Uncovering Novel Genetic Markers of Vestibular Migraine: A Bioinformatics Approach

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Abstract

Over 1 billion people worldwide are affected by migraines, with the vestibular migraine being one of the most disabling subtypes[1]. Despite its prevalence, the genetic factors that predispose migraines remain poorly understood. This study aims to explore the genetic susceptibility to vestibular migraine using bioinformatics tools to analyze RNA sequencing (RNA-Seq) data. The gene expression profiles were extracted from the genomes from 6 migraine patients and 6 control subjects to identify main upregulated and downregulated genes related to this condition. Using DESeq2 and Gene Set Enrichment Analysis (GSEA), significant gene pathways and their potential roles in this disease were analyzed. Notably, genes such as CARS1, CDH8, and NOMO1 were identified as key contributors, along with enriched pathways like KEGG_T_CELL_RECEPTOR_SIGNALING_PATHWAY and PID_KIT_PATHWAY, which provide new information into the molecular mechanisms of vestibular migraines. By focusing on the molecular causes of vestibular migraine, this research leads to a better understanding of its genetic basis, providing possible targets for future therapeutic strategies. Ultimately, this study highlights the importance of bioinformatics in decoding complex genetic diseases and advancing medicine.

Introduction

A Migraine is a debilitating neurological condition that is characterized by severe headaches accompanied by symptoms that include nausea, phonophobia, photophobia, fatigue, and blurred vision[2]. They can be broadly categorized into two main types: migraines with and without aura[2]. Migraines with aura involve neurological symptoms that precede the headache phase, such as visual disturbances, sensory changes, or motor symptoms[3]. These types of auras typically occur before or during the onset of a migraine[3]. Migraines without aura do not involve these neurological symptoms and are more common, representing 42-60% of the migraine cases[2]. Vestibular migraines, a subtype of migraines, involve headaches in combination with nausea, vomiting, and other vestibular symptoms[3].

The impact of migraines on daily life is profound, often disrupting work, social engagements, and personal responsibilities while decreasing overall productivity and quality of life.[4]. In the United States, self-reported migraines and severe headaches affect approximately 15.3% of the population, with a notable gender disparity of about 20.7% of women and 9.7% of men[4]. The high prevalence highlights the need for an effective treatment. A better understanding of the genetic factors that play a role in migraine susceptibility may lead to the development of new treatments

Genetics as well as environmental factors are known to play a crucial role in the onset of migraines[5]. Genes are segments of DNA that encode instructions for varied biological functions. Genetic variations, or gene variants, may occur when there are changes to a DNA sequence that influence an individual's predisposition to developing certain conditions[5].

The genetic basis of migraines involves both monogenic and polygenic factors, which reflect the complexity of this condition. Monogenic migraines are caused by mutations in a single gene, leading to a clear genetic link[6]. These types of migraines are rare and result in severe forms of this condition. A rare subtype of a migraine caused by a monogenic factor includes Familial Hemiplegic Migraine (FHM)[6]. FHM is associated with mutations in genes such as CACNA1A, ATP1A2, and SCN1A[6]. The gene variants can lead to intense neurological symptoms and severe headaches which are characteristic of FHM[6]. On the other hand, polygenic migraines are caused by a combination of multiple gene variants, known as polygenic inheritance[6]. Most common migraines fall into this category. Recent genome-wide association studies revealed that various genes affect pathways related to neuronal excitability, vascular function, and pain perception[7].

Aspect	Monogenic Migraine	Polygenic Migraine
Definition	Caused by mutations in a single gene.	Caused by a combination of multiple genetic variants.
Prevalence	Rare.	Common.
Examples	Familial Hemiplegic Migraine (FHM).	Migraine without aura and migraine with aura.
Genes Involved	CACNA1A, ATP1A2, SCN1A.	Multiple genes, including those affecting neuronal excitability, vascular function, and pain perception.
Inheritance Pattern	Follows Mendelian inheritance (autosomal dominant in many cases).	Does not follow a clear inheritance pattern; influenced by multiple genetic and environmental factors.
Symptoms	Severe neurological symptoms and intense headaches characteristic of FHM.	Variable symptoms, typically including moderate to severe headaches, sensitivity to light/sound, etc.
Diagnostic Approach	Genetic testing to identify mutations in specific genes.	Genome-wide association studies (GWAS) to identify susceptibility loci.
Impact	Direct and significant due to a single gene mutation.	Smaller, additive effects of multiple genetic variants.

Table 1, Differences between Monogenic and Polygenic Migraines

Understanding both monogenic and polygenic migraines, as shown in Table 1, can provide valuable insight to identify targets for treatments and further recognize how genetic predispositions influence migraine development.

The aim of the research paper is to discover specific biomarkers that may cause migraines by analyzing RNA data from migraine patients and healthy individuals. This study is created to answer the question: What specific biomarkers for migraines can be identified through RNA analysis? The goal of this study is to detect differences in gene activity that may indicate altered protein production in migraine patients compared to controls. These variations could reveal patterns in protein expression and be linked to the onset of migraines. Pinpointing these proteins could offer insights into migraine diagnosis and susceptibility, contributing to a better understanding of the molecular mechanisms in this condition.

Methodology

Genome data was sourced from the National Library of Medicine/National Center for Biotechnology information (NCBI) database[8]. A specific dataset was identified containing six vestibular migraine cases and six healthy control cases. All participants were Asian females, aged 21-53, from China. The human genome collection took place in 2024 at the Central and Clinical Laboratory, Sanya People's Hospital, Hainan Province, China. White Blood cells were used as the tissue source. All migraine patients included in the study were diagnosed with vestibular migraine. This condition is defined by episodic vertigo or dizziness associated with migraine symptoms, including headaches, photophobia and phonophobia[9]. This condition is believed to involve central mechanisms affecting the brain and vestibular pathways[9].

An Amazon Web Services (AWS) account was created, and data requests were submitted for an S3 bucket containing the FASTQ genome sequencing files for each case. After a two-day processing period, the data was downloaded in a CSV format (FASTQ files) and stored in an external hard drive. The sequencing data for each sample was split into two reads per DNA strand and organized into designated folders (ex. MigraineCase1, MigraineCase2, etc.) for downstream processing. The FASTQ files were then processed using Kallisto, a pseudo-alignment tool for RNA-sequencing data. Kallisto was downloaded using Miniconda 3, a process that took about 1 hour. Each sample was aligned individually, which took approximately 4.5 hours per sample.

Alignment is an important step in RNA sequencing analysis that involves mapping the raw sequence data from FASTQ files to a reference genome or transcriptome. The FASTQ files contain raw sequence reads generated from sequencing machines, along with the quality scores for each base. While these raw reads are short and may contain errors, alignment helps place them correctly in the context of the genome. The transcriptome file used for the Kallisto alignment in this study was the "Homo_sapiens.GRCh38.113.chr.gtf" file from the Ensembl website[10]. The alignment generated output files that were sorted into separate folders, organized by "migraine" or "control" and by case. The output files for each alignment included an abundance.h5 file, an abundance.tsv, and a run_info.json file.

Before importing the Kallisto output files into Rstudio for analysis, R (version: 4.4.2) and Rstudio (Version: 2024.12.0+467) were installed. R, the statistical computing environment, was downloaded from the official website, and RStudio, an integrated development environment (IDE) for R, was downloaded from the RStudio website[11]. Once installed, RStudio was used to handle the data processing and analysis of this study. The Kallisto output files were then imported into RStudio using the tximport R package. This tool allowed collection of transcript-level expression into a single gene by sample matrix for downstream differential expression analysis. To map the Ensembl transcript ID's to gene ID's, a GTF file for reference genome was downloaded, and a transcript to gene key was created. The metadata table was then prepared in CSV format, which included columns for the sample ID, condition (ex. "Migraine" or "Control"), and any other relevant variables. This table was imported into RStudio and matched with the expression matrix to ensure consistency through the experiment. In

RStudio, the count matrix, which represents the genes as rows and patient samples as columns, was constructed. The RNA sequencing data for all 12 samples was included in the matrix and divided into migraine and control groups.

For differential expression analysis, several R packages were used: DESeq2, ggplot2, tidyr, and dplyr. A DESeqDataSet object was formed using the "DESeqDataSetFromMatrix" function. Genes with low counts, defined as those with fewer than 10 reads in at least 50% of the samples, across all samples were filtered out to improve the analysis power. The "DESeq" function was used for normalization and statistical testing, with results extracted using the "results()" function. Significant genes were identified based on an adjusted p-value (padj) < 0.05 and an absolute log2 fold change (LFC) >1. The "lfcShrink()" function was used with the "apeglm" method to create LFC change shrinkage for better interpretability, mainly for the genes with low counts[12].

The results were exported to a data frame for visualization. A Volcano plot was created to visualize the differential expression results, with log2 fold change on the x-axis and the negative log10 of adjusted p-values on the y-axis. Color-coding was used in the plot to categorize genes as "Upregulated" (red & on right side), "Downregulated" (red & on left side), or Not Significant (black & blue). Overlapping gene labels were solved using the ggrepel package, making sure the labels for notable genes were readable. Plot aesthetics, with axis labels, title, and legend placement, were adjusted for clarity and professional appearance.

Gene set enrichment analysis (GSEA) was performed to identity pathways significantly associated with upregulated or downregulated genes. The ranked gene list was produced by ordering genes by log2 fold change, making a clean list by filtering out genes with missing or invalid values. The clusterProfiler package was used for GSEA with curated gene sets from KEGG (Kyoto Encyclopedia of Genes and Genomes) and Reactome databases. The KEGG[13] and Reactome databases[14] are resources that provide gene pathways, molecular interactions and biological processes. They are sources for understanding the analysis of gene function and the understanding of cellular mechanisms in various biological contexts[13,14]. The results were organized into a spreadsheet with columns of the pathway name, pval (p-value), padj (adjusted p-value), ES (enrichment score), NES (normalized enrichment score), nMoreExtreme (the genes more extreme than leading edge), Size (the total number of genes in the pathway), and LeadingEdge (the subset of genes supporting most of the pathway's enrichment).

The biological significance of the results was interpreted on the top 10 most upregulated and downregulated genes, and their roles in the contribution of vestibular migraine. The integration of DESeq2 and GSEA results helped build an understanding of the molecular changes in the migraine sample. The important results, such as the volcano plot and enriched pathways, were saved as high-quality images used for later analysis and presentation.

Results

Differential Analysis Reveals Key Insights:

The volcano plot generated from the DESeq2 analysis visualizes the differential expression of genes between migraine patients and control samples. The differential expression analysis refers to the differences in the activity levels of genes of two conditions[15]. The expression level is the amount of RNA a gene produces[15]. Therefore, the volcano plot will help us visualize which genes are expressed more in migraine patients than controls (upregulated), and which genes are expressed more in controls than migraine patients (downregulated).

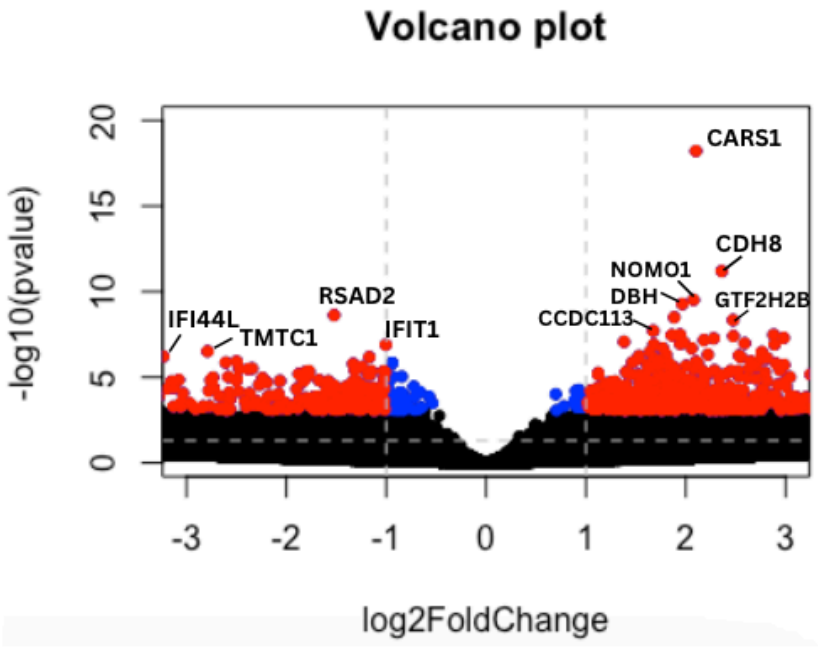

Figure 1, Volcano Plot of Differential Gene Expression Between Migraine and Control Samples including the labels of some outlier genes. Color-coding was used in the plot to categorize genes as "Upregulated" (red & on right side), "Downregulated" (red & on left side), or Not Significant (black & blue).

The x-axis of Figure 1, labeled as log2FoldChange, represents the measure of gene expression changes between the two groups. The y-axis of Figure 1, labeled as -log10(p-value), represents the statistical significance of the observed changes. All of the dots in the volcano plot, whether black, red or blue, are the 34,136 genes that were analyzed in the DESeq2 analysis. Genes located higher on the plot have lower p-values and correspond to those with more significant changes in expression[12].

Key regions on the plot provide additional insights. Genes near the center, where log2FoldChange ≈ 0, show no significant differences in expression between the two conditions; these genes are typically not biologically relevant[12]. Upregulated genes are positioned on the far-right with high statistical significance, indicating their increased activity in migraine patients. Conversely, downregulated genes are positioned on the far-left side with high statistical significance, indicating decreased activity in migraine patients and increased activity in control

patients. The labeled outlier genes will be further explored in their connection to the vestibular migraine condition in the later sections of this study.

GSEA Analysis Reveals Interesting Pathways:

The GSEA results provide insights into the biological pathways altered in migraine patients. This analysis highlights both upregulated and downregulated pathways that could contribute to migraine pathophysiology. The top 10 upregulated pathways show positive Normalized Enrichment Scores (NES), indicating their enrichment in upregulated genes. This is shown in Figure 2.

Rank	Pathway	p-value	padj	ES	NES	Size	LeadingEdge
1	WP_PHOSPHODIESTERASES_IN_NEURONAL_FUNCTION	0.00142	0.02991	0.75463	2.45873	53	107,108...
2	REACTOME_G_PROTEIN_MEDIATED_EVENTS	0.00143	0.02991	0.73722	2.38872	52	107,108...
3	REACTOME_GLUCAGON_SIGNALING_IN_METABOLIC_REGULATION	0.00147	0.02991	0.81625	2.41593	32	107,108...
4	REACTOME_CA_DEPENDENT_EVENTS	0.00147	0.02991	0.79284	2.40266	36	107,108...
5	KEGG_MEDICUS_REFERENCE_ACTH_CORTISOL_SIGNALING_PATHWAY	0.00147	0.02991	0.82434	2.40181	30	107,108...
6	KEGG_MEDICUS_PATHOGEN_HCMV_UL33_TO_GNAI_AC_PKA_SIGNALING	0.00151	0.02991	0.87509	2.41483	24	107,108...
7	KEGG_MEDICUS_VARIANT_MUTATION_INACTIVATED_RASD1_TO_CRHR	0.00151	0.02991	0.86218	2.39721	25	107,108...
8	KEGG_MEDICUS_REFERENCE_GHRHR_PKA_GH_SIGNALING_PATHWAY	0.00152	0.02991	0.87139	2.43302	26	107,108...
9	KEGG_MEDICUS_REFERENCE_TSH_TG_SIGNALING_PATHWAY	0.00152	0.02991	0.86695	2.43382	27	107,108...
10	KEGG_MEDICUS_VARIANT_MUTATION_ACTIVATED_GNAS_TO_CRHR_PKA	0.00154	0.02991	0.88374	2.39418	22	107,108...

Table 2: Spreadsheet of the top 10 upregulated pathways in migraine patients

The upregulated pathways suggest that processes like neuronal function (REACTOME_CA_DEPENDENT_EVENTS), metabolic regulation (REACTOME_GLUCAGON_SIGNALING_IN_METABOLIC_REGULATION) and other signaling pathways are more active in migraine patients[16,17]. Inversely, the top 10 downregulated pathways show negative Normalized Enrichment Scores (NES), indicating their enrichment in downregulated genes. This is presented in Figure 3.

Rank	Pathway	pval	padj	ES	NES	Size	LeadingEdge
1	KEGG_RIBOFLAVIN_METABOLISM	0.002564	0.038486	-0.863689	-2.424984	16	53, 52, ...
2	WP_FATTY_ACID_BIOSYNTHESIS	0.002849	0.040314	-0.809500	-2.444095	22	31, 32, ...
3	WP_CLEAR_CELL_RENAL_CELL_CARCINOMA_PATHWAYS	0.003509	0.043322	-0.489658	-1.923060	83	31, 32, ...
4	WP_AMINO_ACID_METABOLISM	0.003584	0.043322	-0.408052	-1.616120	90	30, 47, ...
5	LEIN_CHOROID_PLEXUS_MARKERS	0.003636	0.043695	-0.410832	-1.624257	92	24, 32, ...
6	PID_ERA_GENOMIC_PATHWAY	0.003650	0.043753	-0.532662	-2.009565	63	21, 322, ...
7	KEGG_T_CELL_RECEPTOR_SIGNALING_PATHWAY	0.003663	0.043812	-0.384957	-1.565325	108	208, 207 ....
8	KEGG_INSULIN_SIGNALING_PATHWAY	0.004149	0.045652	-0.377207	-1.581109	134	31, 32, ...
9	DESERT_PERIPORTAL_HEPATOCELLULAR_CARCINOMA_SUBCLASS_UP	0.004132	0.045652	-0.347947	-1.519439	157	32, 33, ...
10	PID_KIT_PATHWAY	0.003344	0.043322	-0.583090	-2.092892	50	207, 205 ....

Table 3: Spreadsheet of the top 10 downregulated pathways in migraine patients

The downregulated pathways indicate potential disruptions in immune system function (KEGG_T_CELL_RECEPTOR_SIGNALING_PATHWAY), protein metabolism

(KEGG_INSULIN_SIGNALING_PATHWAY), and stress response pathways (PID_KIT_PATHWAY). These findings highlight the complex nature of the migraine, involving the activation of certain signaling pathways and the suppression of others. The interaction between the changes of the metabolic and immune system functions could provide better understanding into the underlying mechanisms of migraine[18,19]. This could allow science to potentially propose therapeutic strategies in targeting these pathways.

Discussion/Analysis

DESeq2 Analysis:

The volcano plot, as seen in Figure 1, revealed significant gene outliers that were differentially expressed in migraine patients. This includes outlier genes on the right side of the plot, such as CARS1, CDH8, NOMO1, and DBH, which are shown to be highly upregulated in migraine patients. CARS1 plays an important role in protein translation and mitochondrial function, and both of these processes are vital in maintaining neuronal energy homeostasis, which is often disrupted in migraine pathophysiology[20,21]. As the specific role of CARS1 in migraines has not been extensively studied, it makes a promising target for future research. As a member of the cadherin family, CDH8 is associated with synaptic adhesion and neuronal connectivity, suggesting its upregulation could influence abnormal neural circuits, which are often observed in vestibular migraines[22]. It presents a new perspective for understanding migraine-induced neural dysfunction. Additionally, NOMO1, known for its regulatory role in suppressing Nodal signaling during development, has been identified as a novel gene of interest[23]. Its upregulation could enhance inflammatory signalling and cellular stress pathways, a process linked to migraine susceptibility[23]. DBH, a gene responsible for converting dopamine to norepinephrine, highlights the link between catecholamine synthesis and vestibular migraines[24,25]. These neurotransmitters control stress responses and vascular regulation, both of these can be applied in the context of migraine-induced dizziness and balance disturbances[26,8].

Additionally, the outlier genes on the left side of the volcano plot, such as RSAD2, IFIT1, TMTC1, and IFI44L are shown to be highly downregulated, signifying that they are suppressed in the migraine patients. RSAD2, an interferon-induced gene involved in immune responses, is connected to inflammation and may contribute to the migraine condition by increasing inflammatory signals[27]. IFIT1, which is involved in antiviral defense, might be disrupted in migraines, impacting the immune control of nerve function[28]. TMTC1 and IFI44L, which are both involved in immune responses, may affect the vestibular system, playing a part in the balance problems seen in vestibular migraines[29,30,9]. These gene suppressions show an overall immune system imbalance that could worsen migraine systems.

GSEA Analysis:

The analysis of the GSEA top 10 upregulated pathways in migraine pathophysiology reveals a complex interplay of various mechanisms. These pathways show which gene pathways are overactive in migraine patients.

The WP_PHOSPHODIESTERASES_IN_NEURONAL_FUNCTION pathway highlights the importance of the enzyme phosphodiesterase-3 (PDE-3) in breaking down cyclic AMP and GMP, which are both regulators of neurotransmitter release and synaptic plasticity, both of which are damaged in migraine patients[31]. Notably, inhibiting PDE-3 has been shown to improve vascular function and reduce headache severity in certain conditions[16,32]. This suggests its potential therapeutic relevance in reducing migraine symptoms by regulating neuronal excitability[32]. Similarly, the REACTOME_G_PROTEIN_MEDIATED_EVENTS pathway supports the G-protein-coupled receptors (GPCRs) in neuronal signaling. GPCRs help regulate neurotransmitters like serotonin, dopamine, and glutamate, all of which affect migraines[33]. Changes in GPCR signaling are linked to increased sensitivity and pain, which are some symptoms of migraines[33]. Migraine medications like triptans target serotonin GPCRs, highlighting the importance of this pathway in treatment[34].

The KEGG_MEDICUS_REFERENCE_ACTH_CORTISOL_SIGNALING_PATHWAY clarifies the connection between stress responses and migraines, where changes in the hypothalamic-pituitary-adrenal axis can increase neuroinflammation and vascular dysfunction[35]. Stress is a well documented trigger for migraines, and the dysregulation of the HPA axis can amplify the stress response[36]. This consequently leads to increased cortisol levels[37]. High levels of cortisol in blood can lead to a weakened immune system and inflammation in the body[37]. KEGG_MEDICUS_VARIANT_MUTATION_INACTIVATED_RASD1_TO_CRHR_PKA_ACTH_SIGNALING_PATHWAY further shows stress hormone dysregulation[38]. The upregulation of RASD1 activity might also influence vascular tone, potentially contributing to the hypersensitivity and pain associated with migraines. RASD1 works to regulate ion channels and inhibit adenylyl cyclase and is often dysregulated in migraines[39]. This pathway expresses the intersection between genetic predispositions and environmental stressors in migraine susceptibility. Targeting RASD1 regulation in possible therapies could help restore vascular tone and balance stress hormones, potentially easing migraine pain and hypersensitivity.

The REACTOME_GLUCAGON_SIGNALING_IN_METABOLIC_REGULATION pathway is essential for energy metabolism and the regulation of glucose levels[40]. Metabolic disturbances, such as hypoglycemia or impaired glucose utilization in the brain, are commonly observed before migraine episodes[41]. Calcium signaling, controlled by the REACTOME_CA_DEPENDENT_EVENTS pathway, is very important in neuronal excitability and synaptic function[42]. Dysregulated calcium-dependent pathways can lead to the hyperexcitability of cortical neurons[42]. This hyperexcitability leads to cortical spreading depression (CSD), where a wave of depolarization is believed to cause migraine aura and trigger the headache[43].

The GSEA top 10 downregulated pathway results highlight which gene pathways may be underactive in migraine patients, giving us more information on molecular disruptions that lead to the development and progression of migraines.

One of the important downregulated pathways found in this study is the KEGG_T_CELL_RECEPTOR_SIGNALING_PATHWAY, which is involved in immune responses. In migraine patients, the reduced activity of this pathway may cause a weakened immune response, resulting in chronic neuroinflammation[44]. This inflammation can trigger neuronal hyperexcitability, which is central to migraine pathophysiology. The underactivity of T cell signalling could hinder the body's ability to manage inflammatory responses, therefore worsening migraine symptoms[45]. Future therapies could target this pathway using immunomodulators to enhance T cell activity, potentially reducing inflammation and migraine severity. The KEGG_INSULIN_SIGNALING_PATHWAY is another pathway that works to regulate glucose metabolism and vascular health using insulin signaling[46]. When this pathway is underactive, it may result in poor regulation of blood vessel function, causing the dizziness or light-headed symptoms commonly linked to vestibular migraines[47,8]. This pathway suggests that metabolic disturbances, especially in insulin sensitivity, may affect the onset of migraines[47].

The PID_KIT_PATHWAY, involved in neuroinflammation and vascular regulation, is also downregulated in migraine patients[48]. The c-Kit receptor directs critical processes such as cell survival and proliferation[49]. Its dysfunction can lead to increased inflammation and damaged vascular responses[49]. This pathway's downregulation triggers heightened pain sensitivity which is observed right before a migraine headache[1]. Treatments could focus on activating the c-Kit pathway with receptor agonists or gene therapies to improve vascular regulation and lower neuroinflammation. The KEGG_RIBOFLAVIN_METABOLISM pathway, containing riboflavin (Vitamin B2) plays a role in mitochondrial function and energy production[18]. Since mitochondrial dysfunction is a known factor in the development of migraines, particularly due to its role in neuronal hyperexcitability, the underactivity of this pathway may show impairment to mitochondrial function[18]. The cellular energy deficits often observed in migraines may be caused by this disruption[50]. Studies have shown that riboflavin supplementation can reduce migraine frequency, showing the importance of this pathway in migraine pathophysiology[50].

Together, these analyses reveal the complex connections of genetic, immune, metabolic, and vascular factors that may lead to migraine predisposition and severity. The identification of key genes and pathways provides valuable information into the molecular disruptions that cause migraine attacks, and may help the development of more effective treatments.

Conclusion

In this study, we aimed to identify the genes and pathways that could provide insight into the mechanisms underlying vestibular migraines, focusing on both migraine and control groups using RNA sequencing data. Kallisto was used for alignment, and DESeq2 was applied for differential gene expression analysis, leading to the pinpointing of several upregulated and downregulated genes. Notably, upregulated genes such as CARS1, CDH8, NOMO1, and DBH

are linked to mitochondrial function, synaptic adhesion, inflammation, and neurotransmitter regulation, while downregulated genes like RSAD2, IFIT1, TMTC1, and IFI44L suggest immune system imbalances. Additionally, gene set enrichment analysis (GSEA) revealed specific pathways that are impacted in vestibular migraine pathology, such as WP_PHOSPHODIESTERASES_IN_NEURONAL_FUNCTION and REACTOME_G_PROTEIN_MEDIATED_EVENTS, affecting neuronal signaling and neurotransmitter release. Downregulated pathways like KEGG_T_CELL_RECEPTOR_SIGNALING_PATHWAY indicate immune dysfunction. These findings provide insight into the genetic and metabolic factors behind vestibular migraines, highlighting potential targets for treatment.

These results demonstrate significant differences in the gene expression between migraine and control groups, highlighting potential biomarkers and biological pathways associated with this condition. These findings would support the deeper understanding of migraines, with implications for targeted therapies and personalized medicine. However, certain limitations should be considered. The small and relatively homogenous sample size, consisting primarily of Asian females, may limit the generalizability of the findings, especially regarding sex and ethnicity. Future studies should aim for larger, more diverse populations to determine if the group chosen for this study has unique genetic markers.

This study offers valuable insights into the genetic and molecular factors that cause vestibular migraines, which could pave the way for new, more personalized treatments. By identifying key upregulated and downregulated genes and pathways, such as those involved in mitochondrial function, neurotransmitter regulation, and immune system imbalance, this research lays the groundwork for potential biomarkers and novel therapeutic targets. Further studies could explore the functional roles of these identified genes and pathways in greater depth. This could potentially lead to the discovery of new treatment strategies tailored to the specific genetic and metabolic profiles of vestibular migraine patients.

References

1. The Facts About Migraine. American Migraine Foundation https://americanmigrainefoundation.org/resource-library/migraine-facts/ (2019).

2. National Institute of Neurological Disorders and Stroke. Migraine. *www.ninds.nih.gov* https://www.ninds.nih.gov/health-information/disorders/migraine (2023).

3. Mayo Clinic. Migraine with aura - symptoms and causes. *Mayo Clinic* https://www.mayoclinic.org/diseases-conditions/migraine-with-aura/symptoms-causes/syc-20352072 (2021).

4. Gawde, P. *et al.* Revisiting Migraine: The Evolving Pathophysiology and the Expanding Management Armamentarium. *Cureus* **15**, (2023).

5. Grangeon, L. *et al.* Genetics of migraine: where are we now? *The Journal of Headache and Pain* **24**, (2023).

6. Oriel Carreño *et al.* Screening of *CACNA1A* and *ATP1A2* genes in hemiplegic migraine: clinical, genetic, and functional studies. *Molecular Genetics & Genomic Medicine* **1**, 206–222 (2013).

7. Hautakangas, H. *et al.* Genome-wide analysis of 102,084 migraine cases identifies 123 risk loci and subtype-specific risk alleles. *Nature Genetics* **54**, 1–9 (2022).

8. National Library of Medicine. National center for biotechnology information. *Nih.gov* https://www.ncbi.nlm.nih.gov/.

9. WebMD Editorial Contributors. What Are Vestibular Migraines? *WebMD* https://www.webmd.com/migraines-headaches/vestibular-migraines (2024).

10. Ensembl. Index of /pub/release-113/gtf/homo_sapiens. Ensembl.org https://ftp.ensembl.org/pub/release-113/gtf/homo_sapiens/ (2024).

11. R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing https://www.r-project.org/ (2024).

12. Zhu, A., Ibrahim, J.G. & Love, M.I. Heavy-tailed prior distributions for sequence count data: removing the noise and preserving large differences. Bioinformatics 35, 2084–2092 (2019).

13. HTG Molecular. Understanding Volcano Plots. HTG Molecular https://www.htgmolecular.com/blog/2022-08-25/understanding-volcano-plots (2022).

14. KEGG: Kyoto Encyclopedia of Genes and Genomes. www.genome.jp https://www.genome.jp/kegg/.

15. Reactome. Home - Reactome Pathway Database. Reactome.org https://reactome.org/ (2019).

16. EMBL-EBI. Differential gene expression analysis | Functional genomics II. *EMBL-EBI Training* https://www.ebi.ac.uk/training/online/courses/functional-genomics-ii-common-technologies-and-data-analysis-methods/rna-sequencing/performing-a-rna-seq-experiment/data-analysis/differential-gene-expression-analysis/.

17. GSEA. WP_PHOSPHODIESTERASES_IN_NEURONAL_FUNCTION. Gsea-msigdb.org https://www.gsea-msigdb.org/gsea/msigdb/cards/WP_PHOSPHODIESTERASES_IN_NEURONAL_FUNCTION.html (2024).

18. Reactome. Glucagon signaling in metabolic regulation. Reactome.org https://www.reactome.org/content/detail/R-HSA-163359 (2025).

19. KEGG. KEGG PATHWAY: Riboflavin metabolism - Reference pathway. Genome.jp https://www.genome.jp/pathway/map00740 (2025).

20. GSEA. LEIN_CHOROID_PLEXUS_MARKERS. Gsea-msigdb.org https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/LEIN_CHOROID_PLEXUS_MARKERS.html (2023).

21. Qi, X.-H. et al. Increased cysteinyl-tRNA synthetase drives neuroinflammation in Alzheimer's disease. Translational Neurodegeneration 13, (2024).

22. Zhao, X.-Y. et al. Mitochondrial Dysfunction in Neural Injury. Frontiers in Neuroscience 13, (2019).

23. Takeichi, M. The cadherin superfamily in neuronal connections and interactions. Nature Reviews Neuroscience 8, 11–20 (2006).

24. Uniprot. Q15155 · NOMO1_HUMAN. Uniprot.org https://www.uniprot.org/uniprotkb/Q15155/entry (2025).

25. DBH gene: MedlinePlus Genetics. Medlineplus.gov https://medlineplus.gov/genetics/gene/dbh/ (2024).

26. Lea, R. A. et al. Evidence for allelic association of the dopamine β-hydroxylase gene (DBH) with susceptibility to typical migraine. Neurogenetics 3, 35–40 (2000).

27. Jacobs, B. & Dussor, G. Neurovascular contributions to migraine: Moving beyond vasodilation. Neuroscience 338, 130–144 (2016).

28. RSAD2 Radical S-adenosyl Methionine Domain Containing 2 [Homo Sapiens (human)] - Gene - NCBI. www.ncbi.nlm.nih.gov https://www.ncbi.nlm.nih.gov/gene/91543 (2025).

29. Fensterl, V. & Sen, G. C. Interferon-Induced Ifit Proteins: Their Role in Viral Pathogenesis. Journal of Virology 89, 2462–2468 (2014).

30. Database, G. TMTC1 Gene - GeneCards | TMTC1 Protein | TMTC1 Antibody. Genecards.org https://www.genecards.org/cgi-bin/carddisp.pl?gene=TMTC1 (2024).

31. IFI44L Gene - GeneCards | IF44L Protein | IF44L Antibody. www.genecards.org https://www.genecards.org/cgi-bin/carddisp.pl?gene=IFI44L (2024).

32. R A Heckman, P. et al. Phosphodiesterase Inhibition and Regulation of Dopaminergic Frontal and Striatal Functioning: Clinical Implications. International Journal of Neuropsychopharmacology 19, pyw030–pyw030 (2016).

33. Al-Khazali, H. M. et al. Effects of PDE-3 inhibition in persistent post-traumatic headache: evidence of cAMP-dependent signaling. The Journal of Headache and Pain 25, (2024).

34. KEGG. KEGG NETWORK: N00782. Kegg.jp https://www.kegg.jp/entry/N00782 (2025).

35. Booth, S. Triptans (Serotonin Receptor Agonists) for Migraine Headaches. WebMD https://www.webmd.com/migraines-headaches/triptans-migraines (2023).

36. KEGG. KEGG NETWORK: N00297. Kegg.jp https://www.kegg.jp/entry/N00297 (2015).

37. Ngala Elvis Mbiydzenyuy & Lihle-Appiah Qulu. Stress, hypothalamic-pituitary-adrenal axis, hypothalamic-pituitary-gonadal axis, and aggression. Metabolic Brain Disease 39, (2024).

38. Kowalick, C. UT Health San Antonio scientist discovers stress-triggered pathway behind migraines - UT Health San Antonio. UT Health San Antonio https://news.uthscsa.edu/ut-health-san-antonio-scientist-discovers-stress-triggered-pathway-behind-migraines (2024).

39. KEGG. KEGG NETWORK: N00325. Kegg.jp https://www.kegg.jp/entry/N00325 (2017).

40. Greenwood, M. P., Mecawi, A. D. S., José Antunes-Rodrigues, Paton, J. F. R. & Murphy, D. Rasd1, a small G protein with a big role in the hypothalamic response to neuronal activation. Molecular Brain 9, (2016).

41. GSEA. REACTOME_GLUCAGON_SIGNALING_IN_METABOLIC_REGULATION. Gsea-msigdb.org https://www.gsea-msigdb.org/gsea/msigdb/cards/REACTOME_GLUCAGON_SIGNALING_IN_METABOLIC_REGULATION (2024).

42. Del Moro, L., Rota, E., Pirovano, E. & Rainero, I. Migraine, Brain Glucose Metabolism and the 'Neuroenergetic' Hypothesis: a Scoping Review. The Journal of Pain 8, (2022).

43. Reactome. Ca-dependent events. Reactome.org https://reactome.org/content/detail/R-HSA-111996?/ (2025).

44. Costa, C. et al. Cortical spreading depression as a target for anti-migraine agents. The Journal of Headache and Pain 14, (2013).

45. KEGG. KEGG PATHWAY: T cell receptor signaling pathway - Homo sapiens (human). www.genome.jp https://www.genome.jp/pathway/hsa04660.

46. Schwaller, F. 10th Biennial National Meeting on Headache. Migraine Science Collaborative https://migrainecollaborative.org/a-neuroimmune-signaling-pathway-drives-chronic-headache?/ (2023).

47. GSEA. KEGG_INSULIN_SIGNALING_PATHWAY. Gsea-msigdb.org https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/KEGG_INSULIN_SIGNALING_PATHWAY.html (2023).

48. Ali, M. et al. The potential impact of insulin resistance and metabolic syndrome on migraine headache characteristics. BMC neurology 22, 422 (2022).

49. GSEA. PID_KIT_PATHWAY. Gsea-msigdb.org https://www.gsea-msigdb.org/gsea/msigdb/cards/PID_KIT_PATHWAY (2023).

50. Drube, S. et al. The receptor tyrosine kinase c-Kit controls IL-33 receptor signaling in mast cells. Blood 115, 3899–3906 (2010).

51. Chen, Y.-S. et al. Effect of Vitamin B2 supplementation on migraine prophylaxis: a systematic review and meta-analysis. Nutritional Neuroscience 9, 1–12 (2021).