139-3p inhibitors under the HG condition, miR-139-3p inhibition significantly reduced GRP78, CHOP, and caspase-12 levels (Figure 4I and J). These aforementioned data confirmed that miR-139-3p inhibition ameliorated HG-induced ERS in podocytes.

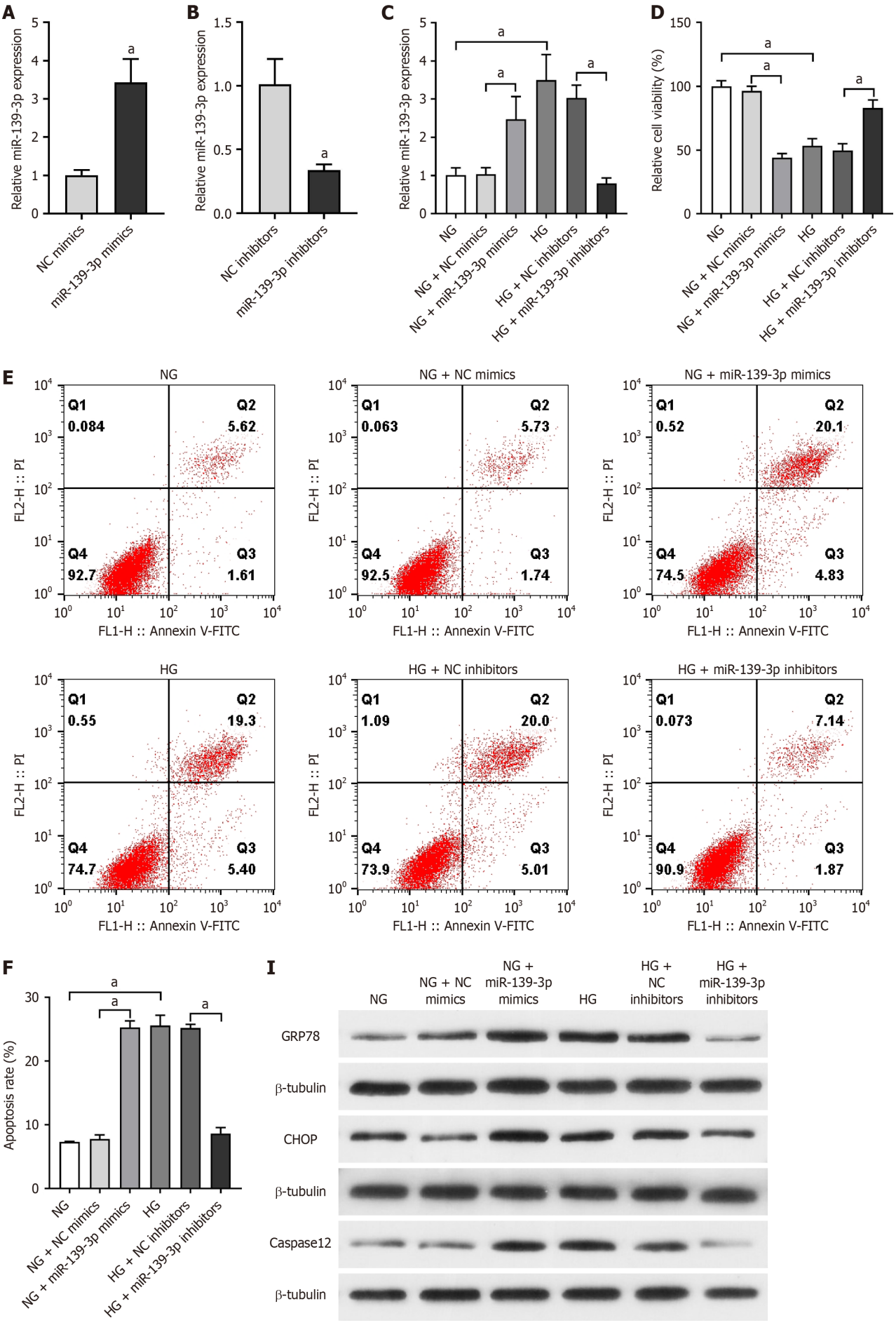
DISCUSSION

Here, we focused on the function and underlying molecular mechanism of lncRNA Pdia3, which is a previously unidentified lncRNA, in hyperglycemia-induced podocyte apoptosis. Next, the interplay between lncRNA Pdia3 and miR-139-3p in podocytes during DN was investigated. LncRNA Pdia3 was significantly downregulated in the HG-cultured podocytes, where in HG simulated a DN microenvironment, compared with the NG-cultured podocytes. More importantly, lncRNA Pdia3 modulated ERS and podocyte apoptosis by serving as a ceRNA of miR-139-3p in DN. Therefore, we hypothesized that lncRNA Pdia3 was a novel podocyte apoptosis regulator through controlling ERS in DN.

LncRNA Pdia3 (Ensembl ID: ENSMUST00000153378), a 400 bp lncRNA, is located in chromosome 2 (chromosome 2: 121,244,364-121,255,082). In this study, lncRNA Pdia3 was first found to be involved in HG-induced podocyte apoptosis. In the HG-cultured podocytes, lncRNA Pdia3 expression was dramatically downregulated, which is relevant to podocyte apoptosis. Moreover, lncRNA Pdia3 overexpression with pcDNA3.1-lncRNA Pdia3 transfection significantly alleviated podocyte apoptosis in the HG-cultured podocytes. The crucial role of lncRNAs in regulating the pathological processes of podocyte apoptosis in DN, such as PVT1[19], lncRNA SPAG5 antisense RNA1 (SPAG5-AS1)[20], and lncRNA MIAT[21], has been confirmed. In our study, lncRNA Pdia3 was first proved to exert a protective effect against podocyte apoptosis.

Notably, lncRNA Pdia3 may be associated with HG-induced ERS. The ER is a key intracellular organelle with multiple functions, which is responsible for protein production, folding, processing, and secretion[22], as well as intracellular calcium storage and lipid production[23]. Newly synthesized proteins are properly folded and structurally corrected in the ER, and then transported to the Golgi apparatus. These folded proteins functioned as secretory or membrane proteins. Thus, maintaining ER homeostasis is crucial for cell survival, differentiation, development, and proliferation[24]. Acute and chronic hyperglycemia disrupts ER homeostasis, causing unfolded protein accumulation in the ER, which is known as ERS[24]. The UPR prevents misfolded protein overloading and restores ER homeostasis. ERS can result in cell death or apoptosis if the UPR system fails to restore the ER balance[25]. Emerging evidence has revealed the crucial role of ERS in regulating DN-related pathological processes[10]. The ER chaperone protein, GRP78, assists with the proper folding and assembly of proteins as a master modulator for UPR. Under ERS, GRP78 preferentially binds to misfolded or unfolded proteins and targets misfolded proteins for degradation[26]. Prolonged or intense stress has induced cell apoptosis by activating various apoptotic pathways, such as caspase-12 and CHOP. LncRNA Pdia3 silencing through siRNA-lncRNA Pdia3 transfection dramatically aggravated ERS in the NG-cultured podocytes in our study, including increasing caspase-12 and CHOP expression. In contrast, lncRNA Pdia3 overexpression through pcDNA3.1-lncRNA Pdia3 transfection dramatically alleviated ERS in the HG-cultured podocytes, including decreasing CHOP and caspase-12 expression. The results revealed that lncRNA Pdia3 overexpression ameliorated podocyte apoptosis by alleviating ERS. Several studies have extensively evaluated the relationship between lncRNAs and podocyte apoptosis, such as lncRNA 1500026-H17Rik[27], KCNQ1OT1[28], CDKN2B-AS1[29], lncRNA Hoxb3os[30], and lncRNA XIST[31]. However, only a few lncRNAs, such as lncRNA TCF7[32], LINC01619[33] and lncRNA TUG1[10], have been reported to be associated with ERS in podocyte apoptosis. LncRNA Pdia3, which is a previously unidentified lncRNA, was confirmed to be a critical regulator of ERS in podocyte apoptosis. LncRNA Pdia3 may become a prospective therapeutic approach for DN prevention or treatment in the future.

The target-mimetic, sponge/decoy function of lncRNAs on miRNAs recently gained widespread research attention[34]. The miRNAs are a class of small noncoding single stranded RNAs of approximately 20-22 nucleotides. These miRNAs negatively modulate gene expression by binding to the target mRNA and subsequently inducing its degradation or suppressing protein translation[35]. LncRNAs served as ceRNAs of miRNA to competitively occupy the shared miRNA binding sequences, thereby causing the modulation of gene expression[36]. Previous studies have elucidated the association between lncRNA and miRNA that is involved in DN progression[37,38], which was a vital action mechanism of lncRNAs. This study revealed that lncRNA Pdia3 overexpression or miR-139-3p inhibition alleviated ERS and podocyte apoptosis in the HG-cultured podocytes, including a decrease in caspase-12 and CHOP expression. Further-


A
Relative miR-139-3p expression
NC mimics
miR-139-3p mimics
B
Relative miR-139-3p expression
NC inhibitors
miR-139-3p inhibitors
C
Relative miR-139-3p expression
NG
NG + NC mimics
NG + miR-139-3p mimics
HG
HG + NC inhibitors
HG + miR-139-3p inhibitors
D
Relative cell viability (%)
E
NG
NG + NC mimics
NG + miR-139-3p mimics
HG
HG + NC inhibitors
HG + miR-139-3p inhibitors
FL2-H :: PI
FL1-H :: Annexin V-FITC
Q1 0.084
Q2 5.62
Q4 92.7
Q3 1.61
Q1 0.063
Q2 5.73
Q4 92.5
Q3 1.74
Q1 0.52
Q2 20.1
Q4 74.5
Q3 4.83
Q1 0.55
Q2 19.3
Q4 74.7
Q3 5.40
Q1 1.09
Q2 20.0
Q4 73.9
Q3 5.01
Q1 0.073
Q2 7.14
Q4 90.9
Q3 1.87
F
Apoptosis rate (%)
I
GRP78
β-tubulin
CHOP
β-tubulin
Caspase12
β-tubulin

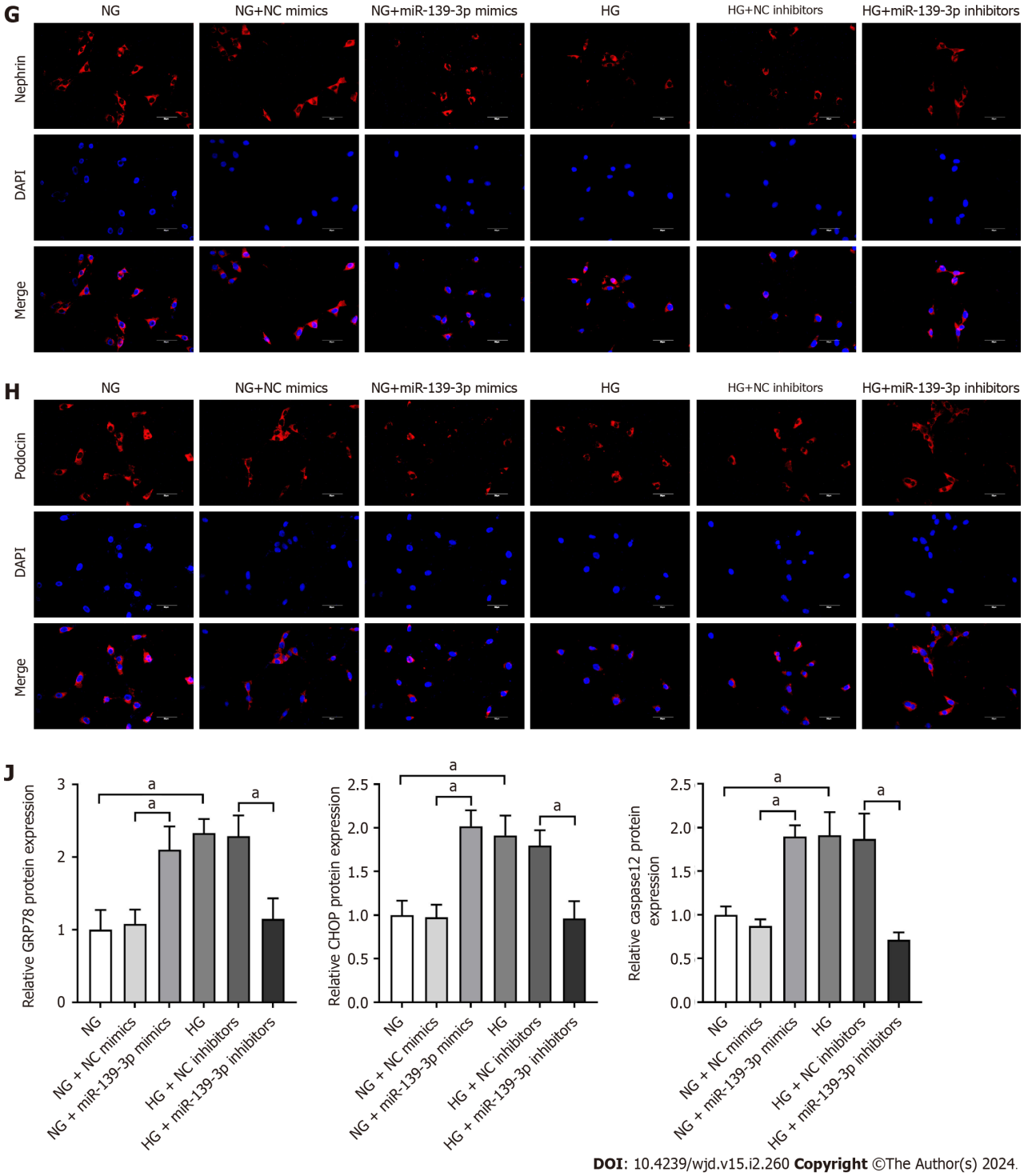


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Figure 4 Inhibition of miR-139-3p attenuated podocyte apoptosis and endoplasmic reticulum stress in high glucose-cultured podocytes. The overexpression of miR-139-3p aggravated podocyte apoptosis and endoplasmic reticulum stress in normal glucose-cultured podocytes. A: The transfection efficiency of miR-139-3p mimics was detected by quantitative real-time polymerase chain reaction (qRT-PCR); B: The transfection efficiency of miR-139-3p inhibitors was detected by qRT-PCR; C: The expression of miR-139-3p was measured by qRT-PCR. U6 served as the loading control; D: Quantitative analysis of the relative cell viability; E: Podocyte apoptosis was detected by flow cytometry; F: Quantitative analysis of cell apoptotic rate; G and H: The expressions of nephrin and podocin in podocytes were analyzed by immunofluorescence. Scale bar = 50 μm; I: The protein expression of endoplasmic reticulum stress-related factors (glucose-regulated protein 78, C/EBP homologous protein, and caspase-12) was analyzed by western blotting. β-Tubulin served as an internal control; J: Quantitative analysis of glucose-regulated protein 78, C/EBP homologous protein and caspase-12. The data were presented as mean ± SD. $^{a}P < 0.05$. lncRNA: Long noncoding RNA; HG: High glucose; NG: Normal glucose; Pdia3: Protein-disulfide isomerase-associated 3; GRP78: Glucose-regulated protein 78; CHOP: C/EBP homologous protein.

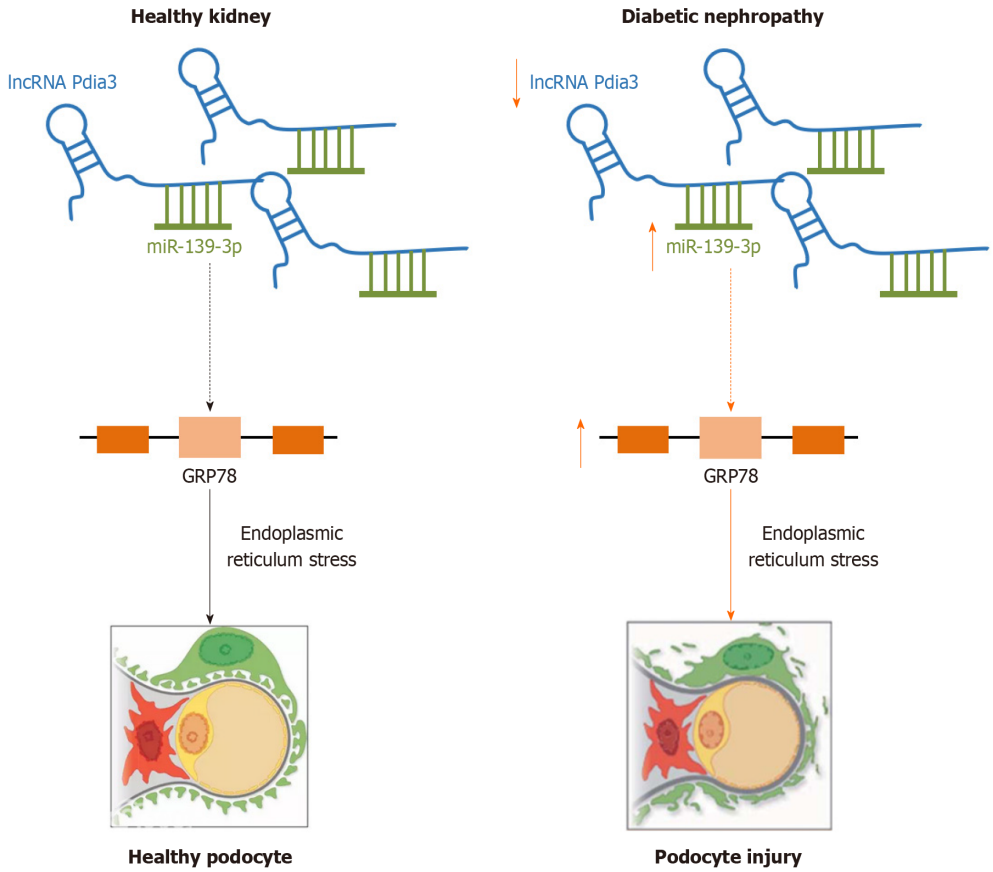

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Figure 5 Mechanistic depiction of the role of long noncoding RNA Pdia3 in endoplasmic reticulum stress and podocyte apoptosis is illustrated. Our finding revealed that long noncoding RNA Pdia3 downregulation induced endoplasmic reticulum stress and podocyte injury by acting as a competing endogenous RNA of miR-139-3p, which led to diabetic nephropathy progression. lncRNA: Long noncoding RNA; Pdia3: Protein-disulfide isomerase-associated 3.

more, the dual luciferase reporter assay verified that lncRNA Pdia3 directly interacts with miR-139-3p. These aforementioned results indicated the importance of lncRNA Pdia3 for preventing HG-induced podocyte apoptosis. LncRNA Pdia3 overexpression alleviated HG-induced podocyte injury and ERS by serving as a ceRNA of miR-139-3p.

CONCLUSION

In conclusion, our study provided evidence that lncRNA Pdia3 downregulation is a significant contributing factor for podocyte apoptosis in DN. LncRNA Pdia3 downregulation could induce ERS and podocyte injury by serving as a ceRNA of miR-139-3p, thereby leading to DN progression (Figure 5). Thus, lncRNA Pdia3 played a substantial role in podocyte apoptosis in DN. So, it might act as a potential therapeutic target and offer an alternative therapy for DN. However, the current study had some limitations. Our study used glucose concentrations (25 mmol/L) to mimic hyperglycemic conditions in podocytes, which might not completely reflect the complex situation in DN patients. In addition, our experiment was conducted by using only mouse podocytes, which might restrict the generalization of the study results. According to their unique genetics, different cell types might respond differently to the same treatment. Whether the findings obtained *in vitro* can be applied to *in vivo* DN needs to be further investigated. We intend to detect the expression and underlying molecular mechanism of lncRNA Pdia3 in DN patients. Our study found that inhibition of miR-139-3p significantly reduced ERS. How does miR-139-3p act on ERS needs to be further investigated. The goal of our research is to produce knowledge that can be applied as widely as possible.

ARTICLE HIGHLIGHTS

Research background

Podocyte apoptosis plays a vital role in proteinuria pathogenesis in diabetic nephropathy (DN). The regulatory relationship between long noncoding RNAs (lncRNAs) and podocyte apoptosis has recently become another research hot

spot in the DN field. LncRNAs are a potential therapeutic target for alleviating DN development to search for novel lncRNAs and alter the expression of specific lncRNAs.

Research motivation

We here investigated lncRNA expression profiles and the associated competing endogenous RNA (ceRNA) network using high-throughput RNA-sequencing (RNA-seq) technologies in normal glucose (5.5 mmol/L, NG group) and high glucose (25 mmol/L, HG group) cultured mouse podocytes. Then, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were conducted to determine the function of differentially expressed lncRNAs.

Research objectives

The present study investigated the function and underlying molecular mechanism of novel lncRNAs in endoplasmic reticulum stress (ERS)-podocyte apoptosis, providing the hope of developing a new and effective therapeutic strategy against DN.

Research methods

Using NG or HG-cultured podocytes, the cellular functions and exact mechanisms underlying the regulatory effects of lncRNA protein-disulfide isomerase-associated 3 (Pdia3) on podocyte apoptosis and ERS were explored. LncRNA Pdia3 and miR-139-3p expression were measured through quantitative real-time polymerase chain reaction. Relative cell viability was detected through the cell counting kit-8 colorimetric assay. The podocyte apoptosis rate in each group was measured through flow cytometry. The interaction between lncRNA Pdia3 and miR-139-3p was examined through the dual luciferase reporter assay. Finally, western blotting was performed to detect the effect of lncRNA Pdia3 on podocyte apoptosis and ERS *via* miR-139-3p.

Research results

LncRNA Pdia3 was down-expressed in HG-cultured podocytes. LncRNA Pdia3 overexpression attenuated podocyte apoptosis and ERS in HG-cultured podocytes. LncRNA Pdia3 regulated podocyte apoptosis by serving as a ceRNA of miR-139-3p. Inhibition of miR-139-3p attenuated podocyte apoptosis and ERS in HG-cultured podocytes.

Research conclusions

This study provided evidence that lncRNA Pdia3 downregulation is a significant contributing factor for podocyte apoptosis in DN. LncRNA Pdia3 downregulation could induce ERS and podocyte injury by serving as a ceRNA of miR-139-3p, thereby leading to DN progression.

Research perspectives

In the future, whether the findings obtained *in vitro* can be applied to *in vivo* DN needs to be investigated. We intend to detect the expression and underlying molecular mechanism of lncRNA Pdia3 in DN patients.

FOOTNOTES

Author contributions: Chen YX conceived or supervised the study; He YX designed experiments; He YX and Wang T performed experiments; Li WX analyzed data; He YX and Chen YX wrote the manuscript; Chen YX made manuscript revisions; and all authors have read and approve the final manuscript.

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REFERENCES

1 **Gnudi L**, Coward RJM, Long DA. Diabetic Nephropathy: Perspective on Novel Molecular Mechanisms. *Trends Endocrinol Metab* 2016; **27**: 820-830 [PMID: 27470431 DOI: 10.1016/j.tem.2016.07.002]
2 **Talas ZS**, Ozdemir I, Yilmaz I, Gok Y. Antioxidative effects of novel synthetic organoselenium compound in rat lung and kidney. *Ecotoxicol Environ Saf* 2009; **72**: 916-921 [PMID: 18222543 DOI: 10.1016/j.ecoenv.2007.11.012]
3 **Talas ZS**, Ozdemir I, Ciftci O, Cakir O, Gulhan MF, Pasaoglu OM. Role of propolis on biochemical parameters in kidney and heart tissues against L-NAME induced oxidative injury in rats. *Clin Exp Hypertens* 2014; **36**: 492-496 [PMID: 24490594 DOI: 10.3109/10641963.2013.863322]
4 **Dai H**, Liu Q, Liu B. Research Progress on Mechanism of Podocyte Depletion in Diabetic Nephropathy. *J Diabetes Res* 2017; **2017**: 2615286 [PMID: 28791309 DOI: 10.1155/2017/2615286]
5 **Tagawa A**, Yasuda M, Kume S, Yamahara K, Nakazawa J, Chin-Kanasaki M, Araki H, Araki S, Koya D, Asanuma K, Kim EH, Haneda M, Kajiwara N, Hayashi K, Ohashi H, Ugi S, Maegawa H, Uzu T. Impaired Podocyte Autophagy Exacerbates Proteinuria in Diabetic Nephropathy. *Diabetes* 2016; **65**: 755-767 [PMID: 26384385 DOI: 10.2337/db15-0473]
6 **Lenoir O**, Jasiek M, Hénique C, Guyonnet L, Hartleben B, Bork T, Chipont A, Flosseau K, Bensaada I, Schmitt A, Massé JM, Souyri M, Huber TB, Tharaux PL. Endothelial cell and podocyte autophagy synergistically protect from diabetes-induced glomerulosclerosis. *Autophagy* 2015; **11**: 1130-1145 [PMID: 26039325 DOI: 10.1080/15548627.2015.1049799]
7 **Podgórski P**, Konieczny A, Lis Ł, Witkiewicz W, Hruby Z. Glomerular podocytes in diabetic renal disease. *Adv Clin Exp Med* 2019; **28**: 1711-1715 [PMID: 31851794 DOI: 10.17219/acem/104534]
8 **Sankrityayan H**, Oza MJ, Kulkarni YA, Mulay SR, Gaikwad AB. ER stress response mediates diabetic microvascular complications. *Drug Discov Today* 2019; **24**: 2247-2257 [PMID: 31430543 DOI: 10.1016/j.drudis.2019.08.003]
9 **Lei J**, Zhao L, Zhang Y, Wu Y, Liu Y. High Glucose-Induced Podocyte Injury Involves Activation of Mammalian Target of Rapamycin (mTOR)-Induced Endoplasmic Reticulum (ER) Stress. *Cell Physiol Biochem* 2018; **45**: 2431-2443 [PMID: 29554648 DOI: 10.1159/000488231]
10 **Shen H**, Ming Y, Xu C, Xu Y, Zhao S, Zhang Q. Deregulation of long noncoding RNA (TUG1) contributes to excessive podocytes apoptosis by activating endoplasmic reticulum stress in the development of diabetic nephropathy. *J Cell Physiol* 2019; **234**: 15123-15133 [PMID: 30671964 DOI: 10.1002/jcp.28153]
11 **Hetz C**, Zhang K, Kaufman RJ. Mechanisms, regulation and functions of the unfolded protein response. *Nat Rev Mol Cell Biol* 2020; **21**: 421-438 [PMID: 32457508 DOI: 10.1038/s41580-020-0250-z]
12 **Cao Y**, Hao Y, Li H, Liu Q, Gao F, Liu W, Duan H. Role of endoplasmic reticulum stress in apoptosis of differentiated mouse podocytes induced by high glucose. *Int J Mol Med* 2014; **33**: 809-816 [PMID: 24503896 DOI: 10.3892/ijmm.2014.1642]
13 **Li M**, Ni W, Zhang M, Liu S, Chen M, Hong X, Ma Y, Yu X, Wang W, Yang M, Hua F. MicroRNA-30/Cx43 axis contributes to podocyte injury by regulating ER stress in diabetic nephropathy. *Ann Transl Med* 2020; **8**: 1674 [PMID: 33490186 DOI: 10.21037/atm-20-6989]
14 **Fan Y**, Zhang J, Xiao W, Lee K, Li Z, Wen J, He L, Gui D, Xue R, Jian G, Sheng X, He JC, Wang N. Rtn1a-Mediated Endoplasmic Reticulum Stress in Podocyte Injury and Diabetic Nephropathy. *Sci Rep* 2017; **7**: 323 [PMID: 28336924 DOI: 10.1038/s41598-017-00305-6]
15 **Zhang Y**, Gao X, Chen S, Zhao M, Chen J, Liu R, Cheng S, Qi M, Wang S, Liu W. Cyclin-dependent kinase 5 contributes to endoplasmic reticulum stress induced podocyte apoptosis *via* promoting MEKK1 phosphorylation at Ser280 in diabetic nephropathy. *Cell Signal* 2017; **31**: 31-40 [PMID: 28024901 DOI: 10.1016/j.cellsig.2016.12.009]
16 **Kunz M**, Wolf B, Fuchs M, Christoph J, Xiao K, Thum T, Atlan D, Prokosch HU, Dandekar T. A comprehensive method protocol for annotation and integrated functional understanding of lncRNAs. *Brief Bioinform* 2020; **21**: 1391-1396 [PMID: 31578571 DOI: 10.1093/bib/bbz066]
17 **He Y**, Chen Y. The potential role of lncRNAs in osteoporosis. *J Bone Miner Metab* 2021; **39**: 341-352 [PMID: 33566207 DOI: 10.1007/s00774-021-01205-6]
18 **Chen Y**, He Y, Zhou H. The potential role of lncRNAs in diabetes and diabetic microvascular complications. *Endocr J* 2020; **67**: 659-668 [PMID: 32404556 DOI: 10.1507/endocrj.EJ19-0574]
19 **Liu DW**, Zhang JH, Liu FX, Wang XT, Pan SK, Jiang DK, Zhao ZH, Liu ZS. Silencing of long noncoding RNA PVT1 inhibits podocyte damage and apoptosis in diabetic nephropathy by upregulating FOXA1. *Exp Mol Med* 2019; **51**: 1-15 [PMID: 31371698 DOI: 10.1038/s12276-019-0259-6]
20 **Xu J**, Deng Y, Wang Y, Sun X, Chen S, Fu G. SPAG5-AS1 inhibited autophagy and aggravated apoptosis of podocytes *via* SPAG5/AKT/mTOR pathway. *Cell Prolif* 2020; **53**: e12738 [PMID: 31957155 DOI: 10.1111/cpr.12738]
21 **Zhang M**, Zhao S, Xu C, Shen Y, Huang J, Shen S, Li Y, Chen X. Ablation of lncRNA MIAT mitigates high glucose-stimulated inflammation and apoptosis of podocyte *via* miR-130a-3p/TLR4 signaling axis. *Biochem Biophys Res Commun* 2020; **533**: 429-436 [PMID: 32972755 DOI: 10.1016/j.bbrc.2020.09.034]
22 **Schwarz DS**, Blower MD. The endoplasmic reticulum: structure, function and response to cellular signaling. *Cell Mol Life Sci* 2016; **73**: 79-94 [PMID: 26433683 DOI: 10.1007/s00018-015-2052-6]
23 **Sozen E**, Ozer NK. Impact of high cholesterol and endoplasmic reticulum stress on metabolic diseases: An updated mini-review. *Redox Biol* 2017; **12**: 456-461 [PMID: 28319895 DOI: 10.1016/j.redox.2017.02.025]
24 **Mustapha S**, Mohammed M, Azemi AK, Jatau AI, Shehu A, Mustapha L, Aliyu IM, Danraka RN, Amin A, Bala AA, Ahmad WANW, Rasool

AHG, Mustafa MR, Mokhtar SS. Current Status of Endoplasmic Reticulum Stress in Type II Diabetes. *Molecules* 2021; **26** [PMID: 34299638 DOI: 10.3390/molecules26144362]

25 **Wang M**, Kaufman RJ. Protein misfolding in the endoplasmic reticulum as a conduit to human disease. *Nature* 2016; **529**: 326-335 [PMID: 26791723 DOI: 10.1038/nature17041]

26 **Ye R**, Jung DY, Jun JY, Li J, Luo S, Ko HJ, Kim JK, Lee AS. Grp78 heterozygosity promotes adaptive unfolded protein response and attenuates diet-induced obesity and insulin resistance. *Diabetes* 2010; **59**: 6-16 [PMID: 19808896 DOI: 10.2337/db09-0755]

27 **Xia J**, Sun W, Dun J. LncRNA 1500026H17Rik knockdown ameliorates high glucose-induced mouse podocyte injuries through the miR-205-5p/EGR1 pathway. *Int Urol Nephrol* 2023; **55**: 1045-1057 [PMID: 36306049 DOI: 10.1007/s11255-022-03396-x]

28 **Fei B**, Zhou H, He Z, Wang S. KCNQ1OT1 inhibition alleviates high glucose-induced podocyte injury by adsorbing miR-23b-3p and regulating Sema3A. *Clin Exp Nephrol* 2022; **26**: 385-397 [PMID: 34997887 DOI: 10.1007/s10157-021-02173-x]

29 **Xiao M**, Bai S, Chen J, Li Y, Zhang S, Hu Z. CDKN2B-AS1 participates in high glucose-induced apoptosis and fibrosis *via* NOTCH2 through functioning as a miR-98-5p decoy in human podocytes and renal tubular cells. *Diabetol Metab Syndr* 2021; **13**: 107 [PMID: 34649592 DOI: 10.1186/s13098-021-00725-5]

30 **Jin J**, Gong J, Zhao L, Li Y, He Q. LncRNA Hoxb3os protects podocytes from high glucose-induced cell injury through autophagy dependent on the Akt-mTOR signaling pathway. *Acta Biochim Pol* 2021; **68**: 619-625 [PMID: 34648253 DOI: 10.18388/abp.2020_5483]

31 **Long B**, Wan Y, Zhang S, Lv L. LncRNA XIST protects podocyte from high glucose-induced cell injury in diabetic nephropathy by sponging miR-30 and regulating AVEN expression. *Arch Physiol Biochem* 2023; **129**: 610-617 [PMID: 33332155 DOI: 10.1080/13813455.2020.1854307]

32 **Liu H**, Sun HL. LncRNA TCF7 triggered endoplasmic reticulum stress through a sponge action with miR-200c in patients with diabetic nephropathy. *Eur Rev Med Pharmacol Sci* 2019; **23**: 5912-5922 [PMID: 31298342 DOI: 10.26355/eurrev_201907_18336]

33 **Bai X**, Geng J, Li X, Wan J, Liu J, Zhou Z, Liu X. Long Noncoding RNA LINC01619 Regulates MicroRNA-27a/Forkhead Box Protein O1 and Endoplasmic Reticulum Stress-Mediated Podocyte Injury in Diabetic Nephropathy. *Antioxid Redox Signal* 2018; **29**: 355-376 [PMID: 29334763 DOI: 10.1089/ars.2017.7278]

34 **Paraskevopoulou MD**, Hatzigeorgiou AG. Analyzing MiRNA-LncRNA Interactions. *Methods Mol Biol* 2016; **1402**: 271-286 [PMID: 26721498 DOI: 10.1007/978-1-4939-3378-5_21]

35 **Ha M**, Kim VN. Regulation of microRNA biogenesis. *Nat Rev Mol Cell Biol* 2014; **15**: 509-524 [PMID: 25027649 DOI: 10.1038/nrm3838]

36 **Thomson DW**, Dinger ME. Endogenous microRNA sponges: evidence and controversy. *Nat Rev Genet* 2016; **17**: 272-283 [PMID: 27040487 DOI: 10.1038/nrg.2016.20]

37 **Zhang J**, Song L, Ma Y, Yin Y, Liu X, Luo X, Sun J, Wang L. lncRNA MEG8 Upregulates miR-770-5p Through Methylation and Promotes Cell Apoptosis in Diabetic Nephropathy. *Diabetes Metab Syndr Obes* 2020; **13**: 2477-2483 [PMID: 32765026 DOI: 10.2147/DMSO.S255183]

38 **Li F**, Dai B, Ni X. Long non-coding RNA cancer susceptibility candidate 2 (CASC2) alleviates the high glucose-induced injury of CIHP-1 cells *via* regulating miR-9-5p/PPARγ axis in diabetes nephropathy. *Diabetol Metab Syndr* 2020; **12**: 68 [PMID: 32774472 DOI: 10.1186/s13098-020-00574-8]

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ORIGINAL ARTICLE

Basic Study

Assessment of pathogenicity and functional characterization of *APPL1* gene mutations in diabetic patients

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Abstract

BACKGROUND

Adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1 (APPL1) plays a crucial role in regulating insulin signaling and glucose metabolism. Mutations in the *APPL1* gene have been associated with the development of maturity-onset diabetes of the young type 14 (MODY14). Currently, only two mutations [c.1655T>A (p.Leu552*) and c.281G>A p.(Asp94Asn)] have been identified in association with this disease. Given the limited understanding of MODY14, it is imperative to identify additional cases and carry out comprehensive research on MODY14 and *APPL1* mutations.

AIM

To assess the pathogenicity of *APPL1* gene mutations in diabetic patients and to characterize the functional role of the APPL1 domain.

METHODS

Patients exhibiting clinical signs and a medical history suggestive of MODY were screened for the study. Whole exome sequencing was performed on the patients as well as their family members. The pathogenicity of the identified *APPL1* variants was predicted on the basis of bioinformatics analysis. In addition, the pathogenicity of the novel *APPL1* variant was preliminarily evaluated through *in vitro* functional experiments. Finally, the impact of these variants on APPL1 protein expression and the insulin pathway were assessed, and the potential mechanism underlying the interaction between the APPL1 protein and the insulin receptor was further explored.

RESULTS

A total of five novel mutations were identified, including four missense mutations (Asp632Tyr, Arg633His, Arg532Gln, and Ile642Met) and one intronic mutation (1153-16A>T). Pathogenicity prediction analysis revealed that the Arg532Gln was pathogenic across all predictions. The Asp632Tyr and Arg633His variants also had pathogenicity based on MutationTaster. In addition, multiple alignment of amino acid sequences showed that the Arg532Gln, Asp632Tyr, and Arg633His variants were conserved across different species. Moreover, in *in vitro* functional experiments, both the c.1894G>T (at Asp632Tyr) and c.1595G>A (at Arg532Gln) mutations were found to downregulate the expression of APPL1 on both protein and mRNA levels, indicating their pathogenic nature. Therefore, based on the patient's clinical and family history, combined with the results from bioinformatics analysis and functional experiment, the c.1894G>T (at Asp632Tyr) and c.1595G>A (at Arg532Gln) mutations were classified as pathogenic mutations. Importantly, all these mutations were located within the phosphotyrosine-binding domain of APPL1, which plays a critical role in the insulin sensitization effect.

CONCLUSION

This study provided new insights into the pathogenicity of *APPL1* gene mutations in diabetes and revealed a potential target for the diagnosis and treatment of the disease.

Key Words: Adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1; Maturity-onset diabetes of the young; Bioinformatics analysis; Gene mutation; Domain

Core Tip: We identified five new mutations in the adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1 (*APPL1*) gene, a critical regulator of insulin signaling and glucose metabolism, in maturity-onset diabetes of the young type 14 patients. We conducted bioinformatics and functional experiments and showed that two mutations were pathogenic, resulting in reduced expression of the APPL1 protein and mRNA. All mutations were in the phosphotyrosine-binding domain of APPL1, which is important for its insulin-sensitizing effect. This study gave new insights into the pathogenicity of *APPL1* mutations in diabetes and revealed potential targets for improved diagnosis and treatment strategies.

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INTRODUCTION

Maturity-onset diabetes of the young (MODY) is a rare form of hereditary monogenic diabetes caused by single gene mutations, constituting approximately 1%-2% of all diabetes cases[1,2]. A total of 14 MODY phenotypes have been identified, exhibiting significant heterogeneity in their clinical presentations. Notably, approximately 80% of MODY cases are initially misdiagnosed as either type 1 diabetes mellitus (T1DM) or T2DM[3,4].

MODY14, characterized by mutations in the adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1 (*APPL1*) gene, represents one of the least known MODY subtypes. Currently, only two related mutations have been reported, namely [c.1655T>A (p.Leu552*) and c.281G>A p.(Asp94Asn)][5-7]. To date, our understanding of MODY14 remains limited. To enhance our comprehension of MODY14 and *APPL1* mutations, it is crucial to identify additional cases, conduct comprehensive research, and consolidate knowledge in this field. By doing so, we can gain a deeper understanding of the underlying mechanisms and clinical implications of MODY14, ultimately paving the way for improved diagnostic and therapeutic strategies.

The *APPL1* gene, situated on chromosome 3p14.3, has 23 exons[8]. It exhibits widespread expression in numerous human tissues, such as pancreas, liver, adipose tissue, brain, and muscle[9,10]. APPL1 serves as a multifunctional adaptor protein, playing an important role in distinct signal transduction and membrane trafficking pathways. Structurally, it contains three primary domains: A Bin-Amphiphysin-Rvs (BAR) domain; a pleckstrin homology (PH) domain; and a phosphotyrosine-binding (PTB) domain[11]. These domains facilitate interactions with various signaling molecules and receptors, thereby regulating intracellular signaling pathways. The BAR domain can recognize and deform membranes with curvature and regulate intracellular trafficking and vesicle formation[12]. The PH domain can bind to phosphoinositides, such as phosphatidylinositol-3,4,5-trisphosphate, and target APPL1 to the plasma membrane, where it participates in various signaling pathways[13]. Meanwhile, the PTB domain can interact with adiponectin receptors 1/2, tropomyosin receptor kinase A, and other molecules, mediating intracellular signal transduction[14-16].

Current studies indicate that APPL1 is an important mediator of insulin sensitization. APPL1 can facilitate the binding of insulin receptor (IR) substrates (IRS) to IR, thereby activating PI3K/Akt signaling pathway and augmenting insulin

sensitivity[17]. Notably, in this process, the PTB domain can interact with IR and promote insulin signal transduction. In addition, APPL1 participates in adiponectin signaling by binding to adiponectin receptors, thereby enhancing lipid oxidation and glucose uptake[18,19]. In summary, further exploration of the interaction and regulatory network of APPL1 with other signaling molecules is warranted. Further, more clinical evidence is required to elucidate the precise role and underlying of APPL1 in diabetes and other metabolic diseases.

In this study, we identified five novel *APPL1* mutations, including four missense mutations and one intron mutation. To enhance our understanding of MODY14, we performed bioinformatics analysis and *in vitro* experiments to characterize the functional impact of these mutations. Based on the experimental results and literature review, we discussed their implications for diagnosis, treatment, and molecular pathogenesis. Notably, this article was the first to report cases of MODY14 in Asia on an international scale. Moreover, our study has identified the largest number of *APPL1* mutations, providing important data for *APPL1* mutation research. By enriching the gene database of MODY14, our discoveries provide new insights into the molecular mechanism and clinical management of this rare diabetes, ultimately guiding optimal treatment strategies, prognosis predictions, and genetic counseling for affected families.

MATERIALS AND METHODS

Study design

The purpose of this study was to determine the pathogenic status of suspected MODY diabetes in patients and evaluate the effects of novel *APPL1* mutations on disease development. We performed whole-exome sequencing (WES) to identify patients carrying *APPL1* gene mutations and conducted bioinformatics analysis of these mutations. Then, we conducted *in vitro* experiments to verify the pathogenicity of these mutations. Finally, the molecular mechanisms and signaling pathways involved in MODY pathogenesis were elucidated in this study.

Ethical considerations

This study adhered to the ethical principles outlined in the Declaration of Helsinki of 1964, along with its subsequent revisions and equivalent ethical standards. Prior to participation, informed consent was obtained from each participating patient or their legal guardian. The Ethics Committee of Shandong Provincial Hospital approved this study.

Patients

The study cohort consisted of 5 patients from five pedigrees. Patients meeting any of the following criteria were enrolled for WES: Younger than 30-years-old; had a family history of diabetes; or had negative insulin antibodies. Then, the clinical history and blood samples of patients were collected for further pathogenicity analysis.

Bioinformatic analysis

To assess the potential pathogenicity of the identified mutations, MutationTaster (https://www.mutationtaster.org/), Poly Phen-2 (http://genetics.bwh.harvard.edu/pph2/index.shtml), Revel (https://sites.google.com/site/revelgenomics), and IntSplice (https://www.med.nagoya-u.ac.jp/neurogenetics/IntSplice/index.html) were utilized. Specifically, MutationTaster, Poly Phen-2, and Revel were used for pathogenicity analysis of missense mutations, while IntSplice was used for pathogenicity analysis of introns. The visualization of multiple sequence alignment and sequence conservation extent were performed by Clustal X and GENEDOC. In addition, we searched the AlphaFold Protein Structure Database (https://alphafold.com/) for the structure of wild-type (WT) APPL1 and IR. The ClusPro server (https://cluspro.org) was used to analyze protein-protein docking. PyMOL software was employed to visualize the spatial structure and changed residues of APPL1. All graphics were created using Biorender (https://www.biorender.com/).

Mutation analysis

Genomic DNA was extracted from blood leucocytes from all study participants using Tiangen Biotech DNA kit. We performed WES on blood DNA and applied the SeqCap EZ MedExome Target Enrichment Kit (Roche NimbleGen) to capture human exons and adjacent introns after fragmenting, ligating, amplifying, and purifying genomic DNA. DNA sequencing was carried out using Illumina HiSeq platform, and the resulting data were aligned to the Hg19 reference genome. Mutation calls were made using NextGENe. The identified mutations were further verified by Sanger sequencing.

Plasmid construction and transfection

WT and mutant human *APPL1* plasmids (transcript ID: NM_012096.3) were generated by the transient overexpression vector GV141 (GeneChem, China)[20]. HEK293 cells were transfected with the plasmids and cultured in complete medium supplemented with 10% fetal bovine serum (Excell, FSD500, South America), penicillin, and streptomycin. Cells were seeded in six-well plates once they reached 80%-90% confluence. Transfection was performed when the degree of cell fusion reached 70%-90%. We added 2 μg of corresponding plasmids to each well of the six-well plate and transfected them into HEK293 cells. The transfection operation was performed followed the instructions of the Lipofectamine 3000 (Invitrogen, American) transfection kit. To ensure optimal transfection efficiency, the process was carried out on a sterile bench (SW-CJ-IC dual person purification workbench).

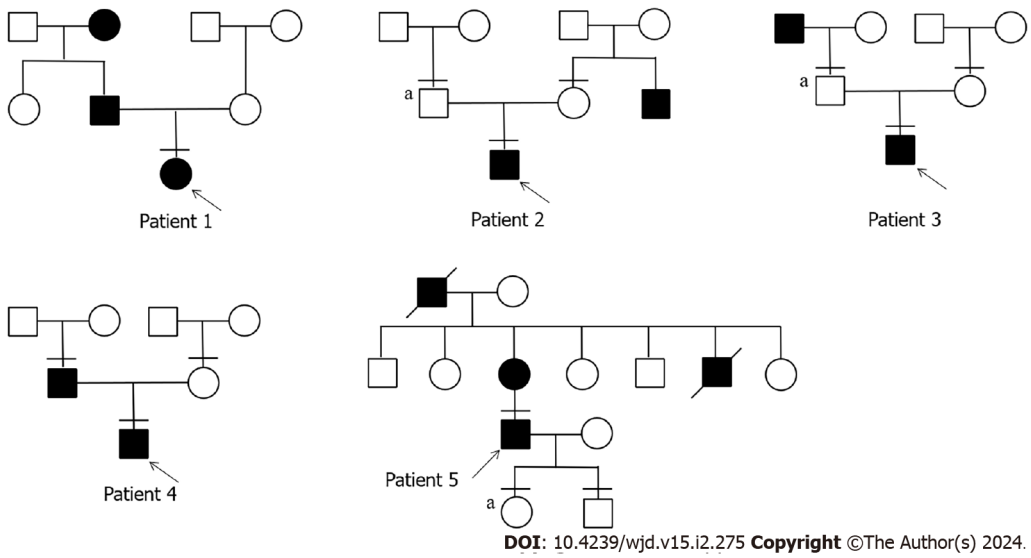

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Figure 1 Pedigree of 5 diabetes patients with novel adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1 variants. Males and females are represented by squares and circles, respectively. The black padding suggests that the patient has diabetes, the arrow represents the progenitor, and the horizontal line indicates a patient who has undergone full exon sequencing. "a" indicates that the patient has the same mutation as the proband.

Real-time PCR

After transfection, cells were collected after 24 h and lysed with Trizol (TaKaRa, Japan). Chloroform was added to separate RNA from DNA and proteins. The RNA was precipitated with isopropanol and washed several times with 75% ethanol. The RNA concentration was measured by nanodrop software after extraction. To convert mRNA into complementary DNA, reverse transcription was performed following the instructions of the reverse transcription kit manual (TaKaRa, Japan) using Mastercycler5333 PCR instrument (Eppendorf, Germany). Next, Bestar SybrGreen qPCR mastermix, PCR Forward Primer, PCR Reverse Primer, DNA template, and ddH_2O were mixed well in a 96-well plate. Finally, qPCR was performed on a real-time fluorescence quantitative PCR instrument (Roche, United States).

Immunoblot analysis

RIPA and PMSF (Shanghai Shenneng Gaming Company, China) were mixed at a ratio of 99:1 in the six-well plate after 48 h of transfection. Lysis buffer was added to each well, and the cells were scraped with a cell scraper. The lysate was transferred to EP tubes and incubated on ice for 20 min. The lysate was centrifuged for 15 min to extract protein. The protein concentration was determined using an enzyme-linked immunosorbent assay. Then, loading buffer was added to the protein samples and boiled for 10 min. Proteins were separated with different molecular weights by electrophoresis on a 10% SDS-polyacrylamide gel. The membrane was transferred and blocked in 5% milk (skimmed milk powder purchased from Yili Group, China) for 1 h. Next, primary antibodies (Flag mouse anti 1:1000, β-actin mouse anti 1:7500) were added overnight at 4 °C. After recovering the primary antibodies, the membrane was washed with TBST for 10 min × 3 times. The secondary antibodies (mouse anti 1:5000) were added and incubated for 1 h. After washing the film, it was developed under the Alpha Fluorochem Q imaging analysis system (United States).

Statistical analysis

The experimental data was analyzed using SPSS software (Version 25.0). Measurement data were presented as mean ± SD and analyzed using an independent samples *t*-test. Statistical significance was defined as a P value < 0.05.

RESULTS

Clinical characteristics

We studied 5 patients with *APPL1* variants, four of whom had experienced elevated fasting blood glucose before the age of 25, while the fifth patient developed diabetes at the age of 39 (Table 1). Patient 1 was diagnosed the diabetes at the age of 13, with a family history of diabetes in both his grandmother and father (Figure 1). His grandmother continued taking oral hypoglycemic drugs, while his father managed diabetes through diet control, resulting in normalized blood glucose level. Patient 1 had obvious polyuria, polydipsia, polyphagia, and diabetic ketoacidosis at the onset of the disease. Insulin antibody testing yielded negative results. The patient used insulin therapy after diagnosis, and then his insulin autoantibodies and islet cell antibodies turned positive after 8 years of insulin therapy.

Table 1 Clinical features of patients with adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1 mutation

Patient	*APPL1* variant	Sex	Age of onset in yr	BMI in kg/m^2	HbA1C 4%-6%	FBG 4.4-6.1 mmol/L
1	c.1894G>T (p. Asp632Tyr)	Female	13	14.98	11	8.25
2	c.1898G>A (p. Arg633His)	Male	21	36.63	6.9	8.3
3	c.1595G>A (p. Arg532Gln)	Male	13	20.62	14.2	11.69
4	c.1926A>G (p. Ile642Met)	Male	12	20.52	8.3	8.46
5	c.1153-16A>T (p.?)	Male	39	25.91	6.1	7.66

APPL1: Adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1; BMI: Body mass index; FBG: Fasting blood glucose; HbA1C: Hemoglobin A1c.

Table 2 Pathogenicity analysis of adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1 gene mutations

Patient	*APPL1* variant	MutationTaster pathogenicity and probability value	PolyPhen-2 pathogenicity and score	Revel pathogenicity and score
1	c.1894G>T (p. Asp632Tyr)	Disease causing (0.995)	Benign (0.285)	Benign (0.120)
2	c.1898G>A (p. Arg633His)	Disease causing (0.999)	Benign (0.052)	Benign (0.140)
3	c.1595G>A (p. Arg532Gln)	Disease causing (1.000)	Probably damaging (1.000)	Probably damaging (0.477)
4	c.1926A>G (p. Ile642Met)	Polymorphism (0.998)	Benign (0.007)	Benign (0.038)
5	c.1153-16A>T (p.?)	Polymorphism (1)	-	-

APPL1: Adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1.

Patient 2 developed diabetes at the age of 21. WES of his family revealed that his father had no diabetes but also carried the same mutation. Only his uncle had diabetes in his family. Additionally, patient 2 presented with obesity and ketoacidosis at the onset of the disease. After the ketoacidosis subsided, the patient shifted to diet control.

Patient 3 developed diabetes at the age of 13 and had a diabetic grandfather. Although the patient's father carried the same mutation, he remained unaffected by the disease. The patient also had ketoacidosis when he developed diabetes. He stopped taking medication after 2 mo of treatment with insulin combined with oral drugs and now only controls glucose by diet.

Patient 4 developed diabetes at the age of 12, and only his father had diabetes in his family. It is worth noting that the patient's blood glucose reached 19.81 mmol/L 2 h after a meal, accompanied by hyperinsulinemia (insulin > 300.00 mU/L 2 h after a meal). The patient relied on oral medication at the onset of the disease and transitioned to glucose control through diet and increased exercise.

Patient 5 developed diabetes at the age of 39. Both his mother and grandfather had diabetes. Patient 5 had a son and a daughter. His daughter carried the variant but as of the writing of this article had not shown any symptoms of diabetes. Patient 5 had been taking oral hypoglycemic drugs since he was diagnosed with diabetes.

Identification of novel variants in the APPL1 gene

We identified five variants, of which Asp632Tyr, Arg633His, Arg532Gln and Ile642Met mutations are missense mutations, and 1153-16A>T is an intron mutation (Figure 2A). The Asp632Tyr, Arg633His, and Ile642Met variants are located in exon 21, while the Arg532Gln variant is located in exon 17. The 1153-16A>T intronic mutation is upstream of exon 14. These four missense mutations are located in the PTB domain of APPL1, which can bind to the IR and regulate the insulin signaling pathway (Figure 2B). The Arg532Gln, Asp632Tyr, and Arg633His variants all caused changes in the surface potential of APPL protein, while the Ile642Met variant had no obvious abnormality. Among them, the Arg532Gln and Arg633His variants resulted in a decrease in positive surface potential, while the Asp632Tyr variant led to the elimination of negative surface potential (Figure 2C). These changes in potential indicate that the mutation may disrupt the interaction of APPL1 with other macromolecules. Moreover, amino acid mutations can influence protein function and folding by altering hydrophilicity (Supplementary Figure 1).

Bioinformatic analysis

To assess the pathogenicity of the four missense mutations, we employed MutationTaster, PolyPhen-2, and Revel for prediction analysis. Remarkably, all three software tools consistently predicted the Arg532Gln variant as pathogenic, with

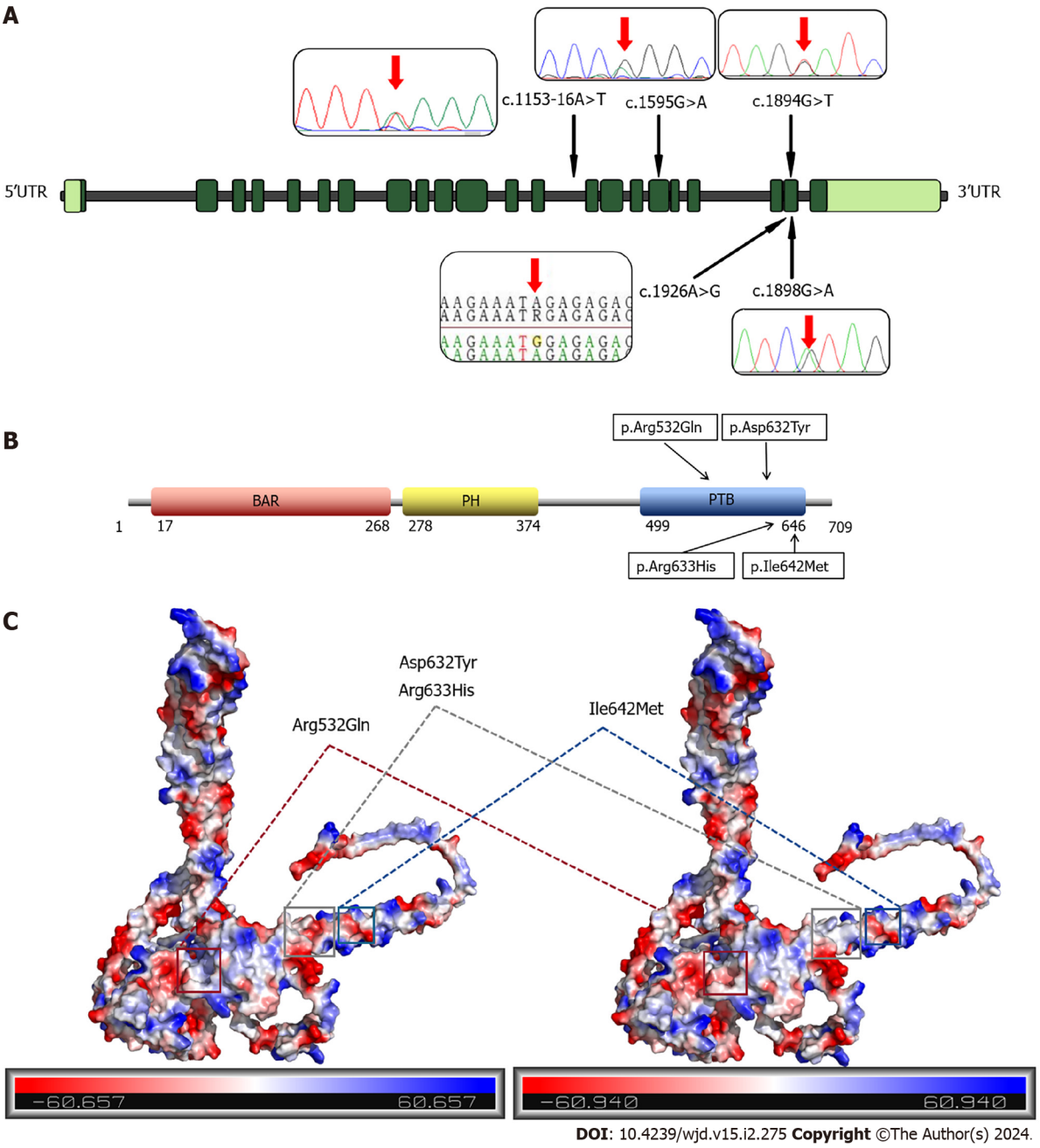

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Figure 2 Distribution of mutation sites in adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1 and adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1 protein and potential changes in mutation sites. A: Exon and mutation site distribution of adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1 (*APPL1*) gene; B: Domain and mutation site distribution of APPL1 protein; C: Potential change of mutated APPL1 protein. BAR: Bin-Amphiphysin-Rvs; PH: Pleckstrin homology; PTB: Phosphotyrosine-binding; UTR: Untranslated region.

MutationTaster indicating a high likelihood of pathogenicity (Table 2). The three prediction outcomes for the Ile642Met variant were all benign. Moreover, the Asp632Tyr and Asp632Tyr variants were both pathogenic in MutationTaster and benign in PolyPhen-2 and Revel. Multiple alignments of amino acid sequences demonstrated that the residues Asp632Tyr, Arg633His, and Arg532Gln were conserved across various species. This implies that they may exert a detrimental impact on the structure and function of the protein, reinforcing their potential pathogenicity. However, we noticed that in multiple species, the amino acid at position 642 of APPL1 was not isoleucine, but methionine, as in our patients' mutation, indicating that this site may not have a significant influence on the function or structure of the protein (Figure 3). Additionally, the pathogenicity analysis of the intronic mutations showed that the prediction results of MutationTaster and IntSplice were not pathogenic.

Functional study of WT and mutant APPL1 in vitro

The experiment was used to confirm the pathogenicity of four missense mutations. As shown in Figure 4A, the mRNA expression level of *APPL1* Asp632Tyr variant decreased by 98% (P = 0.001) compared with WT-*APPL1*, while the

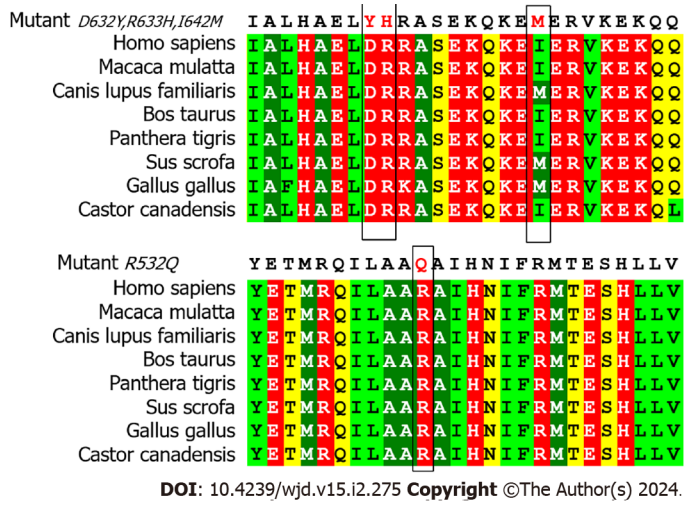

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Figure 3 Conservation of mutation sites in multiple species.

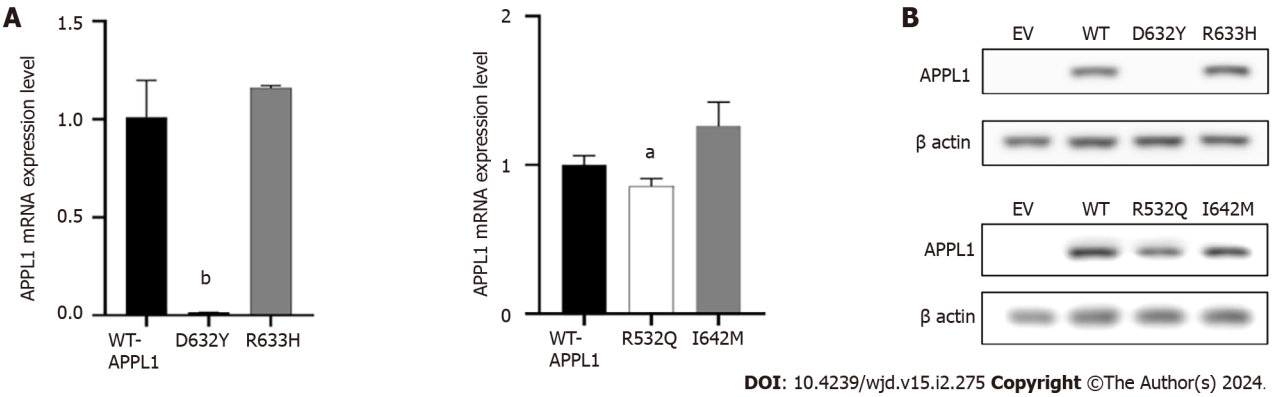

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Figure 4 mRNA and protein expression at mutation sites. A: mRNA expression at the mutation site; B: Protein expression at the mutation site. [a]*P* < 0.05; [b]*P* < 0.001. APPL1: Adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1; EV: Empty vector; WT: Wild-type.

Arg532Gln variant decreased by 14% (P = 0.035), indicating that both mutations at these sites resulted in reduced expression of *APPL1* mRNA. The Arg633His and Ile642Met variants did not cause significant changes in *APPL1* mRNA expression. In the experiment, we also observed that the expression of mutant proteins was consistent with mRNA expression. Compared with WT APPL1, the protein band of the Asp632Tyr-APPL1 variant disappeared, indicating that this variant would prevent the expression of APPL1 (Figure 4B). In addition, the protein expression of the Arg532Gln variant was significantly reduced compared with WT APPL1, indicating that this mutation also inhibited the expression of APPL1 protein. There was no significant change in APPL1 protein expression when mutated at Arg633His or Ile642Met, suggesting that these two mutations may not affect the expression of APPL1 protein.

APPL1 pathway analysis and protein docking prediction

To further elucidate the role of APPL1, we searched for APPL1-related protein pathways in the STRING database. Our analysis revealed that the insulin-related pathway protein AKT had the highest binding affinity with APPL1 (Figure 5A). In the AKT pathway, APPL1 could also bind to IR, which plays a role in insulin sensitization by interacting with the PTB domain where our four missense mutations were located. As shown in the Figure 5B, the NPEY motif of IR (with TYR-999 as the phosphorylation site) might interact with the amino acids between β5 and C-terminal helix of the PTB domain. Notably, the Asp632Tyr mutation is in the closest proximity to this binding site. Based on this observation, we speculate that this site might be associated with the interaction between APPL1 and IR.

DISCUSSION

MODY14 is an extremely rare form of inherited diabetes caused by mutations in the *APPL1* gene. So far, only two variants [c.1655T>A (p.Leu552*) and c.281G>A p.(Asp94Asn)] of *APPL1* were found to be associated with MODY14. In this study, we identified four novel missense mutations and one intronic mutation in APPL1, representing the largest number of de novo APPL1 mutations reported so far. For the first time, we demonstrated that two missense mutations

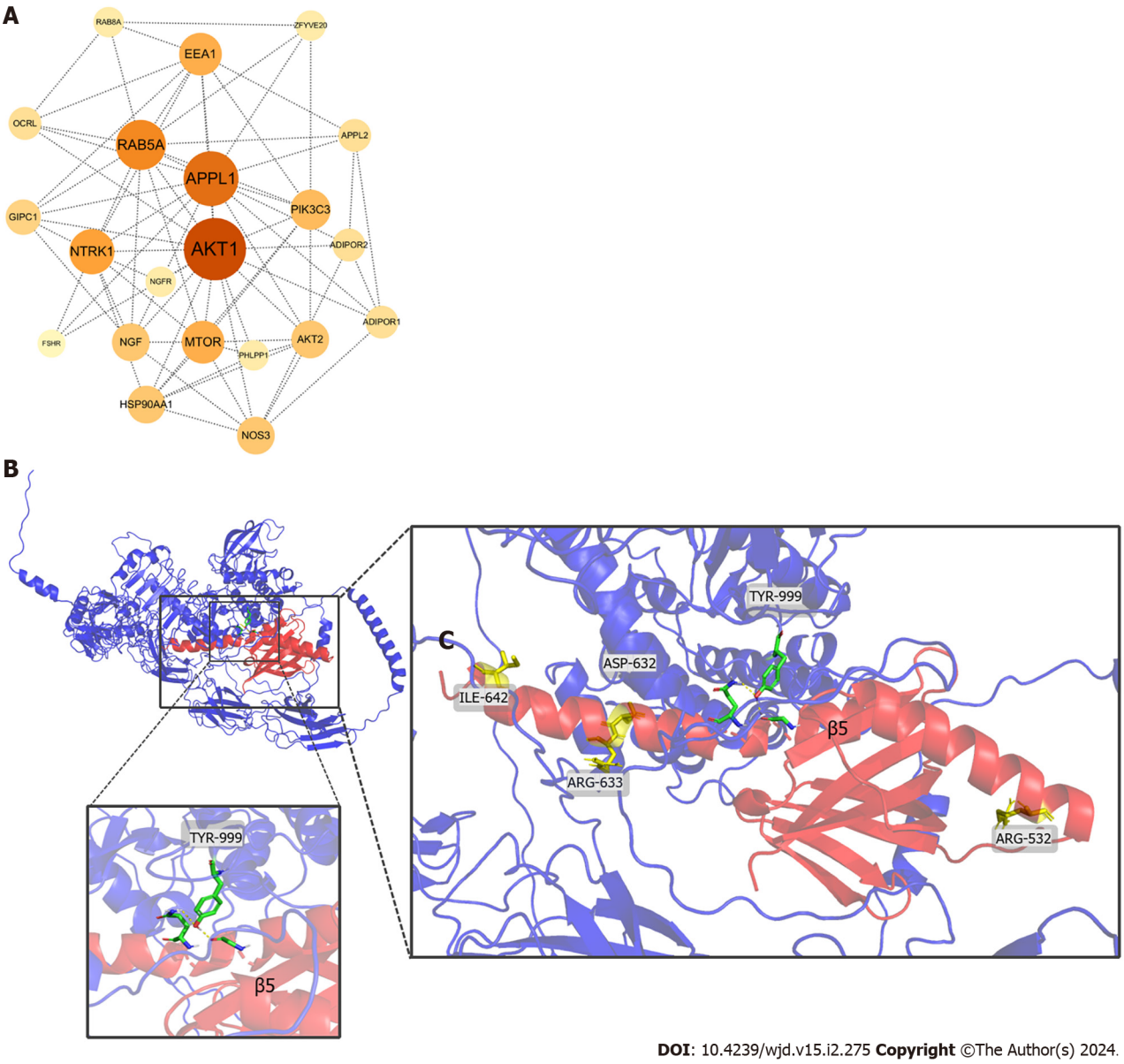

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Figure 5 Adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1-related protein networks and the docking between adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1 and insulin receptor. A The adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1 (APPL1)-related protein network. The degree of binding between proteins is indicated by the colors from yellow to orange on the nodes. The larger the node, the darker the color, and the higher the degree of binding to the APPL1 protein. The edge shows the association of protein-protein; B: The binding of the phosphotyrosine binding domain of APPL1 to insulin receptor proteins. Red is the phosphotyrosine binding domain, and blue is the insulin receptor protein.

[c.1894G>T (p.Asp632Tyr) and c.1595G>A (p.Arg532Gln)] in *APPL1* are pathogenic. Bioinformatics analysis provided compelling evidence for their deleterious effects. In addition, we further investigated the role of *APPL1* in insulin signaling and elucidated its potential molecular mechanisms.

To determine whether the *APPL1* variants of the patients were related to their diabetes symptoms, we performed a comprehensive analysis combining their clinical manifestations and *in vitro* functional experiments. Preliminary experimental validation showed that the mutations carried by patients 1 (carrying mutation Asp632Tyr) and 3 (carrying mutation Asp632Tyr) were pathogenic. Considering their age of onset, family history, and the results of bioinformatics analysis, these 2 patients were diagnosed with MODY14. Interestingly, patient 1 had some insulin antibodies turned positive after many years of insulin therapy, suggesting a subsequent development of T1DM. On the other hand, the father of patient 3 carried the mutation but did not develop the disease, indicating potential incomplete penetrance of the mutation. Patients 2 and 4 were young at the onset of the disease and only needed medication or diet control. However, their family history did not align well with the autosomal dominant inheritance pattern. Pathogenicity prediction and functional testing both indicated that their *APPL1* variants were non-pathogenic. Therefore, based on the comprehensive analysis, we speculated that patients 2 and 4 were more supportive of the diagnosis of T2DM, especially patient 4, who was overweight at the onset of the disease. We hypothesized that overeating and obesity may contribute to an earlier onset of T2DM in this patient. In addition, patient 5 had a more obvious family history of diabetes but an older age of

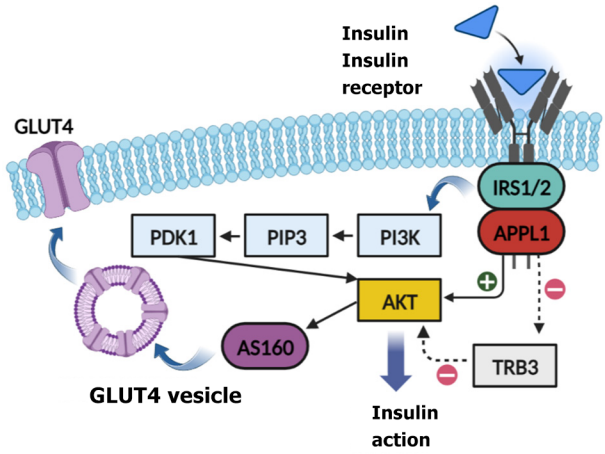

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Figure 6 Role of adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1 in the insulin pathway. AKT: Serine/threonine kinase Akt; APPL1: Adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1; AS160: Akt substrate of 160 kDa; GLUT4: Glucose transporter type 4; IRS1/2: Insulin receptor substrate 1/2; PDK1: Pyruvate dehydrogenase (acetyl-transferring) kinase isozyme 1; PI3K: Phosphatidylinositol 3 - kinase; PIP3: Phosphatidylinositol-3,4,5-trisphosphate; TRB3: Tribble homolog 3.

onset. Taking the pathogenicity analysis into consideration, we propose that patient 5 aligns more closely with the diagnosis of T2DM. Therefore, among the five mutations, c.1894G>T (p.Asp632Tyr) and c.1595G>A (p.Arg532Gln) were pathogenic mutations, and patients carrying these mutations had MODY14.

Among the three major domains of the APPL1 protein, we found that the four missense mutations were all located in the PTB domain, which can bind to both AKT and IR (mainly). We considered that the pathogenicity of the mutation sites was related to the reduced sensitizing effect of APPL1 in the insulin pathway. After insulin binds to its receptor, APPL1 carries IRS1 and IRS2 to the IR and promotes the binding of the IR and IRS by directly interacting with the IR through its PTB domain[17,21] (Figure 6). The peptide binding site in most PTB domains is located between strand β5 of the central β sandwich and the C-terminal helix[22,23]. When we docked APPL1 and the IR, we found that the β subunit of the IR also contained an NPXY motif (NPEY), then the PTB domain docked with it to facilitate the subsequent transmission of insulin signals. Therefore, the mutation of pathogenic sites in the PTB domain of APPL1 may affect the binding of the IR and IRS, leading to an impaired insulin signaling pathway as well as increased blood glucose and insulin resistance. Furthermore, despite the adjacent location of the Asp632Tyr and Arg633His variants, their pathogenicity differs. This observation suggests that the Asp632 site may play a crucial role in binding to proteins associated with the insulin pathway.

In addition, the BAR domain of APPL1 can also enhance insulin-stimulated AKT phosphorylation by directly binding to AKT and competitively inhibiting Tribbles homolog 3 (mainly), thereby achieving the effects of lowering blood glucose (activating AKT to inhibit glucagon-induced hepatic glucose production, promoting glucose transporter type 4 translocation and cellular glucose uptake) and insulin resistance[24-27]. It is noteworthy that all the MODY14 patients we identified had mutations located in the PTB domain. Among the previously reported MODY14 patients, the c.1655T>A (p.L552*) mutation was also located in the PTB domain, indicating a high aggregation of mutations in the PTB domain [7]. This suggests that compared to the BAR domain, the PTB domain may play a more significant role in insulin pathway signal transduction.

Although mutations in *APPL1* are relatively rare, recent advancements in exploring its molecular mechanisms and physiological functions have highlighted its key role in regulating glucose metabolism. Through its PTB domain, APPL1 interacts with AdipoR1 and AdipoR2, facilitating the transmission of adiponectin-stimulated signals to downstream targets[28]. In addition, APPL1 may provide a way of communication between the adiponectin and insulin signaling pathways, mediating the sensitization effect of insulin on muscle glucose disposal[18,19]. A study showed that APPL1 can counteract the high-fat diet-induced insulin resistance and hepatic glucose metabolism disorder, and improve blood glucose levels and insulin sensitivity in mice. Therefore, APPL1 may serve as a potential target for treating diabetes[11]. However, it is worth noting that a study reported that the expression of APPL1 in the muscle of T2DM rats was reduced, leading to weakened insulin-induced AKT signal activation[29]. To some extent, this consolidates the key role of APPL1 in regulating muscle insulin signaling and metabolism, but in this study, patients 2 and 4 who likely had T2DM did not show a reduction in APPL1 expression in the *in vitro* functional experiments.

This study also had some limitations. First, the functional experiments did not fully replicate the real physiological environment and conditions. Hence, we cannot completely rule out the possibility that these mutations might impact the interaction of APPL1 with other proteins or small molecules. Additionally, we did not verify the pathogenicity of the intronic mutation through functional experiments. We also failed to obtain blood samples from some family members for genetic testing. This may result in imprecise estimates of the mode of inheritance and penetrance of the mutation, and the existence of potential epistatic or modifier factors cannot be definitively determined. In the future, a broader range of cell lines or animal models are needed for *in vitro* and *in vivo* experiments to further investigate the impact of *APPL1* gene mutations on the insulin signaling pathway and other metabolic pathways. Our study only serves as an initial investigation of the pathogenic mechanism of MODY14. At the protein level, aberrantly folded proteins can be degraded by the

ubiquitin system, while RNA silencing or nonsense-mediated decay can diminish the level or stability of mRNA encoding aberrant proteins, thereby attenuating the production of aberrant proteins. These aspects warrant further investigation in subsequent research. Future studies should encompass more extensive epidemiological and statistical analysis, as well as detailed investigations in molecular biology and systems biology, to reveal the role and regulation of *APPL1* gene mutations in the complex metabolic network.

CONCLUSION

In summary, this study identified five novel *APPL1* variants, of which c.1894G>T (p.Asp632Tyr) and c.1595G>A (p.Arg532Gln) were pathogenic variants for MODY14. We believe that the PTB domain of APPL1 plays a significant role in the insulin signaling pathway in MODY14 patients. This study provided novel insights and evidence for further elucidating the pathogenicity of the *APPL1* gene in MODY14 and provided new targets and strategies for developing new diagnostic and therapeutic methods.

ARTICLE HIGHLIGHTS

Research background

Mutations in the adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1 (*APPL1*) gene have been associated with the development of maturity-onset diabetes of the young type 14 (MODY14), a rare form of monogenic diabetes. So far, only two mutations [c.1655T>A (p.Leu552*) and c.281G>A p.(Asp94Asn)] have been found to be related to this disease. Due to the limited knowledge of MODY14, it is necessary to identify more cases and conduct a comprehensive study of MODY14 and *APPL1* mutations. In this study, we discovered five new *APPL1* gene mutations by whole exome sequencing (WES) and bioinformatics analysis, of which two were confirmed to be pathogenic mutations by *in vitro* functional assays. These mutations were all located in the phosphotyrosine binding (PTB) domain of APPL1, which has a significant impact on insulin sensitivity.

Research motivation

This study aimed to identify the pathogenicity and functional role of *APPL1* gene mutations in diabetes. It mainly identified and evaluated the pathogenicity of *APPL1* gene mutations and explored the effects of these mutations on APPL1 protein expression and insulin signaling pathway. This will provide potential targets for the diagnosis and treatment of MODY14 and will provide new clues for the interaction mechanism of the APPL1 protein and insulin receptor.

Research objectives

The main objective of this study was to evaluate the pathogenicity of *APPL1* gene mutations in diabetic patient and to characterize the functional role of APPL1 domains. By WES and bioinformatics analysis, five novel *APPL1* gene mutations were identified, among which c.1894G>T (at Asp632Tyr) and c.1595G>A (at Arg532Gln) were confirmed as pathogenic mutations by *in vitro* functional experiments.

Research methods

This study used WES to sequence all the exons in the genome that encode proteins, thus discovering variants associated with diseases. Then, bioinformatics analysis was used to align and predict the sequencing results, thus evaluating the pathogenicity and conservation of the variants. The pathogenicity was further verified by *in vitro* functional experiments.

Research results

Our study identified five novel *APPL1* gene mutations, among which c.1894G>T (at Asp632Tyr) and c.1595G>A (at Arg532Gln) were confirmed as pathogenic mutations by *in vitro* functional experiments. Both mutations are located in the PTB domain of APPL1, which has an important impact on insulin sensitivity. The results showed that the mutations can reduce the expression level of APPL1 protein, thus affecting the activation of the insulin signaling pathway and the regulation of glucose metabolism.

Research conclusions

APPL1 gene mutations c.1894G>T (at Asp632Tyr) and c.1595G>A (at Arg532Gln) are pathogenic in diabetes, and these mutations are located in the PTB domain of APPL1, which has an important impact on insulin sensitivity.

Research perspectives

In the future, the structure and function of APPL1 protein can be further studied, especially the mechanism of action of the PTB domain and the binding mode and regulatory effect of APPL1 protein with the insulin receptor. In addition, the effect of *APPL1* gene mutations on the clinical manifestations and treatment response of diabetic patients can be verified. More effective methods and criteria for the diagnosis and treatment of MODY14 can be provided.

FOOTNOTES

Co-corresponding authors: Kun-Xia Li and Chao Xu.

Author contributions: Li KX and Xu C are the co-corresponding authors of this manuscript; Xu C acted as a guarantor for the research design and manuscript revision; Li KX designed the theme of the article; Shi P carried out the experiments and wrote the manuscript; Tian Y contributed to the experiment implementation and data collection; Xu F edited and reviewed the content of the manuscript; Liu LN and Wu WH contributed to the experiment implementation and data analysis; Shi YZ, Dai AQ, and Fang HY performed bioinformation analysis and graph drawing; and all authors read and approved the final manuscript.

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REFERENCES

1 **Samadli S**, Zhou Q, Zheng B, Gu W, Zhang A. From glucose sensing to exocytosis: takes from maturity onset diabetes of the young. *Front Endocrinol (Lausanne)* 2023; **14**: 1188301 [PMID: 37255971 DOI: 10.3389/fendo.2023.1188301]
2 **Delvecchio M**, Pastore C, Giordano P. Treatment Options for MODY Patients: A Systematic Review of Literature. *Diabetes Ther* 2020; **11**: 1667-1685 [PMID: 32583173 DOI: 10.1007/s13300-020-00864-4]
3 **Ivanoshchuk DE**, Shakhtshneider EV, Rymar OD, Ovsyannikova AK, Mikhailova SV, Fishman VS, Valeev ES, Orlov PS, Voevoda MI. The Mutation Spectrum of Maturity Onset Diabetes of the Young (MODY)-Associated Genes among Western Siberia Patients. *J Pers Med* 2021; **11** [PMID: 33477506 DOI: 10.3390/jpm11010057]
4 **Nkonge KM**, Nkonge DK, Nkonge TN. The epidemiology, molecular pathogenesis, diagnosis, and treatment of maturity-onset diabetes of the young (MODY). *Clin Diabetes Endocrinol* 2020; **6**: 20 [PMID: 33292863 DOI: 10.1186/s40842-020-00112-5]
5 **Broome DT**, Pantalone KM, Kashyap SR, Philipson LH. Approach to the Patient with MODY-Monogenic Diabetes. *J Clin Endocrinol Metab* 2021; **106**: 237-250 [PMID: 33034350 DOI: 10.1210/clinem/dgaa710]
6 **Kant R**, Davis A, Verma V. Maturity-Onset Diabetes of the Young: Rapid Evidence Review. *Am Fam Physician* 2022; **105**: 162-167 [PMID: 35166506]
7 **Prudente S**, Jungtrakoon P, Marucci A, Ludovico O, Buranasupkajorn P, Mazza T, Hastings T, Milano T, Morini E, Mercuri L, Bailetti D, Mendonca C, Alberico F, Basile G, Romani M, Miccinilli E, Pizzuti A, Carella M, Barbetti F, Pascarella S, Marchetti P, Trischitta V, Di Paola R, Doria A. Loss-of-Function Mutations in APPL1 in Familial Diabetes Mellitus. *Am J Hum Genet* 2015; **97**: 177-185 [PMID: 26073777 DOI: 10.1016/j.ajhg.2015.05.011]
8 **Ivanoshchuk DE**, Shakhtshneider EV, Rymar OD, Ovsyannikova AK, Mikhailova SV, Orlov PS, Ragino YI, Voevoda MI. Analysis of APPL1 Gene Polymorphisms in Patients with a Phenotype of Maturity Onset Diabetes of the Young. *J Pers Med* 2020; **10** [PMID: 32854233 DOI: 10.3390/jpm10030100]
9 **Cheng KK**, Lam KS, Wu D, Wang Y, Sweeney G, Hoo RL, Zhang J, Xu A. APPL1 potentiates insulin secretion in pancreatic β cells by enhancing protein kinase Akt-dependent expression of SNARE proteins in mice. *Proc Natl Acad Sci U S A* 2012; **109**: 8919-8924 [PMID: 22566644 DOI: 10.1073/pnas.1202435109]
10 **Wu KKL**, Long K, Lin H, Siu PMF, Hoo RLC, Ye D, Xu A, Cheng KKY. The APPL1-Rab5 axis restricts NLRP3 inflammasome activation through early endosomal-dependent mitophagy in macrophages. *Nat Commun* 2021; **12**: 6637 [PMID: 34789781 DOI: 10.1038/s41467-021-26987-1]
11 **Cheng KK**, Iglesias MA, Lam KS, Wang Y, Sweeney G, Zhu W, Vanhoutte PM, Kraegen EW, Xu A. APPL1 potentiates insulin-mediated inhibition of hepatic glucose production and alleviates diabetes *via* Akt activation in mice. *Cell Metab* 2009; **9**: 417-427 [PMID: 19416712 DOI: 10.1016/j.cmet.2009.03.013]
12 **Peter BJ**, Kent HM, Mills IG, Vallis Y, Butler PJ, Evans PR, McMahon HT. BAR domains as sensors of membrane curvature: the amphiphysin BAR structure. *Science* 2004; **303**: 495-499 [PMID: 14645856 DOI: 10.1126/science.1092586]
13 **Mitsuuchi Y**, Johnson SW, Sonoda G, Tanno S, Golemis EA, Testa JR. Identification of a chromosome 3p14.3-21.1 gene, APPL, encoding an adaptor molecule that interacts with the oncoprotein-serine/threonine kinase AKT2. *Oncogene* 1999; **18**: 4891-4898 [PMID: 10490823 DOI: 10.1038/sj.onc.1203080]

14 **Ye S**, Luo Y, Lu W, Jones RB, Linhardt RJ, Capila I, Toida T, Kan M, Pelletier H, McKeehan WL. Structural basis for interaction of FGF-1, FGF-2, and FGF-7 with different heparan sulfate motifs. *Biochemistry* 2001; **40**: 14429-14439 [PMID: 11724555 DOI: 10.1021/bi011000u]

15 **Zeng L**, Kuti M, Mujtaba S, Zhou MM. Structural insights into FRS2α PTB domain recognition by neurotrophin receptor TrkB. *Proteins* 2014; **82**: 1534-1541 [PMID: 24470253 DOI: 10.1002/prot.24523]

16 **Liu Z**, Xiao T, Peng X, Li G, Hu F. APPLs: More than just adiponectin receptor binding proteins. *Cell Signal* 2017; **32**: 76-84 [PMID: 28108259 DOI: 10.1016/j.cellsig.2017.01.018]

17 **Ryu J**, Galan AK, Xin X, Dong F, Abdul-Ghani MA, Zhou L, Wang C, Li C, Holmes BM, Sloane LB, Austad SN, Guo S, Musi N, DeFronzo RA, Deng C, White MF, Liu F, Dong LQ. APPL1 potentiates insulin sensitivity by facilitating the binding of IRS1/2 to the insulin receptor. *Cell Rep* 2014; **7**: 1227-1238 [PMID: 24813896 DOI: 10.1016/j.celrep.2014.04.006]

18 **Deepa SS**, Dong LQ. APPL1: role in adiponectin signaling and beyond. *Am J Physiol Endocrinol Metab* 2009; **296**: E22-E36 [PMID: 18854421 DOI: 10.1152/ajpendo.90731.2008]

19 **Artimani T**, Najafi R. APPL1 as an important regulator of insulin and adiponectin-signaling pathways in the PCOS: A narrative review. *Cell Biol Int* 2020; **44**: 1577-1587 [PMID: 32339379 DOI: 10.1002/cbin.11367]

20 **Wu H**, Shu M, Liu C, Zhao W, Li Q, Song Y, Zhang T, Chen X, Shi Y, Shi P, Fang L, Wang R, Xu C. Identification and characterization of novel carboxyl ester lipase gene variants in patients with different subtypes of diabetes. *BMJ Open Diabetes Res Care* 2023; **11** [PMID: 36634979 DOI: 10.1136/bmjdrc-2022-003127]

21 **Saito T**, Jones CC, Huang S, Czech MP, Pilch PF. The interaction of Akt with APPL1 is required for insulin-stimulated Glut4 translocation. *J Biol Chem* 2007; **282**: 32280-32287 [PMID: 17848569 DOI: 10.1074/jbc.M704150200]

22 **Li J**, Mao X, Dong LQ, Liu F, Tong L. Crystal structures of the BAR-PH and PTB domains of human APPL1. *Structure* 2007; **15**: 525-533 [PMID: 17502098 DOI: 10.1016/j.str.2007.03.011]

23 **Uhlik MT**, Temple B, Bencharit S, Kimple AJ, Siderovski DP, Johnson GL. Structural and evolutionary division of phosphotyrosine binding (PTB) domains. *J Mol Biol* 2005; **345**: 1-20 [PMID: 15567406 DOI: 10.1016/j.jmb.2004.10.038]

24 **Prudente S**, Sesti G, Pandolfi A, Andreozzi F, Consoli A, Trischitta V. The mammalian tribbles homolog TRIB3, glucose homeostasis, and cardiovascular diseases. *Endocr Rev* 2012; **33**: 526-546 [PMID: 22577090 DOI: 10.1210/er.2011-1042]

25 **Schenck A**, Goto-Silva L, Collinet C, Rhinn M, Giner A, Habermann B, Brand M, Zerial M. The endosomal protein Appl1 mediates Akt substrate specificity and cell survival in vertebrate development. *Cell* 2008; **133**: 486-497 [PMID: 18455989 DOI: 10.1016/j.cell.2008.02.044]

26 **Mîinea CP**, Sano H, Kane S, Sano E, Fukuda M, Peränen J, Lane WS, Lienhard GE. AS160, the Akt substrate regulating GLUT4 translocation, has a functional Rab GTPase-activating protein domain. *Biochem J* 2005; **391**: 87-93 [PMID: 15971998 DOI: 10.1042/BJ20050887]

27 **Saito T**, Okada S, Shimoda Y, Tagaya Y, Osaki A, Yamada E, Shibusawa R, Nakajima Y, Ozawa A, Satoh T, Mori M, Yamada M. APPL1 promotes glucose uptake in response to mechanical stretch *via* the PKCζ-non-muscle myosin IIa pathway in C2C12 myotubes. *Cell Signal* 2016; **28**: 1694-1702 [PMID: 27478065 DOI: 10.1016/j.cellsig.2016.07.010]

28 **Mao X**, Kikani CK, Riojas RA, Langlais P, Wang L, Ramos FJ, Fang Q, Christ-Roberts CY, Hong JY, Kim RY, Liu F, Dong LQ. APPL1 binds to adiponectin receptors and mediates adiponectin signalling and function. *Nat Cell Biol* 2006; **8**: 516-523 [PMID: 16622416 DOI: 10.1038/ncb1404]

29 **Kido K**, Ato S, Yokokawa T, Sato K, Fujita S. Resistance training recovers attenuated APPL1 expression and improves insulin-induced Akt signal activation in skeletal muscle of type 2 diabetic rats. *Am J Physiol Endocrinol Metab* 2018; **314**: E564-E571 [PMID: 29406784 DOI: 10.1152/ajpendo.00362.2017]

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ORIGINAL ARTICLE

Basic Study

Duodenal-jejunal bypass improves hypothalamic oxidative stress and inflammation in diabetic rats *via* glucagon-like peptide 1-mediated Nrf2/HO-1 signaling

Huai-Jie Wang, Li-Bin Zhang, Si-Peng Sun, Qing-Tao Yan, Zhi-Qin Gao, Fang-Ming Fu, Mei-Hua Qu

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Abstract

BACKGROUND

Type 2 diabetes mellitus (T2DM) is often accompanied by impaired glucose utilization in the brain, leading to oxidative stress, neuronal cell injury and inflammation. Previous studies have shown that duodenal jejunal bypass (DJB) surgery significantly improves brain glucose metabolism in T2DM rats, the role and the metabolism of DJB in improving brain oxidative stress and inflammation condition in T2DM rats remain unclear.

AIM

To investigate the role and metabolism of DJB in improving hypothalamic oxidative stress and inflammation condition in T2DM rats.

METHODS

A T2DM rat model was induced *via* a high-glucose and high-fat diet, combined with a low-dose streptozotocin injection. T2DM rats were divided into DJB operation and Sham operation groups. DJB surgical intervention was carried out

on T2DM rats. The differential expression of hypothalamic proteins was analyzed using quantitative proteomics analysis. Proteins related to oxidative stress, inflammation, and neuronal injury in the hypothalamus of T2DM rats were analyzed by flow cytometry, quantitative real-time PCR, Western blotting, and immunofluorescence.

RESULTS

Quantitative proteomics analysis showed significant differences in proteins related to oxidative stress, inflammation, and neuronal injury in the hypothalamus of rats with T2DM-DJB after DJB surgery, compared to the T2DM-Sham groups of rats. Oxidative stress-related proteins (glucagon-like peptide 1 receptor, Nrf2, and HO-1) were significantly increased ($P < 0.05$) in the hypothalamus of rats with T2DM after DJB surgery. DJB surgery significantly reduced ($P < 0.05$) hypothalamic inflammation in T2DM rats by inhibiting the activation of NF-κB and decreasing the expression of interleukin (IL)-1β and IL-6. DJB surgery significantly reduced ($P < 0.05$) the expression of factors related to neuronal injury (glial fibrillary acidic protein and Caspase-3) in the hypothalamus of T2DM rats and upregulated ($P < 0.05$) the expression of neuroprotective factors (C-fos, Ki67, Bcl-2, and BDNF), thereby reducing hypothalamic injury in T2DM rats.

CONCLUSION

DJB surgery improve oxidative stress and inflammation in the hypothalamus of T2DM rats and reduce neuronal cell injury by activating the glucagon-like peptide 1 receptor-mediated Nrf2/HO-1 signaling pathway.

Key Words: Duodenal jejunal bypass surgery; Type 2 diabetes mellitus; Neuron apoptosis; Inflammatory; Oxidative stress; Hypothalamic injury

Core Tip: Duodenal jejunal bypass (DJB) increases serum glucagon-like peptide 1 (GLP-1) Levels and enhances brain glucose utilization, playing a positive role in the treatment of diabetes. The GLP-1 signal may play a significant role after DJB surgery in brain injury related to type 2 diabetes mellitus (T2DM). In the current study, DJB surgery increased the serum levels of GLP-1 and upregulated the expression of GLP-1 receptor and antioxidant signaling proteins (Nrf2 and HO-1) in the hypothalamic tissue of T2DM rats. DJB reduced the expression of hypothalamic inflammatory and nerve cell injury-related factors. Therefore, DJB surgery improve oxidative stress and inflammation in the hypothalamus of T2DM rats and reduce neuronal cell injury by activating the GLP-1-mediated Nrf2/HO-1 signaling pathway.

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a condition characterized by high blood glucose and insulin resistance. It accounts for approximately 90% of adult diabetes patients worldwide[1,2]. Long-term uncontrolled diabetes conditions with high blood glucose levels and whole-body insulin resistance are associated with neurodegeneration[3,4]. This often results in impaired glucose utilization and energy metabolism, leading to oxidative stress in the brain[5-9]. Prolonged oxidative stress in the brain can eventually lead to complications, such as inflammation of the central nervous system (CNS) and neuronal cell apoptosis[10,11].

Metabolic surgery (also known as bariatric surgery) has been proven to have a beneficial effect on the management of type 2 diabetes and obesity[12,13]. For individuals with diabetes, this surgical procedure leads to substantial weight loss, improved management of blood glucose levels, and reduced reliance on medication[14-16]. Duodenal jejunal bypass (DJB) surgery is one of the procedures based on metabolic surgery and can be used to investigate the mechanisms of metabolic surgery in the treatment of diabetes mellitus[17,18]. DJB surgery significantly improves peripheral glucose metabolism in T2DM rats, but its effects on diabetes-induced central brain injury remain unclear[19,20].

Glucagon-like peptide 1 (GLP-1) is a hormone produced by L cells in the gastrointestinal tract, that serves multiple biological functions[21]. The combination of this hormone and its receptor, GLP-1 receptor (GLP-1R), can increase insulin secretion, enhance glucose metabolism, and play an important role in controlling blood glucose homeostasis[22]. Ruze *et al*[23] showed that central GLP-1 improved cerebral glucose uptake in obese and diabetic rats after DJB[23]. In addition to its metabolic effects, GLP-1 has also been shown to have neuroprotective effects, and upregulation of GLP-1 can protect cells from oxidative stress caused by hyperglycemia and limit brain inflammation[24,25]. Kim *et al*[26] demonstrated that the activation of GLP-1R can enhance the expression of proteins related to oxidative stress and the cell metabolism

regulatory protein NRF2, and upregulate antioxidant enzymes such as HO-1 and SOD to alleviate oxidative stress[26-28]. According to research by Shan Y *et al*[29], GLP-1R agonists decrease blood-brain barrier breakdown and brain inflammation in an astrocyte-dependent manner[29].

In our previous studies, we found that DJB can increase serum GLP-1 Levels and enhance brain glucose utilization, playing a positive role in the treatment of diabetes[30-32].Therefore, GLP-1 might play a role in DJB surgery for T2DM-related brain injury. In the current study, we investigated the role and mechanism of GLP-1 signaling in neuronal cell injury and inflammation in the hypothalamus of T2DM rats treated with the DJB procedure. This study can provide new insights into the role and molecular mechanisms of metabolic surgery in the treatment of diabetes and its central complications.

MATERIALS AND METHODS

Animals and treatment

The male Wistar rats (n = 40, 8 wk old) used in this study were obtained from Jinan Pengyue Animal Ltd. (Pengyue, Jinan, China). Rats were housed in individually ventilated cages with 12-h light/dark cycles at 21-23 °C and 30%-40% humidity. The rats had free access to food and water. T2DM rat models were established using a high-fat and high-glucose diet induction paired with streptozotocin intraperitoneal injection (35 mg/kg, Sigma, United States), with random blood glucose > 16.7 mmol/L. We randomly divided the T2DM rats into two groups: The DJB group (T2DM-DJB, n = 10) and the Sham group (T2DM-Sham, n = 10). Normal control rats (control group, n = 10) were fed a normal diet. All the research related to the use of animals in this study has complied with all relevant national regulations and institutional policies for the care and use of animals. The research plan was followed by the National Natural Science Foundation of China, No. 82070856, and conducted in Laboratory Animal Center of Weifang Medical University. The experimental protocols were approved by the Institutional Animal Care and Use Committee (No. 2021SDL574) and Institutional Review Board (No. 2020SDL074).

The detailed surgical and nursing procedures of the animals were performed as previously described[31]. For the DJB operation, distal pyloric transection was performed, and the duodenal stumps were closed with nylon sutures (6-0, Zhejiang, China). The distal pylorus was anastomosed to the distal stump of the jejunal limb after the jejunum was transected 10 cm from the ligament of Treitz. The proximal stump of the small intestine was connected to the digestive limb 15 cm from the distal end of the gastrointestinal anastomosis. Sham operations were performed to transect the gastrointestinal tract and create an in-situ anastomosis, mimicking DJB surgery. The determination of glucose homeostasis pre- and postoperatively was performed as previously described[31].

Tissue processing and preparation

Six weeks after the operation, rats were sacrificed following the completion of testing for blood glucose homeostasis indicators, including blood glucose, serum GLP-1, and insulin levels. A portion of the hypothalamic tissues was collected from the rat hemisphere and frozen in liquid nitrogen for analysis of mRNA and protein expression. The other cerebral hemispheres of the rats were fixed in 4% formalin for immunohistochemistry (IHC) and immunofluorescence (IF) assays.

The expression of differential hypothalamic proteins analyzed by protein chip assay

Hypothalamic tissues were dissected and immediately placed into the SDT lysate buffer (100 mM Tris-HCL, 2% SDS, 100 mM DTT, pH 7.6.) and then homogenized. The quality of the supernatant proteins was analyzed using SDS-PAGE. The supernatant of the hypothalamus homogenate was prepared using filter-aided sample preparation filtration. The preparations were lyophilized and then redissolved in a 40 μL solution of 0.1% formic acid using a C18 cartridge. Peptide segments were separated using the Easy-nLCNano lift flow system and analyzed with the Q Exactive™ Plus Mass Spectrometer. Quantitative analysis was performed using the label-free quantitation algorithm in this project. The significance level of protein enrichment for a specific gene ontology (GO) term or Kyoto encyclopedia of genes and genomes (KEGG) pathway was assessed using Fisher's exact test. Using Matplotlib software, we categorized the samples and proteins, and generated hierarchical clustering heatmaps simultaneously.

Western blotting analysis

Hypothalamus supernatants containing 30 μg of proteins were separated by 12% SDS-PAGE at 110 V for 2 h. The proteins were transferred onto a nitrocellulose membrane at 300 mA for 2 h at 4 °C. TBS pH 8.0 with 0.5% Tween 20 was used to block the membranes for 1 h. After each membrane was incubated with the primary antibody overnight at 4 °C (Supplementary Table 1), it was washed three times with TBS for 30 min. The appropriate secondary antibody was then applied to the membrane and incubated for one hour at 23 °C. Protein analysis was conducted using western blotting detection equipment from Bio-Rad (United States) and ImageJ software (United States).

Flow cytometry

An ultrasonic sonicator (Virsonic 60) was used to lyse 50 mg of rat hypothalamus tissue. Cytokines interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-17A, tumor necrosis factor α, interferon (IFN)-γ, and IFN-αwere measured in hypothalamic homogenate supernatants using cytokines combined detection kit (Jiangxi Cell-Gene Biotech CO., Ltd) and were analyzed by FCAP Array 3.0 software (BD Biosciences) after collection of events in a flow cytometer (Beckman Coulter).

Immunohistochemistry and immunofluorescence assays

Sections were dewaxed, rehydrated, and then blocked with 10% fetal bovine serum for 30 min before undergoing an overnight incubation at 4 °C with the primary antibodies. Sections were incubated with a horse radish peroxidase-conjugated secondary antibody after being washed with phosphate buffered solution. The slides were exposed to diaminobenzidine for five minutes at room temperature prior to IHC examination. Hematoxylin was used for nuclear counterstaining. For the IF experiment, the slides were first incubated with the primary antibody. Then, the sections were incubated with goat anti-rabbit IgG H&L (Alexa Fluor® 488) (ab150077, 1:1000) secondary antibody for one hour. 4', 6-Diamidino-2-Phenylindole dye (blue) was used for nuclear staining. Image acquisition was performed using a microscope (IX71, Olympus, Japan). The primary antibodies are listed in Supplementary Table 1.

Quantitative real-time PCR

Total RNA was extracted from the hypothalamus using TRIzol reagent (Thermo Fisher, United States) and then reverse transcribed to cDNA using a reverse transcription kit (TOYOBO, Japan). SYBR Green (GeneCopoeia, United States) was used in the quantitative real-time (qRT)-PCR reaction, which was performed on a Roche Diagnostics (Germany) machine and analyzed using the Bio-Rad system. Supplementary Table 2 contains a list of the primer sequences that were used.

Statistical analysis

The data are presented as the mean ± SD by Graph Pad Prism 8.0. Differences between pre and post operation were evaluated with a *t*-test. One-way ANOVA followed by Tukey's test was used to analyze differences between multiple groups. $P < 0.05$ indicated statistical significance.

RESULTS

The expression of different proteins in the hypothalamus after DJB intervention

The results of blood glucose and insulin detection pre and post operation showed that DJB surgery can significantly improve glucose homeostasis and alleviate insulin resistance in T2DM rats (Supplementary Figure 1). To investigate the expression of different proteins in the hypothalamus of rats between the T2DM-DJB and T2DM-sham groups, we conducted a quantitative proteomics (label-free) analysis. The results showed that at least 120 proteins were significantly altered (Figure 1A). KEGG enrichment and GO analysis revealed significant differences in signaling pathways and proteins related to oxidative stress, inflammation, and neuronal cell injury between the two groups of rats (Figure 1B and C).

DJB surgery inhibits hypothalamic oxidative stress in rats with T2DM by activating the Nrf2/HO-1 signaling pathway

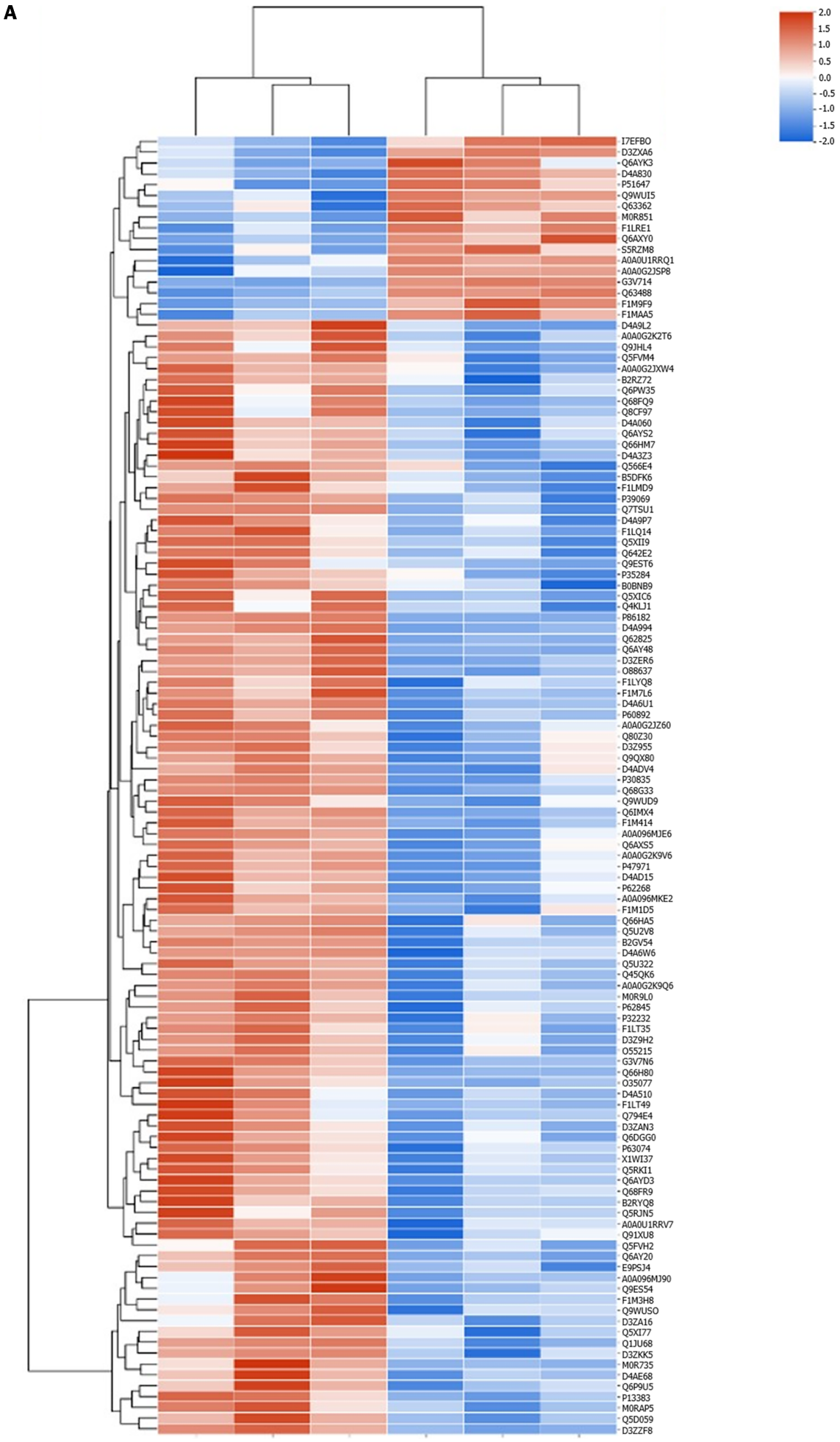
The effects of oxidative stress on various stages of diabetic encephalopathy have been demonstrated in recent studies, and supporting antioxidant stress therapy is beneficial for improving complications of diabetes in the central nervous system [6]. We assessed the redox status of hypothalamic tissue using multiple assays (Figure 2A and B). The levels of MDA decreased, while the content of SOD increased in the hypothalamus of the T2DM-DJB rats after the operation, compared to the T2DM-Sham rats. Nrf2 is involved in antioxidation by upregulating the expression of HO-1 and is inhibited under high glucose (HG) conditions, resulting in inflammatory responses and cell injury[33]. We evaluated the effects of DJB surgery on the expression of Nrf2 and HO-1 in the hypothalamus of rats with T2DM. As shown in Figure 2C-E, the number of Nrf2- and HO-1- positive cells in the hypothalamus of T2DM-DJB rats decreased significantly after the DJB operation. The qRT-PCR and western blot data indicated a significant increase in Nrf2 and HO-1 expression following DJB surgery in the hypothalamus of T2DM rats (Figure 2F and G). These results indicate that DJB surgery improves diabetic hyperglycemia-induced hypothalamic oxidative stress in T2DM rats, possibly achieved by activating the Nrf2/HO-1 signaling pathway.

DJB surgery exerts antioxidant effects by activating hypothalamic GLP-1 signaling in T2DM rats

GLP-1R has been shown to be expressed in neurons in key regions of the brain and has neuroprotective and anti-inflammatory properties, which enhance the expression of the oxidative stress regulatory protein Nrf2/HO-1 to alleviate oxidative stress[26]. We measured serum GLP-1 Levels and the expression of hypothalamic GLP-1R in each group of rats. The results showed that the fasting serum GLP-1 Level of T2DM-DJB rats after DJB surgery was significantly higher than that of T2DM-Sham rats (Figure 3A). In addition, we found that GLP-1R was highly expressed in the hypothalamic tissue of T2DM-DJB rats after DJB surgery (Figure 3B-D). These results indicate that DJB surgery enhances the expression of Nrf2/HO-1 by upregulating GLP-1 signaling in T2DM rats and plays a role as an antioxidant.

DJB blocks the activation of NF-κB signaling and inhibits the production of proinflammatory cytokines in the hypothalamus of T2DM rats

Oxidative stress triggers an inflammatory response by activating the NF-κB signaling pathway, which regulates the release of numerous inflammatory cytokines[34]. To determine whether DJB blocked oxidative stress-induced NF-κB activation, we used western blotting and qRT-PCR to compare the total NF-κB and its phosphorylation levels in the hypothalamus of the three groups of rats. Figure 4A-C shows that the expression of NF-κB and p-NF-κB in the hypothalamus of T2DM-DJB rats was significantly reduced compared to that in T2DM-Sham rats after DJB surgery. To confirm whether DJB surgery could ameliorate the inflammatory state of the hypothalamus by inhibiting NF-κB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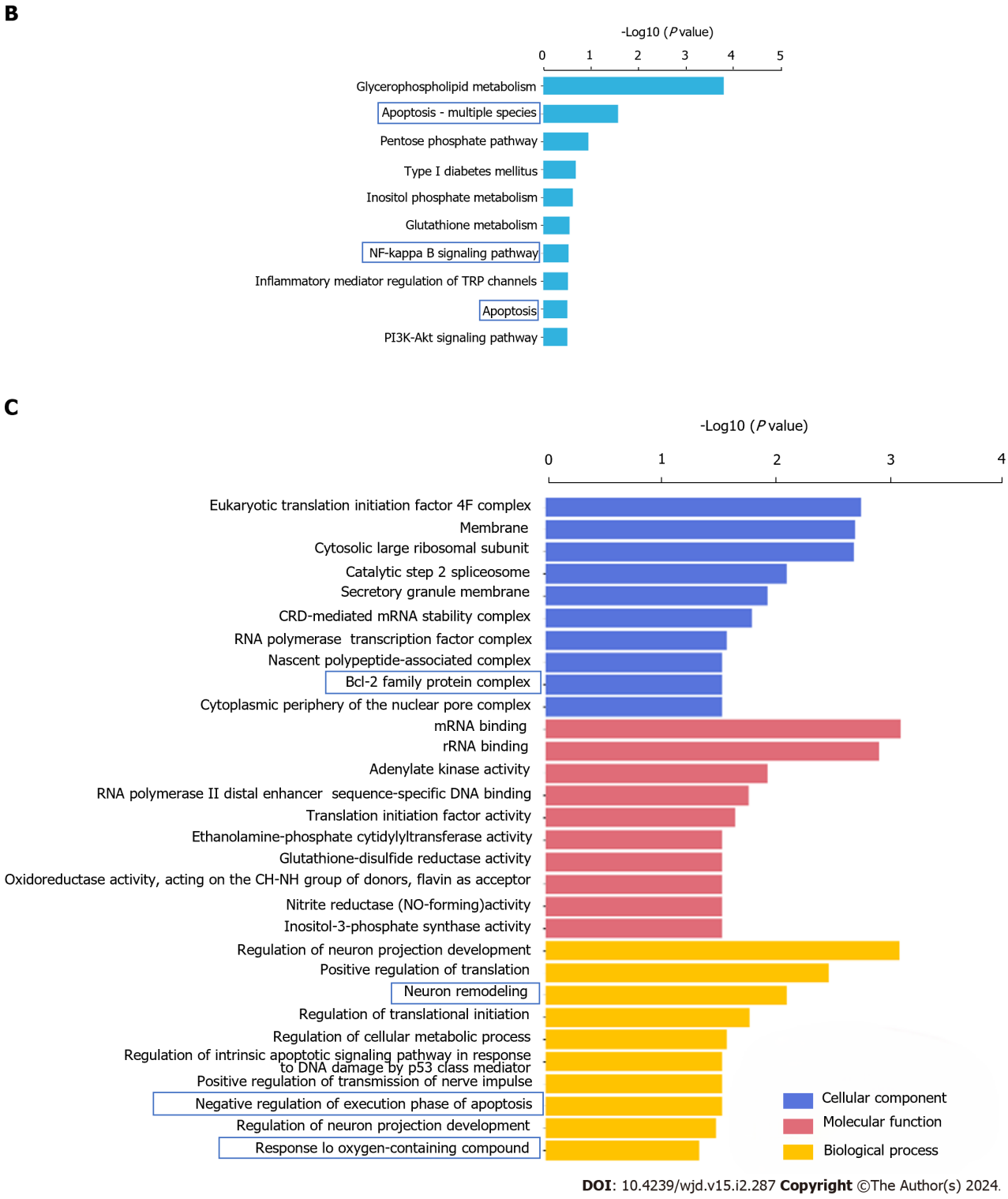

DOI: 10.4239/wjd.v15.i2.287

Figure 1 The expression of differential proteins in the hypothalamus after duodenal jejunal bypass intervention. A: Heat map of the expression of the 120 DEPs between the T [type 2 diabetes mellitus (T2DM)-Sham] rats and D [T2DM-duodenal jejunal bypass (DJB)] rats. The colored column represents the sample number, the row name indicates the DEGs, each rectangle in the graph corresponds to the expression value of a sample, red indicates high expression and blue indicates low expression; B: Statistics of significantly enriched Kyoto encyclopedia of genes and genomes pathways (T2DM-Sham *vs* T2DM-DJB); C: GO term statistics for significantly enriched genes (T2DM-Sham *vs* T2DM-DJB).

signaling, we used flow cytometry to measure the levels of the proinflammatory cytokines IL-1 and IL-6. As shown in Figure 4D-F, DJB surgery resulted in a significant reduction in the abnormally high expression of IL-1β and IL-6 observed in the hypothalamic tissue of T2DM-DJB rats. The qRT-PCR and Western blot results showed a similar trend in the gene and protein expression of IL-1β, and IL-6 as observed in the flow cytometry assay (Figure 4G-I). These findings suggest that DJB surgery significantly inhibits the NF-κB signaling pathway, effectively improving the inflammatory state of the hypothalamus.

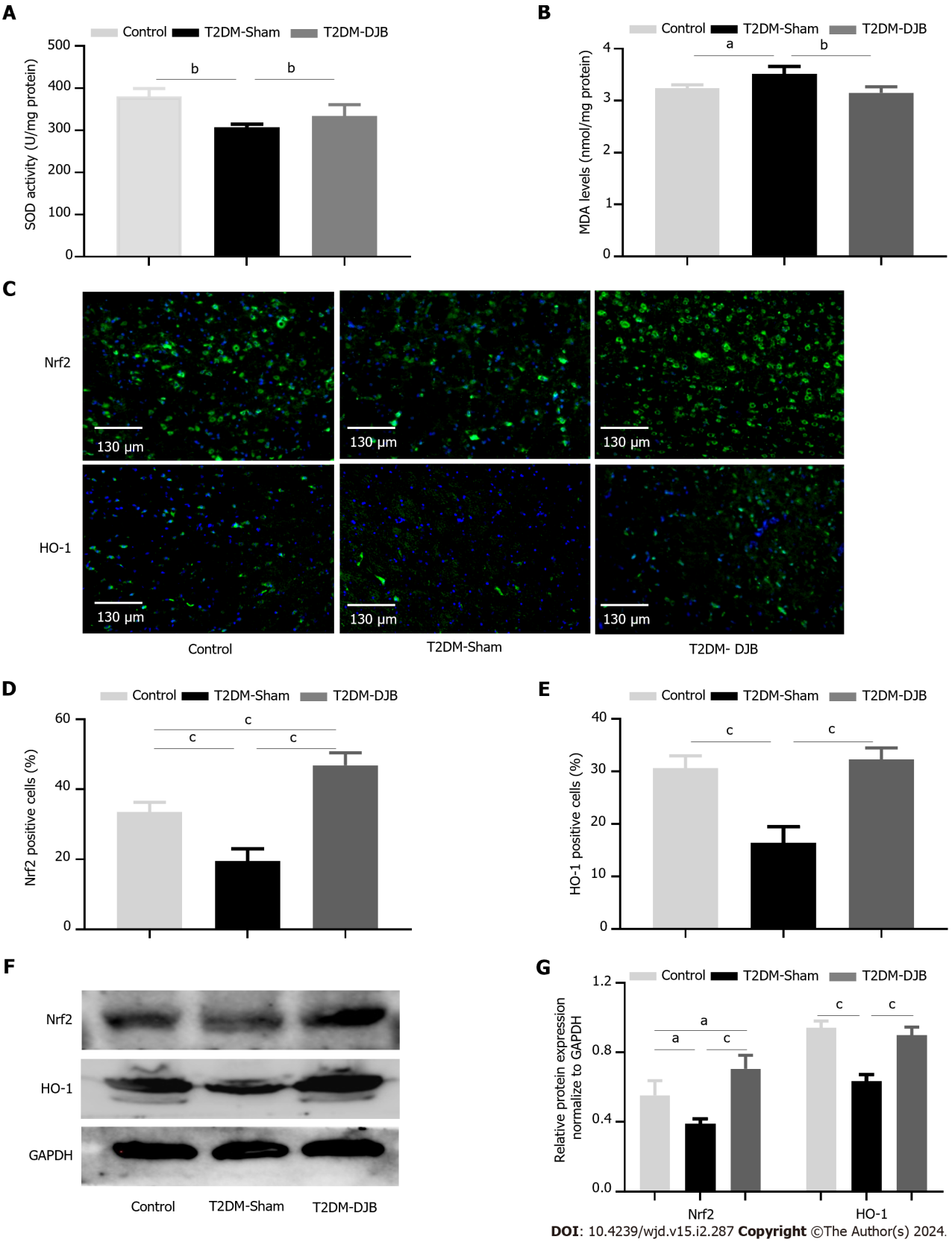

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Figure 2 Duodenal jejunal bypass surgery inhibits hypothalamic oxidative stress in rats with type 2 diabetes mellitus by activating the Nrf2/HO-1 signaling pathway. A: Analysis of SOD content in the hypothalamus of rats; B: Analysis of MDA content in the hypothalamus of rats; C: Nrf2 and HO-1 expression in the hypothalamus detected by immunofluorescence (scale bar, 130 μm); D: The percentage of cells expressing Nrf2; E: The percentage of cells expressing HO-1; F: Expression levels of Nrf2 and HO-1 in hypothalamus detected by Western blotting; G: The quantitative densitometric analysis of Nrf2 and HO-1. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$. T2DM: Type 2 diabetes mellitus; DJB: Duodenal jejunal bypass.

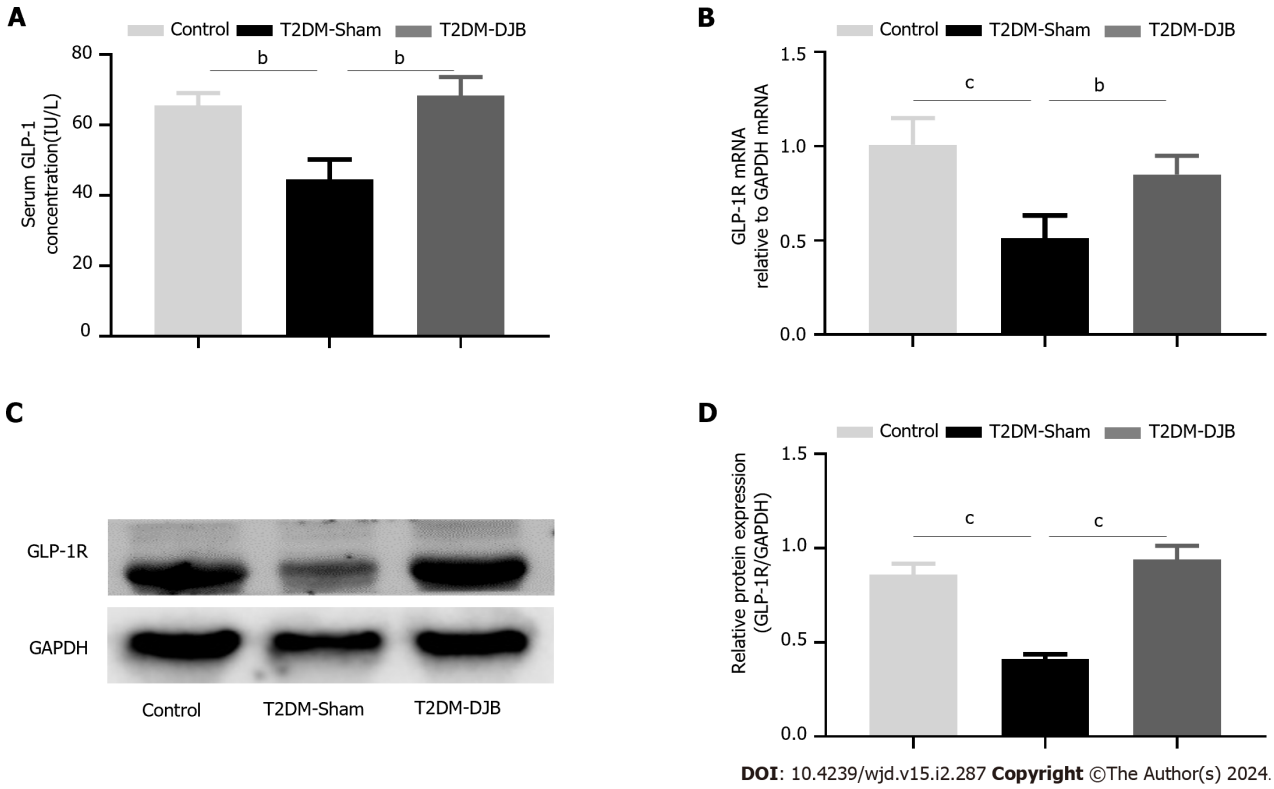

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Figure 3 Duodenal jejunal bypass surgery increases type 2 diabetes mellitus glucagon-like peptide 1 signals and enhances the expression of Nrf2/HO-1. A: Serum glucagon-like peptide 1 (GLP-1) secretion of rats in the three groups; B: The quantitative real-time PCR results of GLP-1 receptor (GLP-1R) expression; C: Expression levels of GLP-1R in the hypothalamus detected by Western blotting; D: The quantitative densitometric analysis of GLP-1R. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$. T2DM: Type 2 diabetes mellitus; DJB: Duodenal jejunal bypass; GLP-1: Glucagon-like peptide 1; GLP-1R: Glucagon-like peptide 1 receptor.

DJB surgery improves hypothalamic nerve injury induced by oxidative stress

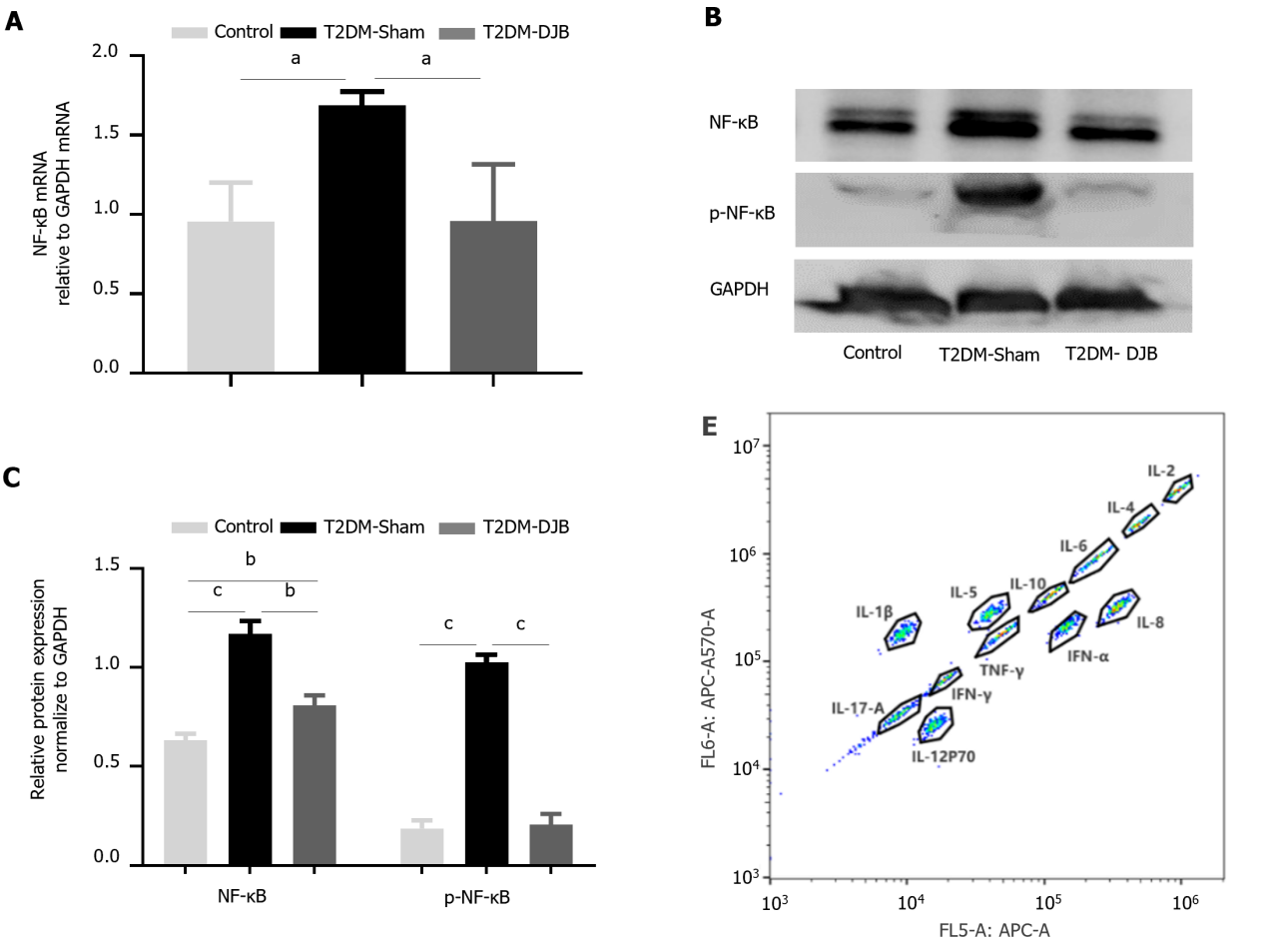
Glial fibrillary acidic protein (GFAP) is a specific marker of mature astrocytes, and its elevated expression indicates the onset of CNS injury[35]. To determine whether DJB surgery ameliorates oxidative stress-induced neuronal injury in the hypothalamus of T2DM rats, the number of GFAP-positive glial cells was determined, and the expression levels of GFAP were measured using IF, qRT-PCR and western blot analysis. The number of GFAP-positive astrocytes was significantly reduced in T2DM-DJB rats after DJB surgery compared to T2DM-Sham rats (Figure 5A and B). The expression of GFAP mRNA and protein in the hypothalamus was significantly reduced in T2DM-DJB rats after DJB surgery, as indicated by qRT-PCR and western blot analysis (Figure 5C-E). These results suggest that DJB surgery ameliorates oxidative stress-induced hypothalamic neuronal cell injury.

DJB surgery improves diabetes-induced hypothalamic cell apoptosis in T2DM rats

Oxidative stress induced by diabetes mellitus is a key factor in promoting apoptosis[24]. To evaluate the impact of DJB surgery on diabetes-induced hypothalamic cell apoptosis, we analyzed the expression of Cleaved-caspase-3, Caspase-3, and Bcl-2 in the hypothalamus of three groups of rats using qRT-PCR and western blot techniques. As depicted in Figure 6, the expression of Cleaved-caspase-3 and Caspase-3 was significantly reduced, whereas the expression of Bcl-2 was increased in the hypothalamus of T2DM-DJB rats following DJB surgery, in comparison to T2DM-sham rats. These results confirm that DJB surgery can inhibit the apoptosis of hypothalamic cells and reduce oxidative stress damage.

DJB surgery promotes hypothalamic neurogenesis in T2DM rats

The occurrence of CNS degenerative diseases in diabetic patients is probably caused by a decrease in activated neurons and an increase in cell apoptosis. A common method of evaluating a cell's proliferation capacity is to stain it with Ki67. Immunohistochemical analysis showed that T2DM-DJB rats had significantly more Ki67-positive cells in the hypothalamus post operation than T2DM-Sham rats (Figure 7A and B). The C-fos proteins are often used as markers of neuronal activation. IHC staining indicated a significant increase in the number of hypothalamic C-fos positive cells in T2DM-DJB rats compared to T2DM-Sham rats (Figure 7C and D). DJB surgery significantly increased the expression of C-fos and BDNF in the hypothalamus of T2DM-DJB rats compared to T2DM-Sham rats, according to the qRT-PCR and western blot analysis (Figure 7E-H). These results suggest that DJB surgery promotes hypothalamic cell growth and neuronal activation in T2DM rats.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Control
T2DM-Sham
T2DM-DJB
NF-κB mRNA relative to GAPDH mRNA
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a
B
NF-κB
p-NF-κB
GAPDH
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T2DM- DJB
C
Relative protein expression normalize to GAPDH
b
c
b
c
c
NF-κB
p-NF-κB
E
FL6-A: APC-A570-A
FL5-A: APC-A
IL-2
IL-4
IL-6
IL-5
IL-10
IL-1β
IL-8
IFN-α
TNF-γ
IFN-γ
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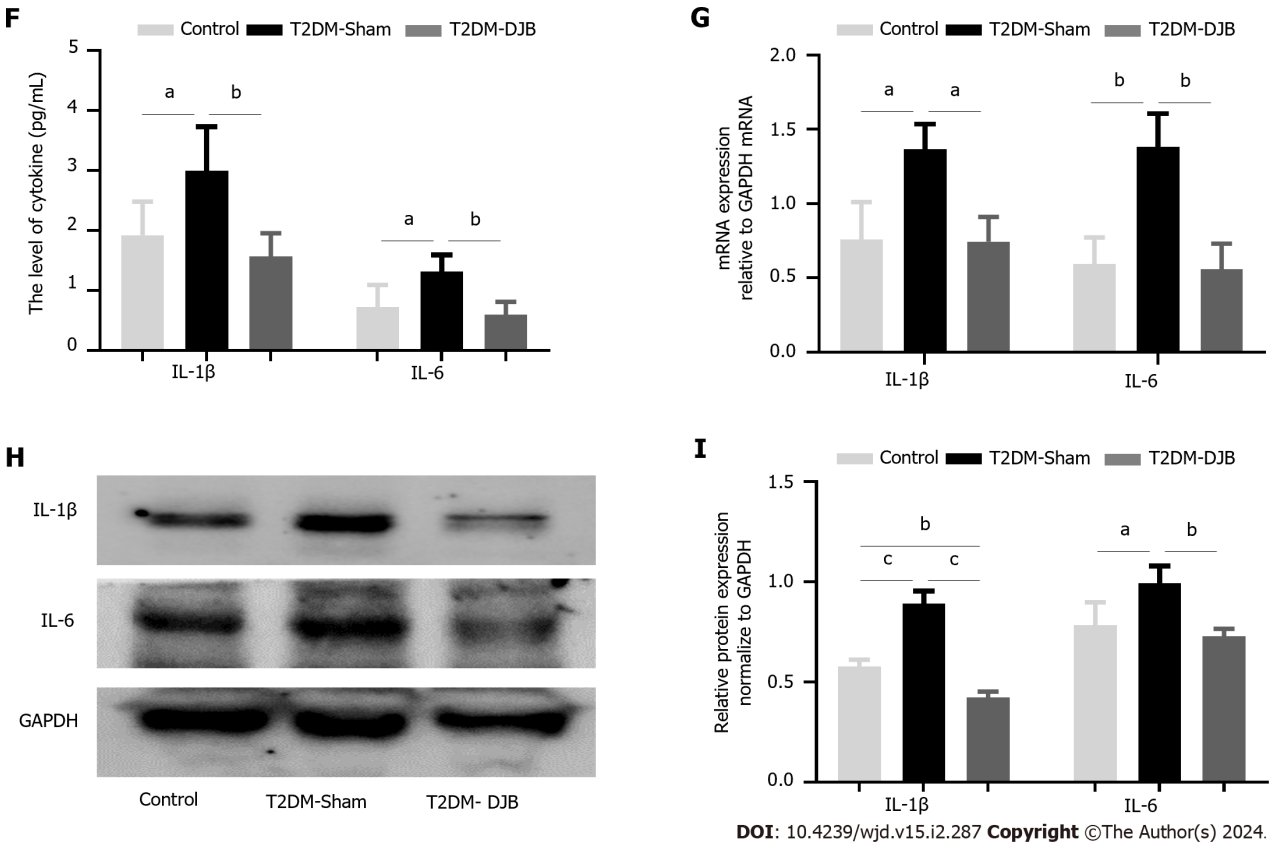

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Figure 4 Duodenal jejunal bypass inhibits the production of proinflammatory cytokines by blocking the activation of NF-κB signaling in the hypothalamus of type 2 diabetes mellitus rats. A: The quantitative real-time (qRT)-PCR results of NF-κB and p-NF-κB expression; B: The expression levels of NF-κB and p-NF-κB detected by Western blotting; C: The quantitative densitometric analysis of NF-κB and p-NF-κB; D: Flow cytometry results for standards; E: Flow cytometry results for samples; F: Flow cytometry results for the levels of proinflammatory cytokines interleukin (IL)-1β and IL-6; G: The qRT-PCR results of IL-1β and IL-6 expression; H: Expression levels of IL-1β and IL-6 detected by Western blotting; I: The quantitative densitometric analysis of IL-1β and IL-6. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$. T2DM: Type 2 diabetes mellitus; DJB: Duodenal jejunal bypass; IL: Interleukin.

DISCUSSION

Diabetic encephalopathy is one of the chronic complications of T2DM. The World Epidemiological Survey showed that patients with T2DM usually have by mild to moderate brain dysfunction[36,37]. Growing evidence has demonstrated that an imbalance in glucose homeostasis in T2DM can exacerbate oxidative stress and tissue inflammation in the brain[38]. Long-term brain inflammation is typically the main cause of neuronal cell apoptosis, resulting in mild cognitive impairment and even neurodegenerative diseases[39,40].

Previous studies have shown that metabolic surgery is effective for weight loss, remission of T2DM, and improvements in brain glucose metabolism[23,41].However, the role and mechanisms of DJB surgery in alleviating diabetes-induced central brain injury are unclear. In this study, we analyzed the differential protein expression in the hypothalamus between the T2DM-Sham group and the T2DM-DJB group of rats using quantitative proteomics (label-free). We found that at least 120 proteins in the hypothalamic tissues exhibited significant changes. KEGG enrichment and GO analysis revealed significant differences in signaling proteins associated with oxidative stress, inflammation, and neurological damage in the hypothalamic tissues of the two groups of rats. Therefore, DJB surgery may improve hypothalamic injury in diabetic rats by modulating oxidative stress, inflammation, and neuronal survival.

Recent studies have confirmed the impact of oxidative stress on various stages of diabetic encephalopathy, supporting the idea that treatments targeting antioxidant stress can help improve CNS complications associated with diabetes[42]. Our results showed that DJB surgery significantly increased the level of SOD and inhibited MDA production in the hypothalamus of T2DM rats, thereby effectively reducing hypothalamic oxidative stress. The Nrf2/HO-1 signaling pathway is a significant regulator of oxidative stress[43,44]. Yang *et al*[45] found that HG induced inflammation and apoptosis in cerebral microvascular endothelial cells, which may be a result of inhibiting the Nrf2/HO-1-mediated antioxidant pathway[45]. Hence, we examined the impact of DJB surgery on the hypothalamic expression of Nrf2 and HO-1 in rats with T2DM. The expression of Nrf2 and HO-1 in the hypothalamus of T2DM rats was significantly upregulated after DJB surgery. Studies have shown that GLP-1 is involved in regulating oxidative stress and inflammation, in addition to its metabolic effects[46]. Kim *et al*[26] demonstrated that EX4 exerts antioxidant effects and reduces damage to pancreatic β-cells by activating the Nrf2 signaling pathway[26,47]. In this study, DJB surgery increased the serum levels of GLP-1 and upregulated the expression of GLP-1R in the hypothalamic tissue of T2DM rats. DJB surgery reduces hypothalamic oxidative stress in rats with T2DM by activating the GLP-1-mediated Nrf2/HO-1 signaling

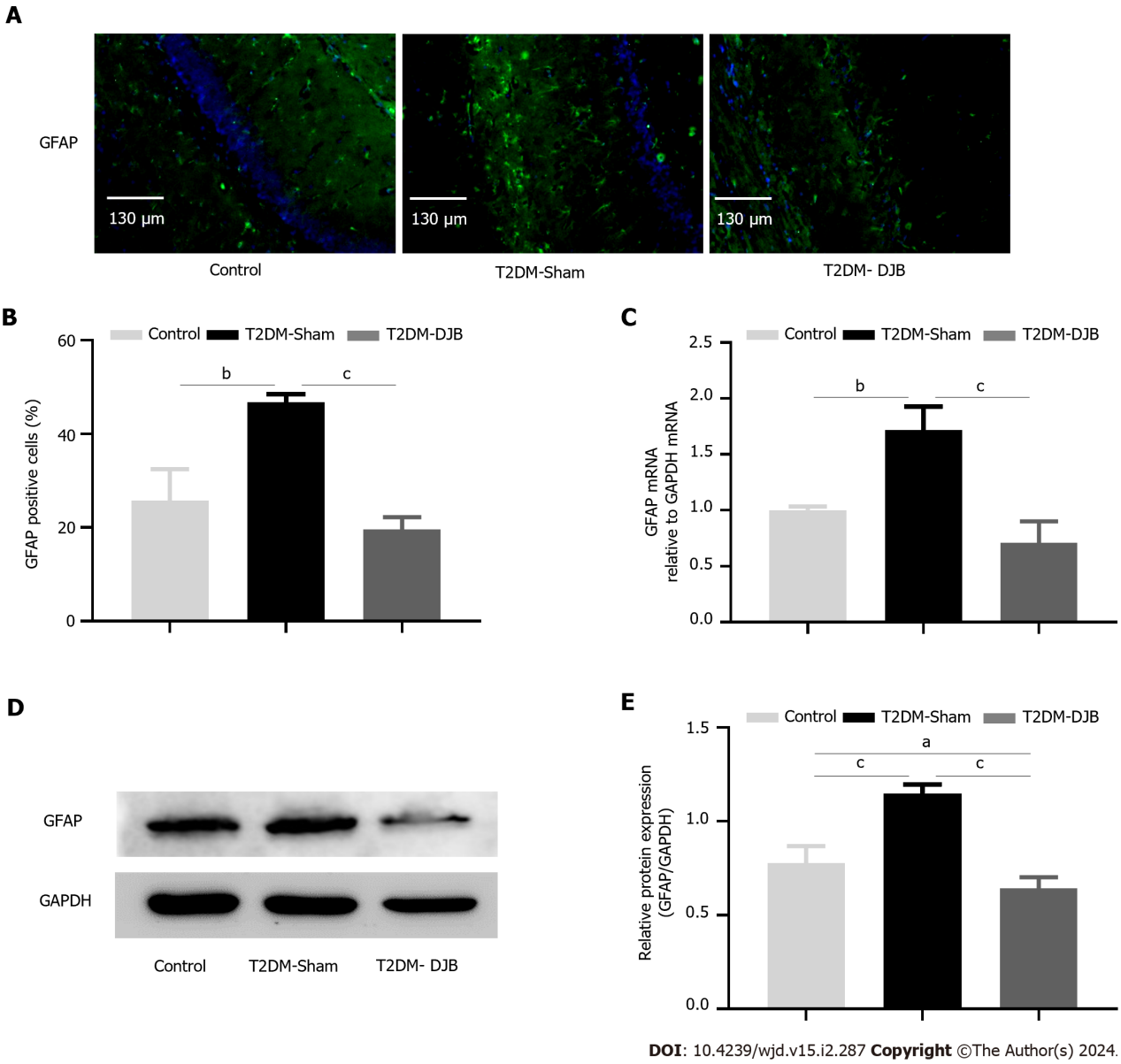

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Figure 5 Duodenal jejunal bypass surgery improves hypothalamic cell injury induced by oxidative stress. A: The expression of glial fibrillary acidic protein (GFAP) in the hypothalamus determined by immunohistochemistry (scale bar, 130 μm); B: The percentage of cells expressing GFAP; C: The quantitative real-time PCR results of GFAP expression in the hypothalamus; D: Expression levels of GFAP detected by Western blotting; E: The quantitative densitometric analysis of GFAP. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$. T2DM: Type 2 diabetes mellitus; DJB: Duodenal jejunal bypass; GFAP: Glial fibrillary acidic protein.

pathway.

Oxidative stress triggers the massive secretion and release of proinflammatory cytokines in the body, ultimately leading to an inflammatory response, which is probably mediated by NF-κB signaling activation[48]. Nrf2 activation inhibits the accumulation of ROS and reduces NF-κB activation, thereby suppressing the inflammatory response. Tu *et al* [49] demonstrated that GEN, a novel agonist of GLP-1R, provides protection against the hyperglycemia-induced inflammatory response in Müller cells and the retinal blood barrier in DR mice. This protective effect is primarily attributed to the upregulation of the Nrf2 antioxidant signaling pathway through GLP-1R[49]. The levels of inflammatory cytokines and NF-κB activation in the hypothalamus of the three groups of rats were assessed using western blot, flow cytometry, and qRT-PCR to investigate the role of DJB in hypothalamic inflammation induced by oxidative stress in T2DM rats. Therefore, DJB surgery improved the hypothalamic inflammatory state in T2DM rats by inhibiting NF-κB signaling activation and reducing the secretion of proinflammatory cytokines.

Increased expression of the astrocyte biomarker GFAP in the brains of diabetic patients is believed to be a significant indication of CNS damage caused by neuroinflammation and tissue changes[35,50]. HG conditions significantly increased the expression of GFAP in Müller cells, as demonstrated by both *in vivo* and *in vitro* studies. Liraglutide treatment reduced oxidative stress and downregulated GFAP expression in Müller cells, protecting them from HG-induced injury [51]. To investigate the effect of DJB surgery on hypothalamic cell injury in diabetic rats, we evaluated the expression of GFAP protein and compared the differences in the number of GFAP-positive cells in hypothalamic tissues among the three groups of rats. The experimental results showed that DJB significantly reduced GFAP expression in the

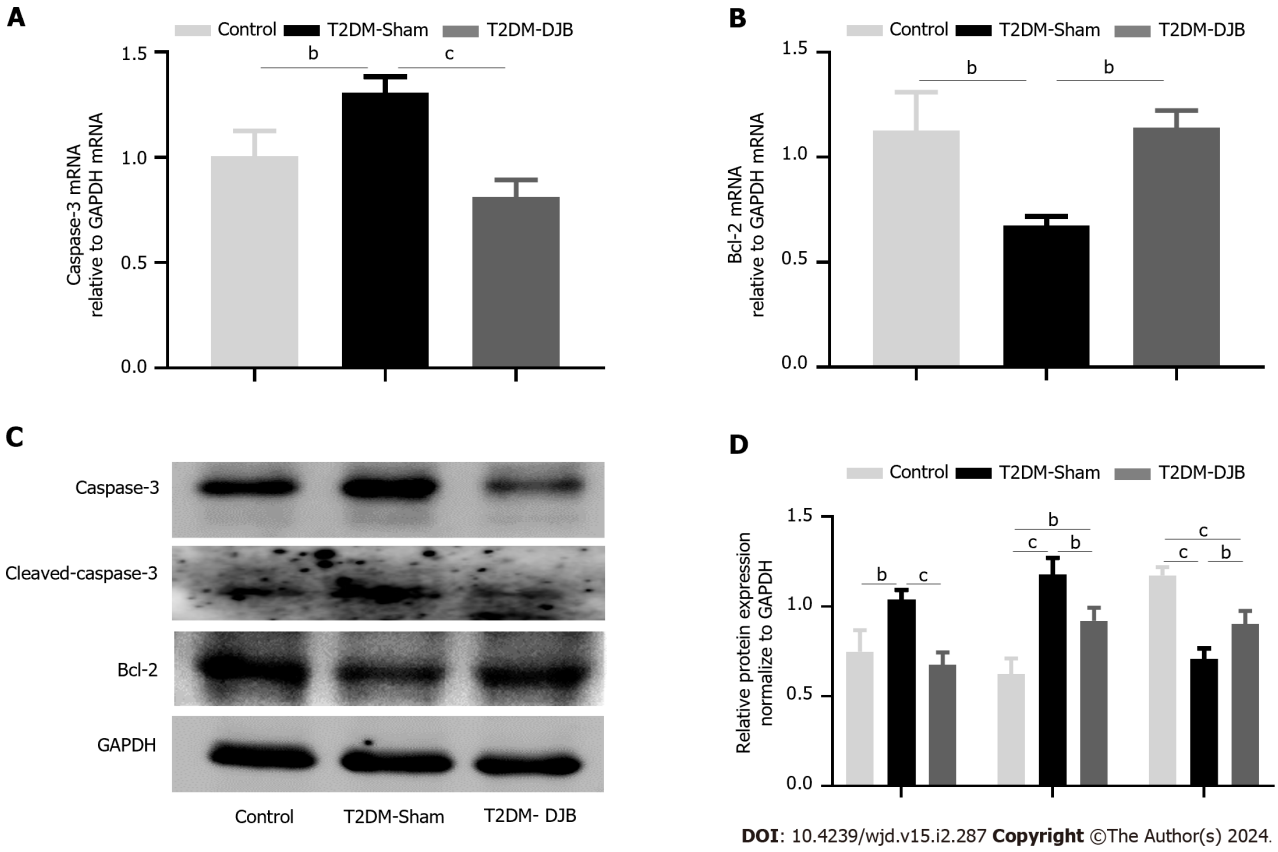

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Figure 6 Duodenal jejunal bypass surgery improves diabetes-induced hypothalamic cell apoptosis in type 2 diabetes mellitus rats. A: The quantitative real-time (qRT)-PCR results of Caspase-3 expression; B: The qRT-PCR results of Bcl-2 expression; C: Expression levels of apoptosis-related proteins in the hypothalamus by Western blotting; D: The quantitative densitometric analysis of apoptosis-related proteins. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$. T2DM: Type 2 diabetes mellitus; DJB: Duodenal jejunal bypass.

hypothalamus of T2DM rats and alleviated hypothalamic cell injury.

Oxidative stress induced by chronic hyperglycemia in diabetes mellitus is an important factor that promotes neuronal cell apoptosis[52,53]. Growing evidence suggests that Caspase-3 activation is essential for apoptosis because it regulates the translocation and activation of Bcl-2 family proteins, thereby promoting the final stages of apoptosis[50,54]. In this study, DJB surgery significantly upregulated the expression of Bcl-2 in the hypothalamus of rats with T2DM, downregulated the expression of Caspase-3, and inhibited the apoptosis of hypothalamic cells. We evaluated the expression of Ki67, C-fos, and BDNF, which are associated with neuronal proliferation, differentiation, and maturation, in diabetic rats to determine the effects of DJB surgery on neuronal injury[55]. We found that the number of C-fos positive and Ki67 positive nerve cells in the hypothalamus was significantly reduced in diabetic rats, accompanied by downregulation of hypothalamic BDNF expression. However, DJB significantly reversed these changes. These data suggest that DJB can inhibit hypothalamic cell apoptosis in T2DM rats, promote neurogenesis, and reduce diabetes-induced neuronal cell damage.

In the present study, we demonstrated that DJB enhanced hypothalamic antioxidant activity and alleviated hypothalamic neuronal apoptosis and inflammation in T2DM rats, partly through the upregulation of peripheral GLP-1 secretion (Figure 8). Since enteric GLP-1 can act on the CNS through two pathways (neural or humoral), our current study did not involve the specific pathways through which enteric GLP-1 affects central brain injury[56]. Further investigation is needed to determine the influence of enteric neural or endocrine pathways after DJB surgery on the amelioration of diabetes-induced central brain injury by GLP-1.

CONCLUSION

DJB surgery can improve oxidative stress and inflammation in the hypothalamus of T2DM rats and reduce neuronal cell injury. This improvement is achieved by upregulating the GLP-1-mediated Nrf2/HO-1 signaling pathway.

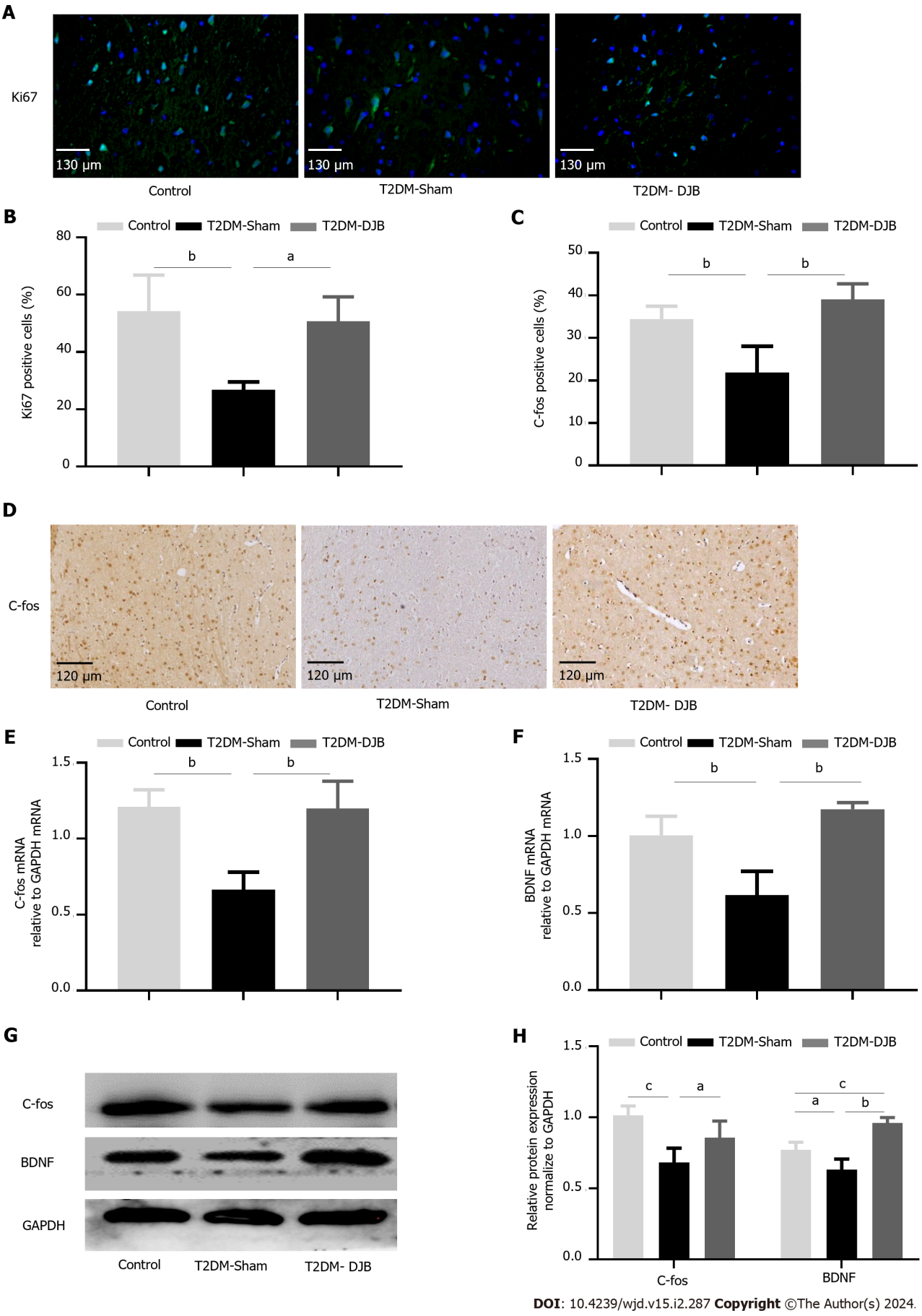

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Figure 7 Duodenal jejunal bypass surgery promotes hypothalamic neurogenesis in type 2 diabetes mellitus rats. A: Immunofluorescence

analysis the expression of Ki67 (scale bar, 130 μm); B: The percentage of cells expressing Ki67; C: The percentage of cells expressing C-fos; D: Immunofluorescence analysis the expression of C-fos (scale bar, 120 μm); E: The quantitative real-time (qRT)-PCR results of C-fos expression; F: The qRT-PCR results of BDNF expression; G: Expression levels of C-fos and BDNF detected by Western blotting. The quantitative densitometric analysis of C-fos and BDNF. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$. T2DM: Type 2 diabetes mellitus; DJB: Duodenal jejunal bypass.

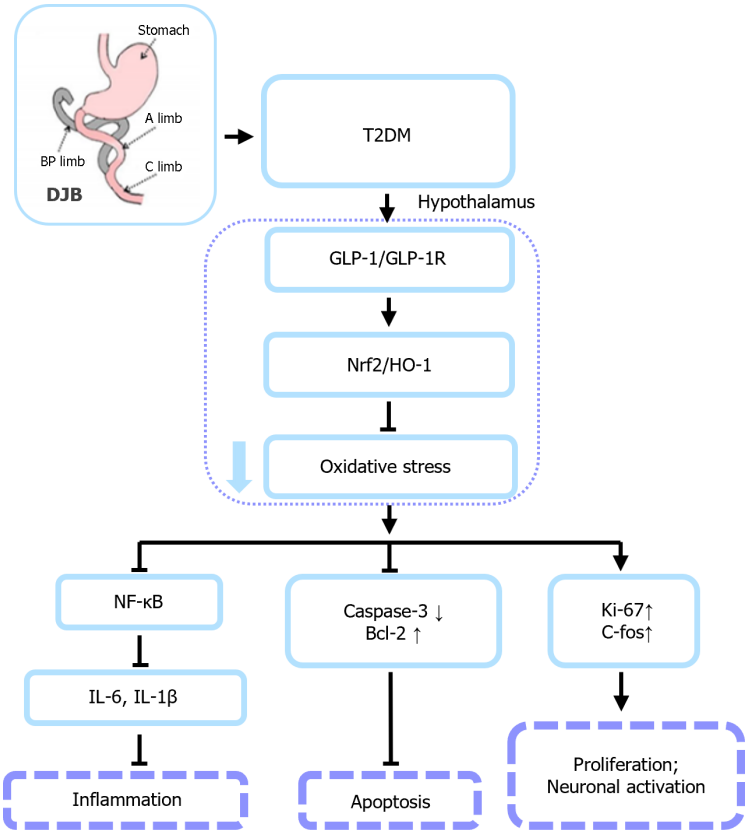

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Figure 8 A schema summarizing the protective effects of duodenal jejunal bypass surgery on hypothalamic cell injury induced by diabetes. Duodenal jejunal bypass (DJB) surgery significantly reduces hypothalamic oxidative stress injury and inflammation in type 2 diabetes mellitus rats by a mechanism depending on glucagon-like peptide 1 (GLP-1)-mediated activation of Nrf2/HO-1 signaling pathway. DJB therapy effectively inhibits hypothalamic oxidative stress and inflammatory damage caused by diabetes by GlP-1-mediated activation of Nrf2/HO-1 to inhibit oxidative stress damage in the hypothalamus. In addition, DJB inhibits inflammation by upregulating Nrf2 expression, activating the Nrf2/HO-1 axis, and inhibiting the NF-κB pathway. T2DM: Type 2 diabetes mellitus; DJB: Duodenal jejunal bypass; GLP-1: Glucagon-like peptide 1; GLP-1R: Glucagon-like peptide 1 receptor; IL: Interleukin.

ARTICLE HIGHLIGHTS

Research background

Type 2 diabetes mellitus (T2DM) is often accompanied by impaired glucose utilization in the brain, leading to oxidative stress, neuronal injury and inflammation. Previous studies have shown that duodenal jejunal bypass (DJB) surgery significantly improves brain glucose metabolism in T2DM rats, but its role in brain injury and the underlying mechanisms are still unclear.

Research motivation

DJB can increase serum glucagon-like peptide 1 (GLP-1) Levels and enhance brain glucose utilization, playing a positive role in the treatment of diabetes. Therefore, GLP-1 signaling may play a significant role after DJB surgery in alleviating T2DM-related brain injury.

Research objectives

To investigate the role and metabolism of DJB in improving hypothalamic oxidative stress and inflammation condition in T2DM rats.

Research methods

A T2DM rat model was induced *via* a high-glucose, high-fat diet, and a low-dose streptozotocin injection. T2DM rats

underwent DJB surgery or Sham surgery. Differential expression of hypothalamic proteins and genes was analyzed by protein microarray, flow cytometry, quantitative real-time PCR, western blot, and immunofluorescence.

Research results

Protein microarray results showed significant differences between the T2DM-Sham rats and the T2DM-DJB rats in signaling proteins related to oxidative stress, inflammation, and neuronal injury. DJB surgery increased the serum levels of GLP-1 and upregulated the expression of GLP-1 receptor and antioxidant signaling proteins (Nrf2 and HO-1) in the hypothalamic tissue of T2DM rats. DJB also reduced the expression of hypothalamic inflammatory and nerve cell injury-related factors, playing a neuroprotective role and reducing hypothalamic injury.

Research conclusions

DJB surgery improve oxidative stress and inflammation in the hypothalamus of T2DM rats and reduce neuronal cell injury by activating the GLP-1-mediated Nrf2/HO-1 signaling pathway.

Research perspectives

Further investigation is needed to determine the influence of enteric neural or endocrine pathways after DJB surgery on the amelioration of diabetes-induced central brain injury by GLP-1.

FOOTNOTES

Co-corresponding authors: Fang-Ming Fu and Mei-Hua Qu.

Author contributions: Qu MH and Fu FM had equal contribution to this paper; Qu MH and Fu FM designed the research scheme and directed the relevant experimental techniques and methods; Wang HJ, Zhang LB, Sun SP, Yan QT, Gao ZQ performed the research and data analysis; Wang HJ, Qu MH analyzed the data and wrote the manuscript; All authors have read and approve the final manuscript. Qu MH and Fu FM contributed to the experimental design. Qu primarily developed the research direction and experimental hypothesis based on literature and previous research. Fu FM participated in the design of the experimental verification scheme. Qu MH and Fu FM jointly supervised the modeling and duodenal jejunal bypass surgery-related experiments. They are co-corresponding authors of this study, and there is no conflict of interest between them.

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REFERENCES

1 **Magliano DJ**, Boyko EJ; IDF Diabetes Atlas 10th edition scientific committee. IDF DIABETES ATLAS [Internet]. 10th ed. Brussels: International Diabetes Federation, 2021 [PMID: 35914061]

2 **Roomi AB**, Al-Salih RMH, Ali SA. The Effect Insulin Therapy and Metformin on Osteoporosis in Diabetic Post-Menopausal Iraqi Women. *Indian J Public Health Res Dev* 2019; **10**: 1544 [DOI: 10.5958/0976-5506.2019.00935.5]

3 **Buse JB**, Wexler DJ, Tsapas A, Rossing P, Mingrone G, Mathieu C, D'Alessio DA, Davies MJ. 2019 Update to: Management of Hyperglycemia in Type 2 Diabetes, 2018. A Consensus Report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care* 2020; **43**: 487-493 [PMID: 31857443 DOI: 10.2337/dci19-0066]

4 **Maciejczyk M**, Żebrowska E, Chabowski A. Insulin Resistance and Oxidative Stress in the Brain: What's New? *Int J Mol Sci* 2019; **20** [PMID: 30781611 DOI: 10.3390/ijms20040874]

5 **Sun Y**, Ma C, Sun H, Wang H, Peng W, Zhou Z, Pi C, Shi Y, He X. Metabolism: A Novel Shared Link between Diabetes Mellitus and Alzheimer's Disease. *J Diabetes Res* 2020; **2020**: 4981814 [PMID: 32083135 DOI: 10.1155/2020/4981814]

6 **Miao C**, Chen H, Li Y, Guo Y, Xu F, Chen Q, Zhang Y, Hu M, Chen G. Curcumin and its analog alleviate diabetes-induced damages by regulating inflammation and oxidative stress in brain of diabetic rats. *Diabetol Metab Syndr* 2021; **13**: 21 [PMID: 33602334 DOI: 10.1186/s13098-021-00638-3]

7 **Zhang Z**, Zhou H, Zhou J. Neuritin inhibits astrogliosis to ameliorate diabetic cognitive dysfunction. *J Mol Endocrinol* 2021; **66**: 259-272 [PMID: 33729996 DOI: 10.1530/JME-20-0321]

8 **Elsharkawy RE**, Abdel Azim GS, Osman MA, Maghraby HM, Mohamed RA, Abdelsalam EM, Ebrahem EE, Seliem NMA. Peripheral Polyneuropathy and Cognitive Impairment in Type II Diabetes Mellitus. *Neuropsychiatr Dis Treat* 2021; **17**: 627-635 [PMID: 33658784 DOI: 10.2147/NDT.S284308]

9 **Burillo J**, Marqués P, Jiménez B, González-Blanco C, Benito M, Guillén C. Insulin Resistance and Diabetes Mellitus in Alzheimer's Disease. *Cells* 2021; **10** [PMID: 34069890 DOI: 10.3390/cells10051236]

10 **Maciejczyk M**, Żebrowska E, Zalewska A, Chabowski A. Redox Balance, Antioxidant Defense, and Oxidative Damage in the Hypothalamus and Cerebral Cortex of Rats with High Fat Diet-Induced Insulin Resistance. *Oxid Med Cell Longev* 2018; **2018**: 6940515 [PMID: 30271528 DOI: 10.1155/2018/6940515]

11 **Dhaliwal J**, Dhaliwal N, Akhtar A, Kuhad A, Chopra K. Tetramethylpyrazine Attenuates Cognitive Impairment Via Suppressing Oxidative Stress, Neuroinflammation, and Apoptosis in Type 2 Diabetic Rats. *Neurochem Res* 2022; **47**: 2431-2444 [PMID: 35665448 DOI: 10.1007/s11064-022-03640-x]

12 **Roth AE**, Thornley CJ, Blackstone RP. Outcomes in Bariatric and Metabolic Surgery: an Updated 5-Year Review. *Curr Obes Rep* 2020; **9**: 380-389 [PMID: 32607822 DOI: 10.1007/s13679-020-00389-8]

13 **Sandoval DA**, Patti ME. Glucose metabolism after bariatric surgery: implications for T2DM remission and hypoglycaemia. *Nat Rev Endocrinol* 2023; **19**: 164-176 [PMID: 36289368 DOI: 10.1038/s41574-022-00757-5]

14 **Leonardou AS**, Karystianos C, Argyropoulos C, Nikiforidis GC, Kalfarentzos F, Alexandrides TK. Restoration of high-frequency glucose-entrained insulin oscillations in obese subjects with type 2 diabetes after biliopancreatic diversion. *Surg Obes Relat Dis* 2016; **12**: 1539-1547 [PMID: 27425836 DOI: 10.1016/j.soard.2016.04.009]

15 **Soong TC**, Lee MH, Lee WJ, Almalki OM, Chen JC, Wu CC, Chen SC. Long-Term Efficacy of Bariatric Surgery for the Treatment of Super-Obesity: Comparison of SG, RYGB, and OAGB. *Obes Surg* 2021; **31**: 3391-3399 [PMID: 33993423 DOI: 10.1007/s11695-021-05464-0]

16 **Fink J**, Seifert G, Blüher M, Fichtner-Feigl S, Marjanovic G. Obesity Surgery. *Dtsch Arztebl Int* 2022; **119**: 70-80 [PMID: 34819222 DOI: 10.3238/arztebl.m2021.0359]

17 **Rubino F**, Marescaux J. Effect of duodenal-jejunal exclusion in a non-obese animal model of type 2 diabetes: a new perspective for an old disease. *Ann Surg* 2004; **239**: 1-11 [PMID: 14685093 DOI: 10.1097/01.sla.0000102989.54824.fc]

18 **Kashihara H**, Shimada M, Yoshikawa K, Higashijima J, Nakao T, Nishi M, Takasu C. Duodenal-jejunal bypass changes the composition of the gut microbiota. *Surg Today* 2017; **47**: 137-140 [PMID: 27412617 DOI: 10.1007/s00595-016-1373-x]

19 **Shen SC**, Lee WJ, Kasama K, Seki Y, Su YH, Wong SK, Huang YM, Wang W. Efficacy of Different Procedures of Metabolic Surgery for Type 2 Diabetes in Asia: a Multinational and Multicenter Exploratory Study. *Obes Surg* 2021; **31**: 2153-2160 [PMID: 33523416 DOI: 10.1007/s11695-021-05239-7]

20 **Wu W**, Lin L, Lin Z, Yang W, Cai Z, Hong J, Qiu J, Lin C, Lin N, Wang Y. Duodenum Exclusion Alone Is Sufficient to Improve Glucose Metabolism in STZ-Induced Diabetes Rats. *Obes Surg* 2018; **28**: 3087-3094 [PMID: 29790129 DOI: 10.1007/s11695-018-3291-z]

21 **Müller TD**, Finan B, Bloom SR, D'Alessio D, Drucker DJ, Flatt PR, Fritsche A, Gribble F, Grill HJ, Habener JF, Holst JJ, Langhans W, Meier JJ, Nauck MA, Perez-Tilve D, Pocai A, Reimann F, Sandoval DA, Schwartz TW, Seeley RJ, Stemmer K, Tang-Christensen M, Woods SC, DiMarchi RD, Tschöp MH. Glucagon-like peptide 1 (GLP-1). *Mol Metab* 2019; **30**: 72-130 [PMID: 31767182 DOI: 10.1016/j.molmet.2019.09.010]

22 **Hutch CR**, Sandoval D. The Role of GLP-1 in the Metabolic Success of Bariatric Surgery. *Endocrinology* 2017; **158**: 4139-4151 [PMID: 29040429 DOI: 10.1210/en.2017-00564]

23 **Ruze R**, Xu Q, Liu G, Li Y, Chen W, Cheng Z, Xiong Y, Liu S, Zhang G, Hu S, Yan Z. Central GLP-1 contributes to improved cognitive function and brain glucose uptake after duodenum-jejunum bypass on obese and diabetic rats. *Am J Physiol Endocrinol Metab* 2021; **321**: E392-E409 [PMID: 34370593 DOI: 10.1152/ajpendo.00126.2021]

24 **Candeias E**, Sebastião I, Cardoso S, Carvalho C, Santos MS, Oliveira CR, Moreira PI, Duarte AI. Brain GLP-1/IGF-1 Signaling and Autophagy Mediate Exendin-4 Protection Against Apoptosis in Type 2 Diabetic Rats. *Mol Neurobiol* 2018; **55**: 4030-4050 [PMID: 28573460 DOI: 10.1007/s12035-017-0622-3]

25 **Pelle MC**, Zaffina I, Giofrè F, Pujia R, Arturi F. Potential Role of Glucagon-like Peptide-1 Receptor Agonists in the Treatment of Cognitive Decline and Dementia in Diabetes Mellitus. *Int J Mol Sci* 2023; **24** [PMID: 37511061 DOI: 10.3390/ijms241411301]

26 **Kim MH**, Kim EH, Jung HS, Yang D, Park EY, Jun HS. EX4 stabilizes and activates Nrf2 *via* PKCδ, contributing to the prevention of oxidative stress-induced pancreatic beta cell damage. *Toxicol Appl Pharmacol* 2017; **315**: 60-69 [PMID: 27939242 DOI: 10.1016/j.taap.2016.12.005]

27 **Liao Z**, Zhang J, Liu B, Yan T, Xu F, Xiao F, Wu B, Bi K, Jia Y. Polysaccharide from Okra (Abelmoschus esculentus (L) Moench) Improves Antioxidant Capacity *via* PI3K/AKT Pathways and Nrf2 Translocation in a Type 2 Diabetes Model. *Molecules* 2019; **24** [PMID: 31108940 DOI: 10.3390/molecules24101906]

28 **Yoo J**, Cho IJ, Jeong IK, Ahn KJ, Chung HY, Hwang YC. Exendin-4, a glucagon-like peptide-1 receptor agonist, reduces hepatic steatosis and endoplasmic reticulum stress by inducing nuclear factor erythroid-derived 2-related factor 2 nuclear translocation. *Toxicol Appl Pharmacol* 2018; **360**: 18-29 [PMID: 30253173 DOI: 10.1016/j.taap.2018.09.032]

29 **Shan Y**, Tan S, Lin Y, Liao S, Zhang B, Chen X, Wang J, Deng Z, Zeng Q, Zhang L, Wang Y, Hu X, Qiu W, Peng L, Lu Z. The glucagon-like peptide-1 receptor agonist reduces inflammation and blood-brain barrier breakdown in an astrocyte-dependent manner in experimental stroke. *J Neuroinflammation* 2019; **16**: 242 [PMID: 31779652 DOI: 10.1186/s12974-019-1638-6]

30 **Jiang B**, Wang H, Li N, Yan Q, Wang W, Wang Y, Xue H, Ma S, Li X, Diao W, Pan R, Gao Z, Qu MH. Role of Proximal Intestinal Glucose Sensing and Metabolism in the Blood Glucose Control in Type 2 Diabetic Rats After Duodenal Jejunal Bypass Surgery. *Obes Surg* 2022; **32**: 1119-1129 [PMID: 35080701 DOI: 10.1007/s11695-021-05871-3]

31 **Li N**, Yan QT, Jing Q, Pan RY, Wang HJ, Jiang B, Li XJ, Wang Y, Dong JH, Wang XJ, Zhang MJ, Meng QG, Li XZ, Liu ZJ, Gao ZQ, Qu MH. Duodenal-Jejunal Bypass Ameliorates Type 2 Diabetes Mellitus by Activating Insulin Signaling and Improving Glucose Utilization in the Brain. *Obes Surg* 2020; **30**: 279-289 [PMID: 31605365 DOI: 10.1007/s11695-019-04153-3]

32 **Lv R**, Du L, Zhang L, Zhang Z. Polydatin attenuates spinal cord injury in rats by inhibiting oxidative stress and microglia apoptosis *via* Nrf2/HO-1 pathway. *Life Sci* 2019; **217**: 119-127 [PMID: 30481506 DOI: 10.1016/j.lfs.2018.11.053]

33 **He T**, Shen H, Zhu J, Zhu Y, He Y, Li Z, Lu H. Geniposide attenuates cadmiuminduced oxidative stress injury *via* Nrf2 signaling in osteoblasts. *Mol Med Rep* 2019; **20**: 1499-1508 [PMID: 31257486 DOI: 10.3892/mmr.2019.10396]

34 **Shukla V**, Mishra SK, Pant HC. Oxidative stress in neurodegeneration. *Adv Pharmacol Sci* 2011; **2011**: 572634 [PMID: 21941533 DOI: 10.1155/2011/572634]

35 **Coleman ES**, Dennis JC, Braden TD, Judd RL, Posner P. Insulin treatment prevents diabetes-induced alterations in astrocyte glutamate uptake and GFAP content in rats at 4 and 8 wk of diabetes duration. *Brain Res* 2010; **1306**: 131-141 [PMID: 19822133 DOI: 10.1016/j.brainres.2009.10.005]

36 **Rivas AB**, Lopez-Picado A, Salas-Butrón MDR, Terleira A, Sanchez Pernaute A, Torres Garcia AJ, Moreno Lopera C, Chicharro LM, Bandrés F, Rubio Herrera MA, Portolés A, Vargas E. Effect of Roux-en-Y gastric surgery on ciprofloxacin pharmacokinetics: an obvious effect? *Eur J Clin Pharmacol* 2019; **75**: 647-654 [PMID: 30649602 DOI: 10.1007/s00228-018-02623-8]

37 **Riederer P**, Korczyn AD, Ali SS, Bajenaru O, Choi MS, Chopp M, Dermanovic-Dobrota V, Grünblatt E, Jellinger KA, Kamal MA, Kamal W, Leszek J, Sheldrick-Michel TM, Mushtaq G, Meglic B, Natovich R, Pirtosek Z, Rakusa M, Salkovic-Petrisic M, Schmidt R, Schmitt A, Sridhar GR, Vécsei L, Wojszel ZB, Yaman H, Zhang ZG, Cukierman-Yaffe T. The diabetic brain and cognition. *J Neural Transm (Vienna)* 2017; **124**: 1431-1454 [PMID: 28766040 DOI: 10.1007/s00702-017-1763-2]

38 **Ye S**, Xie DJ, Zhou P, Gao HW, Zhang MT, Chen DB, Qin YP, Lei X, Li XQ, Liu J, Cheng YX, Yao YC, Cai B, Shen GM. Huang-Pu-Tong-Qiao Formula Ameliorates the Hippocampus Apoptosis in Diabetic Cognitive Dysfunction Mice by Activating CREB/BDNF/TrkB Signaling Pathway. *Evid Based Complement Alternat Med* 2021; **2021**: 5514175 [PMID: 34211563 DOI: 10.1155/2021/5514175]

39 **Song Y**, Ding W, Bei Y, Xiao Y, Tong HD, Wang LB, Ai LY. Insulin is a potential antioxidant for diabetes-associated cognitive decline *via* regulating Nrf2 dependent antioxidant enzymes. *Biomed Pharmacother* 2018; **104**: 474-484 [PMID: 29793180 DOI: 10.1016/j.biopha.2018.04.097]

40 **Sabari SS**, Balasubramani K, Iyer M, Sureshbabu HW, Venkatesan D, Gopalakrishnan AV, Narayanaswamy A, Senthil Kumar N, Vellingiri B. Type 2 Diabetes (T2DM) and Parkinson's Disease (PD): a Mechanistic Approach. *Mol Neurobiol* 2023; **60**: 4547-4573 [PMID: 37118323 DOI: 10.1007/s12035-023-03359-y]

41 **Rebelos E**, Immonen H, Bucci M, Hannukainen JC, Nummenmaa L, Honka MJ, Soinio M, Salminen P, Ferrannini E, Iozzo P, Nuutila P. Brain glucose uptake is associated with endogenous glucose production in obese patients before and after bariatric surgery and predicts metabolic outcome at follow-up. *Diabetes Obes Metab* 2019; **21**: 218-226 [PMID: 30098134 DOI: 10.1111/dom.13501]

42 **Zuliani I**, Urbinati C, Valenti D, Quattrini MC, Medici V, Cosentino L, Pietraforte D, Di Domenico F, Perluigi M, Vacca RA, De Filippis B. The Anti-Diabetic Drug Metformin Rescues Aberrant Mitochondrial Activity and Restrains Oxidative Stress in a Female Mouse Model of Rett Syndrome. *J Clin Med* 2020; **9** [PMID: 32492904 DOI: 10.3390/jcm9061669]

43 **Fan J**, Li L, Qu P, Diao Y, Sun Y. κopioid receptor agonist U50488H attenuates postoperative cognitive dysfunction of cardiopulmonary bypass rats through the PI3K/AKT/Nrf2/HO1 pathway. *Mol Med Rep* 2021; **23** [PMID: 33649775 DOI: 10.3892/mmr.2021.11933]

44 **Habotta OA**, Abdeen A, Roomi AB, Elgndy AI, Sorour SM, Morsi MH, Kamal KM, Ibrahim SF, Abdelrahaman D, Fericean L, Banatean-Dunea I, Ghamry HI, El-Nablaway M, Atawia RT, Abdelhady D. Nootkatone Mitigated Melamine-Evoked Hepatotoxicity by Featuring Oxidative Stress and Inflammation Interconnected Mechanisms: In Vivo and In Silico Approaches. *Toxics* 2023; **11** [PMID: 37755794 DOI: 10.3390/toxics11090784]

45 **Yang MY**, Fan Z, Zhang Z, Fan J. MitoQ protects against high glucose-induced brain microvascular endothelial cells injury *via* the Nrf2/HO-1 pathway. *J Pharmacol Sci* 2021; **145**: 105-114 [PMID: 33357768 DOI: 10.1016/j.jphs.2020.10.007]

46 **Oh YS**, Jun HS. Effects of Glucagon-Like Peptide-1 on Oxidative Stress and Nrf2 Signaling. *Int J Mol Sci* 2017; **19** [PMID: 29271910 DOI: 10.3390/ijms19010026]

47 **Farkas E**, Szilvásy-Szabó A, Ruska Y, Sinkó R, Rasch MG, Egebjerg T, Pyke C, Gereben B, Knudsen LB, Fekete C. Distribution and ultrastructural localization of the glucagon-like peptide-1 receptor (GLP-1R) in the rat brain. *Brain Struct Funct* 2021; **226**: 225-245 [PMID: 33341919 DOI: 10.1007/s00429-020-02189-1]

48 **Ma X**, Ma J, Leng T, Yuan Z, Hu T, Liu Q, Shen T. Advances in oxidative stress in pathogenesis of diabetic kidney disease and efficacy of TCM intervention. *Ren Fail* 2023; **45**: 2146512 [PMID: 36762989 DOI: 10.1080/0886022X.2022.2146512]

49 **Tu Y**, Li L, Zhu L, Guo Y, Du S, Zhang Y, Wang Z, Zhu M. Geniposide Attenuates Hyperglycemia-Induced Oxidative Stress and Inflammation by Activating the Nrf2 Signaling Pathway in Experimental Diabetic Retinopathy. *Oxid Med Cell Longev* 2021; **2021**: 9247947 [PMID: 34938383 DOI: 10.1155/2021/9247947]

50 **Qin L**, Chong T, Rodriguez R, Pugazhenthi S. Glucagon-Like Peptide-1-Mediated Modulation of Inflammatory Pathways in the Diabetic Brain: Relevance to Alzheimer's Disease. *Curr Alzheimer Res* 2016; **13**: 1346-1355 [PMID: 27033055 DOI: 10.2174/1567205013666160401114751]

51 **Ren X**, Sun L, Wei L, Liu J, Zhu J, Yu Q, Kong H, Kong L. Liraglutide Up-regulation Thioredoxin Attenuated Müller Cells Apoptosis in High Glucose by Regulating Oxidative Stress and Endoplasmic Reticulum Stress. *Curr Eye Res* 2020; **45**: 1283-1291 [PMID: 32180468 DOI: 10.1080/02713683.2020.1737137]

52 **Xiong J**, Yang J, Yan K, Guo J. Ginsenoside Rk1 protects human melanocytes from H(2)O(2)induced oxidative injury *via* regulation of the PI3K/AKT/Nrf2/HO1 pathway. *Mol Med Rep* 2021; **24** [PMID: 34558653 DOI: 10.3892/mmr.2021.12462]

53 **Guo Z**, Wan X, Luo Y, Liang F, Jiang S, Yuan X, Mo Z. The vicious circle of UHRF1 down-regulation and KEAP1/NRF2/HO-1 pathway impairment promotes oxidative stress-induced endothelial cell apoptosis in diabetes. *Diabet Med* 2023; **40**: e15026 [PMID: 36510823 DOI: 10.1111/dme.15026]

54 **Li WC**, Yao SP, Zhang J, Liu WB, Liu J, Geng CK. Low-dose lipopolysaccharide protects nerve cells against spinal cord injury *via* regulating the PI3K-AKT-Nrf2 signaling pathway. *Biochem Cell Biol* 2021; **99**: 527-535 [PMID: 34424795 DOI: 10.1139/bcb-2020-0641]

55 **Luo C**, Fan H, Li S, Zou Y. Therapeutic of Candesartan and Music Therapy in Diabetic Retinopathy with Depression in Rats. *Evid Based Complement Alternat Med* 2021; **2021**: 5570356 [PMID: 33833815 DOI: 10.1155/2021/5570356]

56 **Kabahizi A**, Wallace B, Lieu L, Chau D, Dong Y, Hwang ES, Williams KW. Glucagon-like peptide-1 (GLP-1) signalling in the brain: From neural circuits and metabolism to therapeutics. *Br J Pharmacol* 2022; **179**: 600-624 [PMID: 34519026 DOI: 10.1111/bph.15682]

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LETTER TO THE EDITOR

Diabetes is affecting everyone everywhere

Parul Chawla Gupta, Mona Duggal, Arvind Kumar Morya

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Abstract

The article titled "Accessibility and Utilization of Healthcare Services Among Diabetic Patients: Is Diabetes a Poor Man's Ailment?" gave insights into a pandemic systemic disease known as diabetes mellitus. This modern-era pandemic affects everyone, regardless of their financial background. As a result, diabetes is not a systemic disease which just involves people of low socioeconomic status.

Key Words: Diabetes; Incidence and prevalence; Diabetes mellitus

Core Tip: Diabetes is fast becoming a chronic debilitating disease due to poor glycemic control by the patients. We have done a short research on the incidence and prevalence of diabetes mellitus and found that it is equally affecting the developed as well as developing countries. This metabolic disorder affects many organs of the body like kidney, eye, heart, liver, brain and skin.

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TO THE EDITOR

The article titled "Accessibility and Utilization of Healthcare Services Among Diabetic Patients: Is Diabetes a Poor Man's Ailment?" is very well-written. Diabetes affects everyone, regardless of their social background. As a result, diabetes is not an illness which just involves people of low socioeconomic status. Diabetes patients demand additional medical treatments and services than non-diabetic patients due to their increased risk of co-morbidities, inadequate glycemic control, and repeated hospitalizations. Regardless of the encouraging increase in the figures of diabetes individuals taking medical treatment because of increased knowledge, several personal and institutional issues continue to hinder access[1].

The dominance of diabetes amongst high-income people has been linked to physical sedentary habits. In contrast, the frequency of diabetes among low-income people has been linked to poor diet and a lack of funds to manage the negative consequences of diabetic diseases. Diabetes control requires an easy approach to medical treatment providers. According to the existing research, the total sum of patients gaining approach to medical care amenities has grown with time. Yet, various variables have been found in the literature search which impedes patient access to the existing medical care treatments. However, some patients cannot receive these services, so the fundamental goals of providing such treatments are jeopardized. As a result, the health of diabetes patients suffers, particularly in patients from low-income families in developing nations. The primary goal of all medical services is to increase the use of medical care services, and this article has shown that diabetic patients use these services partly, even while the fraction of people who use these amenities is negligible. The level of service is relatively poor. Medical services for diabetes care are lacking in many impoverished nations, and healthcare amenities have been stated to be overstressed, particularly in low-income nations, due to the increased number of diabetics. A cross-sectional study in Southwest China found that the prevalence of prediabetes as well as diabetes was greater amongst urban elderly persons than their rural counterparts, as they had a higher prevalence of obesity, central obesity, and physical inactivity[2]. In the National Health Interview Survey, it was discovered that diabetes was much more common in low-pay populaces. Another study that studied factors influencing the consumption of healthcare facilities related to diabetes encompassed an absence of information on both the disorder and the necessity for screening, economic causes, institution-based constraints, absence of syringes and testing apparatus, high wait periods at eye hospitals, overcrowded clinics, and distress of the anticipated discomfort[3]. It has been noticed that older adults with diabetes use emergency facilities and few outpatient amenities much more than the younger population. According to research done by Shalev *et al*[4], gender affects consumption of health facilities, with females with diabetes using added healthcare amenities than their male counterparts[5].

To improve diabetes patients' access to healthcare services, policies and intervention programmes should be developed and focused on reducing the current barriers that impede diabetic patients' contact with healthcare facilities. Both government and non-governmental organizations must concentrate on refining the value of diabetes services, elementary healthcare facilities, and health awareness programmes to simplify the delivery of effective care to diabetic patients[6,7].

FOOTNOTES

Co-first authors: Parul Chawla Gupta and Mona Duggal.

Author contributions: Morya AK designed and formulated the study; Gupta PC and Duggal M did research and wrote the paper; Morya AK did the editing of the manuscript.

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REFERENCES

1 **Eseadi C**, Amedu AN, Ilechukwu LC, Ngwu MO, Ossai OV. Accessibility and utilization of healthcare services among diabetic patients: Is diabetes a poor man's ailment? *World J Diabetes* 2023; **14**: 1493-1501 [PMID: 37970126 DOI: 10.4239/wjd.v14.i10.1493]
2 **Zhao Y**, Li HF, Wu X, Li GH, Golden AR, Cai L. Rural-urban differentials of prevalence and lifestyle determinants of pre-diabetes and diabetes among the elderly in southwest China. *BMC Public Health* 2023; **23**: 603 [PMID: 36997910 DOI: 10.1186/s12889-023-15527-9]
3 **Piyasena MMPN**, Murthy GVS, Yip JLY, Gilbert C, Peto T, Premarathna M, Zuurmond M. A qualitative study on barriers and enablers to

uptake of diabetic retinopathy screening by people with diabetes in the Western Province of Sri Lanka. *Trop Med Health* 2019; **47**: 34 [PMID: 31139011 DOI: 10.1186/s41182-019-0160-y]

4 **Shalev V**, Chodick G, Heymann AD, Kokia E. Gender differences in healthcare utilization and medical indicators among patients with diabetes. *Public Health* 2005; **119**: 45-49 [PMID: 15560901 DOI: 10.1016/j.puhe.2004.03.004]

5 **Buja A**, Caberlotto R, Pinato C, Mafrici SF, Bolzonella U, Grotto G, Baldovin T, Rigon S, Toffanin R, Baldo V. Health care service use and costs for a cohort of high-needs elderly diabetic patients. *Prim Care Diabetes* 2021; **15**: 397-404 [PMID: 33358612 DOI: 10.1016/j.pcd.2020.12.002]

6 **Mutyambizi C**, Booysen F, Stokes A, Pavlova M, Groot W. Lifestyle and socio-economic inequalities in diabetes prevalence in South Africa: A decomposition analysis. *PLoS One* 2019; **14**: e0211208 [PMID: 30699173 DOI: 10.1371/journal.pone.0211208]

7 **Itumalla R**, Kumar R, Perera B, Elabbasy MT, Kumar Cg S, Kundur R. Patient's Perception of Diabetes Care Services in Hail, Kingdom of Saudi Arabia. *Health Psychol Res* 2022; **10**: 38119 [PMID: 36168641 DOI: 10.52965/001c.38119]

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