World Journal of *Medical Genetics*

World J Med Genet 2023 June 2; 11(2): 8-27





Published by Baishideng Publishing Group Inc

WJMG

World Journal of Medical Genetics

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ABOUT COVER

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The WJMG is now abstracted and indexed in Reference Citation Analysis, China National Knowledge Infrastructure, China Science and Technology Journal Database, and Superstar Journals Database.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: Yu-Xi Chen; Production Department Director: Xiang Li; Editorial Office Director: Jin-Lei Wang.

NAME OF JOURNAL	INSTRUCTIONS TO AUTHORS
World Journal of Medical Genetics	https://www.wjgnet.com/bpg/gerinfo/204
ISSN	GUIDELINES FOR ETHICS DOCUMENTS
ISSN 2220-3184 (online)	https://www.wjgnet.com/bpg/GerInfo/287
LAUNCH DATE	GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
December 27, 2011	https://www.wignet.com/bpg/gerinfo/240
FREQUENCY	PUBLICATION ETHICS
Continuous Publication	https://www.wjgnet.com/bpg/GerInfo/288
EDITORS-IN-CHIEF	PUBLICATION MISCONDUCT
Raffaele Palmirotta	https://www.wjgnet.com/bpg/gerinfo/208
EDITORIAL BOARD MEMBERS	ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/2220-3184/editorialboard.htm	https://www.wignet.com/bpg/gerinfo/242
PUBLICATION DATE June 2, 2023	STEPS FOR SUBMITTING MANUSCRIPTS https://www.wjgnet.com/bpg/GerInfo/239
COPYRIGHT	ONLINE SUBMISSION
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World J Med Genet 2023 June 2; 11(2): 8-20

DOI: 10.5496/wjmg.v11.i2.8

Basic Study

ISSN 2220-3184 (online)

ORIGINAL ARTICLE

Genome-wide associations, polygenic risk, and Mendelian randomization reveal limited interactions between John Henryism and cynicism

Richard R Chapleau

Specialty type: Genetics and heredity

Provenance and peer review: Unsolicited article; Externally peer reviewed

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0 Grade B (Very good): 0 Grade C (Good): C Grade D (Fair): 0 Grade E (Poor): 0

P-Reviewer: Arumugam VA, India

Received: February 8, 2023 Peer-review started: February 8, 2023 First decision: April 28, 2023 Revised: April 28, 2023 Accepted: May 22, 2023 Article in press: May 22, 2023 Published online: June 2, 2023



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Abstract

BACKGROUND

John Henryism (JH) is a strategy for dealing with chronic psychological stress characterized by high levels of physical effort and work. Cynicism is a belief that people are motivated primarily by self-interest. High scores on the JH scale and cynicism measures correlate with an increased risk of cardiovascular disease. High cynicism is also a hallmark of burnout syndrome, another known risk factor for heart disease.

AIM

To evaluate possible interactions between JH and cynicism hoping to clarify risk factors of burnout.

METHODS

We analyzed genetic and psychological data available from the Database of Genotypes and Phenotypes for genome-wide associations with these traits. We split the total available samples and used plink to perform the association studies on the discovery set (n = 1852, 80%) and tested for replication using the validation set (n = 465). We used scikit-learn to perform supervised machine learning for developing genetic risk algorithms.

RESULTS

We identified 2, 727, and 204 genetic associations for scores on the JH, cynicism and cynical distrust (CD) scales, respectively. We also found 173 associations with high cynicism, 109 with high CD, but no associations with high JH. We also produced polygenic classifiers for high cynicism using machine learning with areas under the receiver operator characteristics curve greater than 0.7.

CONCLUSION



We found significant genetic components to these traits but no evidence of an interaction. Therefore, while there may be a genetic risk, JH is not likely a burnout risk factor.

Key Words: Cynicism; Burnout syndrome; John Henryism; Genome-wide association study; Polygenic risk score; Machine-learning

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Core Tip: This study evaluates the interaction of a job-related cardiovascular disease risk factor (John Henryism) and the development of occupational burnout (specifically the cynicism and cynical distrust components). Genome-wide associations and statistical genetics revealed that while John Henryism is not a risk factor for burnout syndrome, there are independent genetic risk factors for both John Henryism and cynicism. These new results provide additional tools to industrial and occupational psychologists, as well as cardiologists, to help reduce burnout incidence.

Citation: Chapleau RR. Genome-wide associations, polygenic risk, and Mendelian randomization reveal limited interactions between John Henryism and cynicism. *World J Med Genet* 2023; 11(2): 8-20 URL: https://www.wjgnet.com/2220-3184/full/v11/i2/8.htm DOI: https://dx.doi.org/10.5496/wjmg.v11.i2.8

INTRODUCTION

First described formally by Freudenberger[1] and later expanded upon by Maslach and Jackson[2], burnout syndrome is generally considered to be a response to long-term occupational stress[3]. The most widely used definition of burnout syndrome is a 3-component syndrome comprised of emotional exhaustion, depersonalization or cynicism, and feelings of low professional efficacy or personal[4]. High levels of burnout syndrome components have been associated with increased disease prevalence including a heightened risk of cardiovascular disease[5,6], impaired cognitive function[7], increased sleep disorders[8,9], and even type II diabetes and hyperlipidemia[10,11]. Clearly, the impacts of burnout syndrome extend beyond just performance in the workplace and can adversely affect health, well-being, and quality of life.

Another response to chronic social stressors is John Henryism (JH), a coping mechanism by which an individual exerts increased effort to overcome stress[12]. As with burnout syndrome, the effects of JH affect both the individual's health and the workplace. Also, like the effects of burnout syndrome, JH can have a negative impact on cardiovascular health, hypertension, and increased rates of alcoholism[13-16]. Paradoxically, high levels of JH have been associated with lower levels of depressive symptoms in African Americans of varying socioeconomic status, suggesting a protective effect on mental health[17]. These findings suggest a nuanced interaction between JH and overall health and well-being.

The literature on the genetic contribution to burnout syndrome and JH is limited. Preliminary work regarding the heritability of cynicism (one of the three components of burnout syndrome) was reported in the late 1980's to early 1990's and revealed mixed results. One twin study of cynical hostility revealed a non-significant genetic component[18]. In contrast, three other twin studies using the Cook Medley Hostility Scale and the MMPI showed significant heritability[19-21]. The genetic heritability of JH also has limited reports, with one estimate suggesting up to 35% of the variability is explained by genetic factors[22,23]. To the best of our knowledge, there have not been any genome-wide or candidate gene studies performed to assess the genetic contribution to these traits.

Here we report the results of our study investigating the relationship between JH and cynicism. We hypothesized that JH would exert a causal influence on cynicism. We came to this hypothesis because of the nature of JH as a coping mechanism for dealing with discriminatory acts and the skepticism and negative view of others inherent to cynicism. We tested this hypothesis through the statistical approach called Mendelian randomization[24], where genetic associations with JH are considered for their independent influence on cynicism. In order to take this approach, we first performed a genome-wide association study (GWAS) to identify genetic variables for JH and cynicism independently. We also extended those GWAS results to develop polygenic risk scores (risk scores considering multiple genetic variants) for each trait and assessed if the higher genetic risk in one trait correlated to higher levels of the other trait. To our knowledge, this study is the first to report genetic associations with any of the three outcomes.

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MATERIALS AND METHODS

This study was reviewed and approved by WCG IRB (Study number 1332892) for human subjects research oversight. All data were obtained from the National Institutes of Health's Database of Genotypes and Phenotypes (dbGAP). We used data from the Coronary Artery Risk Development in Young Adults (CARDIA) Study (dbGAP study accession phs000285)[25]. The CARDIA cohort study design was a longitudinal study, but we used the data in a retrospective fashion. We followed the STREGA guidelines available from the EQUATOR network[26]. The guideline table with annotations regarding how we addressed each point is available as Supplementary material.

Psychological trait definitions

JH was measured by the 12-item John Henryism Active Coping Scale, and responses were reversecoded. JH was calculated as a mean of the responses. As our goal was to observe the effect of having above-average JH, we defined a dichotomous variable of JH as individuals with scores above the median (49) as having high JH[13,15]. Individuals with scores at or below the median were considered average or below average JH. We also performed tests using the JH score as a continuous, meancentered variable. The JH data were obtained from dbGAP accession number phv001133534.v2.p2.c1. Cynicism and cynical distrust (CD) were measured as 12- and 8-item subscales of the Cook-Medley Hostility Scale (CMHS), respectively[27-29]. Mean-centered continuous variables and median-adjusted dichotomous variables were created for cynicism and CD in the same manner as for JH, resulting in four CMHS-derived variables (continuous cynicism, high/Low cynicism, continuous CD, and high/Low CD). The CMHS data were obtained from dbGAP accession number phv00113478.v2.p2.c1. As not all participants completed all questionnaires, missing data were filled in as the mean. Processed data were then split into a validation set (20%, n = 623) and the remainder were used for training (Figure 1). Statistical analyses were performed in R version 4.2.0.

Power analysis

We performed a power analysis for our study to determine if the sample sizes available in the CARDIA cohort were sufficient to identify significant genetic associations. We assumed a 10% effect allele frequency (EAF) in the control population, 20% type-II error rate (beta) and 5% type-I error rate (alpha). We used the observed case-control ratios for each condition to calculate the statistical power. We report the results of the analysis as the minimum EAF in the case population to achieve 80% power in Table 1 alongside the cohort characteristics.

Genetic data pre-processing

Microarray (Affymetrix Genome-Wide Human SNP 6.0 Array) genotype data were obtained from the CARDIA study (dbGAP accession numbers phg000092.v2 and phg000098.v2). Genetic data were preprocessed to ensure uniformity from the original plink data. Briefly, the binary plink file sets were merged and filtered for autosomal genotypes with less than 10% missing genotype calls (sample or locus), a minor allele frequencies threshold was set at 1%, and a Hardy-Weinberg equilibrium threshold of 0.0001 was used for filtering out spurious variants. After pre-processing, they were split into the training and validation sets using the sample identifiers defined in the phenotype data splitting.

Genome-wide association studies

We conducted GWAS using plink 1.9 evaluating only the total scores (not scores for the questionnaire responses) and high/Low status for each trait, totaling 6 phenotypes. We used the following command for the association: *plink --bfile <training dataset prefix> --memory 15000 --pheno <phenotype filename>.csv -- all-pheno --assoc --pfilter 0.001 --adjust qq-plot --out <output filename>. For assessing the replication of associations in continuous variables traits, we repeated the GWAS in plink restricting the input variants to only those candidates identified in the discovery phase. For dichotomous traits, the set of candidate single nucleotide polymorphisms (SNPs) identified as significantly associated with the trait (P < 5 \times 10^{-8}) or suggestively associated (P < 5 \times 10^{-6}) were then evaluated by chi-square test using the test dataset. Additionally, each analysis was repeated conditioning upon the highest associated SNP.*

Polygenic risk score calculation

Our method of PRS development was using the machine learning package scikit-learn[30]. We used four supervised classifiers [Ridge, multi-layer perceptron, random forest, and k-nearest neighbors (KNN)] and iterated through the relevant parameter space for each classifier (*e.g.*, number of neighbors for KNN). The classifiers were trained on the training set (n = 1852) and validated using the test dataset (n = 465). Each classifier was evaluated using the area under the receiver operator characteristics (ROC) curve (AUC) and the model with the best AUC was saved for each classifier. Finally, a ROC curve comparing the best models of each classifier was created.

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Table 1 Breakdown of samples by dichotomous traits, n (%)						
	John Henryism	Cynicism	Cynical distrust			
Training high	877 (47.4)	724 (39.1)	680 (36.7)			
Training not high	975	1128	1172			
Case:Control ratio	1:1.1	1:1.6	1:1.7			
Minimum EAF, %	14.5	15.7	16.0			
Validation high	246 (52.9)	180 (38.7)	171 (36.8)			
Validation not high	219	285	294			

EAF: Effect allele frequency.



DOI: 10.5496/wjmg.v11.i2.8 Copyright © The Author(s) 2023.

Figure 1 Flow diagram for data processing. Rectangles are datasets with sample sizes in parentheses and ovals are processes. dbGAP: Database of Genotypes and Phenotypes; GWAS: Genome-wide association studies; LCV: latent causal variable; MR: Mendelian randomization; PRS: Polygenic risk score.

Mendelian randomization

We performed two-sample Mendelian randomization (2SMR) estimates using the TwoSampleMR R package[24]. We used the MR Egger regression[31], inverse variance weighted estimator[32], weighted median estimator[33], and Wald ratio estimator[34] algorithms. Instrumental variables (IV, SNPs associated with the exposure) were extracted by *P* value thresholds 5×10^{-6} and 5×10^{-8} . We excluded SNPs in strong linkage disequilibrium (LD) to reduce bias and used a clumping process with European samples from the 1000 Genomes Project ($r^2 < 0.001$, window size = 10000). If SNPs identified in the exposure dataset were not in the outcome dataset, proxy SNPs in LD ($r^2 > 0.9$) were used as instrumental variables. For the sensitivity analysis, we performed heterogeneity testing using Cochran's Q and *I*² analyses[35] and tested pleiotropy on the weighted median estimation results[33].

Statistical analysis

Statistical analyses for assessing variant associations were performed in R. To confirm the association of the variants identified for the high cynicism and high CD variables, we created a matrix containing the allele counts for each candidate variant. We then performed a chi-square analysis using the chisq.test function within base R using the allele count matrix as the input argument. The statistical analyses of the PRS estimators were performed in the scikit-learn package. As the class allocation displayed only slight imbalance (control/case group sample size ratios of 1:2.1, 1:2.5, and 1:2.7 for JH, cynicism, and CD, respectively), we used the ROC AUC for evaluating performance of the PRS classification models.

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RESULTS

Characteristics of the dataset

The initial phenotype dataset consisted of 3111 samples (Figure 1). The median scores for JH, cynicism, and CD were 49, 6, and 3, respectively. Score ranges were 26 to 60 for JH, 0 to 13 for cynicism, and 0 to 8 for CD. There were 1497 (48.1%) samples with high JH scores (JH > 49), 1228 (39.5%) with high cynicism (> 6), and 1163 (37.4%) with high CD scores (> 3). We found minimal correlation between JH and cynicism or CD (Pearson r = 0.159 and 0.118, respectively). After splitting the data into training (80%) and validation (20%) sets, the median scores were 49, 6, and 3, respectively, for the training set and 50, 5, and 3, respectively, for the validation set. The percent of samples above the population median scores was 47.1%, 40.0%, and 37.8%, respectively, for the training set and 52.3%, 37.4%, and 35.6%, respectively, for the validation set. T-tests showed that the distribution of samples in the subsets was not statistically different from the original distribution (JH training vs original P = 0.52; JH validation vs original P = 0.12; cynicism training vs original P = 0.48; cynicism validation vs original P = 0.08; CD training vs original P = 0.62; and CD validation vs original P = 0.22). Evaluating the Shapiro-Wilk normality test in R reveals that none of the six variables are normally distributed (all have $P < 2.2 \times 10^{-16}$) . The Shapiro-Wilk W constants for the distributions of quantitative scores were 0.980, 0.935, and 0.970 for JH, CD, and cynicism, respectively, while the W constants for the dichotomous traits were 0.635, 0.615, and 0.622, respectively. The skewness of the three continuous traits was -0.511, 0.118, and 0.373, respectively, while the kurtosis values were 3.11, 1.01, and 2.18, respectively. From these results, we see that the data are generally positively skewed and primarily platykurtic.

The merged genetic dataset contained 2466 samples (1162 from accession phg000092 and 1441 from accession phg000098, with 137 overlapping between the two studies), of which 978 (42.1%) were male. The two datasets contained 909662 and 720622 markers, respectively. Following SNP filtering, there were 561045 variants remaining (153333 for missingness, 13410 below 1% MAF, and 144454 not passing the Hardy-Weinberg filter). 136 samples were removed for having greater than 10% missing genotype calls, creating a final dataset of 2330 samples. The total genotyping rate for the dataset was 99.4%. After splitting samples using the training and validation sets defined in the phenotype stage, there were 1852 samples in the training set, 465 samples in the validation set, and 13 (0.6%) of the samples with genetic data did not have phenotype data (Table 1).

GWAS Results

Our discovery phase GWAS analysis of 1852 samples revealed 25 candidate variants with Bonferronicorrected P values below 5×10^{-8} associated with JH, 28926 candidates associated with cynicism, and 14134 candidate associations with CD (Figure 2). For each of the candidate variants, we performed a second GWAS on the test sample set (n = 465) using only the candidate SNPs identified in the first analysis. We found that 2 SNPs replicated as associated with the quantitative JH trait (P = 0.002), 727 replicated for association with cynicism ($P = 1.73 \times 10^6$), and 204 replicated for association with CD (P = 3.54×10^{-6}).

Similar to how we analyzed the continuous variables, we identified in the dichotomous variable analysis 3 candidate variants associated with high JH, 708 associations with high cynicism, and 17507 associations with high CD. After evaluating for replication of significance (P < 0.05) in the validation set of 465 samples, we found 0, 173, and 109 significantly associated variants that replicated in our test set for JH, cynicism, and CD, respectively. Of the 173 replicated variants associated with cynicism, 19 were located across 9 distinct loci (defined as within a 250000-base pair window on the same chromosome) and the other 154 were distinct variants. We also observed that 79 of the 173 high cynicism-associated variants were also associated with the quantitative trait, while none of the high CD-associated variants were present in the quantitative trait CD list. Lists of all candidate SNPs and replicated SNPs are available from the corresponding author upon reasonable request.

ML-based polygenic risk scores

We used the replicated SNPs for each condition cynicism and CD to evaluate the predictive capability for each, addressing the question of whether genetic variants associated with high JH, for example, were predictive of high cynicism. As no SNPs replicated for high JH, we used the candidate SNPs. Using the scikit-learn software package, we performed the nine tests for each input/output combination (e.g., cynicism vs CD, cynicism, and JH) with the four classification methods. Our results (Figure 3, Table 2) reveal that PRS algorithms based on genetic variants associated with high cynicism are predictive of high cynicism (AUC range = 0.696-0.732) and high CD (AUC range = 0.652-0.684), whereas algorithms trained on genetic markers of high JH or high CD are not predictive for any trait. These classifiers would be considered to be acceptable predictors[36] with AUC values near 0.7, these results show that cynicism and CD are genetically related, reinforcing the psychological relationship, and that JH is a distinct trait deriving from different genetic contributions.

Two-sample Mendelian randomization

Our two-sample Mendelian randomization analyses using the GWAS summary statistics previously



Table 2 Area under the curve values for ML-based polygenic risk algorithms						
Input <i>vs</i> output pair	Ridge	MLP	RFC	KNN		
JH vs JH	0.524	0.542	0.545	0.550		
JH vs cynicism	0.638	0.634	0.649	0.660		
JH vs CD	0.653	0.656	0.651	0.630		
Cynicism vs JH	0.482	0.533	0.565	0.563		
Cynicism vs cynicism	0.732	0.728	0.696	0.712		
Cynicism vs CD	0.681	0.676	0.671	0.684		
CD vs JH	0.526	0.545	0.546	0.563		
CD vs cynicism	0.563	0.556	0.577	0.580		
CD vs CD	0.516	0.516	0.526	0.533		

JH: John Henryism; CD: Cynical distrust; MLP: Multi-layer perceptron; RFC: Random forest classifier; KNN: K-nearest neighbors. Bold values are the greatest area under the receiver operator characteristic curve for the input-output pairing.

> described revealed significant causal relationships between all three traits (Figure 4). The odds ratios (Figure 4A) and beta coefficients (Figure 4B) for the relationships were highly similar, with odds ratios ranging from 2.0 (for the causal effect of JH on cynicism and the causal effect of CD on JH) to 3.3 (for the inverse effects), with the CD/cynicism relationship near 2.6. The mendelian randomization (MR) comparisons involved 4680 variants for the JH/cynicism relationship, 4262 variants for the JH/CD relationship, and 131185 variants for the CD/cynicism relationship. We performed pleiotropy and heterogeneity tests to assess the sensitivity of the MR estimates. We found significant pleiotropy in all cases, with the Egger intercepts significantly different from zero (Figure 4C)[37].

DISCUSSION

Our results from genetic analyses suggest that there is not a significant relationship between JH and cynicism. These observations are consistent with previously published literature using only psychometric approaches. Adams and co-workers[38] demonstrated that JH scores were not significantly correlated with the Cook Medley Hostility Scale, of which we used the cynicism subscale herein. In contrast, a large study investigating the effects of various psychosocial factors on chronic kidney disease found that JH and hostility exhibited inverse risk factor loading[39]. Finally, initial reports from the CARDIA study suggest a weak but significant correlation between hostility and effortful coping (r =0.14, P < 0.05), especially among younger, less educated individuals that were more likely to consume alcohol and be smokers[40].

Although our findings do not substantiate a relationship between JH and cynicism, and subsequently burnout syndrome, they do suggest that there is a genetic component to both outcomes. Research among the general population has shown that hostility is an independent risk factor for cardiovascular disease[41], which has also been reinforced with evidence in population-specific work on African Americans^[42]. Our results suggest that a significant predisposition to hostility and cynicism, when coupled with chronic occupational stress and hostility, could lead to elevated levels of burnout and significant long-term health decline.

Our assessment of causal effects revealed significant pleiotropy in the relationships, suggesting that any interaction which may exist is indirect. Our results do not support a causal effect, but neither do they provide sufficient evidence to refute any such relationship. Indeed, our assessment using multiple MR estimators provides limited support that there is a relationship between these traits, but that such an interaction may not be mediated by genetics. We used the MR Egger estimate to evaluate violations of the instrumental variable assumptions. We found that these assumptions are not valid in our analysis (the Egger intercepts were significantly non-zero). However, the magnitude of the impact of any deviations must be small as none of the six beta coefficients determined by the MR Egger method were outliers from the other four estimates (i.e., all MR Egger estimates were within the 95% confidence interval of the mean observed beta coefficient). Therefore, it appears that using the MR Egger estimator did not introduce additional bias or increase the Type I error rate[32].

Limitations

There are three major limitations of our study. First, the pleiotropic analysis suggests that there are confounders involved in the interaction and, therefore, this pleiotropy highlights one limitation of our





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Figure 2 Manhattan and quantile-quantile plots of genome-wide associations. A: Manhattan plot for cynical distrust score; B: Manhattan plot for high cynical distrust; C: QQ plot for cynical distrust score; D: QQ plot for high cynical distrust; E: Manhattan plot for cynicism score; F: Manhattan plot for high cynicism; G: QQ plot for cynicism score; H: QQ plot for high cynicism; I: Manhattan plot for John Henryism score; J: Manhattan plot for high John Henryism; K: QQ plot for John Henryism score; L: QQ plot for high John Henryism. Black dots = candidate associations from the training set (*n* = 1852); green dots = associations replicated in the verification set (*n* = 465); blue line = genome-wide suggestive association (*P* = 5 × 10⁻⁶); red line = genome-wide significant association (*P* = 5 × 10⁻⁸).

study in that we did not consider covariates in the initial analysis. The CARDIA study included many demographic and phenotypic datasets from which covariates could be identified. As the outcomes we measured tend to change with age[40], at least age at the time the questionnaire could be used as a future covariate. Indeed, the weak correlations reported by Albanese were largely derived from stratified data. While it was beyond the scope of our initial view of the interactions between genetics, JH, and cynicism to assess covariates, additional covariates could include gender, race, or personality, among others.

A second limitation is that the sample size is relatively small for a large-scale genomics study. Our discovery phase was comprised of only 1852 samples and our replication phase consisted of another 465 samples. While an *a priori* power analysis revealed that these sample sizes should have been powered to identify significant associations, it would be prudent to repeat these observations in larger populations to confirm which, if any, variants remain significantly associated with JH, cynicism, and/or CD. Due to this sample size limitation, the generalizability of our results should be considered alongside larger-scale studies, especially with regard to burnout syndrome, for which not much is published in the genetic literature.

Finally, there is a significant deviation from normality in all of the datasets, as determined by analyzing qq-plots (Figure 2). Considering the non-normal distribution of the data and the large sample distribution within the dataset, this deviation from normality was expected (both Cook-Medley and JH scores were previously acknowledged to have skewed distributions)[40]. What was unexpected was the relatively large number of significantly associated variants for cynicism and CD found in both the discovery and replication phases. Viewed alone, the large number of associated variants is cause for skepticism in the results. However, when taken into consideration along with the predictive ability of ML algorithms (Figure 3) developed based upon these replicated variants, the evidence for a significant genetic contribution to cynicism and CD becomes stronger.

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Figure 3 Receiver operator characteristics curves for genetic classification algorithms. A: High cynical distrust classifier predicting high cynical distrust; B: High cynical distrust classifier predicting high cynicism; C: High cynical distrust classifier predicting high John Henryism; D: High cynicism classifier predicting high cynical distrust; E: High cynicism classifier predicting high cynicism; F: High cynicism classifier predicting high John Henryism; G: High John Henryism classifier predicting high cynical distrust; H: High John Henryism classifier predicting high cynical distrust; I: High John Henryism classifier predicting high John Henryism. Each panel shows four predictors (orange = K-nearest neighbors, green = random forest, navy = multi-layer perceptron, teal = ridge classification).



Figure 4 Results from Mendelian randomization. A: Average odds ratios and 95% confidence intervals of the causal relationships between traits as determined by Mendelian randomization (MR); B: Average beta coefficients and standard errors from four MR methods; C: Egger intercepts, standard errors, and P values (italic) from pleiotropy analyses.

CONCLUSION

In conclusion, our results suggest that high levels of cynicism and CD have a significant genetic component and there may some genetic component to levels of JH. This genetic component of cynicism and CD appears to have some common effects as polygenic risk scores developed to classify individuals with high scores in one trait are reasonably effective at classifying individuals with the other trait (AUC



> 0.6). Our results are insufficient to determine if this correlation is based upon causation, however, as there is a significant amount of observed pleiotropy in the MR analysis, suggesting the existence of confounding variables.

ARTICLE HIGHLIGHTS

Research background

Our hypothesis was that cynicism, a distrustful attitude that assumes people are mainly driven by selfinterest, would result from John Henryism (JH), a coping strategy that involves working hard and exerting high levels of effort to deal with chronic stress. We reasoned that as JH is a way of handling discrimination, and that cynicism involves skepticism and negativity towards others, the two traits would be related. Furthermore, both JH and cynicism are linked to a higher risk of cardiovascular disease, while cynicism also often accompanies burnout syndrome, another cardiovascular risk factor.

Research motivation

Rates of burnout are increasing broadly across the globe. Burnout can cause physiological and emotional distress. Understanding the role of stress coping skills such as JH may help clarify the role of occupational stress in overall health and well-being.

Research objectives

The present study aimed to determine if JH is correlated with the cynicism component of burnout by using approaches from statistical genetics.

Research methods

Genotype and phenotype data from the "CARDIA Cohort" study were obtained from the Database of Genotypes and Phenotypes. Genome-wide association studies (GWAS) were performed in plink version 1.9 on the log-normalized JH, cynicism, and cynical distrust (CD) phenotypes as well as a binary "high/ Low" trait for each phenotype using a two-stage discovery and replication approach. The GWAS results were then used to develop polygenic risk score (PRS) algorithms using supervised machine learning in scikit-learn. Significant variants identified in the discovery stage were tested for replication by performing GWAS in a second, independent set of data restricting variants only to those candidates identified in discovery (for continuous variables) or through chi-square testing for the binary variable. The performance of the PRS algorithms at classifying individuals as "high" or "low" for the phenotype was evaluated in scikit-learn using the area under the receiver operator characteristics curve.

Research results

The GWAS identified significant variant associations with JH (2), cynicism (727), or CD (204) scores and with high cynicism (173) or CD (109). PRS classifiers were successfully developed for cynicism and CD (AUC > 0.65), but not for JH. Neither of the classifiers for cynicism or CD could predict JH traits, nor could the JH classifier predict cynicism or CD.

Research conclusions

There are genetic variants associated with each trait, however JH active coping does not appear to be correlated with cynicism or CD levels.

Research perspectives

The genetic associations with these phenotypes suggest that further research could provide insight into how each trait results in health impacts such as cardiovascular disease.

ACKNOWLEDGEMENTS

The authors wish to thank Mr. Billy Thompson, Ms. CharLee Martin, Mr. Eric Rigby, and Mr. Warren Fridy for their assistance in coordinating data access and security. The authors also wish to thank the participants and the original depositors of the CARDIA study.

FOOTNOTES

Author contributions: The author confirms sole responsibility for the following: study conception and design, data collection, analysis and interpretation of results, and manuscript preparation.



Institutional review board statement: The study was reviewed and approved by the WIRB-Copernicus Group Institutional Review Board (Protocol Number: 1332892).

Conflict-of-interest statement: The author declares no conflict of interest.

Data sharing statement: Summary statistics for significant variants are available from the corresponding author at richard.chapleau@nsas-llc.com.

ARRIVE guidelines statement: The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

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S-Editor: Liu JH L-Editor: A P-Editor: Liu JH

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World J Med Genet 2023 June 2; 11(2): 21-27

DOI: 10.5496/wjmg.v11.i2.21

ISSN 2220-3184 (online)

CASE REPORT

Mosaicism of a novel variant in the ANKRD11 gene in a child with a mild KBG phenotype: A case report

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Specialty type: Endocrinology and metabolism

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0 Grade B (Very good): B, B Grade C (Good): 0 Grade D (Fair): 0 Grade E (Poor): 0

P-Reviewer: Mijwil MM, Iraq; Zhang XQ, China

Received: February 6, 2023 Peer-review started: February 8, 2023 First decision: April 28, 2023 Revised: May 3, 2023 Accepted: May 19, 2023 Article in press: May 19, 2023 Published online: June 2, 2023



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Abstract

BACKGROUND

KBG syndrome is likely underdiagnosed because of mild and non-specific features in some affected patients especially before the upper permanent central incisors eruption at about the age of 7-8 years. Somatic mosaicisms are usually recognized in the parents only after a typically affected son is diagnosed with KBG syndrome. We describe for the first time the mosaicism of a novel variant in a child with a mild KBG phenotype.

CASE SUMMARY

Our patient presented at 24 mo of age with short stature, hand abnormalities, facial dysmorphism and mild developmental delay. Pituitary hypoplasia and central hypothyroidism were also detected. By next generation sequencing (NGS) analysis we found a novel deletion in the ANKRD11 gene (c.4880_4893del.), that



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can be classified as likely pathogenic for the syndrome, with the percentage of mutated allele of 36%. We considered this finding as causative of the mild and non-specific phenotype for KBG syndrome in our patient, as previously reported in adults. A heterozygous variant in *HESX1* gene, classified as variant of uncertain significance, but suspected of causing pituitary hypoplasia and hormonal deficiency, was also found. The patient started levothyroxine and growth hormone treatment.

CONCLUSION

The increased use of NGS analysis may expand the phenotypic spectrum of KBG syndrome because it allows genetic diagnosis of somatic mosaicisms also in children.

Key Words: ANKRD11; KBG; Mosaic; HESX1; Child; Case report

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Core Tip: Somatic mosaicisms of KBG syndrome are usually recognized in the parents only after a typically affected son is diagnosed. We report for the first time the case of a somatic mosaicism for KBG syndrome diagnosed in a child with a mild and non-specific phenotype. The increased use of next generation sequencing allows a genetic diagnosis of this mosaic form in children expanding the phenotypic spectrum of the KBG syndrome.

Citation: Franceschi R, Rivieri F, Novelli A, Ferretti D, Anesi A, Soffiati M, Porretti G, Maines E, Mucciolo M, Radetti G. Mosaicism of a novel variant in the *ANKRD11* gene in a child with a mild KBG phenotype: A case report. *World J Med Genet* 2023; 11(2): 21-27 **URL:** https://www.wjgnet.com/2220-3184/full/v11/i2/21.htm

DOI: https://dx.doi.org/10.5496/wjmg.v11.i2.21

INTRODUCTION

"KBG" represents the initials of the last name of the first three families diagnosed with the syndrome and is a rare genetic disease (OMIM 148050). Common manifestations are macrodontia (especially of the upper central incisors), typical facial features, short stature, skeletal anomalies, hearing loss, global developmental delay, and intellectual disability[1-4]. The transmission of this disease is autosomal dominant, and is caused by either heterozygous ANKRD11 point mutations (OMIM 611192) or microdeletion in chromosome 16q24.3 including the ANKRD11 gene[5] or ANKRD11 intragenic duplication[6]. The ANKRD11 gene encodes an ankyrin repeat containing protein (ANKRD11) which is indispensable in neuron proliferation and acts as a transcriptional repressor by two transcriptional repression domains (RDs: RD1, aa 318–611; and RD2, aa 2369–2663) and promoting transcription through one activation domain (AD), aa 1851–2145[1]. Since 1975, over 200 KBG patients have been described[1].

KBG syndrome is likely underdiagnosed because of mild and non-specific features in some affected patients especially before the upper permanent central incisors eruption at about the age of 7-8 years[2, 7]. Macrodontia of the permanent upper incisors is a typical finding, making diagnosis prior to the eruption of these teeth a challenge[2]. According to the latest diagnostic criterion, KBG syndrome should be considered in a patient with cognitive delay/learning difficulties, speech delay or behavioral anomalies with at least two major criteria or one major and two minor criteria[2]. Major criteria are: (1) Macrodontia or phenotypic features of KBG in child with primary dentition; (2) height < 10th centile; (3) recurrent middle ear infections and/or hearing loss; and (4) 1st degree relative with KBG syndrome. Minor criteria are: Brachydactyly or relevant hand anomaly; epilepsy; cryptorchidism; feeding difficulties; palate abnormalities; autism; large anterior fontanelle and/or delayed closure. A phenotypic variability among KBG patients has been observed intra- and interfamilial, and between patients with the 16q24.3 microdeletion compared to those harboring *ANKRD11* gene mutations[1]. Somatic mosaicisms have been reported in the parents after a typically affected son was diagnosed with KBG syndrome, and exhibited a milder phenotype, suggesting that KBG phenotypes in adults might be dose-dependent[5-7].

Here we describe for the first time in a child a mosaicism of a novel variant in the *ANKRD11* gene. The patient had a mild KBG phenotype and the diagnosis was performed by NGS analysis, providing insights into the spectrum of mosaic mutations.

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CASE PRESENTATION

Chief complaints

The proband came to our attention at the age of 24 mo, owing to postnatal growth retardation (Supplementary Figure 1).

History of present illness

The boy was born at term (41 wk) after a pregnancy achieved with egg fertilization by intracytoplasmic sperm injection. Birth weight was 3830 g (0.84 SD), length 53 cm (1.38 SD), head circumference 34 cm (-0.76 SD). Non-consanguineous parents had a normal stature (father 179 cm, mother 182 cm, mid-parent sex-adjusted target height 187 cm).

History of past illness

Not informative.

Personal and family history

Not informative.

Physical examination

His height was 89 cm (-2 SD), his weight was 12.6 Kg (-2 SD), and his head circumference was 50 cm (0 SD). Clinical examination revealed facial dysmorphisms, including tall forehead, widely spaced eyes, bushy eyebrows, left palpebral ptosis, prominent and anteverted ears, facial asymmetry. Skeletal anomalies included short fingers with fifth finger clinodactyly. He showed delay/cognitive impairment as assessed by Griffith's scale.

Laboratory examinations

Routine chemistry turned out as normal. ACTH was 16.7 pmol/L (normal range 5-25), cortisol 286.9 nmol/L (normal range 250-550), and prolactin 13.2 ug/L (normal range 4-15); insulin like growth factor 1 (IGF-1) 4.84 nmol/L (normal range 1.70-30.46), fT4 was repeatedly low: 9.8-10.2 pmol/L (normal range 12-22) with inappropriately normal TSH: 2.3-4.79 mU/L (normal range 0.2-4.5). Thyroid Ultrasound revealed an in situ and normal sized gland. An arginine stimulation test elicited a reduced peak of growth hormone (GH) peak 2.28 ng/mL and IGF-1 was 8.76 nmol/L (2.61-45.36). Considering growth retardation, psychomotor delay and dysmorphic features, clinical exome sequencing analysis was performed.

Imaging examinations

At 36 mo, bone age corresponded to 3 mo for the carpus and 12-16 mo for the phalanges, in the presence of mild malformation of the intermediate phalanx of the fifth finger (Supplementary Figure 2). He presented extra tarsal persistence of chalazion, with sub-palpebral hematomas. Pituitary magnetic resonance imaging (MRI), revealed a hypoplastic gland (Figure 1) with a normal pituitary stalk. Brain MRI excluded central nervous system abnormalities.

FINAL DIAGNOSIS

Exome sequencing analysis identified a deletion of 14 nucleotides in the *ANKRD11* gene, c.4880_4893delCCGCCGTCGTCTG. The deletion is a mosaic with the percentage of mutated allele of 36%. At protein level the deletion determines the introduction of a premature stop codon p.Ala1627GluTer9. The frameshift variant, not present in the father DNA, is not reported in the gnomAD database, and has never been described in literature. According to current the American College of Medical Genetics and Genomics (ACMG) guidelines[8], the variant can be classified as likely pathogenetic (class 4) for KBG syndrome.

Exome sequencing analysis also identified three variants: (1) A heterozygous variant in *HESX1* gene (c.541A>G, p.Thr181Ala, NM_003865.3), inherited from the father, who presented with normal height, TSH and fT4. This variant has been reported as heterozygous and pathogenetic in a patient with isolated growth hormone deficiency and pituitary hypoplasia[9] and in a patient with combined pituitary hormone deficiency, anterior pituitary hypoplasia, and ectopic posterior lobe[10]; in silico analysis suggests that this missense variant does not affect protein structure/function and according to current ACMG guidelines[8], the variant can be classified as variant of uncertain significance (VUS); (2) a hemizygous VUS in *ATRX* gene (c.189G>A, p.Glu63Glu, NM_000489.5); and (3) a heterozygous VUS in *NIPBL* gene (p.Ile1982Leu, p.Ile1982Leu). The latest two variants are not present in the father DNA.

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Figure 1 Pituitary magnetic resonance imaging revealed a hypoplastic gland with a normal pituitary stalk.

TREATMENT

Once the diagnosis of central hypothyroidism was confirmed, treatment with levothyroxine was started. After GH test, we started GH treatment that changed growth trajectory (Supplementary Figure 1).

OUTCOME AND FOLLOW-UP

The patient is on follow up at our outpatient clinic, he is now 7 years old, and he started GH six months ago.

DISCUSSION

KBG syndrome is a rare autosomal dominant disorder characterized by short stature, delay/cognitive impairment and distinctive craniofacial characteristics. It shows a wide spectrum of clinical phenotypes and it is likely underdiagnosed because of mild and non-specific features in some affected patients especially before the upper permanent central incisors eruption at about the age of 7-8 years. Here we present, for the first time in literature, the mosaicism (36%) of a novel variant in the ANKRD11 gene that underlies a mosaic KBG phenotype in a child.

This finding led us to conclude that the variant was acquired at a postzygotic level, and is classifiable as likely pathogenetic for KBG syndrome.

Somatic mosaicism is usually recognized in the parents only after a typically affected son is diagnosed with KBG syndrome, because patients with somatic mosaicisms exhibited a milder phenotype. The phenotypic effect of mosaic ANKRD11 haploinsufficiency might be dose-dependent[4] and some experiences in the literature confirm this hypothesis (Table 1).

Nevertheless, recent emerging evidence also suggests that somatic mosaicism is found in apparently healthy individuals and increases with age[11].

Our patient presented with short stature (-2SD and mid-parent sex-adjusted target height of 187 cm), that is very common among patients with KBG syndrome, being found in 40%-77% of affected patients [12]. We reported typical but milder features of KBG syndrome[12]: Dysmorphic features (widely spaced eyes, bushy eyebrows, ptosis and large protruding ears), delayed bone age, hand anomalies (clinodactyly and brachydactyly), mild developmental delay and mild ocular involvement (anisotropy and left eye exodeviation). Major problems as epilepsy, intellectual disability, spinal-costal anomalies, heart defects, hearing loss, kidney abnormalities, or feeding problems, were not presented by our patient[3].

Interestingly, our patient presented with extra tarsal persistence of chalazion, with sub-palpebral hematomas; skin and hair abnormalities have been previously reported in KBG syndrome: one patient with a tendency to skin bruising, and delayed wound healing, and another with keloid scarring[3].

Primary subclinical hypothyroidism has been described in KBG syndrome[12], but our patient presented with secondary (pituitary) hypothyroidism. Our patient presented also pituitary hypoplasia,



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Ref.	Sex and age	Molecular analysis	% mosaicism	Phenotype	Phenotype in relative with the same non-mosaic mutation	
Our case	2 yr	Deletion (c.4880_4893del.)	36	Short stature, hand abnormalities, facial dysmorphism, mild developmental delay	-	
Khalifa et al[5], 2013	Female, 31 yr (mother)	Microdeletion 16q24.3	38	Round face, brachy- cephaly, macrodontia, abnormal dentition with malposition and extra teeth, brachy- dactyly, postaxial polydactyly, partial syndactyly between the 2 nd and 3 rd toes, short stature, learning difficulty	Female, 11 yr, Multiple congenital abnormalities including patent foramen ovale, umbilical hernia, hypospadias with chordee, penile-scrotal fusion, intestinal malrotation, chronic interstitial pulmonary disease, febrile seizure, pharyngeal dysphagia, developmental delay, dysmorphic features (round face, epicanthic folds, hypertelorism, broad arched eyebrows with synophrys, a flat nasal bridge, and a relatively small nose with a bulbous tip), brachycephaly, short neck, macrodonzia, dental malocclusion, chronic otitis media, partial syndactyly between the 2 nd and 3 rd toes, delayed bone age	
Crippa <i>et al</i> [6], 2015	NA (mother)	Microduplication 16q24.3 (chr16:89,350931-89439639, hg19)	5	Mild facial dysmorphisms, similar to those of her children, and a nasal voice	Male, 17 yr. Short, stature, moderate intellectual disability, facial dysmorphisms including long triangular face, frontal bossing, arched and bushy eyebrows with slight synophrys, large and prominent ears, broad and high nasal bridge with bulbous nasal tip, anteverted nares, long philtrum, macrodontia of central incisors, and a nasal voice, brachymetacarpia, third- degree vesicoureteral reflux	Female, 13 yr. Short, stature, moderate intellectual disability, facial dysmorphisms including long triangular face, frontal bossing, arched and bushy eyebrows, large and prominent ears, broad and high nasal bridge with bulbous nasal tip, anteverted nares, long philtrum, macrodontia of central incisors, and a nasal voice, brachymetacarpia, ureterocele associated with duplex pelvicalyceal district
Guo et al[7], 2022	Female, 30-35 yr (mother)	c.5227C>T p. (Gln1743*)	Only 2 out of 298 sequencing reads for this variant found in her blood	History of miscarriage, mild facial features, (<i>e.g.</i> , synophrys, thick eyebrow, wide nasal bridge, prominent nasal tip) with speech delays and seizures in childhood	Male, 5-10 yr. More severe phenotypic features in comparison to the mother, history of seizures and concurrent speech and motor delays, mitral valve repair at around one year of age, abdominal migraines	

NA: Not available

up to now reported as associated to KBG in only one patient[4] who presented with hypogonadotropic hypogonadism at 15 years, and GH deficiency.

Mutations in the transcription factor *HESX1* can cause several congenital pituitary defects, ranging from isolated growth hormone deficiency [9,13] to septo-optic dysplasia (SOD) with panhypopituitarism [14]; most patients carry mutations at the heterozygous state, invariably associated with reduced penetrance, and generally show a milder phenotype than the rare homozygous patients[9,15]. According to us, in our patient pituitary hypoplasia, central hypothyroidism and GH deficit might be explained by the variant in HESX1 gene.

CONCLUSION

Table 1 Cases of mosaic KBG phenotype reported in the literature

In conclusion, we reported for the first time in literature the case of a somatic mosaicism for KBG syndrome, diagnosed in a child with a mild and non-specific phenotype that included short stature, hand abnormalities, distinctive facial dysmorphism and mild developmental delay, in absence of macrodontia consistent with his age. A heterozygous variant in HESX gene, strongly suspected of causing pituitary hypoplasia and hormonal deficiency was also found.

KBG syndrome is likely underdiagnosed because of mild and non-specific features in some affected patients; mosaic forms are even more challenging. Our case underlines that the recognition of mosaicism is important not only for establishing a diagnosis, but also for assessing recurrence risk and for providing genetic counseling to the family. Our paper increases awareness of mild forms of KBG syndrome in children and underlines the importance of NGS analysis for an early genetic diagnosis of



mosaic forms.

ACKNOWLEDGEMENTS

We appreciate the father for his collaboration.

FOOTNOTES

Author contributions: Franceschi R, Rivieri F, Maines E, Anesi A, Soffiati M, Porretti G, and Radetti G followed the patient up; Novelli A, Ferretti D and Mucciolo M performed the genetic test; Franceschi R, Radetti G, Maines E and Mucciolo M drafted the manuscript; All authors critically reviewed and edited the manuscript, and approved the final version as submitted.

Informed consent statement: Informed written consent was obtained from the father of the patient for publication of this report. The father refused consent to publish child's picture.

Conflict-of-interest statement: The authors declare that they have no conflict of interest to disclose.

CARE Checklist (2016) statement: The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist (2016).

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S-Editor: Liu JH L-Editor: A P-Editor: Liu JH

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