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Whole-Exome Sequencing Identifies Susceptibility Genes for Vestibular Migraine: A Preliminary Study

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Title

Whole-Exome Sequencing Identifies Susceptibility Genes for Vestibular Migraine: A Preliminary Study

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WW and TC performed the study design. XZ acquired and analyzed the data. XZ and TC drafted the manuscript. QD, CW, XH, JL and JY revised the manuscript. All authors read and approved the final manuscript.

Abstract

Objective

Vestibular migraine (VM) is a central nervous system disorder characterized by recurrent episodes of vertigo accompanied by migraine symptoms. It exhibits high genetic heterogeneity, an unclear molecular pathogenesis, and a lack of specific biomarkers. This study aimed to utilize whole-exome sequencing (WES) to screen for pathogenic genetic variants associated with VM, identify susceptibility genes, and elucidate their regulatory pathways. These efforts provide an experimental basis for clarifying the genetic pathogenesis of VM and for developing precision diagnostic

biomarkers and targeted therapeutic strategies.

Methods

A total of 59 VM patients (53 females and 6 males; mean age: 49.27 ± 12.77 years) meeting the diagnostic criteria of the Bárány Society and 177 healthy controls were recruited from Tianjin First Central Hospital. Genomic DNA was extracted from peripheral blood for WES. Initial variant screening was conducted based on allele frequency, variant location, and protein deleteriousness predictions. Candidate variants were subsequently validated via Sanger sequencing. Functional enrichment and signaling pathway analyses of the candidate genes were performed using the Metascape database. A P-value < 0.05 was considered statistically significant.

Results

Females accounted for 89.83% of the VM cohort. All patients experienced moderate-to-severe headaches, with a high prevalence of unilateral pulsating pain, photophobia, phonophobia, motion sickness, and cochlear symptoms such as tinnitus and aural fullness. Through sequencing screening and Sanger validation, 16 candidate susceptibility genes for VM were identified. The mitochondrial metabolism gene NDUFB7 exhibited the highest mutation frequency (10.17%). Functional enrichment analysis revealed three statistically significant core pathways: monoatomic cation transmembrane transport, axon guidance, and the SYNDECAN 2 signaling pathway. The core susceptibility genes were categorized into four functional classes: mitochondrial energy metabolism (NDUFB7, TSFM); ion channel encoding (CACNA1I, KCNV2); neurodevelopment and cytoskeletal remodeling regulation (DOCK6, EPB41, EPHB2, GSN); and lysosomal homeostasis and immune regulation (HEXA, RAG1). Pathway interaction analysis indicated that mitochondrial energy metabolism dysfunction, neuronal ionic homeostasis imbalance, and aberrant axonal development and synaptic scaffolding regulation collectively contribute to VM pathogenesis.

Conclusion

The WES analysis of this VM cohort confirms that VM is a complex genetic disorder co-regulated by multiple genes, expanding the spectrum of candidate

pathogenic variants for VM. The pathogenesis of VM is mediated synergistically by multiple pathways, involving pathological processes such as mitochondrial energy metabolism dysfunction, ion channel dysfunction, and imbalances in neurodevelopmental and cytoskeletal regulation. These findings hold significant theoretical and clinical value, providing a foundation for elucidating the diverse clinical phenotypes of VM, distinguishing genotypic characteristics among different VM subtypes, and developing precision diagnostic biomarkers and potential therapeutic targets.

Keywords: Vestibular Migraine; Whole Exome Sequencing; Susceptibility Gene; Mitochondria; Ion Channel

Introduction

Vestibular Migraine (VM) is a neurological disorder characterized by the co-occurrence of recurrent episodic vertigo and migraine symptoms. In 2012, it was officially included in the third edition of the International Classification of Headache Disorders (ICHD-3) beta version by the International Headache Society (IHS) [1]. Its clinical manifestations involve vestibular dysfunction lasting from minutes to 72 hours (including spontaneous vertigo, positional vertigo, and visually induced vertigo, among others), often accompanied by migraine-like headaches, photophobia/phonophobia, and other typical migraine features [2, 3]. Epidemiological studies indicate that the prevalence of VM in the general population ranges from 4% to 5.7%, while its incidence is significantly higher among migraine patients, reaching 10.3%. Women are notably more affected than men (female-to-male ratio 1:3–5) [4], making VM the second most common cause of central vertigo after benign paroxysmal positional vertigo (BPPV). Although the diagnostic criteria for VM have been progressively refined, its pathophysiological mechanisms remain incompletely understood. Currently, the ICHD-3 diagnosis of VM still primarily relies on clinical phenotypes, lacking objective biomarkers, which poses challenges in differentiating it from conditions such as Méni ère’s disease and vestibular paroxysmia [5].

Significant progress has been made in the genetic research of migraine. Genome-

wide association studies (GWAS) have identified over 40 common susceptibility loci, involving genes related to neuronal excitability regulation (e.g., TRPM8, PRDM16), synaptic transmission (e.g., CACNA1A, ATP1A2), and vascular function (e.g., NOS3)[6]. Among these, multiple studies have demonstrated a strong association between TRPM8 and migraine risk, making it a candidate gene for VM[7, 8]. Mutations in ion channel genes such as CACNA1A and SCN1A can lead to familial hemiplegic migraine (FHM)[9, 10]. However, these studies predominantly focus on known functional genes or pathways, presenting certain limitations. On one hand, candidate gene studies are constrained by prior knowledge and may overlook unknown pathogenic genes. On the other hand, while GWAS enables genome-wide scanning, the high genetic heterogeneity of VM and insufficient sample sizes often result in weak association signals, making it difficult to pinpoint causative genes. Additionally, the clinical phenotypes of VM are highly diverse, and different subtypes may have distinct genetic underpinnings, which traditional research methods struggle to systematically elucidate."

Whole Exome Sequencing (WES) technology targets approximately 2% of the protein-coding regions in the human genome, enabling efficient detection of various genetic variants, including single nucleotide variants (SNVs) and insertions/deletions (Indels). This makes it a powerful tool for identifying pathogenic genes in rare and complex diseases [11, 12]. In recent years, WES has been successfully applied to uncover disease-causing genes in neurological disorders such as epilepsy [13] and Alzheimer's disease [14], demonstrating significant potential in elucidating disease genetic mechanisms. Given this, the present study employs WES in a cohort of VM patients. By systematically analyzing the exonic regions of VM patients and healthy controls, we aim to screen for genetic variants associated with VM pathogenesis, preliminarily map susceptibility genes, and provide a theoretical foundation for further clarifying the genetic mechanisms of VM and exploring potential therapeutic targets.

Materials and methods

Participants

This study enrolled 59 VM patients (53 females, 6 males; mean age 49.27±12.77 years) who visited the Department of Otolaryngology at Tianjin First Central Hospital between January 2023 and November 2024. VM diagnosis was established according to the Bárány Society diagnostic criteria[3]. Differential diagnoses excluded peripheral vestibular disorders (e.g., Ménière's disease, BPPV, sudden deafness with vertigo, Hunt's syndrome, labyrinthitis) and central vestibular pathologies (e.g., head trauma, stroke, or other central balance disorders). All procedures adhered to the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants after full explanation of the study's purpose and potential implications. The study protocol was approved by the Ethics Review Committee of Tianjin First Central Hospital (Approval No.: YC-BY-LC-2023-0020).

Genetic testing and WES

Following informed consent, peripheral venous blood samples were collected from probands. Genomic DNA was extracted and subjected to PCR amplification. Exonic regions were captured using targeted sequence capture probes (MyGenostics GenCap), followed by sequencing on the BGI DNBSEQ-T7 platform. Raw sequencing data were collected for subsequent analysis.

Variants Selected

Variant screening strategy for detecting potentially pathogenic mutations

After sequencing, the raw data were filtered by cutadaptor software. The clean reads were mapped to the UCSC hg19 human reference genome using the parameter BWA of Sentieon software.The duplicated reads were removed using the parameter driver of Sentieon software, and the parameter driver is used to correct the base, so that the quality value of the base in the reads of the final output BAM file can be closer to the real probability of mismatch with the reference genome, and the mapped reads were used for the detection of variation. The sequencing data were screened as shown in the flowchart in Fig. 1. First, all variants were initially screened based on their frequency, location, and variant effect, according to the following criteria: (1) the minor allele frequency (MAF) < 0.01 in both the 1000 Genomes Project and the overall cohort of gnomAD; (2) the MAF in the East Asian (EAS) subpopulation of gnomAD <0.01; (3) Vlocated in exons or splice sites; (4) nonsynonymous variants; and (5) not in

MyGenostics InNormal database; After that, the rest mutations should be the potential pathogenic mutations for further analysis.

Bioinformation analysis

Genetic variation verification and functional assessment were further conducted through multiple approaches. BAM alignment visualization was performed to verify the accuracy of variant calling and exclude false-positive variations. Missense variants were evaluated via protein function prediction software, including Sorting Intolerant from Tolerant (SIFT), Polymorphism Phenotyping v2 (PolyPhen2), MutationTaster, and Mendelian Clinically Applicable Pathogenicity (M-CAP), to assess the potential impact of variants on protein function. A deleterious effect on protein function was identified when ≥3 of the following conditions were met: (1) SIFT score ≤0.05; (2) PolyPhen2 prediction of probably damaging (score ≥0.909) or possibly damaging (score 0.447–0.909); (3) MutationTaster prediction of disease causing automatic or disease causing; (4) GERP++ > 2; and (5) M-CAP score > 0.025.

Sanger sequencing

Potentially pathogenic variants were further validated via Sanger sequencing. Primers targeting the candidate regions were designed with the online Primer 3 tool (https://primer3.org/). Raw sequencing reads were analyzed using Polyphred software. After eliminating false-positive signals from Sanger sequencing, the retained variants were ultimately identified as candidate pathogenic variants.

Statistical analysis

Gene Ontology (GO) enrichment analyses were performed based on Matescape database to predict the clinical phenotypes.Categorical variables were presented as number (percentage). Comparisons of categorical data were made by chi-square test. $p<0.05$ was considered as statistically significant. All statistical analyses were performed using SPSS V25.0 for statistics and GraphPad Prism 10 for visualization.

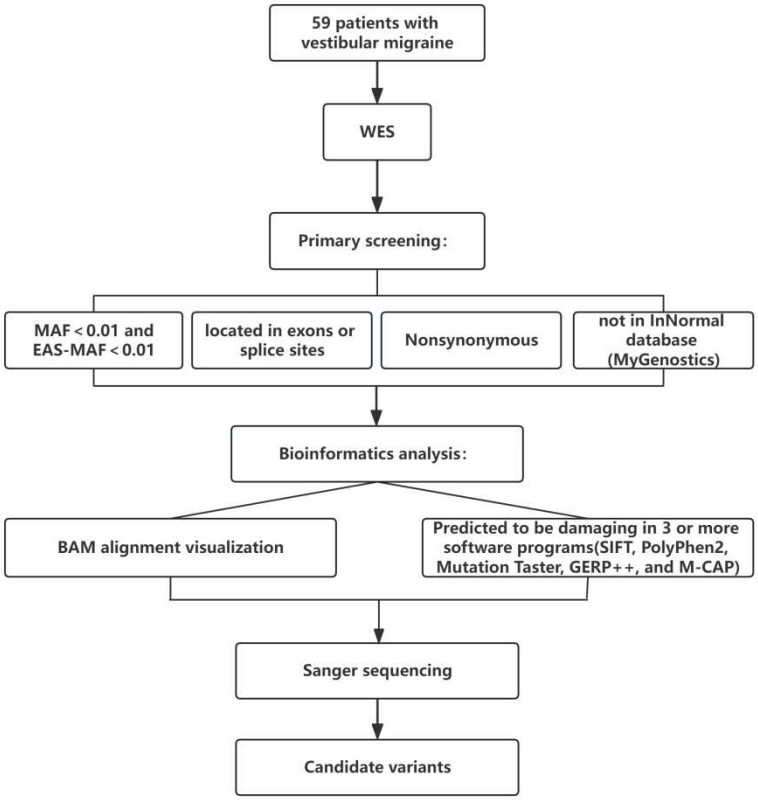


Figure 1. Flow chart of screening and analysis of candidate variants.

Results

1、General demographic characteristics of subjects

In this study, a total of 59 VM patients and 280 healthy controls were enrolled (Table 1). Among the 59 confirmed VM cases, the duration of episodes ranged from 2 months to 40 years, with attack frequencies varying from 4 times per year to 5 times per month. The distribution of headache symptoms in the cohort was as follows: one sided location(96.61%), pulsating quality (74.58%), moderate or severe pain intensity (100%), and aggravation by routine physical activity (84.75%). Photophobia/phonophobia was reported in 77.97% of cases, visual aura in 55.93%, motion sickness in 74.58%, and cochlear symptoms (e.g., tinnitus, aural fullness) in 66.10%. Comorbid conditions such as diabetes and hypertension were present in 6.78% and 18.64% of patients, respectively. The clinical characteristics of the VM cohort are detailed in Table 2.

Table 1. Demographic information for individuals with WES data

Cohort	Number			Mean age (SE)		
	Female	Male	Total	Female	Male	Total

VM case	53	6	59	49.6±12.7	46.2±13.4	49.3±12.8
Control	82	95	177	41.0±5.4	43.8±6.2	42.5±6.0

Table 2. Clinical feature of subjects

Variables		Result
Number		59
Age (yr)		49.3±12.8
Sex	Male n (%)	6 (10.17)
	Female n (%)	53 (89.83)
Headache characteristics	one sided location n (%)	57 (96.61)
	pulsating quality n (%)	44 (74.58)
	moderate or severe pain intensity n (%)	59 (100)
	aggravation by routine physical activity n (%)	50 (84.75)
Photophobia and phonophobia		46 (77.97)
Visual aura		33 (55.93)
Motion sickness		44(74.58%)
Cochlear symptoms		39 (66.10)
Accompanying diseases	Diabetes mellitus n (%)	4 (6.78)
	Hypertension n (%)	11 (18.64)

2、Identification of variants from whole exome sequencing

A total of 16 pathogenic variant loci of 16 genes were identified by WES (Figure 2). In 59 VM patients, each patient carries 0 to 6 candidate pathologic variants. Variations of the following genes were identified in more than 3 patients: NDUFB7 (10.17%, n=6), HEXA (8.47%, n=5), DOCK6 (6.78%, n=4), EPB41 (6.78%, n=4), CACNA1I (5.08%, n=3), EPHB2 (5.08%, n=3), GSN (5.08%, n=3), LAMA3 (5.08%, n=3), RPL3L (5.08%, n=3), SBF2 (5.08%, n=3), TSFM (5.08%, n=3), DNAH10 (6.78%, n=4), KIF9 (6.78%, n=4), KCNV2 (5.08%, n=3), OPLAH (5.08%, n=3), RAG1 (5.08%, n=3).

The molecular information for the pathogenic 16 susceptibility variant loci from 16 genes associated with VM are presented in Table 3 (only variants with mutation counts ≥3 in the VM cohort are listed). All 16 variants were verified by Sanger sequencing, and all results were consistent with those from WES analysis. The forward and reverse primers for direct polymerase chain reaction (PCR) sequencing of these variants are compiled in Supplementary File 1.

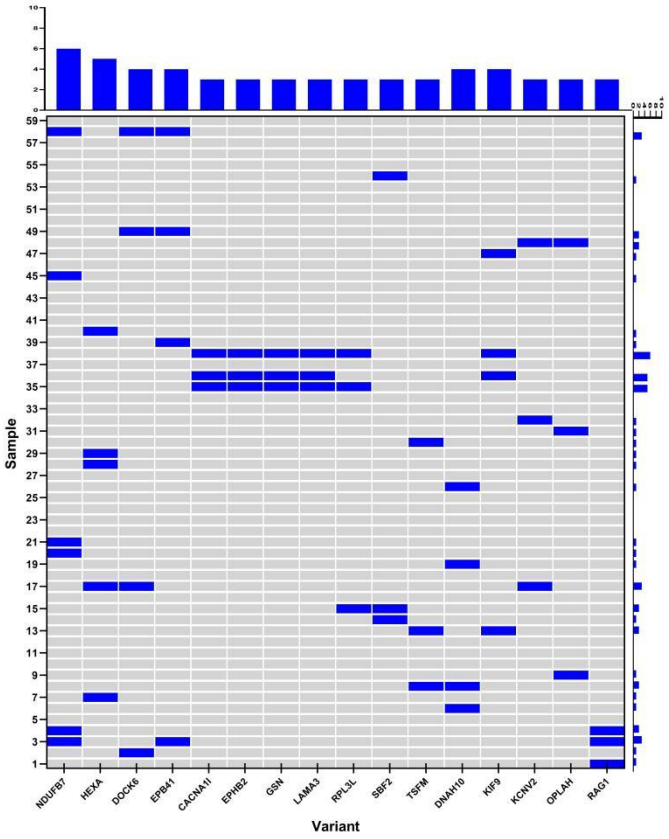


Figure 2. Susceptibility genes variant of vestibular migraine identified by whole exome sequencing.

Gene annotation:NDUFB7, HEXA, DOCK6, EPB41, CACNA1I, EPHB2, GSN, LAMA3, RPL3L, SBF2, TSFM, DNAH10, KIF9, KCNV2, OPLAH, RAG1.

Table 3. Molecular information for the pathogenic variants of genes associated with VM.

Gene	Genomic pos.(hg19)	Ref. transcript	Mutation type	Nucleotide changes	Amino acid changes	Mutation frequency		p value	OR	MAF	EAS-MAF	Gene region	Variants effect	SIFT	PolyPhen2	Mutation Taster	GERP++	M-CAP
						case（n=59）	Contral（n=177）											
NDUFB7	chr19:14677043-14677043	NM_004146.6	SNV	c.316C>G	p.Arg106Gly	10.17%(n=6)	0.56%(n=1)	0.001	14.296	0.005	0.003	exon3	nonsynonymous	0.002	0.829	1.000	4.220	0.096
HEXA	chr15:72645431-72645431	NM_000520.6	SNV	c.548T>A	p.Leu183His	8.47%(n=5)	1.13%(n=2)	0.012	7.084	0.006	0.005	exon5	nonsynonymous	0.000	0.988	1.000	5.590	0.369
DOCK6	chr19:11322772-11322772	NM_020812.4	SNV	c.4547C>T	p.Thr1516Met	6.78%(n=4)	0.56%(n=1)	0.015	9.541	0.003	0.003	exon36	nonsynonymous	0.007	0.891	1.000	4.580	0.044
EPB41	chr1:29391585-29391585	NM_001376013.1	SNV	c.2099G>A	p.Arg700Gln	6.78%(n=4)	0.56%(n=1)	0.015	9.541	0.004	0.004	exon16	nonsynonymous	0.033	0.950	1.000	4.130	0.078
CACNA1I	chr22:40064325-40064325	NM_021096.4	SNV	c.4133C>A	p.Ser1378Tyr	5.08%(n=3)	0.00%(n=0)	0.015	21.991	-	-	exon24	nonsynonymous	0.000	0.998	1.000	4.530	0.526
EPHB2	chr1:23110978-23110978	NM_017449.5	SNV	c.220C>G	p.Arg74Gly	5.08%(n=3)	0.00%(n=0)	0.015	21.991	-	-	exon3	nonsynonymous	0.004	0.974	1.000	5.190	0.040
GSN	chr9:124076297-124076297	NM_198252.3	SNV	c.749A>G	p.Tyr250Cys	5.08%(n=3)	0.00%(n=0)	0.015	21.991	0.001	0.000	exon7	nonsynonymous	0.001	0.834	1.000	5.510	0.202
LAMA3	chr18:21338364-21338364	NM_198129.4	SNV	c.952C>T	p.Arg318Trp	5.08%(n=3)	0.00%(n=0)	0.015	21.991	0.001	0.000	exon7	nonsynonymous	0.006	-	0.899	4.360	0.076
RPL3L	chr16:1997088-1997088	NM_005061.3	SNV	c.700C>T	p.Arg234Cys	5.08%(n=3)	0.00%(n=0)	0.015	21.991	0.000	0.000	exon6	nonsynonymous	0.000	0.992	1.000	3.950	0.185
SBF2	chr11:9850942-9850942	NM_030962.4	SNV	c.3754A>T	p.Ser1252Cys	5.08%(n=3)	0.00%(n=0)	0.015	21.991	0.004	0.004	exon28	nonsynonymous	0.009	0.541	0.843	4.470	0.103
TSFM	chr12:58176996-58176996	NM_005726.6	SNV	c.161G>A	p.Arg54Gln	5.08%(n=3)	0.00%(n=0)	0.015	21.991	0.001	0.001	exon2	nonsynonymous	0.000	0.999	1.000	5.290	0.134
DNAH10	chr12:124399076-124399076	NM_001372106.1	SNV	c.10553C>T	p.Thr3518Met	6.78%(n=4)	1.13%(n=2)	0.036	5.692	0.008	0.006	exon61	nonsynonymous	-	0.998	1.000	5.140	0.066
KIF9	chr3:47318830-47318830	NM_182902.4	SNV	c.35G>T	p.Arg12Leu	6.78%(n=4)	1.13%(n=2)	0.036	5.692	0.009	0.008	exon2	nonsynonymous	0.000	1.000	1.000	4.200	0.268
KCNV2	chr9:2719083-2719083	NM_133497.4	SNV	c.1344G>C	p.Trp448Cys	5.08%(n=3)	0.56%(n=1)	0.049	7.289	0.005	0.005	exon1	nonsynonymous	0.000	1.000	1.000	5.440	0.580
OPLAH	chr8:145113448-145113448	NM_017570.5	SNV	c.734T>C	p.Ile245Thr	5.08%(n=3)	0.56%(n=1)	0.049	7.289	0.009	0.006	exon6	nonsynonymous	-	0.966	-	4.070	0.157
RAG1	chr11:36596427-36596427	NM_000448.3	SNV	c.1573C>T	p.Pro525Ser	5.08%(n=3)	0.56%(n=1)	0.049	7.289	0.005	0.003	exon2	nonsynonymous	0.001	0.997	1.000	4.630	-

3. Function enrichment of susceptibility genes

Functional enrichment and pathway analysis were performed using Metascape (Fig. 3). A total of 3 statistically significant enriched pathways were identified, with 8 genes involved in at least one pathway, which were mainly categorized into three classes: canonical pathways, GO biological processes, and Reactome gene sets. The pathway analysis yielded top 3 clusters with their representative enriched terms with a p-value < 0.01. The GO terms with the greatest number of genes were "PID SYNDECAN 2 PATHWAY" (25.0%, 4 out of 16 genes), "Axon guidance" (18.8%, 3 out of 16 genes), "monoatomic cation transmembrane transport" (18.8%, 3 out of 16 genes).

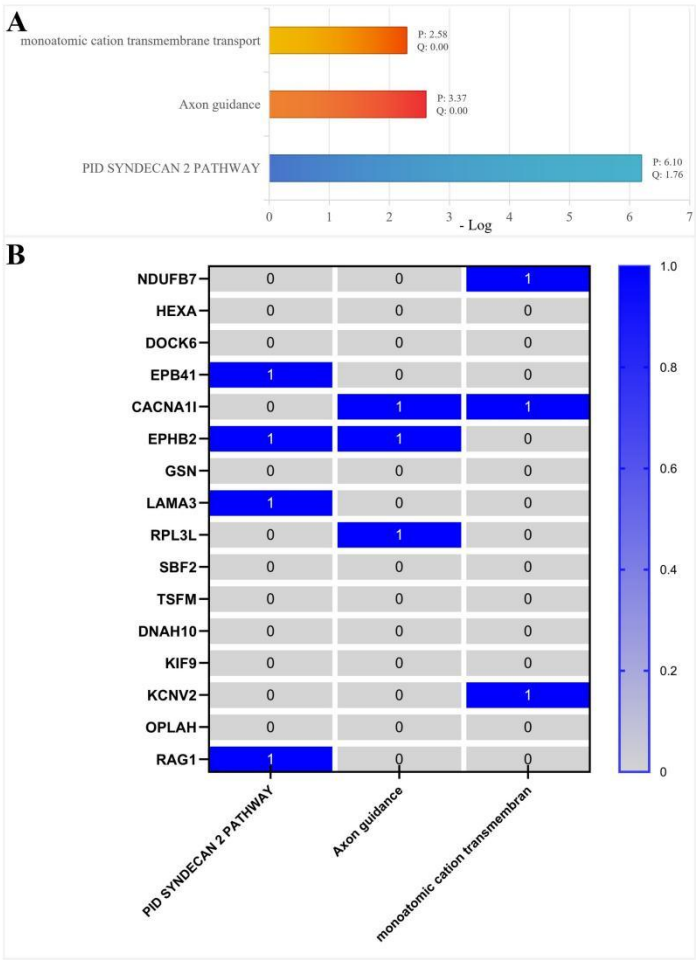


Figure 3. Functional enrichment analysis of susceptibility genes in patients with vestibular migraine. (A) Significance levels of the top 3 significantly enriched pathways identified by MetaScape analysis. The X-axis represents -Log(P value), with higher values indicating greater enrichment significance. (B) Heatmap showing the distribution of 16 mutated genes across the 3 enriched pathways. Blue blocks indicate that the corresponding gene is involved in the pathway (binary representation). The pathways include: PID SYNDECAN 2 PATHWAY, Axon guidance, and monoatomic cation transmembrane transport.

Discussion

Vestibular migraine (VM) is one of the most common central vestibular disorders in clinical practice. It is primarily characterized by recurrent vestibular dysfunction accompanied by migraine-related symptoms, and it exhibits a high population prevalence along with significant genetic heterogeneity[15, 16]. In this study, we performed a systematic genetic analysis using whole-exome sequencing (WES) on 59 VM patients and 177 healthy controls. A total of 16 candidate susceptibility genes for VM were identified and subsequently validated via Sanger sequencing. Furthermore, we systematically elucidated the potential genetic pathogenic pathways of VM across multiple dimensions, including mitochondrial energy metabolism, ion channel function, neurodevelopment and cytoskeletal regulation, cation transmembrane transport, and synaptic signal transmission. These findings provide novel experimental evidence for clarifying the molecular pathogenesis of VM, identifying objective biomarkers, and developing targeted therapeutic strategies.

A recent study identified multiple monogenic disorders associated with migraine, revealing distinct but interconnected mechanisms. Specifically, ion-channel dysfunction (CACNA1A, SCN1A, ATP1A2), vascular impairment (NOTCH3, TREX1), mitochondrial dysfunction (MT-TL1), and circadian dysregulation (CSNK1D) have emerged as critical contributors to migraine pathophysiology[17]. In this study, mutations in the NDUFB7 gene exhibited the highest frequency (10.17%), identifying it as a core high-risk susceptibility gene. NDUFB7 encodes a subunit of the mitochondrial NADH dehydrogenase complex, which is a crucial component of mitochondrial oxidative phosphorylation and directly regulates energy synthesis in central neurons[18]. Mitochondrial dysfunction is associated with migraine [19]. Furthermore, genetic variations in mitochondrial DNA (mtDNA) are increasingly becoming a focal point in migraine etiology research[20, 21]. Recent studies have demonstrated that migraine patients suffer from cerebral energy metabolism disorders, suggesting that mitochondrial dysfunction may be involved in the pathogenesis of migraine[22]. Impaired mitochondrial energy synthesis leads to insufficient neuronal energy supply, which triggers abnormally elevated cortical excitability and induces cortical spreading depression (CSD). This

is also a crucial pathological basis for vertigo and headache attacks in vestibular migraine (VM). Furthermore, mitochondrial dysfunction exacerbates oxidative stress, promotes the release of inflammatory neuropeptides, activates the trigeminovascular system, and aggravates vestibular central nervous system injury[23]. The results of this study indicate that missense mutations in NDUFB7 can lead to abnormal protein structure and function, thereby reducing mitochondrial respiratory efficiency. This causes insufficient energy supply and impaired membrane potential homeostasis in neurons of the vestibular pathway. Consequently, the threshold for neuronal tolerance to stimuli is lowered, predisposing patients to vestibular dysfunction and migraine attacks. Meanwhile, the TSFM gene (with a mutation frequency of 5.08%) is involved in the regulation of mitochondrial transcription and translation. Mutations in TSFM can disrupt the normal assembly of mitochondrial proteins and act synergistically with NDUFB7 mutations to exacerbate mitochondrial impairment. Together, they constitute a pathogenic pathway of mitochondrial metabolic abnormalities in VM. These findings provide molecular genetic evidence that impaired mitochondrial energy metabolism is a crucial genetic basis for VM, offering key targets for the development of mitochondria-targeted prevention and treatment strategies.

Dysfunctional ion channels represent one of the core molecular mechanisms underlying migraine and vestibular migraine (VM) pathogenesis[24]. In this study, we identified two ion channel susceptibility genes, CACNA1I and KCNV2, providing direct evidence for the ion channel hypothesis of VM. CACNA1I belongs to the CACNA gene family and encodes the subunit of T-type voltage-gated calcium channels. The CACNA gene family encodes the alpha subunits of voltage-gated calcium channels, which form the foundation of functional calcium channels and play a pivotal role in key physiological processes of the nervous system, including the regulation of neuronal excitability, synaptic transmission and synaptic plasticity[25]. Previous studies utilizing WES have demonstrated a significantly increased missense variant burden in CACNA1I among patients with hemiplegic migraine, suggesting that variants in this gene may elevate the risk of developing migraine-related disorders[26, 27].

The KCNV2 gene (potassium voltage-gated channel subfamily V member 2) encodes

Kv8.2, a modulatory "silent" subunit which forms heteromeric channels with voltage-gated potassium channels in rod and cone photoreceptors[28]. Kv8.2 possesses the function of regulating cellular excitability and modulating potassium currents[29]. Voltage-gated potassium channels are composed of alpha and beta subunits, wherein the alpha subunit determines the channel's function, while the beta subunit modulates its properties[30]. The Kv8.2 protein encoded by the KCNV2 gene is classified as a type of alpha subunit[31]. Previous studies have confirmed that mutations in this gene cause a monogenic disorder clinically classified as cone dystrophy with supernormal rod response (CDSRR)[32]. Furthermore, pathogenic variants of KCNV2 may contribute to the initiation and progression of hyperexcitability neurological disorders such as epilepsy via inducing gain-of-channel function effects, indicating that this gene also plays a vital role in neuroelectrophysiological regulation [33]. In summary, the abnormal calcium and potassium channel functions mediated by CACNA1I and KCNV2 may jointly participate in the pathophysiological processes of VM by disrupting neuronal membrane potential homeostasis and excitability regulation. These findings further verify that genetic variants in ion channel genes constitute an important genetic pathogenic basis for VM.

In addition to mitochondrial metabolism and impaired ion channel function, the pathways identified by functional enrichment analysis in the present study, including neurodevelopmental regulation, cytoskeletal remodeling, axon guidance and cation transmembrane transport. These findings serve as a crucial complement to elucidating the genetic mechanisms of VM, further refining the multidimensional molecular pathogenesis framework of VM. The susceptibility genes identified in this study, including DOCK6, EPB41, GSN, KIF9, and DNAH10, are primarily involved in microtubule motility, cytoskeletal assembly, and neuronal axon development. They play crucial roles in the construction of the central vestibular neural network, maintenance of neuronal morphology, and neural signal transduction. Notably, as a regulator of Rho GTPases, DOCK6 participates in neuronal migration, axon extension, and synaptic development in the central nervous system by modulating actin cytoskeleton remodeling[34]. Functional variants of this gene may lead to impaired survival and homeostasis of peripheral

nerve axons, thereby triggering related neurological disorders[35]. Variations in EPB41 (erythrocyte membrane protein 4.1) suggest that dysregulation of membrane protein stability may play a critical role in the pathogenesis of VM. Although the classical biological function of EPB41 is to maintain the mechanicochemical properties of the cell membrane by promoting the interaction between spectrin and actin, recent studies indicate that it is also essential for the stable accumulation of various ion channel proteins on the cell surface. For instance, in the heart and nervous system, EPB41 has been shown to directly regulate the membrane localization and trafficking of voltage-gated sodium channels (e.g., NaV1.5) and ligand-gated channels (e.g., GluR1/4). Impairment of its function may lead to reduced density or abnormal distribution of ion channels on the neuronal membrane, thereby prolonging the action potential duration and altering neuronal excitability[36]. This provides a potential molecular mechanism for the abnormal firing observed in the vestibular nuclei of VM patients. As a crucial actin-binding protein, GSN plays a pivotal role in the dynamic assembly and disassembly of the cytoskeleton. Aberrant cytoskeletal remodeling mediated by GSN may impede synaptic vesicle transport and inhibit neurotransmitter release, ultimately disrupting signal transmission from the vestibular nuclei to the cortex[37].

Functional enrichment analysis further revealed significant enrichment of the Axon guidance pathway, primarily involving key candidate genes such as EPHB2 and RPL3L. As a member of the receptor tyrosine kinase family, EPHB2 regulates axonal projection direction during both embryonic development and adulthood, mediating neural circuit remodeling and synaptic plasticity[38]. Beyond its role in maintaining cardiac function, recent studies have demonstrated that RPL3L is significantly associated with cognitive decline and brain atrophy in Alzheimer's disease[39]. This suggests that RPL3L may be involved in the pathological progression of neurodegenerative diseases and could serve as a potential biomarker for disease risk. The co-enrichment of EPHB2 and RPL3L in the Axon guidance pathway not only highlights the molecular basis underlying neural development and synaptic plasticity but also provides crucial candidate targets and theoretical insights for elucidating the pathological mechanisms of neurodegeneration. Furthermore, it suggests that these two genes may

contribute to the pathogenesis of VM by regulating neural circuit remodeling and altering the susceptibility to central nervous system injury. The significant enrichment of the "monoatomic cation transmembrane transport" pathway further corroborates the central role of ionic homeostasis imbalance in the pathogenesis of VM. Beyond the aforementioned CACNA1I and KCNV2, the identification of HEXA and RAG1 is also noteworthy. Although HEXA is primarily associated with lysosomal function, substrate accumulation can trigger severe neurodegenerative disorders, leading to lower motor neuron dysfunction and progressive deterioration of neuroelectrophysiological function[40]. Meanwhile, RAG1 is essential for mediating V(D)J recombination in lymphocytes; studies using Rag1 knockout (Rag1-/-) animal models have demonstrated that adaptive immune cells play a pivotal role in regulating central nervous system inflammation[41]. It is therefore hypothesized that RAG1 variants may indirectly contribute to VM-associated inflammatory responses by disrupting adaptive immune homeostasis. However, this hypothesis warrants further validation through expanded cohort studies and functional experiments. Furthermore, functional enrichment analysis revealed a significant enrichment of the "SYNDECAN 2 signaling pathway." As a crucial postsynaptic scaffolding protein, SYNDECAN 2 plays a pivotal role in regulating synaptic morphological maturation, cell adhesion, and the clustering of neurotransmitter receptors[42]. Dysregulation of this pathway suggests potential microstructural alterations in synaptic plasticity or neural network connectivity between the vestibular nuclei and the cortex in VM patients. This may provide a cellular and biological mechanistic clue for the vestibular-visual integration deficits and associated emotional disorders observed in this population.

This study has several limitations. First, the study was conducted using a single-center, small-sample cohort design. The limited sample size may introduce bias in the screening of rare pathogenic variants; therefore, expanded multi-center cohorts are required to further validate the pathogenic effects of the candidate genes. Second, gene functions and pathogenic pathways were predicted solely through bioinformatics analysis. Although the variants were validated by Sanger sequencing, functional experiments (e.g., using cellular or animal models) are still lacking to elucidate the specific regulatory mechanisms of these genes, and the upstream and

downstream molecular regulatory networks of the candidate genes warrant further investigation. Finally, no correlation analysis was performed between genetic variants and clinical phenotypes, disease severity, or attack frequency, leaving the genotype-phenotype correlations unclear.

In conclusion, this study identified preliminary candidate susceptibility genes for VM using WES, with subsequent functional enrichment analysis performed. The findings suggest that the pathogenesis of VM may involve complex crosstalk among multiple pathways, including mitochondrial energy metabolism dysfunction (NDUFB7, TSFM), ion-channel dysfunction (CACNA1I, KCNV2), and neurodevelopmental and cytoskeletal regulation (DOCK6, EPHB2, GSN). These results provide molecular-level evidence supporting the classification of VM as a complex genetic disorder governed by the synergistic regulation of multiple genes. These discoveries not only offer new perspectives for elucidating the molecular pathogenesis of VM but also provide potential biomarkers and therapeutic targets for developing precision treatment strategies tailored to specific genetic subtypes in the future, such as mitochondrial protectants or ion-channel modulators. Future research should expand the sample size and conduct familial co-segregation validation alongside in vitro and in vivo functional experiments to further clarify the pathogenic mechanisms of key genes. Ultimately, this will facilitate the transition of VM diagnosis from clinical phenotyping to molecular precision diagnosis, paving the way for individualized prevention and treatment.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Human Ethics and Consent to Participate declarations

The studies involving human participants were reviewed and approved by the Ethics Committee of Tianjin First Central Hospital. The patients/participants provided their written informed consent to participate in this study.

Consent to Participate

Written informed consent was obtained from all individual participants included in the study.

Ethics Approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Tianjin First Central Hospital (Approval No.: YC-BY-LC-2023-0020).

Data Availability Statement

The datasets generated and/or analysed during the current study are available in the NCBI Sequence Read Archive (SRA) repository, under accession number PRJNA1524369.

Availability of data and materials

The whole-exome sequencing data generated and/or analysed during the current study have been deposited in the NCBI SRA and are publicly available under the BioProject accession number PRJNA1524369: [https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1524369] (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1524369).

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