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ABOUT COVER

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REVIEW

Neurologic orphan diseases: Emerging innovations and role for genetic treatments

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Abstract

Orphan diseases are rare diseases that affect less than 200000 individuals within the United States. Most orphan diseases are of neurologic and genetic origin. With the current advances in technology, more funding has been devoted to developing therapeutic agents for patients with these conditions. In our review, we highlight emerging options for patients with neurologic orphan diseases, specifically including diseases resulting in muscular deterioration, epilepsy, seizures, neurodegenerative movement disorders, inhibited cognitive development, neuron deterioration, and tumors. After extensive literature review, gene therapy offers a promising route for the treatment of neurologic orphan diseases. The use of clustered regularly interspaced palindromic repeats/Cas9 has demonstrated positive results in experiments investigating its role in several diseases. Additionally, the use of adeno-associated viral vectors has shown improvement in survival, motor function, and developmental milestones, while also demonstrating reversal of sensory ataxia and cardiomyopathy in Friedreich ataxia patients. Antisense oligonucleotides have also been used in some neurologic orphan diseases with positive outcomes. Mammalian target of rapamycin inhibitors are currently being investigated and have reduced abnormal cell growth, proliferation, and angiogenesis. Emerging innovations and the role of genetic treatments open a new window of opportunity for the treatment of neurologic orphan diseases.

Key Words: Neurologic orphan diseases; Gene therapy; Clustered regularly interspaced palindromic repeats/Cas9; Antisense oligonucleotides; Adeno-associated virus; mTOR inhibitors

Core Tip: Neurologic orphan diseases are rare conditions that impact a small percentage of the population. Through new advances in technology and research, the use of genetic treatment for these conditions is increasing. Recent advances in clustered regularly interspaced palindromic repeats/Cas9, adeno-associated viral vectors, antisense oligonucleotides, and mammalian target of rapamycin inhibitors have shown improvements in the care of patients and their quality of life.

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INTRODUCTION

Orphan diseases are rare diseases that affect less than 200000 individuals within the United States[1]. Despite being rare, these diseases affect over 300 million individuals globally[2]. Of the 7000 diseases listed on the National Institute of Health's Office of Rare Diseases site, most are neurological and of genetic origin[3]. 90% of orphan diseases have serious neurological effects[2]. Neurologic orphan diseases are fatal, drastically decrease quality of life, and are defined by long periods of disability[4]. However, diagnosis and treatment of rare central nervous system (CNS) disorders pose a challenge[2]. Lack of access to diagnostic genomic sequencing, screening tests, and specialists contributes to the difficulty of diagnosing and managing neurologic orphan diseases[2]. Most neurologic orphan diseases don't have treatments that prevent disease progression[4]. Additionally, clinical trials investigating neurologic orphan disease therapeutics have the lowest success rate[5]. Global Genes, a rare disease advocacy organization, states that since 2021, a total of $22.9 billion has been invested in research on neurologic orphan diseases, which offers a promising future for patients with these conditions[6].

One study found that the use of whole genome sequencing within clinical practice increases the diagnosis of neurologic orphan diseases[7]. Genetic conditions such as Huntington's disease and Friedreich's ataxia are caused by repeat expansions which can effectively be detected by whole genome sequencing due to its high sensitivity and specificity for repeat expansions[7].

The Patient Identification and Engagement for Rare CNS Disorders initiative is designed to investigate and improve barriers to diagnosis and clinical research trials[2]. This initiative plans to address the underrepresentation of certain groups from clinical trials and to improve access for those individuals in the participation of trials investigating new gene therapy approaches[2]. The future for the diagnosis and management of neurologic orphan diseases looks promising and hopes to improve the efficacy of therapeutic agents by gene targeting methods. Some forms of gene therapy include the use of clustered regularly interspaced palindromic repeats (CRISPR)/Cas9, antisense oligonucleotides (ASO), adeno-associated viruses (AAV), and mammalian target of rapamycin (mTOR) inhibitors.

CRISPR/Cas9 is a form of gene-editing where a guide RNA binds to a target sequence of genomic DNA, followed by the endonuclease, Cas9, binding to the guide RNA. Cas9 then creates a double strand break in the genomic DNA which is then repaired. This process allows for the inclusion or exclusion of desired genes to create a desired mutation[8].

ASO are small molecules that can modify gene expression preventing or altering protein production. If a certain protein is undesired, an ASO can be designed to cause the protein to be terminated or partially expressed and modify it so that it is not harmful[9].

AAV are used as a vector for gene therapy by using a non-enveloped virus engineered to deliver deoxyribonucleic acid (DNA) to targeted cells[10]. This gene therapy has shown preclinical and clinical access in gene replacement, gene silencing, and gene editing[11].

mTOR is a kinase closely correlated with the occurrence of neurodegenerative diseases and tumors in humans. The goal of mTOR inhibition therapies is to block the mTOR signaling pathway that may be contributing to abnormal signal transduction to block the occurrence and development of disease[12].

MUSCULAR DETERIORATION

Spinal muscular atrophy

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disease caused by deletions or mutations in the survival motor neuron (*SMN1*) gene[13]. Specifically, diagnostic testing commonly demonstrates the absence of SMN1 exon 7 on chromosome 5[14,15]. SMA is characterized by progressive muscle weakness and atrophy resulting from

progressive degeneration and irreversible loss of anterior horn cells in the spinal cord[16]. The severity of SMA can range from more mild cases where the onset occurs in adulthood and progresses at a slow rate to more severe cases where the onset can occur in the first months of life and result in respiratory failure[17].

In recent years, new treatment options like gene therapy involving splicing modulation of SMN2 and SMN1 genes and the development of the first approved drugs for SMA treatment have shown promise in treating SMA. When therapy is initiated early, it can significantly alter the natural course of the disease. Current evidence in these treatments is limited to a small scope of patients and more research is needed for conclusive results[17].

Nusinersen was the first drug that received approval for the treatment of SMA. Nusinersen enhances the inclusion of exon 7 in mRNA transcripts of SMN2 by suppressing the binding of certain splicing factors which results in an increase in functional SMN2-mRNA with included exon 7[18-20]. Various studies in infants and young children have displayed improvements in prolonged time of death and improved motor functions[21-23]. Furthering the potential of SMN2 gene alteration, Risdiplam, and Branaplam are oral medications that have been shown to cross the blood-brain barrier and increase the number of full-length SMN proteins[24].

Gene therapy has also shown promise with SMN1-gene replacement. Studies in mice have shown prolonged survival following successful vector delivery of intact SMN1-gene across the blood-brain barrier[25,26]. Zolgensma is a gene therapy medicine that is administered as an intravenous infusion and uses adeno-associated virus vectors to deliver a functional copy of SMN to motor neuron cells[27]. Clinical trials in children treated with Zolgensma have shown improved survival, motor function, and developmental milestones following treatment[28,29]. A summary of emerging treatment options for SMA can be found in Figure 1 below.

Duchenne muscular dystrophy

Duchenne muscular dystrophy (DMD) is the most common hereditary neuromuscular disease and is one of the most severe forms of inherited muscular dystrophy. Symptoms of DMD include severe and progressive muscle wasting, muscle weakness, and difficulty with movement[30]. Late stages of DMD often require the need for assisted ventilation and the use of a wheelchair to perform daily activities and lead to premature death in the mid-twenties due to respiratory muscle weakness or cardiomyopathy[31].

DMD is an X-linked inherited disorder that predominantly affects males. The onset of DMD occurs due to mutations in the dystrophin gene on chromosome Xp21. This results in a ceased production of dystrophin in cardiac and skeletal muscle. The absence of dystrophin results in a loss of myofibril membrane integrity through cycles of necrosis and regeneration. Fibrous connective tissue and fat then replace muscle over time, resulting in the progression of expressed clinical symptoms[30,32].

In recent years, there has been progress in the development of diagnosis and therapeutics for DMD, but the current treatments given do not cure DMD[33]. Daily prednisone treatment is commonly used to increase muscle strength and function, improve pulmonary function, and significantly slow the progression of weakness[34,35]. While this treatment does not cure the disease, it does improve the overall quality of life for patients.

The most advanced therapy is antisense oligonucleotides -mediated exon skipping which has shown promise in clinical application. For this technique, the administration of 20-30 bp long antisense oligonucleotide hybridizes to splice motifs necessary for pre-mRNA processing and mask RNA splicing signals. This leads to the exclusion of the intron and adjacent exon, creating an in-frame mRNA without the targeted exon. This mRNA can then be translated into a truncated and partially functional dystrophin protein[33,36].

CRISPR/Cas technology has been used therapeutically to treat DMD by upregulating a dystrophin homolog, utrophin, to compensate for the lack of dystrophin protein which has been successfully demonstrated in patient cells. In these patients, full-length dystrophin was restored in patient cells carrying duplication mutations[37]. Manipulations resulting from CRISPR-Cas9 were shown to restore the expression of truncated but partially functional dystrophin, improve skeletal and cardiac muscle function, and increase the survival of mdx mice significantly[38].

Emerging vector-mediated gene therapy in mice has been shown to deliver a functional DMD gene to cells lacking dystrophin protein and has been shown to increase exercise capacity[39]. While promising, this technique is challenging due to the very large size of the dystrophin gene and the widespread distribution of muscles[40].

EPILEPSY & SEIZURES

Dravet syndrome

Dravet syndrome (DS), first described by Charlotte Dravet in 1978, is an early-onset epileptic syndrome characterized by a variety of refractory seizures and neurodevelopmental impairment that often persist into adulthood[41,42]. The clinical features of DS typically progress over time, most commonly first presenting as a bilateral tonic-clonic in the first year of life, with half of the patients being febrile[43]. The disease progresses to multiple types of seizures, often leading to poor therapeutic control and the majority of patients (93%) experiencing status epilepticus[43,44]. Additionally, patients with DS will develop neurodevelopmental delay, as well as motor and cognitive impairment that will persist into adulthood. It should be noted, however, the diagnosis is highly clinical as both magnetic resonance imaging (MRI) and electroencephalogram (EEG) studies may be nonspecific[45].

Previously named severe myoclonic epilepsy of infancy, the molecular basis of DS arises from a *de novo* mutation on chromosome 2q24 on the sodium voltage-gated channel alpha subunit (SCN1A) gene[45,46]. Although the role of the SCN1A variant in the pathogenesis of DS has not been fully elucidated, some studies have suggested that the diffuse neuronal hyperactivity in DS patients is correlated with a loss of inhibitory GABAergic interneurons which have an

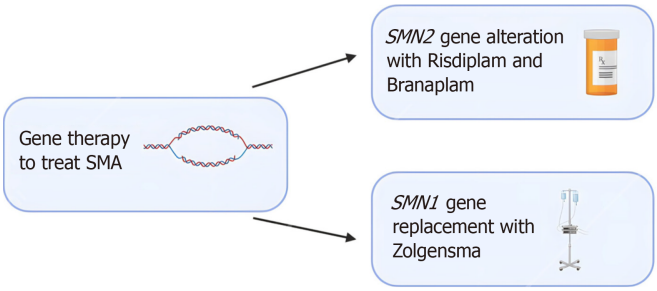

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Figure 1 Emerging gene editing treatment options for spinal muscular atrophy. Risdiplam and Branaplam are oral medications which can cross the blood-brain barrier and increase the number of spinal muscular atrophy (SMA) full length proteins by targeting the *SMN2* gene. For patients that require replacement of the *SMA1* gene, Zolgensma is an intravenous medication that uses an adeno-associated viral vector to deliver a functional copy of the gene. Clinical trials in patients treated with Zolgensma have shown positive outcomes. SMA: Spinal muscular atrophy.

SCN1A mutant in non-coding regions[47,48].

Currently, DS is managed symptomatically with a series of anti-seizure medications (valproic acid being the first line of treatment), though this regimen has variable efficacy[48]. Gene-specific therapies for the causes of DS continue to gradually emerge. One study used Targeted Augmentation of Nuclear Gene Output technology, utilizing ASO, to successfully increase the expression of the SCN1A protein in mice models. In addition to a higher expression of this SCN1A product, the incidence of seizures in these DS mice was significantly reduced[49]. The use of viral vectors to target genes has shown success in some diseases but poses a challenge in DS[50]. The SCN1A coding sequence is 6kb long, which exceeds the carrying capacity of adeno-associated viruses[50]. However, the use of a different viral vector, such as lentiviruses, poses another challenge as it demonstrates a limited spread in neural tissue therefore it cannot effectively treat large brain areas[50]. Another approach to upregulating the expression of SCN1A is through the use of dCas9-based gene activation systems. One study found that the use of dCas9 resulted in the upregulation of SCN1A in brain tissue and cultured neurons[51]. Overall, there have been significant developments in viral vectors and molecular techniques that demonstrate promising results.

Ohtahara syndrome

Ohtahara syndrome, also referred to as Early Infantile Developmental and Epileptic Encephalopathy (EIDEE) syndrome, is a group of devastating pediatric conditions characterized by frequent spasms in neonates and infants leading to severe cognitive and physical disabilities and even death[52,53]. There are numerous causes of Ohtahara syndrome, including structural brain defects, metabolic derangements, and genetic variants. Multiple studies have identified the most common variants associated with Ohtahara syndrome are PRRT2, SCN1A, KCNQ2, and SLC2A1[54]. The extensive distribution of these channelopathies and their various penetrance help explain the evolution of focal seizures to status epilepticus seen in EIDEE[54,55].

Seizure episodes in Ohtahara syndrome are initially treated with anti-seizure medications but are usually only in managing the frequency of seizures. Patients with Ohtahara syndrome with KCNQ2, SCN2A, or SCN8A mutations have been found to respond to sodium channel anti-seizure medications, such as carbamazepine, lacosamide, or phenytoin[56,57]. Additionally, implementing a ketogenic diet has been shown to provide some improvement in many infants[58]. Currently, the rise in genetic testing has allowed identifying the monogenic etiology of various EIDEE. An AAV-based gene replacement therapy has been proposed for Ohtahara syndrome particularly for the transmembrane sodium channel SLD13A5, though these principles could provide insight into future therapeutic targets of Ohtahara syndrome[59]. However, challenges exist for gene therapy due to the limited knowledge of the disease mechanism and progression of Ohtahara syndrome. Further research is needed in patients with Ohtahara syndrome to evaluate the efficacy of gene therapies such as viral vectors. A summary can be found in Figure 2 below.

Lennox-gastaut syndrome

Lennox-gastaut syndrome (LGS) is another severe pediatric epileptic and encephalopathic disorder characterized by severe pediatric seizures, treatment-resistant epilepsy, and cognitive impairments[60,61]. As in various other childhood epilepsy disorders, LGS is caused by several etiologies, including genetic predispositions, anatomical brain abnormalities, hypoxic-reperfusion encephalopathies, meningitis, and head trauma[61]. The majority of children affected by LGS have underlying genetic disorders, often chromosomal syndromes, or *de novo* pathogenic variants[61,62].

As LGS is characterized by seizures resistant to pharmacologic therapy, management often includes a multidisciplinary approach, often including dietary and surgical interventions. In one case series, 50% of children received a greater than 50% reduction in seizure frequency, and almost a quarter of the children achieved a greater than 90% reduction[63]. Additionally, the serotonergic agent fenfluramine is commonly used in LGS and has been shown to have significant benefits for the reduction of generalized tonic-clonic seizures and drop seizures[64]. Fenfluramine increases the level of serotonin in the extracellular compartment and acts as a serotonergic 5-HT2 receptor agonist and an alpha-1 receptor antagonist to decrease anti-epileptic activity. Since LGS seizures evolve over a patient's life, an LGS algorithm detailing several anti-epileptics medications as well as adjunctive therapy including a ketogenic diet, possible surgical resection,

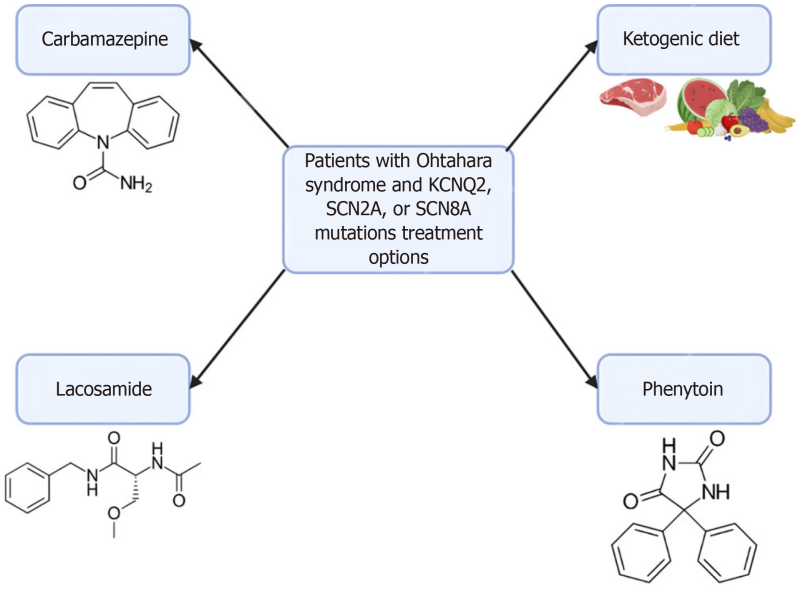

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Figure 2 Treatment options for patients with Ohtahara syndrome. In patients with Ohtahara Syndrome with KCNQ2, SCN2A, or SCN8A mutations, there are several treatment options available. Carbamazepine, lacosamide, and phenytoin are sodium channel anti-seizure medications. These medications don't offer a cure and are currently only used to manage the frequency of seizures. Additionally, implementation of a ketogenic diet has been shown to reduce seizure frequency in infants

and close EEG monitoring[65].

NEURODEGENERATIVE MOVEMENT DISORDER

Friedreich ataxia

In the discourse of neurologic orphan diseases, one important consideration in the context of neurodegenerative movement disorders is Friedreich Ataxia (FRDA). In the second half of the 19th century, its original description by German professor of medicine at Heidelberg, Nikolaus Friedreich, remarked FRDA was a degenerative atrophy of the posterior spinal cord columns[66]. Further, between the years of 1863-1877, Friedreich published the earliest and most extensive works on "Friedreich's ataxia", which provided insightful descriptions of this neurological disorder as being marked with the principal abnormality of axonal thinning without axonal loss of the dorsal spinal roots[66]. Unfortunately, Friedreich received little recognition for his academic effort during his lifetime, however, an anonymous obituary of "perhaps his most important work" details Friedreich's rapid scientific progress and 59 publications[67]. Nonetheless, the full extent of FRDA's etiology couldn't be fully appreciated until direct genetic testing became available in the late 1960s[68]. Thus, the proceeding discussion will review current knowledge on this debilitating condition and evaluate the recent developments in treatment approaches.

As the most common inherited ataxia—defined as the compromised coordination of voluntary muscle movement—FRDA is an autosomal recessive, neurodegenerative disorder involving multiple organ systems including the central and peripheral nervous systems and the cardiovascular system[68,69]. At the molecular level, a gene, X25, encodes a 210-amino acid protein known as "frataxin", or FXN, and has been identified as a critical region susceptible to mutation for the FRDA locus on chromosome 9q13[69]. Specifically, the most common defect leading to the development of FRDA is due to a large, homozygous intronic expansion of guanine–adenine–adenine (GAA) trinucleotide repeats in intron 1 of the said frataxin gene[70,71]. Frataxin, a highly conserved small mitochondrial protein, is required for efficient regulation and homeostasis of cellular iron stores[72]. Consequently, the dysregulation of this critical molecule's function is associated with mitochondrial iron overload, iron-sulfur cluster biosynthesis, and free radical oxidative stress[73,74]. A graphical representation of the molecular etiology and subsequent biological consequences of dysfunctional frataxin protein function can be seen in Figure 3 below.

Clinically, due to frataxin's ubiquitous biochemical role in numerous cellular pathways, humans with frataxin deficiency manifest with dysfunction in the central and peripheral nervous system, heart, skeleton, and even endocrine organs including the pancreas[71]. Regarding FRDA in particular, many of these patients classically present with neuropathologic disabilities including progressive ataxia, peripheral sensory loss, and muscle weakness beginning between the ages of 5 and 15 years old[76]. The aforementioned manifestations are chiefly secondary to neuropathy in the dorsal root ganglia, accompanied by degeneration of both peripheral sensory nerve fibers and posterior columns of the spinal cord[76]. Moreover, non-neurological areas of morbidity include the heart, typically in the form of left ventricular hypertrophy due to mitochondrial proliferation, and the pancreas, with approximately 10% of all FRDA patients developing diabetes mellitus[76,77].

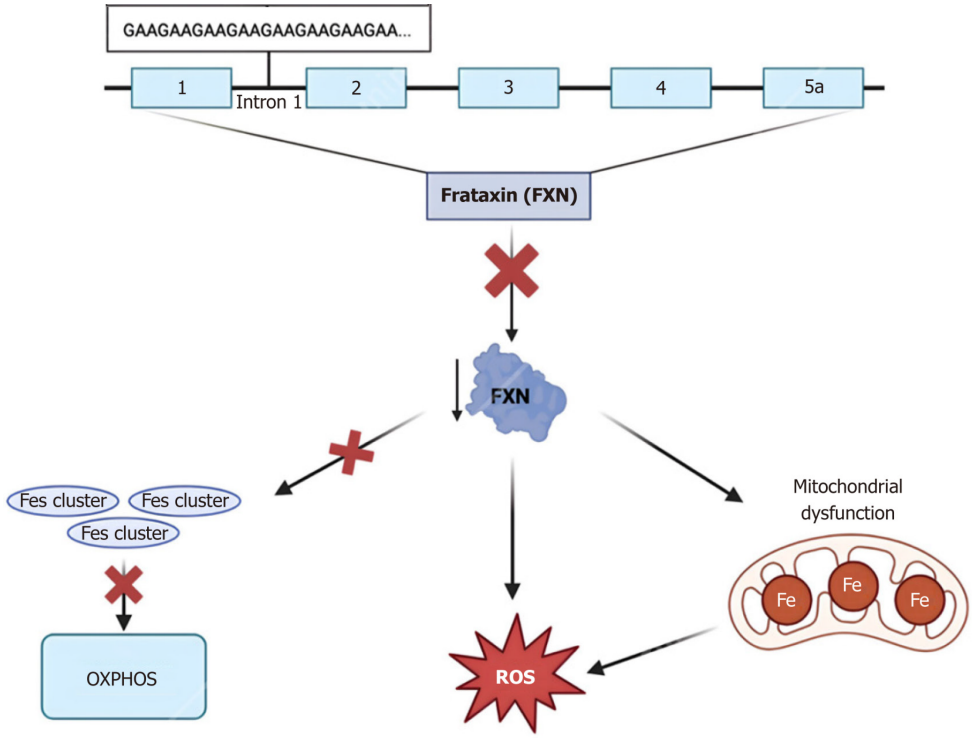

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Figure 3 Frataxin protein dysregulation. The 2100 amino acid protein frataxin is encoded within the first intron of the *FXN* gene on chromosome 9q13. Due to trinucleotide repeat expansions ranging from approximately 44–1700 "GAA" triplet sequences, affected individuals experience numerous characteristic signs and symptoms of Friedreich Ataxia. At the cellular level, dysregulation of this small mitochondrial protein results in the overproduction of iron-sulfur clusters, free radical oxidative stress, and mitochondrial iron overload[75]. FXN: Frataxin; ROS: Reactive oxygen species.

Before the dawn of genetic testing, the constellation of the above-mentioned signs and symptoms as well as MRI was employed in the diagnosis of FRDA[78]. Presently, however, this neurologic disorder is more precisely diagnosed using modern genetic testing such as Southern blot and conventional polymerase chain reaction (PCR) techniques[79]. The accurate diagnosis of FRDA helps in differentiating it from other ataxias and provides a guide for physicians to appropriately tackle the treatment and management of this progressive disorder. Despite the heterogeneity of symptomatic presentation, most patients will lose the ability to independently walk, stand, and/or sit within 10-15 years of initial disease onset[76]. Therefore, there is a persisting call for advancements in techniques and approaches for treating FRDA.

In the past, there were no effective protective pharmacological agents for FRDA, and thus treatment was aimed at symptomatic management and physical therapy for impaired motor function[80]. Further, aggressive surveillance was and still is used in managing progressive cardiomyopathy, arrhythmias, and diabetes mellitus to improve the quality of life for these patients[80,81]. More recently, treatment approaches to address the mitochondrial dysfunction caused by a mutation to the frataxin protein include mitochondrial function enhancers, free radical scavengers, and iron chelators such as coenzyme Q_{10} and its synthetic analog idebenone, as well as vitamin E[82]. Although the employment of such molecules is mechanistically sound, the pre-clinical and clinical trial data on their use has demonstrated little to no therapeutic effect across multiple studies and even worsened enzymatic activity[80]. As of February 2023, however, the first FDA-approved treatment for FRDA became available within the United States. Developed by Reata Pharmaceuticals, Inc., the small molecule Omaveloxolone, or SKYCLARYS™, is an orally active drug aimed at alleviating oxidative stress, mitochondrial damage and dysfunction as a direct result of Nrf2 pathway suppression found in Friedreich patients[83]. As a novel pharmaceutical with early proven safety and effectiveness, Omaveloxolone is a promising emerging option for FRDA treatment.

In addition to symptomatic treatment and management, newer therapeutics for neurological disorders are being developed, evidenced by the rapid increase in research surrounding gene and cell therapy for FRDA between the years 2000 and the present day. Considering the severity of neurophysiological abnormalities is strongly correlated with the size of the GAA repeat expansion implicated in the genetic inheritance of FRDA, many of these novel techniques are aimed at altering this pattern. Specifically, the use of the CRISPR-Cas9 system in GAA expansion-based animal models such as YG8-derived cells and mouse models demonstrated promising results in the successful editing of GAA expansion both *in vitro* and *in vivo*[84]. Contrastingly, instead of altering the primary genetic mutation, other more recent methods of ataxia reversal are aimed at inducing the expression of the frataxin protein itself[85]. Particularly, Piguet *et al*[86] demonstrated the complete reversal of sensory ataxia and cardiomyopathy *via* viral vector-based introduction of AAV-expressing frataxin (AAV-FXN) in cells and mouse models. Finally, other prospective therapeutics look to stimulate the direct transcription of FXN and/or increase FXN mRNA stability *via* interferon administration, or indirectly *via* the stimulation of NRF2—a transcription factor whose levels are intimately associated with FXN mRNA expression[87].

Altogether, these animal-based models hold promising treatment options for future application to clinical medicine, for now, more research is necessary before translation into FRDA human-based clinical trials.

INHIBITED COGNITIVE DEVELOPMENT

Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are the first known examples of diseases relative to imprinted genes. These diseases have their own clinically distinct behavioral, cognitive, and neurologic phenotypes. However, they are still considered "sister disorders" due to the common origin of the disease from the imprint gene abnormalities in the 15q11-q13 region[88]. The difference in the diseases is that with PWS, the contribution is paternal whereas with AS, the contribution is maternal. This parent-of-origin difference is the reason for the variable expression of the disease depending on the sex of the parent from whom the disease is inherited[88,89].

PWS

PWS is suspected in individuals that present with clinical features of hypotonia (during the first few years) that lead to hyperphagia, hypogonadism, short stature, and mild mental retardation[90]. However, regarding the 15q11-q13 region, the abnormality is suggestive of PWS, but not diagnostic. The diagnosis of PWS is established by identifying the abnormal DNA-methylation and maternal-allele imprinting in the Prader-Willi critical region. This can be due to either the deletion of the paternal allele, maternal disomy, and/or imprinting defect causing the absence of the paternal allele [91]. Moreover, point mutation does not cause PWS because it is a factor of multiple gene products. An exception to this rule is when the point mutation is seen in the imprinting control region[92]. In these cases, although very rare, the loss of function has been seen to contribute to many different aspects of the PWS phenotype[93].

The underlying molecular defect in PWS further opens a great scope of molecular treatment and epigenetic therapy. An increasing number of studies are pointing toward gene SNORD116 being the main causative player in PWS[94-96]. Deactivation of the SNORD116 gene is associated with a complex of a zinc finger protein ZNF274 and SETDB1 a histone H3 methyltransferase[97-99]. Thus, CRISPR-Cas systems can be employed to deactivate ZNF274 and/or SETDB1 and increase the expression of SNORD116. One of the drawbacks to this approach is that the SETDB1 is not specific and thus, targeting it can have many off-label systemic effects.

Another approach explored in many studies was to inhibit G9a, another histone H3 methyltransferase, which restored the targeted genes from the silenced maternal chromosome[100-102]. Based on these studies, the use of a G9a inhibitor improved perinatal lethality and poor growth, which ultimately can improve the life span[100-102]. But again, one of the drawbacks of these research studies is that they are all performed with either mouse models or PWS patient-derived cells. Thus, the efficacy of gene therapy in PWS patients is yet to be explored and proven to be beneficial. Moreover, during the development of new drug therapies, focus should be put on off-target effects. Epigenetic drugs are known to have complex and broad effects, and thus, the development of specific molecular therapy for PWS patients is necessary.

AS

AS clinically presents as severe developmental delay, minimal or no speech, difficulty walking, and a unique behavioral phenotype that includes frequent smiling and excessive laughter[103]. As mentioned above, defects in the 15q11.2-q13 chromosome region are the genetic basis of both PWS and AS. In terms of AS however, the maternally expressed allele does not produce functional gene product, and the paternal allele is imprinted. The gene of interest in terms of AS is the UBE3A sequence which is mutated in certain individuals with AS[103].

In terms of epigenetic therapy, restoration of the mutated UBE3A gene expression is more favorable and plausible rather than targeting known activities of the molecule. However, there haven't been any successful ways researched to do so. Thus, expressing the silenced paternal UBE3A has been an adequate alternative. The most common way proposed is by administering topoisomerase-I inhibitors and reactivating the inhibition of paternal UBE3A.

Topoisomerase-I inhibitors like topotecan and irinotecan are FDA-approved chemotherapeutic drugs. Moreover, these drugs are also shown to promote the expression of paternal UBE3A in a dose-dependent manner. The drugs work by reactivating the gene *via* reduced transcription of an imprinted antisense RNA[104-106]. Despite proving their potential, topoisomerase inhibitors are not currently used as a first-line treatment for AS due to their side effects. According to one study, topoisomerase inhibitors also reduced the levels of other genes that are linked to autism, thus increasing the potential of autism characteristics in individuals[107].

Thus, as discussed, all the approved therapies for PWS and AS target the management of symptoms rather than the actual disease. Thus, there is currently no cure for either AS or PWS. Gene and molecular therapies offer a promising way into precision medicine and the development of novel therapies however a lot more research needs to be done regarding offsetting the systemic side effects. A summary of the chromosome 15 abnormalities in PWS and AS is shown in Figure 4 below.

NEURON DETERIORATION

Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder affecting both the upper and lower motor neurons and is the most common motor neuron disease in adults[108-110]. The onset of ALS symptoms typically occurs between

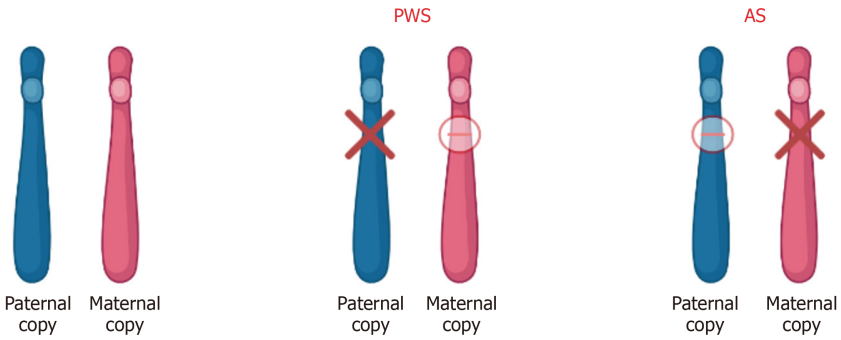

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Figure 4 Chromosome 15 (15q11-13) abnormality in patients with Prader-Willi syndrome and Angelman syndrome. In patients with Prader-Willi syndrome (PWS) and Angelman syndrome (AS), the imprinted gene abnormality is the 15q11-q13 region of chromosome 15. Although the abnormality is in the same region, the difference in disease and phenotype depends on the parental contribution. In PWS, the disease results due to loss of paternal gene expression. In AS, the disease results due to loss of maternal gene expression. PWS: Prader-Willi syndrome; AS: Angelman syndrome.

50-70 years old and is more common in males than females[111-113]. While some individuals can survive over 10 years with ALS, most survive less than 3 years due to the rapid disease progression[114]. Current treatments focus on the treatment of symptoms; however, their effect is often minimal by extending a patient's survival by only three months [112]. Although the etiology of ALS is currently unknown, there does appear to be a genetic component as nearly 10 percent of diagnosed individuals reported having a family member with ALS and are said to have familial ALS (fALS), while the remaining individuals are classified as having sporadic ALS (sALS)[115].

In recent years, the number of genetic mutations linked to ALS has increased. Superoxide dismutase-1 (SOD1) was the first gene discovered to be associated with ALS, and mutations of the SOD1 gene account for 10%-15% of fALS and 1%-2% of sALS[110,115-117]. More recently, mutations to the C90RF72 gene have been associated with 30%-40% of fALS and nearly 6% of sALS[115,116]. Mutations to other genes such as TDP-43, FUS, OPTN, TBK, GRN, NEK1, and C21ORF2 have also been linked to ALS[115,116]. Current evidence suggests that many of these mutations result in the aggregation of misfolded proteins, which then causes ALS[110,112]. Furthermore, knockout models tested on mice have shown that the removal of these genes typically does not result in symptoms of ALS, suggesting that these mutations result in a gain of function[112,116,117].

Current ALS treatments in clinical trials focus on gene therapy as opposed to treatment of the symptoms of ALS. These treatments attempt to replace the mutated gene, inactivate the mutated gene, or introduce a new gene to fight ALS[117]. Tofersen, an ASO, is currently in clinical trials for individuals suffering from ALS because of a SOD1 mutation. Tofersen causes the degradation of SOD1 mRNA, decreasing the amount of SOD1 protein[118]. However, ASOs such as Tofersen are unable to cross the blood-brain barrier and must be injected into the cerebrospinal fluid[117,118]. This can cause many unwanted side effects, such as inflammation, infection, and long and repeated injections[117]. As a result, nanoparticles, such as calcium phosphate lipid nanoparticles, are being developed to reduce the need for direct injection of ASOs into the CSF[117]. Additional research is being conducted on AAV vectors for the modification of genes. The major advantage of AAV vectors is that only one injection is necessary[116]. AAV vectors have the potential to deliver gene-silencing material, such as antisense sequences, to mutated genes such as the SOD1 gene[116]. However, immunoreactivity to AAV has been documented in both animal and human studies presenting future challenges to the use of AAV vectors for ALS treatment[116].

Huntington's disease

Huntington's disease (HD) is a neurodegenerative disorder resulting from the expansion of a CAG trinucleotide sequence on the *HTT* gene which encodes the huntingtin protein[119-121]. For an individual to be at risk for HD, they must have greater than 36 glutamine repeats, and as the number of repeats increases the age of onset typically decreases[120]. HD is inherited in an autosomal dominant manner, with symptoms first appearing in mid-life[121,122]. Individuals with HD experience cognitive, motor, and psychiatric symptoms that are progressive over time[119]. Current treatments for HD focus on treating symptoms but often have limited benefits, and there is no available disease-modifying treatment[121, 123].

The HTT gene encodes the huntingtin protein, which is involved in a variety of cellular functions including cell division, vesicle transportation, and transcription regulation[120]. Repeat CAG trinucleotide sequences found on exon 1 of the HTT gene cause the formation of mutant huntingtin protein aggregates within neurons[124,125]. Individuals who possess 60 or more CAG repeats will develop HD before the age of 20[124,125]. However, the CAG repeat length explains less than 50% of the HD age of onset[124]. Other factors such as glutamic acid polymorphisms and glutamate and N-methyl-D-aspartate receptor polymorphisms also play a role in the onset of HD[124]. These genetic modifiers all present potential targets for genetic treatment for HD.

As a result of the many functions of the huntingtin protein, the embryonic knockout of the *HTT* gene in mice was lethal [120,121]. Furthermore, the inactivation of the HTT gene after birth has been linked to neurological deterioration in mice [121]. Because of this, many HD disease-modifying treatments being researched focus on gene editing as opposed to gene silencing[125]. In a phase 1-2a clinical trial conducted between 2015-2017, an ASO known as IONIS-HTT$_{Rx}$ was studied for

its safety, ability to remain in the CSF, and ability to reduce mutant HTT in the CSF[126]. Upon completion of this trial, it was found that IONIS-HTT_{Rx} did not increase the number of adverse effects and resulted in a dose-dependent reduction of the mutant HTT concentration[126]. However, phase three trials show that IONIS-HTT_{Rx} may cause worse motor and cognitive function[127]. Along with ASOs, RNA interference (RNAi) technology is being developed to treat HD. RNAi technology attempts to decrease the amount of mutant HTT being translated using small non-coding RNAs[127]. This RNAi technology is typically delivered through an AAV. However, this technology is in the early stages of development, with significant testing still needed.

TUMORS

Neurofibromatosis-1

Neurofibromatosis-1 (NF-1), also known as Von Recklinghausen disease, is an autosomal dominant disorder characterized by changes in the nervous system, bones, and skin[128]. Risks associated with NF-1 include bone abnormalities, vasculopathy, and cognitive impairment[128]. NF-1 is also the common type of hamartoma neoplastic syndrome, which is the formation of benign tumorlike malformations consisting of abnormal cells and tissues[128]. Neurofibromatosis also consists of neurofibromatosis type 2 (NF-2) and Schwannmatosis[128]. NF-2 presents with similar cutaneous manifestations as NF-1 but mainly exhibits schwannomas (tumors of the nervous system), meningiomas (tumors of the meninges), and ependymomas (brain and spinal cord tumors)[129]. Of the three kinds of NF, NF-1 accounts for 96% of all cases, NF-2 accounts for 3% and Schwannomatosis accounts for less than 1%[130].

NF-1 is known as an autosomal dominant disorder that affects 1 in 2600 to 3000 individuals. All generations are included. The expression of this disease differs between individuals of the same family and from one affected family to another[128]. The NF-1 gene is located on chromosome 17 and is a tumor suppressor gene. The NF-1 gene produces a product called neurofibromin, which catalyzes the hydrolysis of guanosine triphosphate (GTP)-bound Ras to guanosine diphosphate-bound Ras, ultimately inactivating Ras GTPase[131]. Neurofibromin is expressed in various tissues throughout the body and the function of NF-1 is to modulate the activity of the RAS pathway[132]. This pathway in turn delivers signals from the granulocyte-monocyte colony-stimulating factor to proliferating cells[132]. In turn, the NF-1 protein promotes the conversion of the activated complex, Ras-GTP, to Ras-GDP, the inactivated form[133]. When the NF-1 gene is mutated or deleted, the typical phenotype of NF-1 results due to activation of the Ras-GTP, ultimately resulting in cell growth and prolferation[128]. With this disorder, penetrance is complete[134]. NF-1 has a high degree of variability in clinical presentation, which may include cutaneous, bony, vascular, and cognitive features along with multiple neoplasms. Due to these manifestations overlapping with other genetic conditions, accurate diagnosis of NF1 is important for clinical care and genetic counseling[135]. The mechanism of neurofibromatosis-1 is summarized in Figure 5 below.

New gene techniques were shown to help with NF-1-related pain. A study conducted on this rare autosomal disease suggested that collapsing response mediator protein 2 (CRMP2) is a key target for therapeutic intervention[136]. Moutal *et al*[136] highlighted there is a direct connection between the amount of neurofibromin expressed and pain. CRMP2 regulates the activity of calcium channels and increases the Ca^{2+} current and release in sensory neurons[137]. Additionally, CRMP2 binds to the C-terminus of neurofibromin, suggesting a possible correlation between CRMP2 and the pain experienced by patients with NF-1[136]. In the study, they used clustered regularly interspaced short palindromic repeats of the CRISPR-associated 9 (CAS9 genome), a commonly used DNA editing device[136]. With this, the researchers created a novel rat model of NF-1-related pain[136]. The delivery guide of Cas9 nuclease plasmid was used to generate allele-specific C-terminal truncation of neurofibromin[136]. Additionally, researchers used (S)-LCM, an inactive enantiomer of the drug Vimpat to inhibit CRMP2 phosphorylation, uncoupling CRMP2 from the NF-1 protein [136]. The rats with truncation of neurofibromin showed increases in voltage-gated calcium and sodium resulting in increased nociceptor excitability and behavioral hyperalgesia[136]. As the protein CRMP2 regulates these channels and binds to the C-terminus of neurofibromin, this indicated a possible mechanism underlying NF1 pain[136]. Targeting CRMP2 phosphorylation offers a new therapeutic way to manage the pain associated with NF-1. However, future research is needed in human trials to further investigate the link between CRMP2 and the NF-1 protein.

In children with neurofibromatosis 1, low-grade gliomas are the most common tumor and often result in significant visual loss due to tumor progression along with motor deficits[138]. The current therapeutical agent used for the management of tumor progression is vincristine/carboplatin, or vinblastine, a chemotherapeutic agent[139]. Although this agent prevents tumor progression, restoration of vision or motor function is rare[140]. A new type of therapeutic approach involves the use of mTOR inhibitors[140]. When mTOR is activated, abnormal cell growth results leading to cell proliferation and the formation of new blood vessels through angiogenesis[140]. Everolimus is derived from rapamycin and inhibits angiogenesis, hypoxia-inducible factor 1, and vascular endothelial growth factor production, and ultimately prevents the proliferation of cells[141]. When Everolimus was given to patients with NF-1, the tumor progression was significantly halted and demonstrated sufficient penetration of the blood-brain barrier[141]. This offers a promising therapeutic for children with low-grade gliomas as it is available orally and has minimal toxicities. However, further researcher is needed on Everolimus in combination with other agents to determine if it is the superior therapeutic agent or if a combination offers better results.

Tuberous sclerosis

Tuberous sclerosis is an inherited autosomal dominant neurocutaneous genetic disorder[142]. This genetic disorder affects multiple systems and often presents in children with skin lesions, seizures, and hamartomas of the brain, kidney,

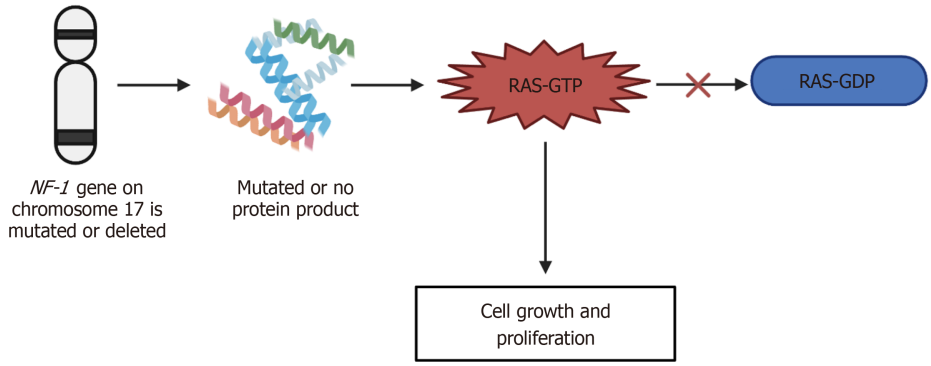

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Figure 5 Mechanism of neurofibromatosis-1. If the *neurofibromatosis-1* gene on chromosome 17 is either mutated or deleted, either no protein product is formed, or a mutated protein is made. This mutation or deletion then results in the inhibition of the conversion of Ras-GTP to Ras-GDP, ultimately resulting in the increased signaling of Ras-GTP and leading to cell growth and proliferation, producing the neurofibromatosis phenotype. GTP: Guanosine triphosphate; GDP: Guanosine 5'-diphosphate; NF-1: Neurofibromatosis-1.

and heart[142]. Additionally, tuberous sclerosis impacts 1 in 5500 individuals. Affected individuals may also present with developmental delays[142]. Cardiac rhabdomyomas of cortical tubers may also be present prenatally[142]. Adulthood signs show osseous, renal, or pulmonary lesions[142]. Skin lesions are found in 90% of patients of all ages whilst, hypopigmented macules are found in early childhood[142]. Ungual fibromas appear during puberty and facial angiofibromas are more commonly found towards adolescence[143].

Tuberous sclerosis disorder arises from four mutations in tuberous sclerosis complex 1 (TSC1) (9q34) and tuberous sclerosis complex 2 (TSC2) (16p13.3) genes which respectively encode hamartin and tuberin[144,145]. This disorder has been known to have a broad spectrum of mutations in both genes[144]. No regions seem more liable to mutations and the frequency is consistently higher in TSC2 rather than in TSC1[144]. 15% of patients admitted who meet clinical criteria for tuberous sclerosis demonstrated no identifiable genetic mutations[143]. The disease itself is caused by a mutation of these genes and causes dysfunction of proteins hamartin or tuberin[143]. Hamartin's role helps control the proliferation of cell growth, division, and cell size[143]. Tuberin functions to regulate cell growth and protein synthesis through the downstream inhibition of mTOR[146]. Therefore, the loss of either of these proteins can lead to the overgrowth of lesions in many vital organs[146].

Gene therapy for tuberous sclerosis type 2 was conducted on a mouse model by delivering an adeno-associated virus (AAv9) which encoded a condensed form of tuberin[147]. A mouse model of TSC2 was generated by AAV-Cre recombinase disruption of Tsc2-floxed alleles at birth, leading to a shortened lifespan (mean 58 d) and brain pathology consistent with TSC[147]. When these mice were injected intravenously on day 21 with AAV9-cTuberin, the mean survival was extended to 462 d with a reduction in brain pathology[147]. This study demonstrated the potential of treating life-threatening TSC2 Lesions with a single intravenous injection of AAV9-cTuberin[147]. Preventions for this disease will vary depending on the developmental stage of the specific individual as tuberous sclerosis has a highly variable clinical course and the prognosis may be uncertain[147]. Options pertaining to tuberous sclerosis are limited to surgery for treating symptoms of tuberous sclerosis related to the growth of hamartomas[148]. Therefore, to better understand the genetic causation for this disease, clinical trials of mammalian target of rapamycin inhibitors, including sirolimus and Everolimus have been conducted[149]. mTOR is an evolutionary conserved serine-threonine kinase that regulates cell growth and cell survival[149]. The connection between TSC and mTOR led to the clinical use of mTOR allosteric inhibitors, Sirolimus and Everolimus[149]. Both sirolimus and Everolimus inhibit mTOR selectively with similar molecular mechanisms but distinct clinical profiles[149]. Everolimus has been approved for subependymal giant cell astrocytomas and renal angiomyolipomas in TSC patients[149]. However, sirolimus is not approved for TSC and has undergone considerable investigation to treat various aspects of TSC. However, the use of sirolimus has been studied in older children and adults with TSC[150-153]. One study published in 2023 investigated the use of sirolimus in children under the age of 2 with tuberous sclerosis complex and found that sirolimus was safe to use in children[154]. The study reported that the most common adverse events due to sirolimus use included anemia, hyperlipidemia, and thrombocytosis, which were able to be managed well[154]. Despite proving its safety through this study, further research is needed into sirolimus to demonstrate safety and efficacy through larger studies and clinical trials, but it offers a promising therapeutic option for patients with tuberous sclerosis.

CONCLUSION

Neurologic orphan diseases impact less than 200000 individuals within the United States and because they're rare, diagnosis and treatment are often difficult. However, in recent years, data has suggested that the use of whole genome sequencing has increased the diagnosis of neurologic orphan diseases, allowing patients who have these conditions to be more easily identified. With new advances in technology, more research is being devoted to developing therapeutic options for patients with neurologic orphan diseases. Such advances in research include the use of AAV vectors which

have shown positive results in SMA, ALS, Friedreich Ataxia, and NF-1. Additionally, the use of CRISPR/Cas has demonstrated promising results in DMD, Dravet syndrome, Friedreich Ataxia, NF-1, and Tuberous Sclerosis. The use of therapeutics that can cross the blood-brain barrier, such as Risdiplam and Branaplam, has increased the number of treatment options available for patients with SMA. Additionally, research into mTOR inhibitors offers a promising option for patients with NF-1 and tuberous sclerosis. Although further research is needed, these treatment options can significantly impact the quality of life and survival of patients with neurologic orphan diseases.

FOOTNOTES

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Personalized clinical managements through exploring circulating neural cells and electroencephalography

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Abstract

BACKGROUND

Since an initial diagnosis of Alzheimer disease (AD) in 1907, early detection, was unavailable through 116 years. Up-regulation of V-Ets erythroblastosis virus E26 oncogene homolog 2 (Ets2) is capable to enhance neuronal susceptibility and degeneration. Protein expression (PE) of Ets2 has functional impact on AD and Down's syndrome, with diverse intensity. PE of Ets2 has an influential pathogenic impact on AD. Clinical aspects of neurological disorders directly interact with psychological maladies. However, deterioration requires an early management including programmed based protection.

AIM

To include cell biology in neuro-genetics; personalized, prognostics, predictive, preventive, predisposing (5xP) platform, accompanied by stratifying brain channels behavior pre- and post-intervention by light music in the AD-patients.

METHODS

Include exploration of PE assay and electroencephalography of brain channels. The processes are applied according to: (1) Triangle style, by application of cellular network; and (2) PE assay of Ets2 in the peripheral blood of the patients with AD, by Manual single cell based analysis, and Flow-cytometry. (1) Applying the Genetic counselling and pedigree analysis; (2) considering the psychological status of the referral cases; (3) considering the macro-and/or micro-environmental factors; (4) performing the required Genetics' analysis; and (5) applying the required complementary test(s).

RESULTS

PE of Ets2 has pathogenic role in AD. PE unmasked the nature of heterogeneity/diversity/course of evolution by exploring Ets2, D1853N polymorphism in Ataxia Telangiectasia mutated gene (ATM), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) and course of evolution at the single cell level of the brain. Ets2 revealed different cellular behavior in the blood and suggested the strategy as 'Gene Product-Based Therapy' and the personalized managements for the patients. PE reflected weak expression of ATM, mosaic pattern of Ets2; remarkable expression of VEGF and EGF by highlighting an early detective platform, considering circulating neural cells (CNCs) and the required molecular investigation, for the target individual(s) predisposed to AD or other neural disease including brain neoplasia. Brain channels-cooperation with diverse/interactive-ratios lead to strategic balancing for improving the life-quality in AD.

CONCLUSION

We highlighted application of the single CNCs and correlated Ratio based between Brain channels by providing the 5xP personalized clinical management model for an early detection and therapy of the patients with AD and their targeted/predisposed relatives. Novel-evolutionary/hypothetic/heterogenic-results in brain-channels offer personalizd/constructive markers with unlimited cooperation in health and disease.

Key Words: Alzheimer; Protein expression; Brain channels; Predictive/early detection

Core Tip: The provided outcomes emphasize on the personalized/classified/functional/single cell based/complementary insights, and systematic strategy in Neuro-Science. The successful bridging approach between Neuro-Science and Medicine requires: (1) The combination of the molecular and functional insights at single cell level; (2) by emphasizing on the course of evolution; and (3) to expedite towards unmasking the functional modifications in the blood stream of the patients with neurological disorders including Alzheimer disease (AD). However, exploration of the circulating neural cells accelerate to unmask the course of evolution by providing the personalized and translatable model to the target based therapy. Let's improve the life quality of the patients with neurological disorders including AD with simply the light music which corresponds with 40 Hz in gamma sensory stimulation therapy. It is essential to differentiate between neurological- and neuro-psychiatric diseases. Surprisingly, we offer an early detection of the stem cells, including the neural CD133 at fetal period, *i.e.,* as early as 8-9 wk, or later through the circulating fetal cells in the maternal blood stream.

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INTRODUCTION

Present introduction provides the multi-directional insights towards the Alzheimer disease (AD), including the function of involved protein expression (PE) at single cell level; and electroencephalography (EEG), to observe the role of light music (LM) on the brain channels, and the interaction between different brain channels. It was also aimed to provide the personalized periodic chart for the predisposed individual and/or patients affected with AD. In addition, an initial description of the histopathology of AD has been provided by Alois Alzheimer[1] in 1907, which is categorized by brain atrophy, an extracellular, cumulated of Aβ peptide (amyloid plaques), loss of neuron and synapse, composition of tau protein, as neurofibrillary ingredient[1]. Regulation of the Human Presenilin-1 Gene by V-Ets erythroblastosis virus E26 oncogene homolog 2 (Ets2) Transcription Factors and p53 play key role in AD-pathogenesis[2]. The decisive role of DNA by controlling 90% of the expression of presenilin 1 gene located in chromosome 21 (-35 to +6) is highlighted. This region harbors an Ets transcription factor-binding motif, and a 2-base pair alteration within the sequence of (GGAA to TTAA) of the Ets2 territory leading to the 90% transcriptional reduction. It was also reported that Ets1/2 transcription factors activate PS1 transcription.

The pathological diagnosis in AD, and accumulation of amyloid, occur almost decades before an initial sign of clinical symptoms[3]. Besides, functional aspect of brain network could be unmasked by magnetoencephalography. Most importantly, application of the non-invasive strategy, as an early detection of the symptom(s) of neuropathology at pre-developmental period of the AD is essential.

Initially, the micro- and macro-environmental factors, including nutrition play, partly, the influential roles in initiation and progression of AD. The most challenging panel in this topic is the late diagnosis and the risky procedures through the exploration of AD and therapy. In addition, EEG is the non-invasive and trustable method to detect synaptic dysfunction and the course of the disease. However, performance of EEG is helpful, but very late.

In addition, the combined strategy to classify the EEG-data, by applying an early functional assay of the target protein(s), at single cell level, and as the personalized analytical procedures in AD is, currently, our ongoing process.

Brain function could be, routinely, examined by EEG in AD with significant resolution[4]. EEG is revealed to be: (1) Non-invasive; (2) unmasks the alphabetic informative codes of the neural territory; (3) recording many required targets; (4) unmasking the health conditions related to the patients' age; and (5) deals with the pharmacological modeling[5,6]. Actually, EEG is capable to unmask the basic prognostic mechanisms in AD. Furthermore, it is proposed that the progression in AD is supposed to be associated to the "functional disconnection"[7].

In the resting phase, the increased and decreased functional connection is indicative of activity for the prefrontal and posterior regions through the alpha band[8,9]. Besides, by considering the decreased function in EEG exploration, its consequences on the connectivity is remarkable which depends on the individuals and technology.

Regarding the genetic test, the Ets2 up-regulation may lead to an increased neuronal degeneration, apoptosis and susceptibility[10]. The *Ets2* gene is involved in two different group of patients, affected at totally diverse age of onset including AD and Down's syndrome (DS)[11].

The primary predisposing/predictive/early detective strategy is the pedigree-based analysis of the proband affected with nervous system/brain disorder(s) including AD. Such approach will cover the candidate relatives of the proband(s) for the screening program. This strategy will unmask: (1) The predisposing factor through the pedigree; and (2) the target and candidate relatives for an early and non-invasive screening.

Circulating neural cells (CNCs) offer: (1) The non-invasive screening; (2) at single cell level; and (3) unmasking the heterogeneity/diversity and evolution.

Ethics play fundamental and supportive role in Genetics and Psychiatry. It facilitates, care and guarantee the benefit of patients in different stages including sampling, research, and clinical managements. Through such platforms, the chain of events including predisposition, prognosis, prediction, prevention (as 4xP aim), diagnosis and personalized therapy are required to be considered. In cell biology, the single cell level play the key role in unmasking the brain's behavior and have complimentary role and functional impact on the neurological system.

The personalized, prognostics, predictive, preventive, predisposing (5xP) strategy would be achievable through the systematic exploration with an early-detection and personalized therapeutic management. Besides, the pedigree-based analysis provides the predictive stage for the relatives of proband, affected with AD at any age.

Personalized screening, at single cell level, with adequate cell analysis, as early as possible, is scientifically trustable. Therefore, it is an urgent aim for both Scientific and Medical platforms to consider an early detection of the neural disorders.

Brain is a sophisticated and transmittable organ. The pathology of the brain is classified and available[3]. By considering Genetics and cell biology, the specific factors in the neural cells play key role in the neural territory. As a result, the manner of current systems have the influential and functional roles on the specific neurological mechanisms at single cell level. Therefore, to unmask the alterations, it requires the most comprehensive exploration at somatic- and genomics level[11].

Brain harbors the multi-channels, which is characterized with the miscellaneous and essential proteins.

Conclusively, neural cells are derived from the pool of homogeneous progenitor cells. Furthermore, environmental factors, affect the genetic variations and predisposing factors. Concerning the required techniques and the essential therapeutic aspects of the neurological disorders, the informative panel including the histo-pathologic, PE at single cell level and the molecular techniques are required.

As the routine manner, the most puzzling information achieved at the global level, which does not provide the essential Information on the diversity and heterogeneity. Furthermore, the single cell based analysis of PE, is capable to unmask the architecture of the expression and co-expression at the final cellular procedure and production.

The routine and global molecular based analysis are, rapidly, used in the neuroscience research and diagnosis, but are not informative to unmask the heterogeneity and diversity of the functional single cell based which has key role on the occurrence of evolution.

As the matter of fact, the roots of different diseases including cancer and non-cancerous are either related to their ancestral lines and sides through the pedigrees, or the rules and natural events at embryonic, and/or fetal, adulthood and elderly durations (Figure 1). However, micro- and macro-environmental factors play the influential role to initiate and developing disorders. Finally, the complicated periodic chart is required to be considered in the management of health and diseases for the index case and their targeted relatives through their pedigrees.

Cancer and Alzheimer are the historical diseases and have, their roots, hidden at the single cells and brain territory.

The physicians and scientists need to take their valuable action as the experienced archeologists, to trace the roots of both diseases, one by one at single cell level and not at global dimension.

Figure 1 presents the flow-chart which may assist the medical team, including clinicians, pharmacologists, nurses, and scientists to achieve the required complementary data, as early as possible. The prompt therapy requires an early diagnosis. At a glance, the matter of fact includes the occurrence and diagnosis of diversity, heterogeneity and evolution. Such a triangle, will direct the medical, pharmaceutics and scientific teams to: (1) The translatable platform; and (2) to plan the personalized clinical management including an early-therapy (Figure 1).

MATERIALS AND METHODS

Ten patients, including six females and four males, affected with AD; and ten healthy individuals are participated in our previous study[11]. The lymphocytes were cultured in RPMI media (Sigma Aldrich, St Louis, MO, United States) for 35

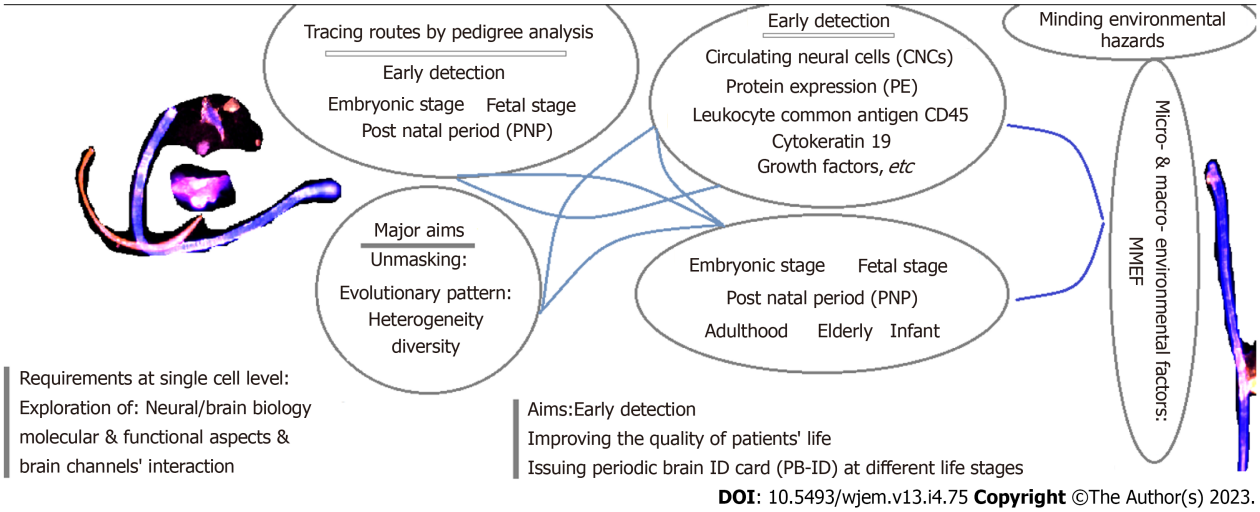

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Figure 1 The required managements for early detection and therapy: A Journey from embryonic and fetal periods to elderly.

min at 37 °C. Then the cells were treated with the hypertonic solution, and were fixed. The lymphocytes were stained with the polyclonal Ets2 antibody (Avivasysbio, CA, United States), washed by Phosphate buffer solution, and then stained with secondary antibody (FITC-conjugated goat-anti-rabbit). Flow-cytometry (FC) assay was performed by BD FACS Calibur FC (BD FACSCALIBUR™ FC-System, United States) and the results were analyzed by Flowjo-7.6. software.

Data was analyzed by SPSS 18 (SPSS Inc, IL, United States) The Spearman and Pearson Correlation Coefficients were also performed. The, *P* value less than 0.05 was considered as significant[11].

The single cell based assay is an essential channel to unmask heterogeneity, diversity, and evolution. Besides, the high cell enumeration is required. There are apparatus; capable to automate, cell analysis, but very few cells is enumerated. Besides, through the manual analysis, broad exploration of multi thousand single cells are analyzable to unmask the new cellular function and their interaction.

PE has been assayed by

Manual single cell based analysis by immunofluorescence (IF), also based on high enumeration, with different magnification, which provides the classified intensity of the PE. However, FC is totally machinery based and non- classified cellular screening according to the degree of intensity of the whole cell population with manual IF, and FC.

Visions and visualization: PE assay and EEG

The applied processes are performed according to the triangle style, by highlighting the power of cellular network, which reflects the association between the PE of Ets2 is also performed in the peripheral blood of the patients with AD, according to the standard flow cytometry technique[11]. The PE assay was capable to unveil the nature of heterogeneity/diversity/course of evolution by exploring D1853N polymorphism in ATM gene, and PE assay of vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) at the single cell level of the brain and through the course of evolution[12,13].

However, there are challenges regarding the CNCs in neurological disorders[14]. Furthermore, an innovative and complementary insight is required for an intensive exploring of the brain channels as well. Buzsáki *et al*[14] have focused on "The origin of extracellular fields and currents-EEG, ECoG, LFP and spikes"[14]. Briefly, the challenges and achieved data in circulating tumor cells, and EEG highlights the involvement of two diverse territories and the related machinery in AD[14,15].

By considering the presence of family history of AD in the related pedigree; CNCs is offered as an applicable biomarker based test: (1) At any age, by only 2 mL of the pregnant maternal peripheral blood to screen the status of target embryo and/or fetus for Ets2 gene and the CNCs; and (2) screening the same test by buccal mucosa or buccal smear of the born child at different stages of his or her life.

RESULTS

PE assay

Analysis of PE was performed in the embryonic and chorionic villus samples; and patients affected with AD. PE is assayed by IF in the peripheral blood samples and 1000-3000 cells were analyzed at single cell level, by the manual exploration. An aborted embryonic sample at the early gestational week has been explored to assay the mode of PE, including neural marker (NE), neural stem cell (CD133) and VEGF (Figure 2A-C). PE at early stage of embryonic period revealed to be high for NE (Figure 2A) and VEGF (Figure 2C). But, the Neural stem cell marker (CD133) is characterized with an absolutely lack of PE, which reflects no sign of stem cell function at early stage of embryo in this aborted sample

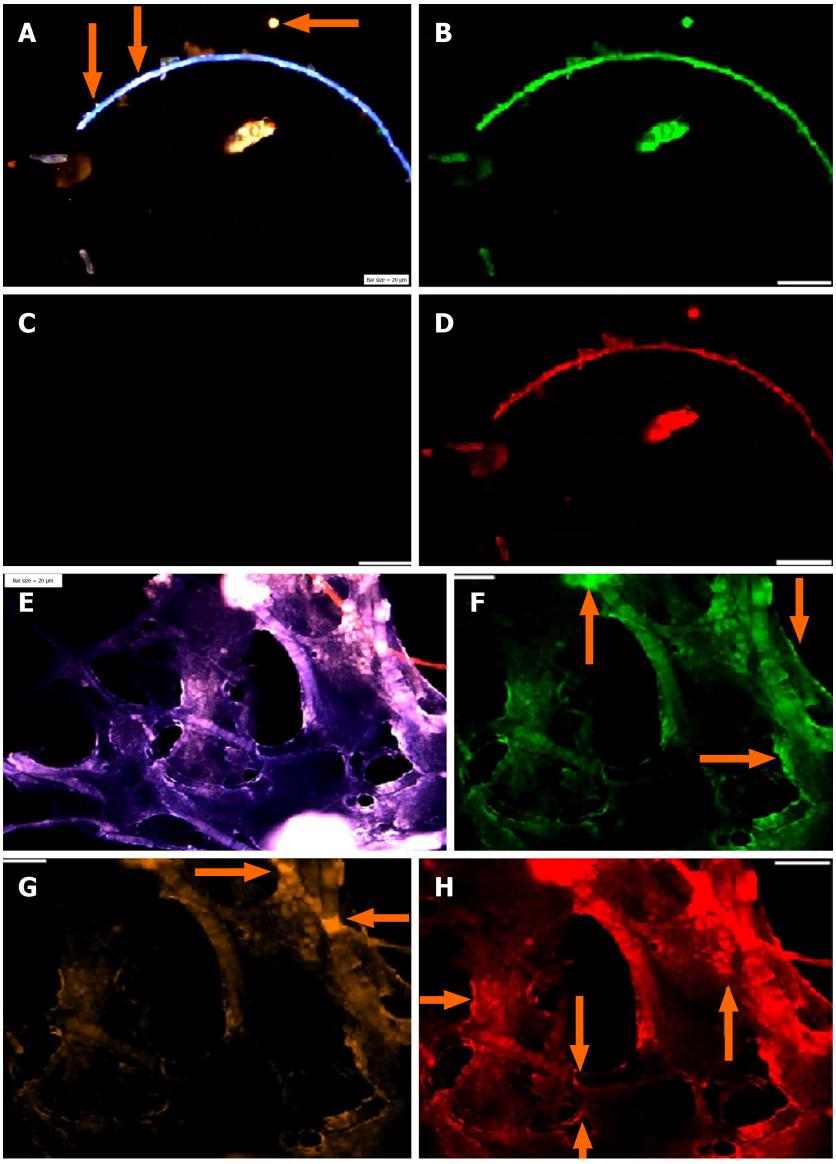

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Figure 2 Circulating neural cells in embryonic and chorionic villus samples. A: Circulated neural cells (CNCs) conjugated with Dapi/Ne/CD133/vascular endothelial growth (VEG); B: Same cells conjugated with fluorescein isothiocyanate (FITC) (Ne) reflecting high protein expression in the limited cells; C: Same cells conjugated with Rpe (CD133), totally lacks expression in stem cells; D: Same cells conjugated with Pe-Cy5, reflecting high protein expression, accompanied by few cells with low expression; E-H: Reflect protein expression in chorionic villus sample including; E: CNCs conjugated with Dapi/Ne/CD133/VEG; F: Same cells conjugated with FITC (Ne) reflecting high protein expression; G: Same cells conjugated with Rpe (CD133), lack of expression is observable, accompanied by few cells with positive CD133; H: Same cells conjugated with Cy5 (VEGF), reflecting high protein expression and remarkable angiogenesis. Bars = 20 μm.

(Figure 2A-D).

Regarding the chorionic villus, sample, the behavior of PE seems to be diverse, the highest PE is traced for VEGF with high angiogenesis in the whole sample (Figure 2E-H); mosaic pattern of PE for the NE is more remarkable (Figure 2B) than VEGF, the stem cell CD133, reveals a notable, low expression, accompanied by few cells with high expression (Figure 2E-H) which reflect the initiative step for activation of CD133 stem cell, and significantly differs from the embryonic sample, lacking any expression of CD133. This is the course of evolution, which could be influenced by micro- and macro environmental factor during the fetal growth. Such behavior could occur for any other involved marker(s) for cancer initiation and/or progression. By projecting the impact of early detection, the initial target to unmask any sign of functional alteration related to the neurological disorders including AD by circulating fetal cells (CFCs), is available. In this regard, we offer an extremely early detection of neural stem cell (CD133), Ets2, and any related key proteins, involved in the initiation and progression of neurological disorders including AD at 8-9 gestational week (Figure 2E-H). Furthermore, screening the evolutionary course of the molecular and functional alteration(s) is possible by the follow-up strategy and only 2-3 mL maternal peripheral blood as often as, is essential. In addition, we provide an early detective platform including the stem cells (CD133) at fetal period, *i.e.*, as early as 8-9 wk through the CFCs/chorionic villus in the maternal blood stream. Besides the key proteins including cyclins B, D, E, EGFs and VEGF could be also analysed. The benefit of such an extremely early screening is to deliberate the preliminary and preventive strategy including micro- and

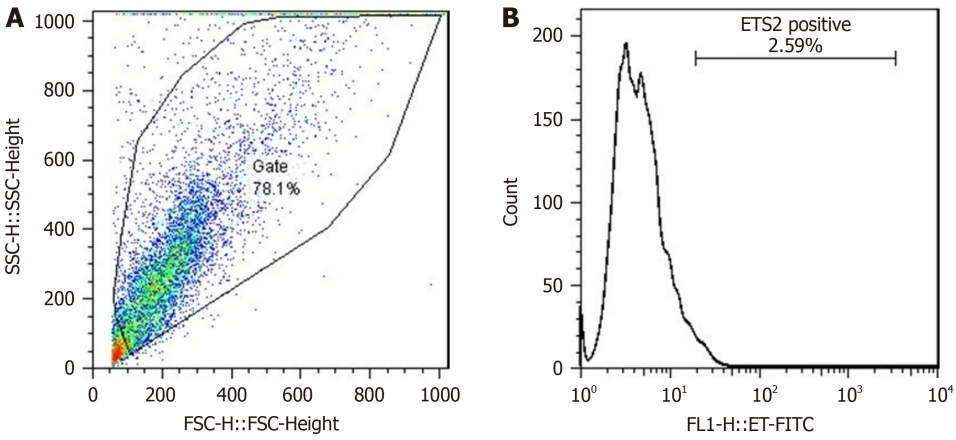

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Figure 3 Flow-cytometry results of erythroblastosis virus E26 oncogene homolog 2 protein expression in a patient affected with Alzheimer disease. A: R1: 78.1 % (7812/10000 cells); B: Erythroblastosis virus E26 oncogene homolog 2: 2.59 % (202/7812 cells). Ets2: Erythroblastosis virus E26 oncogene homolog 2; SSC: Side scatter reflects the internal complexity. Adapted from Reference[11].

macro-environmental factors, counting nutrition. Besides, planning the essential and non-invasive screening, such as EEG at the right time through the offspring's life, under the guide and care of physician could be planned to detect any sign of AD manifestation(s) for applying any essential 5xP aims.

Besides the manual detection by immunofluorescence, automated analysis of PE by FC, is performed for Ets2 protein (Figure 3).

Three proteins including ATM, Ets2 and VEGF are reflective of: (1) Weak expression of ATM as a tumor suppressor gene; (2) mosaic pattern of PE for Ets2; and (3) remarkable expression of VEGF which is indicative of angiogenesis in the neural cells (Figure 4).

The CNCs were explored by IF method for combination of NE, neural stem cell and angiogenesis (NE/CD133/VEGF (Figures 5 and 6); and for Ne/CD133 (Figure 7). The PE of NE/VEGF/Ets2 is also provided (Figure 8). The migrated neural cells with high or low expression are shown with arrows. The micro-vesicle and the vascular section harboring the migrated neural cells are detectable. The CNCs were explored by IF method for combination of NE and neural stem cell (NE/CD133) (Figure 7). The PE of NE/VEGF/Ets2 is also provided (Figure 8). Lower expression of this triangle (NE/VEGF/Ets2) is a reliable predictive/early and non-invasive platform for exploring the status of AD at any stage of life. The CNCs' ratio, between pre- and post- intervention were analyzed based on the PE-screening of cytokeratin 19 (CK19), and leukocyte common antigen (CD45) and EGF. The cells conjugated with VEGF (Rpe), having lower expression (Figure 8), the CNCs' ratio, between pre- and post- intervention were analyzed based on the PE-screening of CK19, and leukocyte common antigen (CD45) and EGF. The personalized insight is considered/and the ratio value is estimated for the cases. The Ratios are characterized as < 1 and > 1 between different channels within the horizontal and vertical axes. Finally, the diverse patterns were classified according to the distribution including clusters or sporadically forms. The CNCs' ratio, between pre- and post- intervention were analyzed based on the PE- screening of CK19, leukocyte common antigen (CD45) and EGF (Figure 9). The confirmation of CNCs was based on the mode of positive $CK19^+$ and negative CD45. The personalized insight is considered. The ratio value is estimated in cases. The Ratios are characterized as < 1 and > 1 between different channels within the horizontal and vertical axes. Finally, the diverse patterns were classified according to the distribution including clusters or sporadically forms.

Conclusively, by aiming an early clinical management, including diagnosis and therapy, the application of an available multi-panels based on: (1) Non-Invasive; (2) repeatable; (3) cost benefit; and (4) safe CNCs sample, in only 2 mL peripheral blood or buccal smear, for an early detection of the target proteins expression in the brain cells could be considered. Let's Revise Alzheimer disease, based on single cell based vocabulary (Figure 10).

Applicability at a glance

The combination of neurology/genetics/cell biology/psychology/ethics is required for the research and clinical management for the neurological disorders. Moreover, the following items harmonize the 5xP by: (1) Applying the Genetic counselling by documenting the patients' pedigree, including the family history of the related diseases, up to 3-4 or more generations; (2) organization of the accustomed plan according to the psychological status of the referral cases; (3) considering the macro-and/or micro-environmental factors in the patients' pedigrees; (4) performing the required Genetics' analysis according to the information, based on the pedigree study; (5) focusing on the apparently normal relatives, having the family history of the relatives affected with the diseases through generations in the pedigree; (6) applying the required complementary test(s); and (7) application of the predictive and early detective strategy for the target suspicious patients with AD and their target relatives.

Genetics/cell biology organize and play the most fundamental roles, by considering a triangle model to unmask the heterogeneity/diversity/evolution. Through such manner, the personalized diagnosis and therapy could be developed. AD as a major aim is selected to explore Ets2. This gene has been explored in two different group of patients affected with neuro-degenerative disorder including AD and DS[11]. Ets2 with its pathogenic role, revealed different cellular behavior

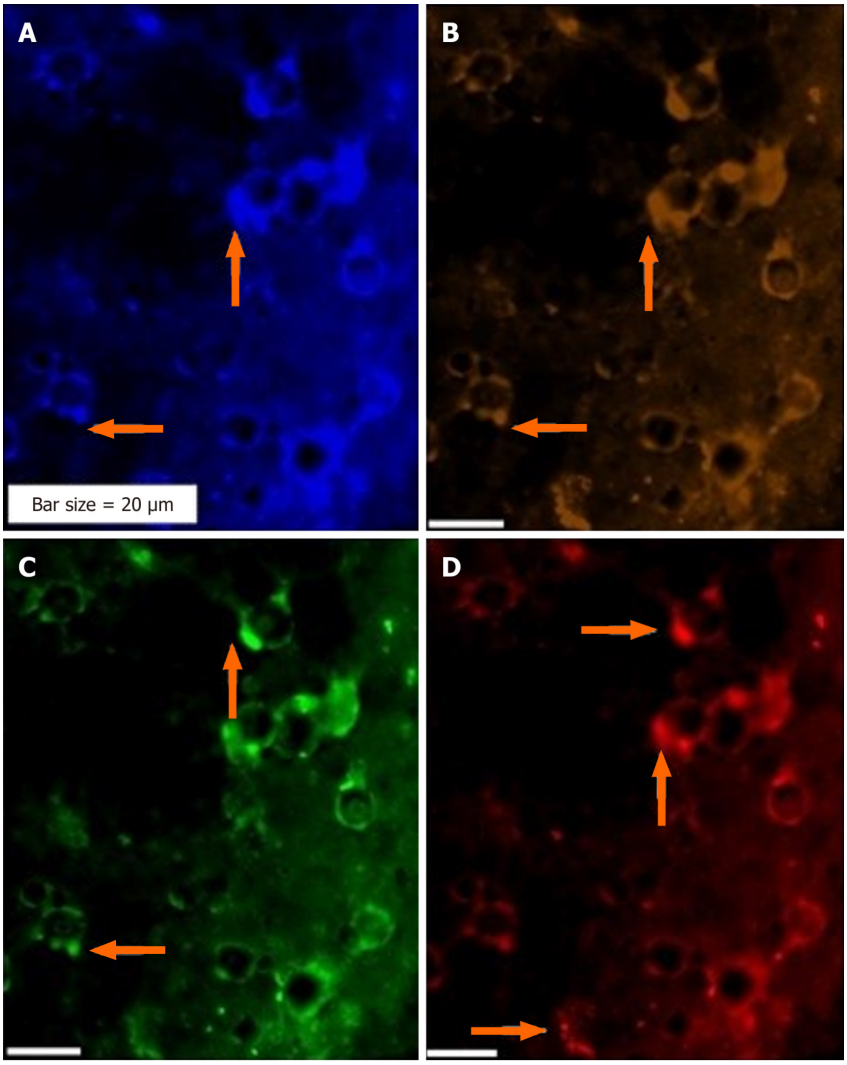

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Figure 4 Protein expression of Ataxia Telangiectasia mutated gene, erythroblastosis virus E26 oncogene homolog 2, and vascular endothelial growth factor in the circulated neural cells from the neural system to the blood stream in a patient with Alzheimer disease. A: Circulated neural cells (CNCs) with dapi; B: CNCs conjugated with Ataxia Telangiectasia mutated (Rpe), reflecting lack or very low expression; C: The same cells conjugated with Ets2 (Fluorescein isothiocyanate) with a mosaic pattern of expression; D: Same cells conjugated with vascular endothelial growth factor (Rpe), reflecting mosaic angiogenesis.

in two different diseases. The results have suggested the strategy as 'Gene Product-Based Therapy' and the personalized managements for both diseases.

DISCUSSION

The information of patients' and controls are provided in Tables 1 and 2. Total cells of 10000 in AD patients were analyzed by FC, but without providing any heterogeneity. The FC-results of Ets2, is provided (Figure 3). PE assay unmasked the nature of heterogeneity/diversity/course of evolution by exploring Ets2, D1853N polymorphism in ATM gene, VEGF, EGF and course of evolution at the single cell level of the brain. The arrangement of Genetics/Cell biology/Psychology/Ethics is essential quadrat-radial format for programming the research and clinical management. The required steps in the Genetics and cell biological programs include: (1) Genetic counselling/pedigree documentation; (2) considering the macro-and/or micro-environmental factors; (3) performing the required Genetics' analysis, based on the pedigree-information; (4) considering the target proband's relatives for the required screening; and (5) applying the required Genetic test(s). Besides, harmonizing the 5xP insights are the essential target in the clinics.

Complementary and comparative insights between different panels of PE assay is the key aim in the patients with AD. PE of Ets2 by IF at analyzable single cell level of the AD patients are provided (Figures 4 and 9). However, PE is a challenging assay and the cells with high magnification are required to: (1) Reflect the clear panorama of the PE; and (2) most importantly, to echo the cellular heterogeneity clearly. The PE of Ets2 is also assayed for ten control individuals (Table 2). Two different single cell- based platforms are indicative of diverse conclusions. In fact, the most diagnostic and

Table 1 Erythroblastosis virus E26 oncogene homolog 2 protein expressions status and clinical features of Alzheimer's disease patients

Case No	Gender	Age, yr	Clinical feature	Family History of AD (years old)	Ets2 protein expression (%)	Total cells evaluated by flow-cytometry
1	F	69	ML	-	146 (3.47)	4207
2	F	57	ML, tremble (hands)	Cousin of paternal father (65)	155 (2.13)	7276
3	F	92	ML, MD	-	121 (1.11)	10900
4	M	76	ML	Half brother (70)	607 (15.8)	3841
5	M	79	ML	-	810 (11)	7363
6	M	67	ML (severe)	-	166 (2.43)	6831
7	M	42	ML (severe)	-	1100 (6.75)	16296
8	F	54	ML	-	228 (3)	7600
9	F	81	ML, HC, BP	Maternal aunt (95)	98 (5.12)	1914
10	F	79	ML	-	541 (18.9)	2862

M: Male; F: Female; ML: Memorial loss; MD: Movement disorder; HC: Heart complication, BP: High blood pressure; AD: Alzheimer disease; Ets2: Erythroblastosis virus E26 oncogene homolog 2; Total cells analyzed in 10 patients: 69090[11].

Table 2 Erythroblastosis virus E26 oncogene homolog 2 protein expressions status in healthy individuals as control for the patients with Alzheimer disease

ID	Age, yr	Ets2 protein expression (%)	Total cells analyzed
C-1	65	2 (0.066)	3025
C-2	56	10 (0.19)	5214
C-3	79	0 (0)	1546
C-4	74	3 (0.065)	4587
C-5	79	6 (0.48)	1232
C-6	62	3 (0.38)	783
C-7	42	0 (0)	923
C-8	54	4 (0.10)	9823
C-9	81	7 (004)	874
C-10	79	3 (0.19)	1542
Total		38 (0.12)	29549

The cells were analysed by flow cytometry: All controls (C) had no family history of Alzheimer disease. C: Control; Ets2: Erythroblastosis virus E26 oncogene homolog 2.

visibility of cellular diversity is achievable by IF through which the territory of the migrated cells from the brain domain is detectable (Figures 3 and 8). The provided figure by IF, clearly, reflect heterogeneity at single cell level.

An extra copy of Ets2 gene in the DNA of brain cells is reported by quantitative densitometry[16] and the provided guidlines[17,18]. Based on this valuable finding, the possible correlation of an extra chromosome 21 in DS as the cause of AD was also reported. In this regard, the expression of Ets2 was also high in DS by: (1) A segmental trisomy model for transcriptome during postnatal development of DS in a mouse model[19]; (2) leading to induction of neural apoptosis [20]; and (3) high-expression of Ets2 led to amplification of the APP gene expression and raising β-amyloid proteins, with consequential brain anomaly in AD and DS[21]. The Most interesting report was related to the beta amyloid gene duplication in both Alzheimer's disease and the case of DS with normal karyotype[21]. It has been, also, emphasized that through the mechanism of an imbalance process for gene dosage, the mental disorder could be evolved through the molecular/cellular machinery in DS[22]. Conclusively, the PE of Ets2, as a remarkable pathogenic factor, has the fundamental role at single cell level of two different disease including AD and DS.

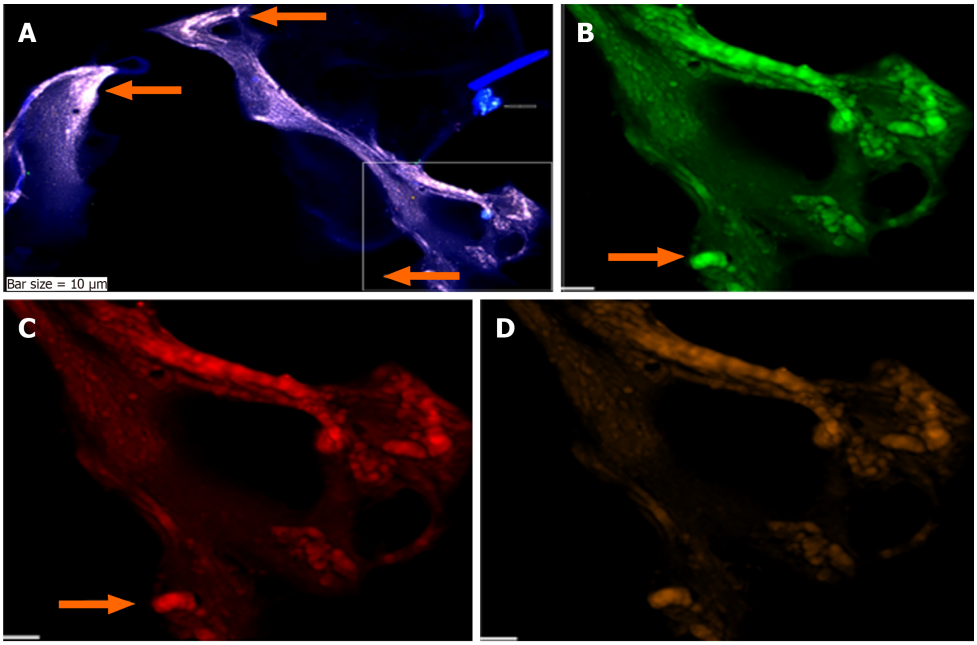

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Figure 5 Protein expression of neural marker, CD133, and vascular endothelial growth factor in the circulated neural cells from the neural system to the blood stream in a patient with Alzheimer disease. A: Merged of dapi/fluorescein isothiocyanate/CD133/vascular endothelial growth factor (VEGF); B: Neural cells, conjugated with FITC with low expression, accompanied by the cells with high expression; C: Neural cells conjugated with Pe-cy5 also with low expression, accompanied by the cells with high expression; D: Cells conjugated with R-phycoerythrin and VEGF, majority of cells reflecting low expression, accompanied with a minor cells with lack of expression.

It is reported that application of "Sensory-Evoked 40-Hz Gamma" facilitates and improves sleeping process and the quality of life in the patients affected with Alzheimer's Disease[23]. Similarly, the light music has also the same impact on the individuals affected with AD as well.

Frontal lobe functions and AD

As a major component of the cerebral cortex, the frontal lobe serves a variety of functions. It is believed that the orbitofrontal cortex (PFC) is involved in the cognitive process of decision-making and other ventromedial prefrontal cortex regions of frontal lobe; it plays a significant role in the regulation of motivation and emotion as well[24,25]. The ventromedial PFC plays a more specialized function in pleasure, happiness, and reward conditioning[26]. Executive function, however, is one of the most significant frontal lobe-related functional groups. Executive functions are typically cognitive processes that help people tackle challenging, unique, and complicated tasks by choosing and fusing actions or thoughts with internal goals and mediating activities over time[27-29]. Working memory, cognitive flexibility, and inhibition are all components of executive function, which is reliant on top-down (*i.e.*, goal-driven) control of distributed processes taking place across the brain. The nature of the processes being controlled determines the precise behavioral output[30]. Key cognitive processes relating to social, emotional, and motivational elements of conduct are carried out by prefrontal cortical areas. Working memory, goal-driven attention, task switching, planning, problem-solving, and the need for novelty are all functions of the dorsal lateral prefrontal cortex. The medial prefrontal cortex is involved in self-awareness, motivation, emotional regulation, and updating goal-directed behavior; the orbitofrontal cortex is involved in personality, inhibition, and emotional and social reasoning. The ventral lateral prefrontal cortex is involved in inhibition, response selection, and monitoring[27]. Dysexecutive syndromes have historically been linked to dorsolateral prefrontal cortex damage, but it is now understood that they can also be caused by a parietal-temporal-frontal system impairment, which is the focus of a specific type of atypical AD. Simple daily tasks requiring executive control and a variety of neuropsychological tests are among the tasks that this dysexecutive Alzheimer phenotype performs poorly on. Disrupted executive control over social, emotional, and motivational elements of behavior characterizes dysexecutive syndromes, which are more closely associated with the frontal lobe[31].

EEG findings in AD

It is crucial to take into account both the rhythms' location in the brain during EEG analysis in addition to the rhythms themselves. For instance, because alpha rhythms represent the activity of several neuronal populations, they have different neurological correlations when they are observed in the occipital, temporal, or frontal cortexes[32].

The majority of EEG studies used to diagnose AD have been based on spectral decomposition of scalp signals in both the resting state with closed eyes and open eyes. We now understand that healthy aging causes changes in brain activity, which are consequently recorded in EEG recordings. They include an increase in delta (1-4 Hz) and theta (4-8 Hz) power, a decrease in background activity, and a slowing of alpha activity (8-13 Hz)[33]. During physiological aging, the

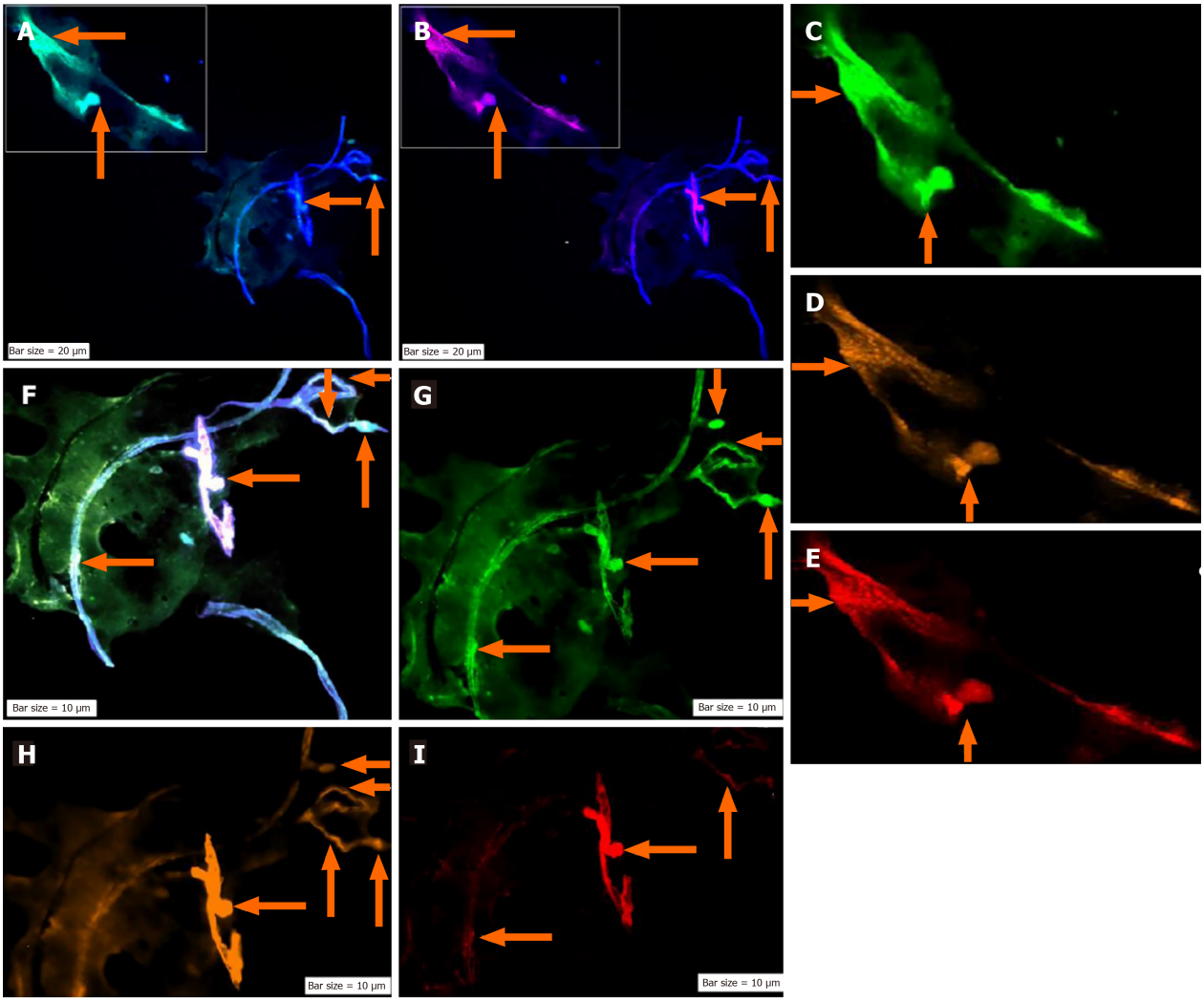

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Figure 6 Protein expression of neural marker/CD133/vascular endothelial growth factor in the circulated neural cells from the neural system to the blood stream in a patient with Alzheimer disease. A: Merged of dapi/Ne (Ne: Neural cells conjugated with dapi/Ne); B: Merged of dapi/Pe-cy5, CD133, Same cells conjugated with fluorescein isothiocyanate reflecting high expression; C: Neural cells, conjugated with fluorescein isothiocyanate (FITC)/Ne; D: Neural stem cells, conjugated with Pe-cy5 with mosaic pattern including the cells with high, very low-, and lacking-expression; E: Conjugated with Pe-cy5/neural cells; F: Co-expression of Dapi/Ne/CD133/VEGF; G: Neural cells conjugated with FITC; H: Vascular endothelial growth factor conjugated with Rpe; I: CD133 conjugated with Pe-cy5. The migrated neural cells with high or low expression are shown with arrows. The micro-vesicle and the vascular section harboring the migrated neural cells are detectable. The micro-vesicle and the vascular section harboring the migrated neural cells are detectable. Ne: Neural cells conjugated with FITC; CD133: Neural stem cells, conjugated with Pe-cy5; VEGF: Vascular endothelial growth factor, conjugated with RPe; The CNCs were explored by IF method for combination of neural marker, neural stem cell for Ne/CD133 (Figure 7).

magnitude of the alpha rhythm reduces in posterior cortical regions, and this is related to the level of general cognitive function[34]. However, numerous studies indicate that compared to age-matched controls, both mild cognitive impairment (MCI) and AD suffer from changes in the pattern of their EEG recordings: Alpha and beta rhythms typically decline, whereas delta and theta oscillations generally increase[35-37]. Such evidences highlight the key application of EEG through the course of disease.

At the AD-initiation, there is an increase in slow waves, notably in the theta rhythm. The earliest indicators of cognitive deterioration and the theta power rise are related[38]. Theta relative power, (which is expressed as the percentage of theta band power to all other bands), is higher in AD than in MCI and higher in MCI than in healthy controls and is associated with worsening performance across all cognitive domains[39]. alpha power is lower in AD than in MCI, and in MCI than in normal people[40]. There is a correlation between the decline in alpha activity and the severity of the illness and the cognitive abnormalities[40]. In addition to spectrum features, EEG also has synchronization features. EEG synchronization also describes how various brain oscillations are adjusted. When two distinct signals lock in phase, become phase-amplitude coupling, or modulate their amplitudes simultaneously (amplitude-amplitude coupling), they are said to be coupled[41]. One of these is spectral coherence measurement, which measures the spectral covariance of activity between two electrode locations. When compared to healthy controls, EEG coherence in AD participants exhibited statistically significant changes[42-44]. Alpha wave coherence in the temporo-parieto-occipital regions decreased in AD patients, although delta wave coherence between the frontal and posterior regions increased[45]. Considering the previously

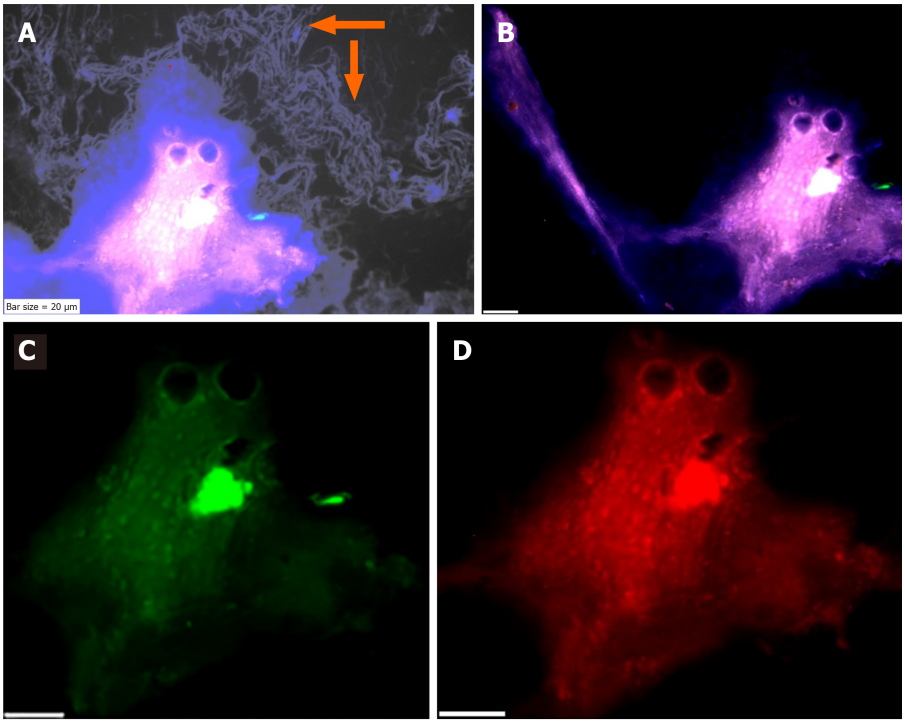

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Figure 7 Protein expression of neural marker, and CD133 in the circulated neural cells from the neural system to the blood stream in a patient with Alzheimer disease. A: Merged of phase contrast/dapi/neural marker (NE)/CD133, in which the presence of beta-Amyloid is remarkable in the background of this image; B: Merged dapi/NE/CD133, reflects the co-expression between NE and CD133; C: Diverse expression of NE, reflects the dissected of only upper section of image with low expression of NE; accompanied by an isolated section with low expression of NE; including group of cells with positive expression; D: Diverse protein function including high and low expression of CD133. The protein expression of NE/vascular endothelial growth factor/erythroblastosis virus E26 oncogene homolog 2 is also provided (Figure 8).

mentioned theory that basal forebrain neurons are severely impaired, all EEG characteristics associated with AD, such as changes in frequency patterns and synchrony measurements, may be caused by the loss of neurons, altered anatomical structure of the neuronal tracts, as well as altered release of neurotransmitters, all of which lead to impairments in neural activity[44].

Highlighted points of view

Neurological disorders are extremely complicated with an influential impact on the whole society including the patients' family and close friends. Therefore, the educational packages are required to create the cooperative surrounded living atmosphere between the patients, family and the friend.

Regarding the muti-dimentional approach of the neurological disorders, including AD, the following facts and directive points of view in the recent review article provided by Tanaka and Vécsei[46]: Differentiating the "neuroprotective and neurodegenerative modules of neurological and neuropsychiatric diseases", highlighting the etiology, available techniques, diagnostic and therapeutic approaches of neurodegenerative diseases.

As the whole, neural engagements can be stabilized by the" neuroplasticity" which is characterized by: (1) The nervous system capabilities to convert other components; (2) based on the status of functions, structural format, and constructive ability; and (3) being malleable, tolerated and adaptive against the non-desirable event and challenges[46].

Brain EEG

Regarding the traditional brain technology for AD- patients, and by focusing on the psychological disorders, the neurodevelopmental aspects of the brain, have not been much unmasked. Indeed, EEG, as a globally trusted screening system, applied to measure the brain motion for a century. EEG affords the brain's electrical motion and measure the electro cortical rhythms[6,7,9,14].

The EEG reports are skillful in: (1) Resting status; and (2) closed eyes using a Delta-med digital EEG acquisition system for 20 min. Scalp electrodes were adjusted according to "the modified International 10 ± 20 system plus 11 additional electrodes by 256 Hz sampling rate". Thirty electrodes were positioned on the scalp include Fp1, Fp2, F7, F3, Fz, F4, F8, FT7, FC3, FC7, FC4, FT8, T3, C3, Cz, C4, T4, TP7, CP3, CPz, CP4, TP8, T5, P3, Pz, P4, T6, O1, Oz, and O2.

Furthermore, by considering especial care about the patients affected with AD at preclinical zone, information including classification up to the diagnostic principles requires the complementary attention[17].

Ratio was calculated between different brain- channels to achieve the manner of motions between different channels at pre- and post-mediation with the LM. Therefore, the LM as a balancing and relaxing the neural targets mediated the Volunteers.

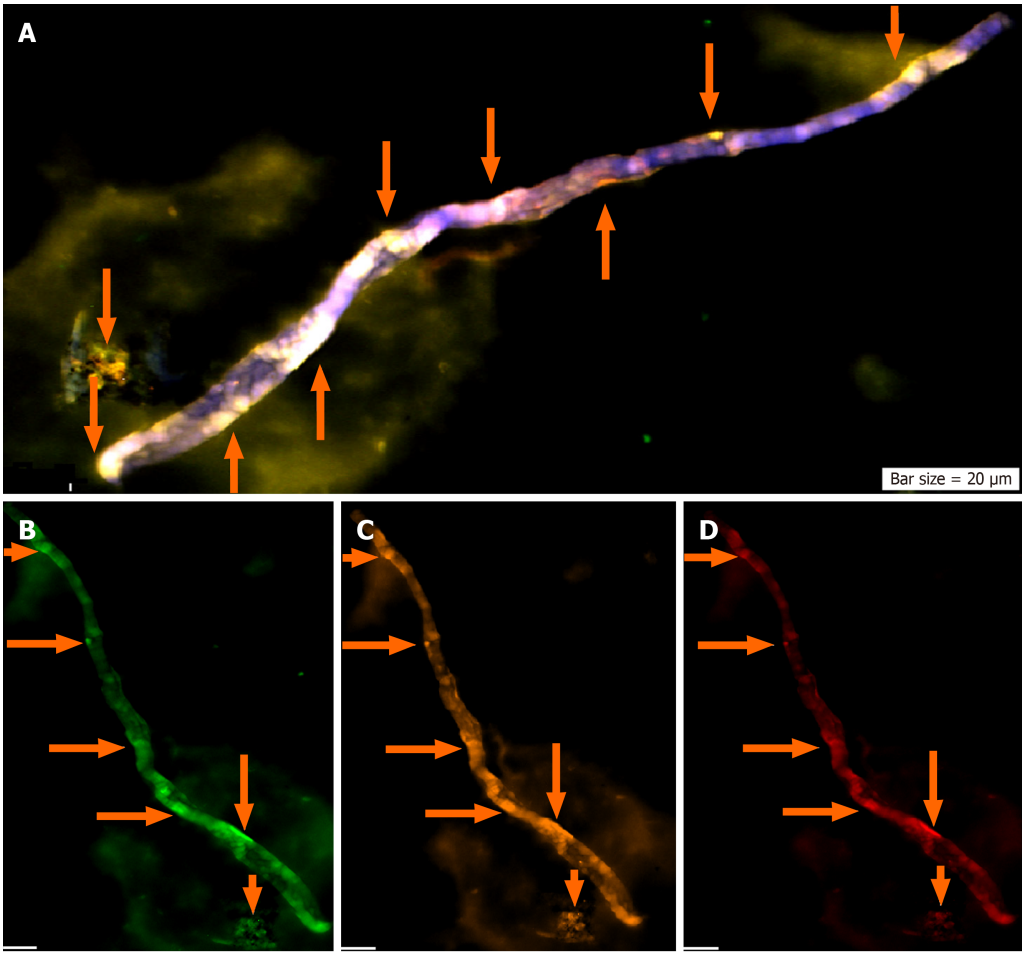

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Figure 8 Protein expression of neural marker/vascular endothelial growth factor/erythroblastosis virus E26 oncogene homolog 2 in the circulated neural cells from the neural system to the blood stream in a patient with Alzheimer disease. A: Circulated neural cells (CNCs) with dapi/neural marker/vascular endothelial growth factor (VEGF)/erythroblastosis virus E26 oncogene homolog 2 (Ets2) reflecting remarkable co-epression between 3 proteins; B: Few CNCs with Ne (conjugated with fluorescein isothiocyanate), reflecting high expression; C: The cells are conjugated with RPe and reflects VEGF with diverse high-, low- expression; D: Cells are conjugated with Pe-cy5 reflects Ets2 with diverse, mostly low-, accompanied by cells with high- and lack of expression.

Complementary notes, challenges and highlights on EEG in brain: Focusing on AD

The history and the benefit of EEG in research and clinics are, approximately, related to the 120 years ago (since 1930s). However, the scientific knowledge lacks the course of evolution through the brain channels. Whatever the Scientists unmask, is required to reflect: (1) The initial/fundamental event(s) through the brain channels; (2) priority of the events; (3) the cascade manner of the alterations; (4) interaction between the channels; (5) stability of the alteration(s); (6) unmasking the resting state of EEG; and (7) unveiling the course of evolution in the human brain channels.

The function and role of Broadman's areas including 64 channels of EEG are directive to unmask the complementary/paralleled function of the brain territory. However, the interaction between the channels requires to be personalized and classified. Any kind of mediation may increase or decrease the activity of alpha and gamma waves, respectively, and affect the brain metabolism and anxiety.

Mediation by LM led us to provide a brain channel based and personalized model, and to unmask the nature of process through the brain channels, including a significant increase in Alpha and Beta bands at frontal, central and occipital lobes of the brain.

Ratio between different brain channels unveil the diverse signal pattern at the pre- and post-mediation with the LM.

As a question, whether any mechanism relies on an interactive system between the brain's channels or not. So, 63 channels were investigated in 5 cases, at pre- and post-mediation with LM.

Based on the *t*-test, a significant increased pattern in alpha wave- and decreased in gamma wave activity, and reduction in the brain metabolism and anxiety were observed in the candidates' EEG ($P < 0.05$). As the result, a significant increase in Alpha and Beta bands of the frontal, central and occipital lobes of the brain were observed. The waves including Alpha 1,2; Beta 1-3, in both eye conditions, either open or close, the power is found between 1 to 1.6. Diversity is remarkable with close eyes than in open eyes condition.

By considering the power ratio of brain signals, the post- *vs* pre-mediation of LM was explored in the candidates. The outcomes of mediation with LM commanded to significant escalation of the power of alpha band wave at Fz, F4, FC2 sites (frontal lobe), Cz (central lobe) and O1 (occipital lobe). The increased activity of Alpha waves in Centro-frontal regions

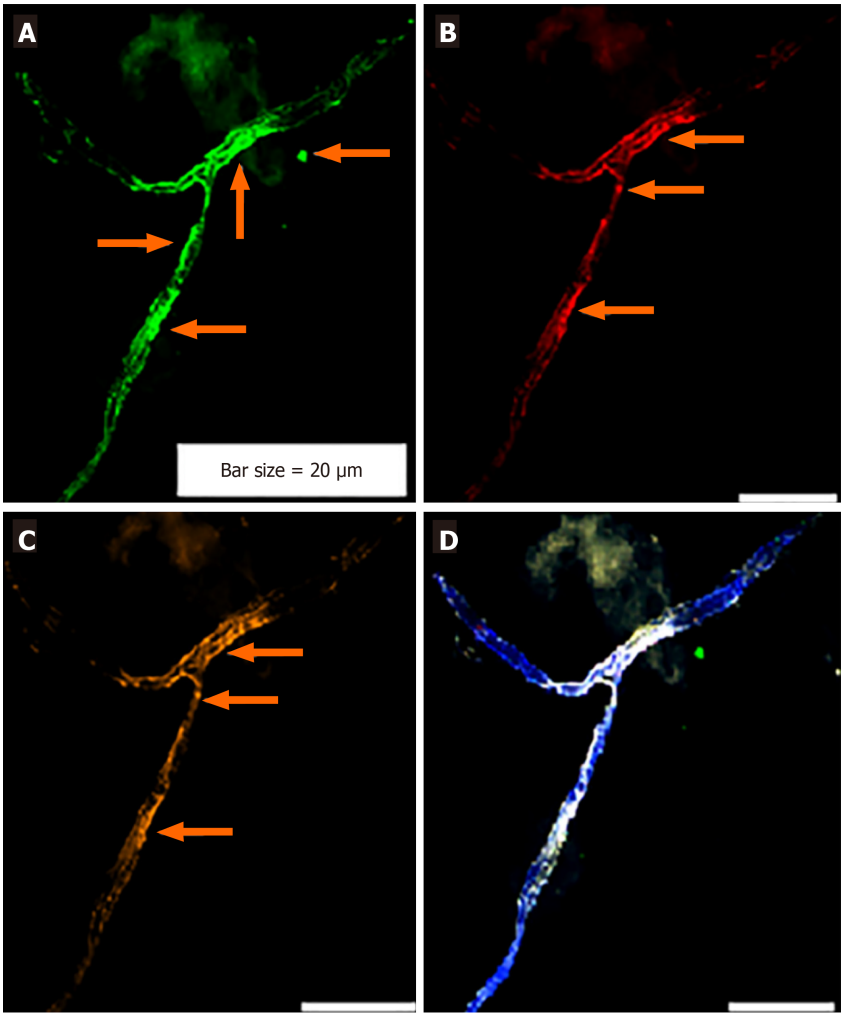

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Figure 9 Protein expression of cytokeratin-19/CD45/endothelial growth factor in circulating neural cells in the blood stream of an apparently healthy individual under mediation with light music. A: The circulating neural cells (CNCs), conjugated with fluorescein isothiocyanate for cytokeratin 19 (CK19); B: Same image with Cy5 for endothelial growth factor (EGF); presenting less cells expressing than CK19; C: Same image with R-phycoerythrin for CD45, and expressing less than CK19; D: Co-expression of dapi/CK19/EGF/CD45. The arrow refers to the CNCs cells with remarkable expression. Presence of CNCs was confirmed by the mode of cytokeratin 19+/CD45.

represents *edited* brain activity, metabolism, and reduced anxiety. Conclusively, the brain requires fewer properties for managing its cognitive task. Data of EEG revealed that the mediation, has significantly led to: (1) Increasing the Alpha and Beta bands of the frontal, central and occipital lobes of the brain; (2) reduction of the brain metabolism and anxiety; and (3) declining the activity of gamma wave in the EEG.

Remarkable diverse pattern was observable between absolute pattern for Gamma 1 and 2 at open eye- (characterized with < 1 absolute power) than close eye-condition (characterized with > 1, ranging between 1-7).

Status of the absolute power ratio in all brain channels sounds as an Informative panel in the brain research and with possible translational impact on personalized classification in Medical Neurology. Exploring of the absolute power ratio in all brain channels could be performed upon the physician's authorization, at different periods of life, including childhood, adulthood and elderly, by issuing the '*Brain ID card*'. Such preliminary and three complementary ID-card pave the way towards tracing the course of *evolution* at brain channels, and the probable connective pathways to the certain disorders. Such Information provides the 5xP package for any preventive, and/or the clinical recommendations to the target patients (Figures 10, 11, 12, and 13).

Conclusively

The provided Ratios are considered as the reliable indices for organizing the behavioral status of brain channels (Figures 10, 11, 12, and 13) to: (1) Guarantee an early detection; (2) being accompanied by performance of PE in the non-invasive tissues, including 2-3 mL peripheral blood, buccal smear and/or mucosa, nail, urine, stool, and/or through performing circulating cells from the target organs/tissues; (3) considering as the personalized, predictive, preventive, and prognostic panel for the patient's relatives within the pedigree; (4) cooperation between different channels with diverse interactive ratios lead to the balancing strategy for improving the life-quality and quantity of the patients with neurological disorders and an apparently normal individuals, by light nusic and any other safe, non-invasive and beneficial

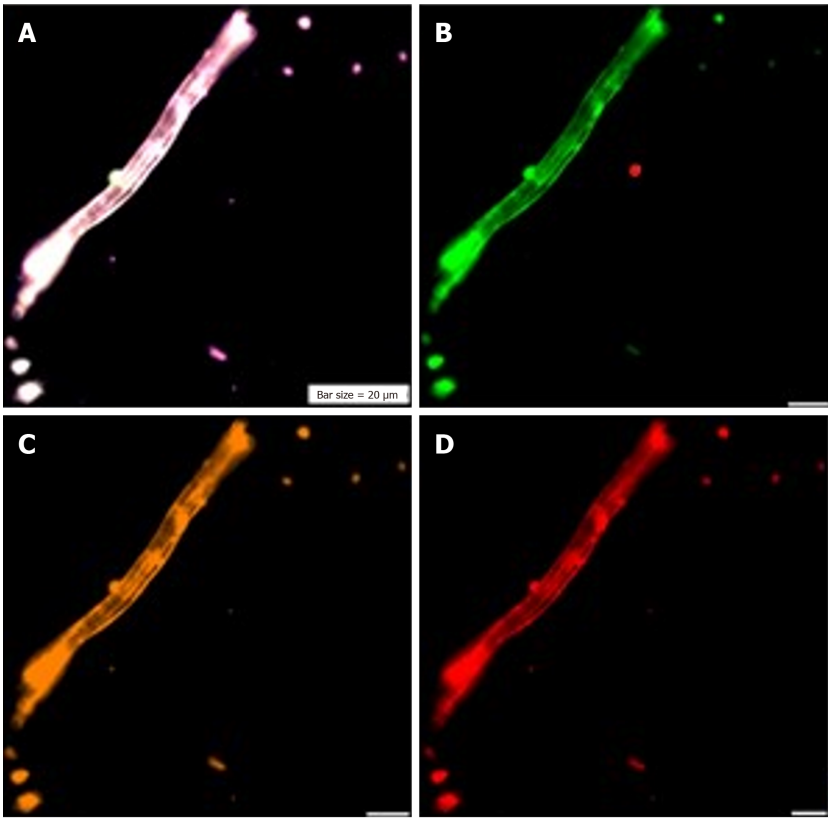

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Figure 10 Circulating neural cells. A: Co-expression of Dapi/NE/CD133/vascular endothelial growth (VEG); B: Neural marker, conjugated with fluorescein isothiocyanate; C: CD133: Neural stem cell, conjugated with R-phycoerythrin; D: Vascular endothelial growth factor, conjugated with Pe-cy5. A-D images present circulating neural cells within the blood stream. Neural marker (B) reflects high expression; neural stem cell (C) and VEGF (D) are characterized with the harmonic and higher protein expression.

intervention(s); and (5) by considering the provided hypothetic based data, heterogenic/diverse results presented the occurrence of evolutionary course within the brain channels. Multi-interaction between different channels, are suggested as the personalized and constructive markers with unlimited cooperation in health and disease.

The influence of the light music on the brain channels reflected the diverse patterns in the absolute power ratio at pre- to post-intervention (Figures 10, 11, 12, and 13).

Power of the brain channels

Mean Power of Delta (HZ) before and 20 min after mediation with LM revealed the significant differences, in the following brain channels: Mean Power of the Delta waves frequency (Hz) at before and after mediation with LM is found to be significant with p-value ranging between 0.013-0.031 for the channels including Fz-, F44, FC2-,Cz- and O1-LE. Furthermore, the mean power of Beta- gamma before and after mediation ranged between 0.001-and 0.048 for the channels Fz-Le; F4-Le; FC2-Le; Cz-Le; and O1-Le of Alpha wave.

It is not documented that whether status of the delta-wave frequency, between the closed and opened eyes in different brain channels is diverse or not. Therefore, we aimed to explore the ratio-pattern between absolute powers in different channels (Figures 10, 11, 12, and 13).

Interaction between different brain channels, either balanced or imbalanced, is a personalized pattern, leading to the new classification in the brain. Exploration of EEG according to the following results and discussion reflect the remarkable diversity between different channels, which could be classified as the unique/personalized classification for the brain behavioral Science (Figures 10, 11, 12, and 13).

Amongst the current diverse status of the delta-wave frequency, 8/9 ratios are characterized with the higher frequency for the closed eyes than in the opened eyes' condition including F8, F7, FP1, T7, C4, F3, and F4. Besides, the diverse status includes the ratio with higher values in the opened eyes including Cz, F3, Fz, Pz, P4, P8, P3, O1 and O2 with less remarkable diversity, but still diversity is notable.

By considering the wave frequency of Delta, the ratio of closed eyes to the open eyes is reflective of: The most stable targets are traced in the C3 (12.23392/12.9445), T8 (CE: 6.965104/6.241952); and P7 (CE: 7.173759/OE: 7.919045) (Figure 11).

The indices as discordance based grouping with < 1 ratio, at post mediation by LM: The F3, Fz, Cz, P3, Pz, P4, P8, O1, O2 are classified as discordance grouping with ratio < 1. With the current diverse status of the Delta-wave frequencies, 8/9 ratios are characterized with the higher frequency for the closed eyes than in the opened eyes' condition including F8,

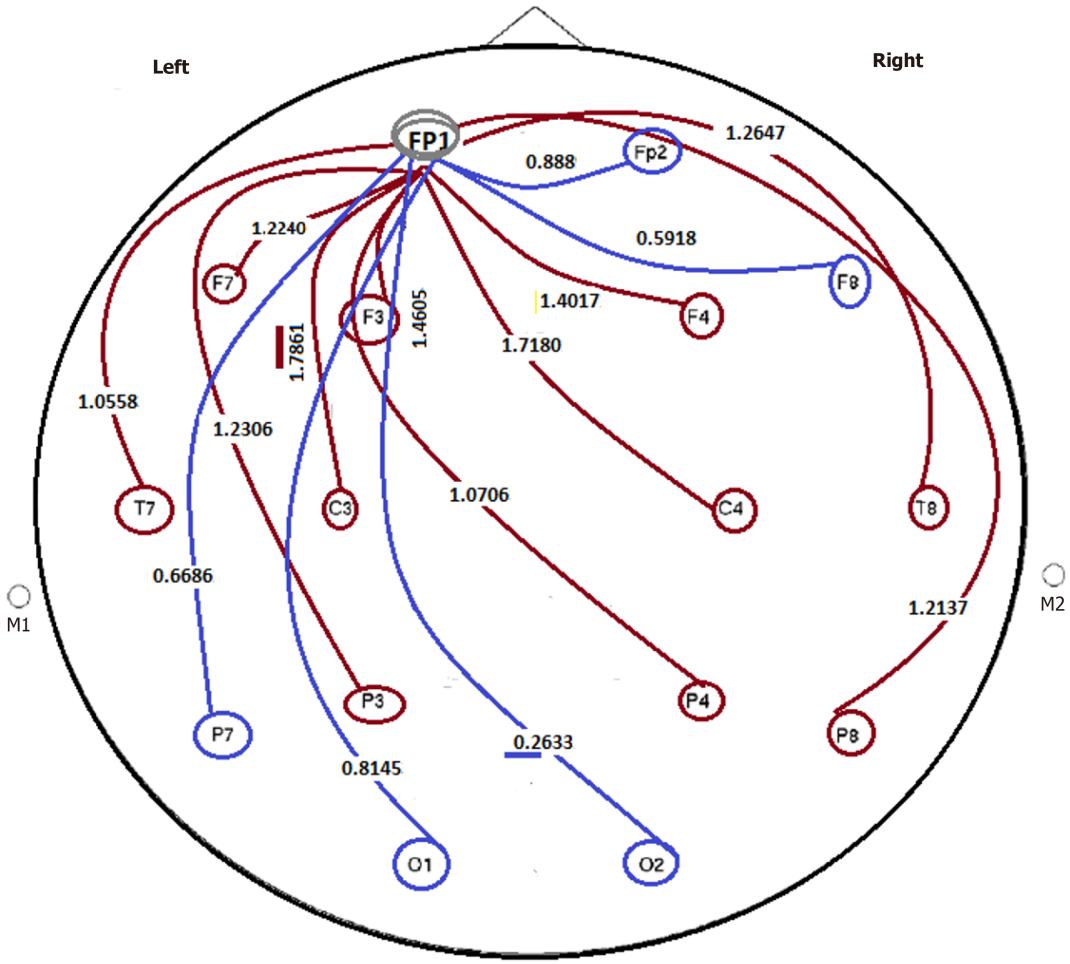

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Figure 11 Power ratio at pre- to post applied condition of closed/open eyes in an apparently healthy individual. By considering the applied condition- model as closed/opened eyes, evaluation was performed by considering the ratio of FP1 to other channels: (1) Minor group: Cooperation from polar section of brain, *i.e.*, between FP1/Fp2 = 0.888; FP1/F8 = 0.5918; FP1/O2 = 0.2633; FP1/O1 = 0.8145; FP1/P7 = 0.6686. All ratio < 1. Range of ratio: 0.2633-0.8145; (2) major group: have diverse destinations including FP1/T8 = 1.2647; FP1/P8 = 1.2137; FP1/F4=1.4017; FP1/C4 = 1.7180; FP1/P4 = 1.0706; FP1/F3 = 1.4605; FP1/C3 = 1.7861; FP1/F7 = 1.2240; FP1/P3 = 1.2306; and FP1/T7 = 1.0558. All ratio > 1. Range of ratio: 1.0558-1.7861. In this image, FP1 is the unique station as the primary initiating action within the brian channels, as octopods. There is a uique pattern as one direction from the upper to lower-section of the brain, including 10 indices less than 1 ratio (the blue lines) and 10 indices higher than 1 (brown lines). Such remarkable diversity is indicative of the well defined as two opposite directions from a unique central station towards 20 destinations. By defining the status of closed- to open eyes including > 1 as relaxation and < 1 as stressful conditions, then the challenging points are related to two distinct-outcomes. But as the matter of fact, the imposed rule is the unique major initiator, as the main station towards two categorized destinations. This regulation is considered as: (1) The correlative/single station, as major initiator/hypothesis; and (2) two distinct Ratio based categories, including > 1 and < 1 (Figure 11). The question is related to the diverse impact on the behavioral, emotional and other related physical and/or mental behaviors. Keeping the stable behavioral balances is the major aim with the reliable, harmless, and available tools such as light music and/or any relaxation method. This data reflects: (1) An EEG, personalized based and reliable categorized hypothesis; (2) with aim of detecting the behavioral characteristics of both group of individuals including an apparently healthy and individuals with either affected with Alzheimer disease, or at primary stages of AD; (3) considering an early detective strategy; and (4) organizing the prognostic, predictive, and preventive management as early as possible. F: Frontal; O: Occipital; C: Central; FC: Between F and C; AF: Between Fp and F; C3,4: Central Lobe; F3,4,7,8,z: Frontal lobe; FP1,2: Pre frontal cortex; PF3,4,7,8: Partial Lobe; O1,2: Occipital lobe; T7,8: Temporal 7,8.

F7, FP1, T7, C4, F3, and F4. Besides, the diverse status includes the ratio with higher values in the opened eyes including Cz, F3, Fz, Pz, P4, P8, P3, O1, and O2 with less remarkable diversity (Figure 11).

By considering the wave frequency of Delta, the ratio of closed eyes to the open eyes is reflective of: The most stable targets are traced in the C3 (12.23392/12.9445), T8 (CE: 6.965104/6.241952), and P7 (CE: 7.3759/OE: 7.919045) (Figures 11 and 12).

Diverse grouping with discordance index: The indices as discordance based grouping with > 1 ratio, at post mediation by LM: F7, Fp2, F8, FP1, T7 in > 1 category, of those F8, and F7, reflect remarkable high ratio than others in this classified group (Figure 12).

Conclusively, remarkable diversity is detectable for the Ratio between closed eye/open eyes after the post mediation by LM.

Descriptive data includes before and after mediation with the LM, at the significant level of the alpha band. Mediation by LM, significantly decreases the power of Beta-Gamma band wave at points Fz, F4, FC2 (forehead areas), Cz (central

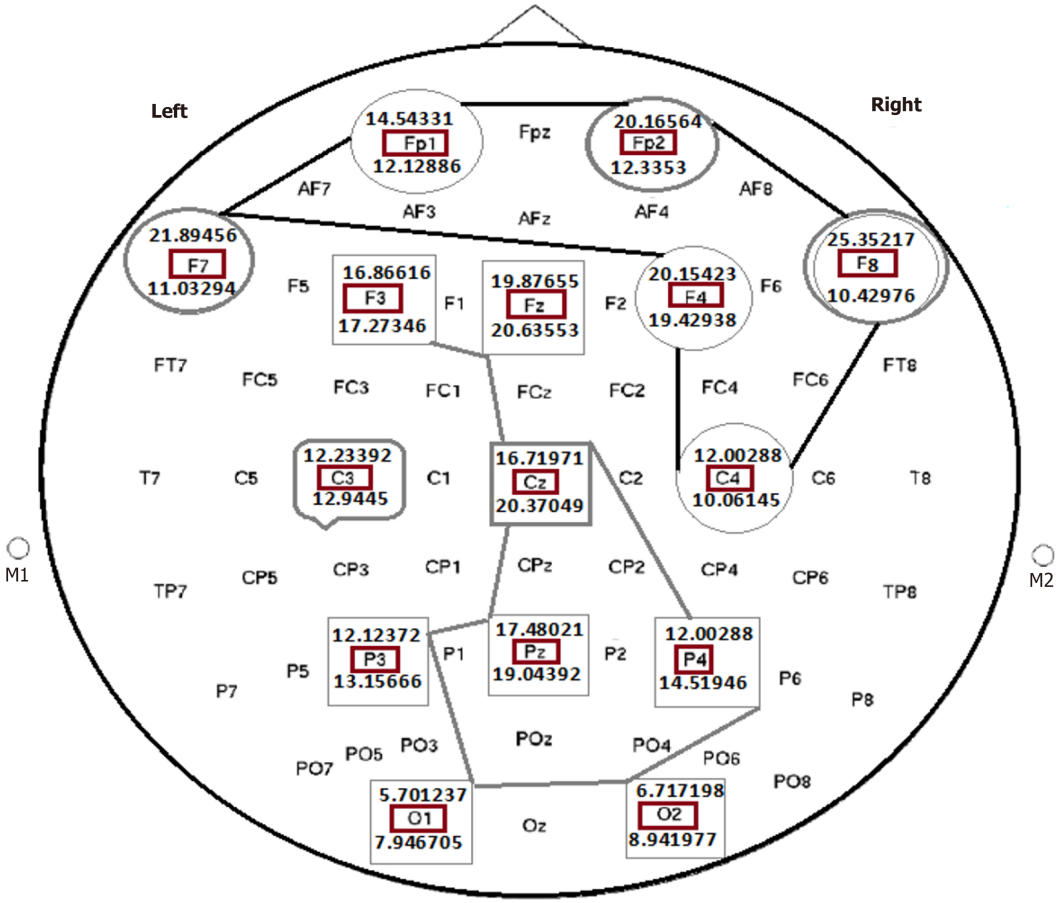

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Figure 12 Status of the absolute power Ratio by considering closed eye to open eye, based on the mediation of light music on the selected brain channels of a healthy individual. This figure includes 19 brain channels. (1) Mean power of delta (HZ) 20 min post mediation with light music; (2) channel's names represent locations of brain including Frontal (F), Occipital, central (C), between F and C, between Fp and F; and (3) the significant value: $P < 0.05$. Figure 12 is indicative of the cooperative/multi-centers/and two distinct categorized correlations including 6 and 8 destinations include: (1) Indices > 1, characterized with diverse degrees of ratio as > 1; and (2) the cooperative cluster involed 8 channels, including F3, Fz, F4, Cz, P3, Pz, P4, O1, nd O2 all with ratio < 1. These channels have impact through the middle section of brain brain from up-to down-destination. Channel C3 is characterized with almost ratio = 1, and it seems that the cooperative manner with other channels, is considered as an independent chanel. Catagories: Closed eyes (CE), opened eyes (OE); CE < OE; CE > OE; CE remarkably > OE; Almost equal CE and OE. This figure includes 19 brain channels. F: Frontal; O: Occipital; C: Central; FC: Between F and C; AF: Between Fp and F; CE: Closed eyes; OE: Opened eyes. By considering 19 channels, the absolute power ratio in all brain channels by considering close eye to open eye, based on the mediation of light music in the brain channels of a healthy individual provided the following results (Figure 13): Diverse grouping with discordance index includes: The indices as discordance based grouping with < 1 ratio, at post mediation by light music, and includes: F3, Fz, Cz, P3, Pz, P4, P8, O1, O2 which are classified as discordance grouping. The indices as concordance based grouping and includes: (1) HZ Pre- and 20 min post mediation with light music; (2) channels' names represent locations of 19 channels in the brain including: C3,4,z: Central Lobe; F3,4,7,8,z: Frontal lobe; FP1,2: Pre frontal cortex; PF3,4,7,8,z: Partial Lobe; (3) the results, based on the ratio of closed eyes to the open eyes is classified as concordance and discordance; and (4) closed eyes, and opened eyes (P value: $P < 0.05$). In upper frame, except F3 with ratio of < 1, the other 9 channels present ratio of > 1. The blue-line group, are reflective of mosaicism including P7, C3 and T8 with equal ratio; Cz with < 1, and C4 with > 1. Interestingly, the most harmonic channels are traced within the middle region (red line includes the cooperative panel) which are characterized with the < 1 ratio between 9 different channels through the horizontal and vertical distributed channels (Figure 13).

region) and O1 (occipital region of the brain). Such a decreased power of beta-gamma reflects the reduced activity and connectivity of brain between frontal/central regions.

Concisely, an increased alpha power and decreased beta-gamma power after mediation of the LM is remarkable. This link between neural oscillations in diverse frequency bands proposed to harmonize neural processing.

Furthermore, concordance and discordance between different channels of brain is the 5xP model after mediation with LM or any selected mediated factor, upon the specialists/physician's recommendation for further planned clinical management(s).

CONCLUSION

Application of CNCs and correlated Ratio based between brain channels by providing the 5xP personalized clinical management model for early detection and therapy of the patients with AD and their targeted/predisposed relatives. Highlights offer an early detective platform by considering neuro-genetics/cell biology, CNCs; molecular investigation,

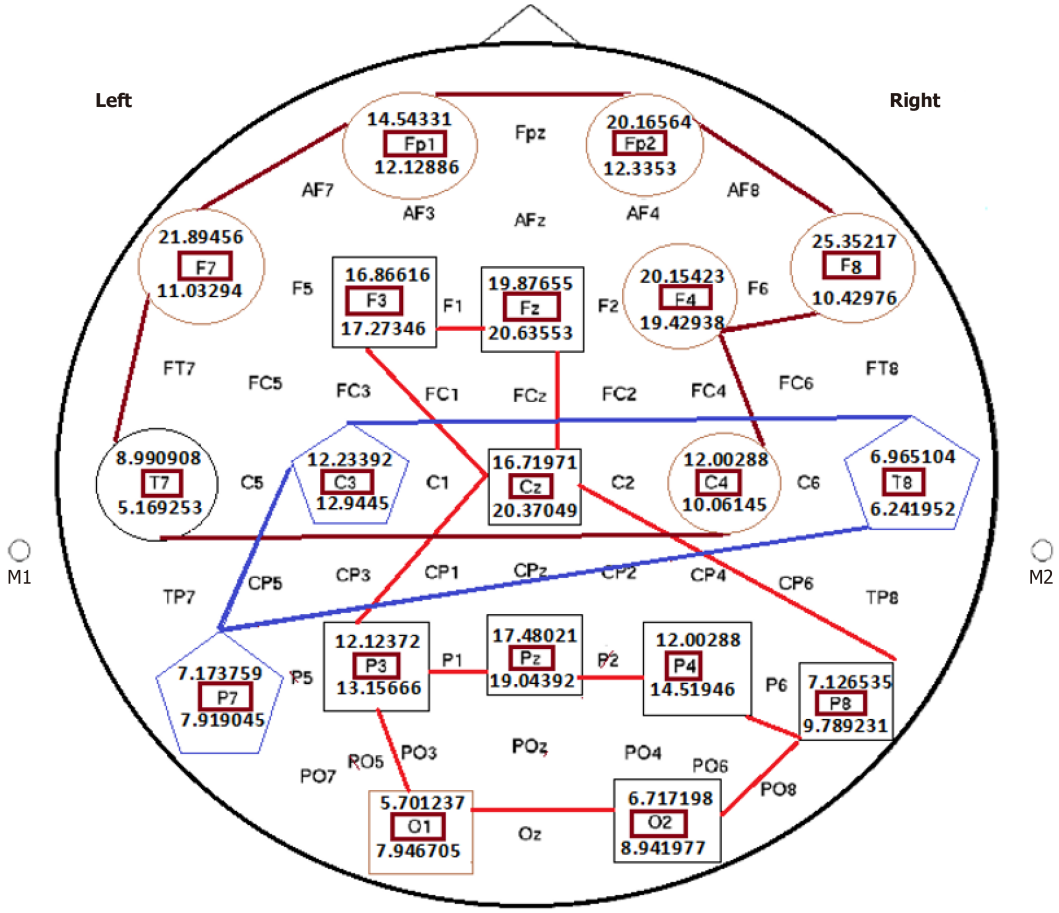

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Figure 13 Status of the absolute power Ratio in all brain channels by considering close eye to open eye, based on the mediation of light music in the brain channels of a healthy individual. This figure includes 19 brain channels. The absolute power ratio in all channels are an Informative frame in the brain research with target based translational impact on personalized classification in Medicine/Neulogy. The cooperative strategy between different channels is rather complicated in which 3 diverse, unique and cooperative platforms are established which could optimize the status of the mental behavior and health status of the target individuals. Brain is governed by multi-centered channels, being capable of influential cooperation.

for the predisposed individuals to AD- or other neural disease. The initiation and functional stage of triangle PE including neural-marker, stem cell (CD133), and VEGF is unmasked not only at the embryonic phase, but through the beginning of chorionic villus period around 8th-10th of gestational weeks. The mediation with LM resulted to remarkable escalation of the power of alpha band wave at frontal lobe including Fz, F4, FC2 sites, Cz in central lobe and O1 in occipital lobe and the increased activity of Alpha waves in Centro-frontal regions.

ARTICLE HIGHLIGHTS

Research background

Circulating neural cells (CNCs) is an essential strategy which offers the *pentagonal* application including the Personalized, Prognostics, Predictive, Preventive, predisposing (5xP) panel in the clinical neurology and Neuro-Science. Therefore, it was aimed to explore the developing picture of CNCs' behavior of the brain cells in the blood stream. CNCs are an available approach for the preventive Medicine and the personalized/target-based therapy.

Research motivation

This strategy includes and offers: (1) Routinizing the CNCs assay through the high and reliable enumeration of the CNCs; (2) providing the required information's of CNCs to the neurological clinics for the patients affected with neurological disorders including Alzheimer disease (AD); (3) providing adequate Information on the application of CNCs for the protocol to the target clinical centers for either their referral patients affected with, or predisposed to AD as promptly as possible; (4) highlighting the safety, rapidness of the process, non-invasiveness of the CNCs exploration; and an early/preventive strategic detection of the AD in the probands and the relatives through their pedigree; and (5) considering the pedigree-based analysis for tracing of any micro- and macro-environmental predisposing factors, including nutrition and the history of the neurological based diseases including AD.

Research objectives
In the Alzheimer-prone families, the history of any related clinical sign of the neurological symptoms including light and/or progressive through different generations of the referral suspicious case, could be candidate for exploring CNCs. If there is any persuasive micro-and/or macro-environmental risks, the target of the proband's offspring may be candidate for the CNCs.

Research methods
The originality of the provided research is based on the Manuel performance and exploration of the single cell based analysis with high enumeration. This strategy is capable to unmask the heterogeneity, diversity and highlights the specific role of the target proteins, and identification of the novel involvement of additional protein through the developmental stages of AD. Furthermore, by respecting the bridging system between Medicine and Science, and according to the primary results of Electroencephalography, as a golden performance, an innovative, complementary and predictive model is provided in this paper. Finally, based on an early detection, and the developmental process through the passed progressive period of AD, the personalized detection, hopefully, leads to the personalized therapy.

Research results
The novel results of the provided manuscript are characterized with: Personalized data; Single cell based strategy; and translatable data for the index case and their relatives; an early detection of the protein expression (PE) and brain channels bridging systems, the personalized therapy, could be translated, either for Alzheimer or the related psychological related to the brain disorders. Hypothetic/heterogenic/diverse results highlight evolutionary course within the brain channels.

Research conclusions
Unmasking the diverse pattern of PE of the migrated cells from brain to the blood stream, by an adequate enumeration of the CNCs based on the Manuel analytical approach. Unveil the heterogenic pattern of the analyzed target proteins' expression. Multi-interaction between brain channels, are considered as the personalizd/constructive markers with unlimited cooperation in health and disease.

Research perspectives
Unmasking the role of other candidate protein(s). Considering the informative publication(s) as the complementary educational package. Bridging between Science and Medicine by considering the pedigree based translational system for neuro-model, as an early detective platform. Suggesting for stablishing the Neuro-clinic with the aims including 'Personalized-Predictive/Early detection/Preventive clinic for the families with 'Alzheimer disease' and in future with neurogenetics disorders'.

FOOTNOTES

Author contributions: Mehdipour P designed the research, analytical strategy and contributed the data on genetics and cell biology; Fathi N and Nosratabadi M, and Mehdipour P contributed equally to this work and analysed data of the brain channels; all authors wrote, have read, and approved the final manuscript.

Institutional review board statement: The study was reviewed and approved by investigation of the fundamental pattern in the brain channels' EEG of five apparently healthy individuals, with ethics ID "IR.IUMS.REC.1399.310".

Institutional animal care and use committee statement: This study was not involved any animal experiments.

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

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

Data sharing statement: No additional data are available.

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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LETTER TO THE EDITOR

Ophthalmologic implications to consider when using hydroxychloroquine to treat COVID-19 and induced arthritis

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Abstract

As the world continues to grapple with the novel coronavirus [coronavirus disease 2019 (COVID-19)], many treatments have been proposed to help alleviate the symptoms and reduce the mortality rate. Hydroxychloroquine (HCQ) is an antimalarial drug that is typically used for several autoimmune, rheumatic, and dermatological conditions. It has also been considered to treat and prevent COVID-19 and subsequent arthritis associated with the infection. This drug is known to cause retinal toxicity, which can lead to vision impairment or loss. While the exact mechanism is not yet fully understood, it is thought to be due to the accumulation of the drug in the retinal pigment epithelium. The risk of toxicity increases with long-term use or with high doses of the drug and is more likely to occur in patients with pre-existing retinal diseases or those who are predisposed to retinal diseases. In this context, several steps can be taken to monitor and minimize the risk of ophthalmological adverse events when using HCQ to treat patients with COVID-19.

Key Words: COVID-19; Hydroxychloroquine; SARS-CoV-2; Retinopathy; Maculopathy; Post-COVID-19 arthritis

Core Tip: Hydroxychloroquine (HCQ) is typically considered when treating rheumatic and autoimmune diseases. It has been currently considered to help treat symptoms of coronavirus disease 2019 and to help alleviate several clinical manifestations after infection. In this letter, several ophthalmological implications that should be taken into consideration when using this drug are discussed. While the drug may be beneficial in treating symptoms, ophthalmological manifestations can be of clinical importance. Proper diagnoses, periodic testing, and correct management of patients in chronic treatment with HCQ can ensure that any potential ophthalmological side effects are minimized.

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TO THE EDITOR

The paper by Bajpai *et al*[1] reports an interesting thorough review of the use of hydroxychloroquine (HCQ) and azithromycin therapy to treat and prevent coronavirus disease 2019 (COVID-19). HCQ is a synthetic derivative of quinine. The drug works by inhibiting the growth of the virus, thus reducing the severity of clinical manifestations of the disease. HCQ binds to the viral protein responsible for replica-ting the virus and preventing the virus from replicating[2]. It has been found to be effective in treating the symptoms of COVID-19, including fever, cough, and difficulty breathing [3]. In addition, HCQ also has anti-inflammatory properties, which can help reduce inflammation caused by the virus. A recent commentary by Swarnakar *et al*[4] based on the review by Bajpai *et al*[1] presented the possible interesting use of HCQ to treat arthritis induced after viral infection. Numerous studies in the literature have shown the benefits offered by HCQ in alleviating debilitating symptoms. The aim of this letter article is to summarize the important ophthalmologic considerations when HCQ is considered in the treatment of COVID-19. The issues regarding whether or not HCQ is effective in treating COVID-19, which was reported in the papers recently published in this journal[1,4], will not be considered in this article. Here, the important potential risk factors and ophthalmological side effects are briefly mentioned, with the aim of reminding clinicians about the ophthalmological considerations and best practices to monitor, diagnose, and manage patients using HCQ. This is imperative to ensure that any potential ophthalmological side effects and toxic damage are minimized.

HCQ is a drug traditionally used to treat and prevent a variety of different conditions. In brief, it is an antimalarial and anti-inflammatory medication that works by inhibiting the growth and spread of certain parasites, bacteria, and viruses. At the molecular level, HCQ works by inhibiting the activity of certain enzymes known as heme polymerases[5]. These enzymes are responsible for the synthesis of heme, a molecule that is essential for the development of some parasites, bacteria, and viruses. By blocking the activity of heme polymerases, HCQ prevents the growth and spread of certain parasites, bacteria, and viruses. HCQ is used to treat a variety of conditions, including malaria, lupus, and rheu-matoid arthritis[6]. In malaria, it is used to prevent and treat the disease, while in lupus and rheumatoid arthritis, it is used to reduce inflammation and relieve symptoms. In addition, HCQ is also used to treat and prevent certain types of malaria.

HCQ is generally considered to be safe and well tolerated, but some people may experience systemic side effects such as nausea, dizziness, and headache. Studies have shown that HCQ can sometimes cause glucose abnormalities, cardiotoxicity (conduction abnormalities, cardiovascular collapse, cardio-myopathy, *etc.*), gastrointestinal effects, neuromyotoxicity, neuropsychiatric events, and dermatologic reactions[7].

There are several important ophthalmologic effects of HCQ[8]. This drug has been shown to influence cellular autophagy and lysosomal activity. HCQ can also interact with membrane stability and alter transcriptional activity and signaling pathways[9]. The ocular side effects include corneal deposits, retinal pigmentary changes, maculopathy, and optic neuritis. These complications need to be closely monitored and managed in patients using high doses and long-term therapy with HCQ. Differential diagnosis, alternative therapies for underlying disorders, proper periodic testing for functional and anatomical toxicity, and treatment options are imperative to limit drug toxicity in patients under chronic HCQ medication.

Corneal deposits are usually seen in patients taking HCQ for more than 5 years and are generally considered benign. Corneal deposits and damage induced by HCQ can vary depending on the dosage and duration of treatment. Damage to the cornea can manifest in the form of corneal opacity and corneal deposits. These deposits are typically referred to as corneal verticillata and are comprised of amor-phous, yellow-white deposits scattered throughout the cornea[10]. Corneal verticillata can cause significant visual impairment if they are dense enough to obscure vision. The mechanism of action by which HCQ induces corneal deposits is not fully understood. It is believed that HCQ accumulates in the corneal epithelium and forms a complex with proteins and lipids, resulting in the formation of deposits[11]. This could also possibly be due to reduced tear turnover and accumulation of the drug in the tear film. Additionally, it is thought that HCQ can interfere with the normal metabolism of the cornea, resulting in the accumulation of lipids and other deposits. The effects of HCQ on the cornea can be severe and can lead to permanent vision loss. Therefore, it is important to monitor patients taking HCQ for the development of corneal deposits and damage. If corneal deposits are found, HCQ should be discontinued and other treatments can be considered.

Retinal pigmentary changes are characterized by granular deposits at the level of the retinal pigment epithelium (RPE) and can lead to decreased visual acuity and progressive visual field loss[12]. Maculo-pathy is a form of retinopathy that is characterized by a bull's eye maculopathy, which is a specific pattern of retinal damage that specifically affects the circular area of damage to the macula. This small area is the central part of the retina responsible for sharp, central vision [13]. The mechanism of bull's eye maculopathy due to HCQ toxicity is not completely understood, but it is thought to involve the drug's effects on the RPE cells due to the accumulation of the drug in the RPE, leading to the formation of pigmentary changes. These RPE cells play a key role in supporting the function of the photoreceptor cells in the retina, and HCQ may disrupt this support system, leading to damage to the photoreceptor cells and the characteristic bull's eye pattern of damage. This can lead to decreased visual acuity and pro-gressive visual field loss.

Diagnosis of bull's eye maculopathy due to HCQ toxicity can be challenging, as it can be difficult to distinguish from other retinal disorders. Differential diagnosis includes other forms of macular degeneration, such as age-related macular degeneration and Stargardt disease, as well as other retinal disorders such as cone dystrophy and macular dystrophy[14]. Testing for HCQ toxicity includes complete ophthalmologic examination with vision testing and dilated fundus assessment, visual field testing, spectral domain optical coherence tomography, fundus autofluorescence imaging, and multi-focal electroretinography[15].

Optic neuritis is an inflammatory disorder of the optic nerve, which can lead to reduced visual acuity, decreased color vision, and decreased peripheral vision. This complication due to HCQ is very rare. Differential diagnosis can be made by magnetic resonance imaging, angiography, blood tests, visual field testing, and visual evoked responses[16]. The mechanism of action is thought to be due to an autoimmune response to the drug, leading to inflammation of the optic nerve[17].

The incidence of HCQ toxicity is dose-dependent, with higher doses and longer duration of treatment increasing the risk of toxicity[18]. The recommended daily dose of HCQ that is generally considered safe is up to 6.5 mg/kg/d, but at higher doses, the risk of toxicity increases. Risk factors include a cumula-tive dose of > 1000 g of HCQ, treatment duration of more than 5 years, preexisting liver or renal dysfunction, being very elderly, and preexisting retinopathy[19].

It is yet unclear how HCQ can cause retinal damage. According to studies, the medication impacts the metabolism of retinal cells and binds to melanin in the RPE, which may help to explain why some people continue to experience side effects even after stopping the prescription. With regards to dysfunction related to toxicity, it is thought that HCQ binds to melanin in the RPE, blocking its function, which can lead to irreversible photoreceptor loss and resulting visual field defects over the afflicted sector of the retina. In some cases, bull's eye configuration can be seen as a ring scotoma on a visual field test when RPE malfunction leading to atrophy occurs across the perifoveal ring when the central fovea is spared. The half-life of HCQ is about 1 mo, with a washout period of about 6 mo. Early diagnosis of HCQ retinal toxicity is crucial to prevent maculopathy from progressing after HCQ use is stopped[20]. Besides retinopathy, the other ocular side effects, which tend to be benign or infrequent with low doses of HCQ, include keratopathy, corneal deposits, punctate/linear corneal opacities, infiltrates, ciliary body deposits, ocular muscular imbalance, lens opacities, papilledema, *etc*[21].

Treatment for HCQ toxicity involves cessation of the drug and monitoring for further progression of retinal damage. The prognosis for patients with bull's eye maculopathy due to HCQ toxicity is variable, with some patients experiencing improvement in visual function after cessation of the drug, while others may experience permanent vision loss[20-22]. In some cases, the damage caused by the drug may be delayed and irreversible, resulting in permanent vision loss. There are currently no Food and Drug Administration-approved treatments for bull's eye maculopathy due to HCQ toxicity [22].

In closing, it is important to remember that HCQ can cause several systemic and ophthalmological side effects, most of which are minor and reversible. The prognosis for patients receiving treatment with HCQ for COVID-19 and associated arthritis is generally good. Further research is needed to evaluate the potential long-term ophthalmological side effects associated with the use of the drug to treat and prevent COVID-19 and associated arthritis. Ophthalmological consider-ations should be evaluated prior to initiating the therapy, including baseline visual acuity, fundus examination, and visual field testing to exclude the presence of underlying preexisting ophthalmic retinal disorders. HCQ should be avoided in patients with ophthalmopathies, including any form of retinopathy. Patients receiving HCQ should be period-ically monitored for potential ophthalmological side effects, including retinopathy, corneal deposits, and papilledema. Immediate termination of HCQ and alternative treatment regimens need to be considered in patients that develop HCQ toxicity.

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LETTER TO THE EDITOR

Update on hydroxychloroquine use in pregnancy

Wassan Nori, Nabeeha Najatee Akram, Raid M Al-Ani

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Abstract

It is well-known that hydroxychloroquine (HCQ) treats malaria, systemic lupus erythematosus, and rheumatoid arthritis in women for its immunomodulatory and anti-inflammatory action. Additionally, HCQ was used in cases with refractory antiphospholipid syndrome. HCQ safety was reinforced in pregnant women owing to insignificant reports of adverse pregnancy outcomes and major congenital malformation. Recently, HCQ was tested in cases with chronic placental inflammation with a promising result of increased life birth; however, its benefit needs further validation. We aimed to highlight the recent updates for HCQ use in various conditions in pregnancy.

Key Words: Pregnancy; Hydroxychloroquine; Preeclampsia; Antiphospholipid syndrome; Chronic placental inflammation; COVID-19

Core Tip: The immunomodulatory, anti-inflammatory, and anti-thrombotic activity of hydroxychloroquine (HCQ), an anti-malarial drug, made it recommendable for rheumatoid arthritis and systemic lupus erythematosus. HCQ was also implemented in refractory antiphospholipid syndrome showing a successful outcome. Recent evidence supports the benefits of its use to outweigh the risk during pregnancy as it reduces the disease activity and the associated adverse pregnancy outcome. Chronic placental inflammation is another condition for which HCQ proved to be helpful. Further investigations are required to verify HCQ's efficacy in chronic placental inflammation as well as its action in reducing the severity of coronavirus disease 2019 in pregnant women.

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TO THE EDITOR

With interest, we read the Bajpai *et al*[1] study published in the *World Journal of Experimental Medicine* (issue 3, volume 12, 2022) that discussed the role of hydroxychloroquine (HCQ) in treating high-risk groups with coronavirus disease 2019 (COVID-19). Indeed, HCQ gained much interest during the current pandemic owing to its anti-inflammatory and immunomodulatory effects[1].

HCQ had an update regarding its use among pregnant, first in autoimmune diseases and its safety profile-second, its therapeutic role in cases with chronic placental inflammation. Finally, we discuss its potential use in pregnant with COVID-19, which is worth mentioning and was not discussed by Bajpai *et al*'s study[1].

HCQ was already used for treating women suffering from malaria, systemic lupus erythematosus (SLE), and rheumatoid arthritis (RA)[2]. In 2020, the American College for Managing SLE, RA for women of reproductive age, advised that those cases should receive HCQ before and throughout pregnancy[3]. In addition, pregnant women with refractory antiphospholipid syndrome may consider HCQ in addition to standard treatment (aspirin and low molecular weight heparin)[4].

HCQ was used as an adjunctive therapy for cases with refractory antiphospholipid syndrome[5]. It is a beneficial role proposed to be mediated *via* anti-thrombotic, antiplatelet, and immunomodulatory properties[6]. Others suggested that HCQ reduces endothelial dysfunction and improves vascular elasticity, thus improving blood flow[7].

Bérard *et al*'s study addressed pregnancy outcomes related to HCQ use in a cohort study that recruited 233748 pregnant women[8]. Interestingly the study showed that HCQ had a good safety profile. There was no increased risk of preterm labor among drug users; the adjusted odd ratio was 1.39, with respective 95% confidence interval (CI): 0.83 to 2.3. As for the low birth weight, the adjusted odd ratio was 1.12, 95%CI: 0.59 to 2.07. Finally, the adjusted odd ratio for major congenital malformation was 1.02, 95%CI: 0.68 to 1.53[8].

Another study confirmed no substantial rise in significant congenital malformations in newborns exposed to HCQ during the first trimester of pregnancy[9]. In line with earlier work[10,11]. These results reinforce that therapy advantage during pregnancy is likely to exceed the risks for the majority of patients with rheumatic disease.

Ye *et al*[12] discussed that HCQ application might alleviate the risk of high lupus activity during pregnancy and the incidence of preeclampsia. However, in their meta-analysis, Hu *et al*[13] found no value of HCQs in reducing preeclampsia in antiphospholipid syndrome. Moreover, HCQ had no value in reducing fetal growth defects in SLE and/or antiphospholipid cases.

The promising results observed with the use of HCQ to treat autoimmunity in pregnancy have laid the foundation for its use in chronic placental inflammation, a condition characterized by the disruption of healthy placental tissue. They can only be confirmed by a post-delivery histopathological examination[14]. Chronic placental inflammation has been linked to severe complications of pregnancy, such as fetal growth restriction, premature labor, and miscarriage[15].

Brady *et al*'s study examined the value of adding HCQ to pregnant women with a positive history of chronic placental inflammation, showing a decrease in disease severity and a trend for a higher live birth rate[16]. There are currently no prospective, informatively constructed, controlled trials on the efficacy of HCQs in these settings, which emphasizes the need for such work. Since some forms of chronic placental inflammation are recurrent, determining the cause is crucial for future pregnancies care.

The use of HCQ in COVID-19 cases will depend upon if the ongoing clinical trials demonstrate significant benefits of HCQ in reducing the incidence or severity of COVID-19[9,17,18]. Even though initial trials utilizing HCQ to treat COVID-19 failed to demonstrate efficacy, pre-exposure preventative trials are yet to be reported[9].

In conclusion, HCQ has demonstrated efficacy in mitigating the activity of autoimmune diseases and some of their adverse pregnancy outcomes while maintaining a favorable safety profile. HCQ has emerged as a potential therapeutic option for cases with chronic placental inflammation, as it enhances live birth rates while decreasing the severity of the associated disease. Nevertheless, the efficacy and safety of HCQ in pregnant individuals with COVID-19 have not been thoroughly assessed. Further research is needed to unveil more applications in practice.

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