Exploratory Human Whole-Genome Analysis in Familial Hemiplegic Migraine

St. Olaf Individual Major Capstone Project

Elise Hachfeld

I. Abstract

Familial hemiplegic migraine (FHM) is a rare, monogenic subtype of migraine characterized by temporary weakness or paralysis on one side of the body. Although pathogenic variants in *CACNA1A*, *ATP1A2*, and *SCN1A* account for many cases, a substantial proportion of clinically diagnosed individuals lack mutations in these canonical genes. This study performed an exploratory whole-genome analysis of an individual with clinically diagnosed FHM to identify additional genetic contributors to the condition.

Whole-genome sequencing data aligned to GRCh38 were analyzed using a bioinformatics pipeline based on Genome Analysis Toolkit (GATK) best-practice workflows. The pipeline included pre-processing, calling of single-nucleotide variants (SNVs) and small insertions/deletions (indels), and variant annotation. Variants on genes in a targeted gene panel of 6 genes and an expanded gene panel of over 220 genes associated with ion transport, neuronal excitability, and related neurological phenotypes were considered as candidates. Filtering was performed based on quality metrics, read depth, predicted functional impact, and population allele frequency.

No rare, high-impact variants were identified in canonical FHM genes. The application of the expanded gene panel identified 39 moderate or high-impact variants in candidate genes. After filtering and manual validation, a subset of variants in biologically relevant genes, including ATP13A5 and ATP8A1 were identified as potential candidates,

although no definitive causal variant was identified. This study highlights both the potential and limitations of exploratory whole-genome analysis in rare disease.

II. Introduction

Migraine is a common neurological condition characterized by recurrent moderate to severe headaches, often accompanied by nausea, phonophobia, and photophobia. It affects approximately 14% of the global population and is one of the leading causes of disability worldwide (Dong et al., 2024). Attacks typically progress through four distinct phases: prodrome, aura, headache, and postdrome. The prodrome phase may include subtle symptoms, such as fatigue, mood changes, or craving, while the headache phase is characterized by unilateral head pain. Approximately 25% of people with migraines experience transient sensory disturbances called auras (Kikkeri & Nagalli, 2024). Migraine aura can include disturbances in the visual field, loss of vision or hearing, abnormal skin sensations, and, in rare cases, motor weakness. Migraine with aura is a clinically distinct subtype of migraine associated with an increased risk of certain neurological conditions, including ischemic stroke (Øie et al., 2020).

Several mechanisms have been proposed to explain the aura symptoms associated with migraine. The leading hypothesis involves a phenomenon called cortical spreading depression (CSD). First described by Aristides Leão in 1944, CSD is a slowly propagating wave of depolarization that moves across the cortical surface at a rate of approximately 2-6 mm/min (Dodick, 2018). Following this depolarization, cortical neurons enter a temporary state of reduced electrical activity. As the wave of depolarization propagates across the cortex, it disrupts normal neuronal function in the affected regions. The progression of aura

symptoms corresponds closely with the propagation of CSD across the cortex. Many patients experience visual aura symptoms first, and functional MRI studies have shown that CSD often originates in the occipital lobe, which is responsible for visual processing (Hadjikhani 2001).

Although the mechanisms underlying CSD are still not fully understood, it is believed that the process is initiated by an increase in extracellular potassium (K+). Elevated extracellular potassium levels disrupt ionic gradients of the cell membrane and promote depolarization. This depolarization is associated with an influx of intracellular sodium (Na+) and calcium (Ca2+), and release of the excitatory neurotransmitter glutamate (Hill et al., 2024). CSD has also been observed in other neurological conditions, such as epilepsy, hemispheric stroke, subarachnoid hemorrhage, and intracerebral hemorrhage (Lauritzen et al., 2010; Hill et al., 2024).

While migraine is most often influenced by both genetic and environmental factors, a rare monogenic subtype of migraine called familial hemiplegic migraine (FHM) follows an autosomal dominant inheritance pattern. FHM is characterized by migraine attacks accompanied by unilateral motor weakness or temporary paralysis during the aura phase (Headache Classification Committee of the International Headache Society, 2018). Pathogenic variants in *CACNA1A*, *ATP1A2*, and *SCN1A* are the primary genetic causes of FHM (Grangeon et al., 2023). These genes encode proteins involved in neuronal ion transport and excitability, including voltage-gated calcium channels, sodium channels, and sodium-potassium pumps. Mutations in these genes can increase neuronal excitability. Experiments in mice carrying mutations in known FHM genes demonstrate increased susceptibility to CSD, suggesting that altered ion channel function may lower the threshold

for initiating CSD (Deghani & Karatas, 2019; van den Maagdenberg et al., 2001). These findings support the hypothesis that dysregulation of neuronal ion channels plays a central role in the pathophysiology of FHM.

Despite these known genetic variants, a substantial portion of patients clinically diagnosed with FHM have no pathogenic mutations in the currently recognized FHM genes, suggesting that additional genetic contributors exist (Grangeon et al., 2023). Several genes implicated in FHM are also associated with epilepsy and other disorders of neuronal hyperexcitability. The canonical FHM genes *CACNA1A*, *ATP1A2*, and *SCN1A* are also involved in hereditary epilepsies such as epileptic encephalopathy, generalized epilepsy with febrile seizures plus (GEFS+), and Dravet syndrome. Given their shared pathophysiological mechanisms, variants in genes associated with epilepsy may also influence susceptibility to FHM. This overlap provides a strong rationale for investigating whether variants in epilepsy-associated genes may contribute to FHM in patients lacking mutations in the canonical FHM genes.

This study aims to explore this hypothesis through whole-genome sequencing of an individual with clinically diagnosed FHM. Specifically, this study investigates whether rare or potentially deleterious variants in genes associated with neuronal hyperexcitability, particularly those associated with epilepsy, may be responsible for FHM. We hypothesize that in the absence of pathogenic variants in known FHM genes, FHM in this case may be associated with variants in genes associated with disorders that have shared pathophysiology.

III. Data and Methods

This study is an exploratory whole-genome analysis of previously collected data from an adult clinically diagnosed with familial hemiplegic migraine (FHM). The proband, or affected individual, experiences recurrent migraine with aura and unilateral motor weakness consistent with FHM. The proband's mother is also diagnosed with FHM, consistent with autosomal dominant inheritance. No developmental abnormalities were present in the proband; however, migraine with aura and childhood epilepsy were reported in maternal relatives. Given the role of ion channel dysfunction in cortical spreading depression, the analysis focused on identifying variants in genes responsible for regulating voltage-gated potassium, sodium, and calcium channels, and related neurological phenotypes.

The subject provided written informed consent for use of their genomic data. The project was reviewed and approved by the St. Olaf College Institutional Review Board and was conducted for research purposes only.

Whole-genome sequencing was performed by a research-grade sequencing laboratory. Genomic DNA was collected using a buccal swab and analyzed on a DNBSEQ T7 sequencer, generating 200 base pair paired-end reads. Binary alignment map (BAM) files aligned to the human reference genome GRCh38 were provided by the laboratory. Only high-quality reads from canonical chromosomes (chr1-22, X, mitochondrial DNA) were retained for analysis. As the proband is biologically female, reads aligned to the Y chromosome were excluded from the analysis due to low coverage and lack of biological relevance. All downstream analysis was conducted using the human GRCh38 reference build (Schneider et al., 2018). Analysis was performed in a Conda environment using GATK v4.6.1.0 (Van der Auwera & Connor, 2020), samtools v1.21 (Danecek et al., 2021), Picard

v3.3.0, Ensembl Variant Effect Predictor v115.2 (McLaren et al., 2016), and Python v3.13.1.

To prioritize biologically relevant regions, two gene panels were constructed. The initial panel prioritized the known FHM genes: *CACNA1A*, *ATP1A2*, and *SCN1A*, and three additional genes that are tentatively associated with FHM: *SLC1A3*, *SLC2A1*, and *ATP1A3* (Grangeon et al., 2023; Paucar et al., 2020; Weller et al., 2014; Potic et al., 2015). An expanded gene panel was also created, including gene families and 7 single genes (Table 1). Five gene families, *CACN*, *ATP*, *SCN*, *SLC*, and *PRRT*, were added because they contain genes that are known or believed to cause FHM. One additional gene family, *KCN*, was added because there are genes within the family that cause both migraines and epilepsy (Lacroix et al., 2024; Ginn et al., 2025; Gertler et al., 2018; Miceli et al., 2022; Miceli et al., 2023). The single genes were chosen because they are known to cause disorders that are associated with migraine or have an overlapping phenotype. In total, over 220 genes were examined: 3 genes known to be connected to FHM, 10 genes that have tentative links to FHM, over 200 genes from the families of the known and tentative genes, and 7 novel genes with an overlapping phenotype.

Initial quality control was performed to assess file integrity and alignment quality. Reads were coordinate-sorted and indexed, and metrics, including alignment statistics and genome-wide coverage, were calculated to evaluate data quality. Data quality metrics were calculated using only chromosomes 1-22 and X. Mean genome-wide coverage was 93.39%, mean read depth was 11.01, mean base quality was 34.77 and mean mapping quality was 54.98. A single read group was added using the Picard function *AddOrReplaceReadGroups and* PCR duplicates were identified and marked using *MarkDuplicates*.

Base Quality Score Recalibration (BQSR) was performed with the *BaseRecalibrator* and *ApplyBQSR* functions using known sites of variation from dbSNP and the 1000 Genomes Project data to adjust for systematic sequencing errors. Variants were subsequently called using GATK *HaplotypeCaller*, producing a Variant Call Format (VCF) file containing both single-nucleotide variants (SNVs) and insertions/deletions (indels). Structural variants (SVs), copy number variants (CNVs), and non-coding regulatory regions were not systematically analyzed and present additional sources of variation not captured in this study.

Variants were separated by type and filtered using hard-filtering thresholds based on GATK best-practice recommendations. Variant Quality Score Recalibration (VQSR) was not performed due to the absence of a large cohort of multiple samples for model training. SNVs were filtered to exclude variants with quality by depth (QD) < 2.0, overall quality (QUAL) < 30.0, strand odds ratio (SOR) > 3.0, Fisher strand bias (FS) > 60.0, mapping quality (MQ) < 40.0, mapping quality rank sum (MQRankSum) < -12.5, or read position rank sum (ReadPosRankSum) < -8.0. Indels were filtered to exclude variants with QD < 2.0, QUAL < 30.0, FS > 200.0, ReadPosRankSum < -20.0, or SOR > 10.0. The cutoffs were adapted from the GATK best practices recommendations (Van der Auwera et al., 2013). Only variants matching all these criteria were retained for downstream analysis.

Variants were annotated using Ensembl Variant Effect Predictor (VEP) in offline mode. Annotation included chromosome, position, reference and alternate alleles, biological consequence, predicted impact, SIFT and PolyPhen scores, and gnomAD population frequency. Variants were further prioritized based on predicted functional impact and population allele frequency. Population frequency from the gnomAD genomic data set was used for filtering if available, otherwise the frequency from exome data was used. Rare

variants (gnomAD population frequency ≤ 0.01) were prioritized. Variants lacking population frequency information from VEP were manually annotated using the gnomAD web interface (Karczewski et al., 2020). To ensure confidence of variant calls, variants were further filtered for read depth ≥10.

Candidate variants were manually validated using the Integrative Genomics Viewer (IGV) (Robinson et al., 2017). Visual inspection of aligned sequencing reads was used to evaluate read depth, strand bias, and mapping consistency, and indicators of alignment artifacts such as soft-clipped bases. Variants showing evidence of poor alignment or low read support were noted. Biological relevance of retained candidate variants was further assessed using GeneCards, dbSNP, and published literature (Stelzer et al., 2016; Sherry et al., 2001). Genes were prioritized based on expression in neurologically relevant tissue types, involvement in ion transport or neuronal signaling, association with migraine or epilepsy phenotypes, and potential relevance to cortical spreading depression.

Type	Name	Description	Rationale
Gene Family	CACN	Calcium voltage-gated channel subunits	Mutations in CACNA1A known to cause FHM[1]
Gene Family	ATP	ATPases	Mutations in ATP1A2 known to cause FHM, mutations in ATP1A3 strongly believed to cause FHM[1]
Gene Family	SCN	Sodium voltage-gated channels	Mutations in SCN1A known to cause FHM[1]
Gene Family	SLC	Solute carrier families	Mutations in SLC1A3 and SLC2A1 strongly believed to cause FHM[1]
Gene Family	PRRT	Dispansins	Mutations in PRRT2 strongly believed to cause FHM[1]
Gene Family	KCN	Potassium channels	Mutations in KCNK18 associated with autosomal dominant migraine with aura[1], mutations in KCNC1[2], KCNT1[3], KCNQ2[4], KCNQ3[5] associated with epilepsy
Gene	PNKD	Myofibrillogenesis Regulator 1	Two families with FHM had mutations in this gene[1]
Gene	CSNK1D	Casein kinase 1 delta	Associated with FASP, a sleep disorder linked to migraines[1]
Gene	ALPK1	Alpha kinase 1	Associated with ROASH syndrome, which causes migraine headaches[1]
Gene	TREX1	Three prime repair exonuclease 1	Associated with RVCL, a small vessel disease which impacts the brain[1]
Gene	COL4A1	Collagen type IV alpha 1 chain	Mutations can cause small vessel disease in the brain[1]
Gene	COL4A2	Collagen type IV alpha 2 chain	Mutations can cause small vessel disease in the brain[1]
Gene	NOTCH3	Notch receptor 3	Associated with CADASIL, a hereditary disorder with migraine symptoms. Included due to strong phenotypic overlap[1]

Table 1: Expanded gene panel used for candidate variant filtering. The expanded panel included established Familial Hemiplegic Migraine (FHM) genes and genes strongly implicated in migraine-associated neurological disorders. Both individual genes and broader gene families were included to capture potentially pathogenic variants with functional relevance to cortical spreading depression. The table summarizes the gene or family, biological function, and relevance to the analysis. [1](Grangeon et al., 2023); [2](Ginn et al., 2025); [3](Gertler et al., 2018); [4](Miceli et al., 2022); [5](Miceli et al., 2023)

IV. Results

Quality control analysis demonstrated high base-level sequencing accuracy and broad genome coverage. The mean base quality score was 34.77, corresponding to a base-calling error rate of approximately 1 in 3,000. Genome-wide coverage reached a mean of 93.39% not including mitochondrial DNA, with most chromosomes exceeding 90% coverage (Figure

1). However, mean read depth was approximately 11.01, which is lower than the benchmark of 30-50x coverage that is typically considered ideal for calling SNVs and indels accurately (Sun et al., 2021). Several chromosomes, including chromosome 22, showed reduced coverage relative to the genome-wide average. These metrics were calculated from the aligned reads of the canonical chromosomes prior to variant calling and filtering, and therefore reflect the quality of the raw data rather than the filtered set of variants.

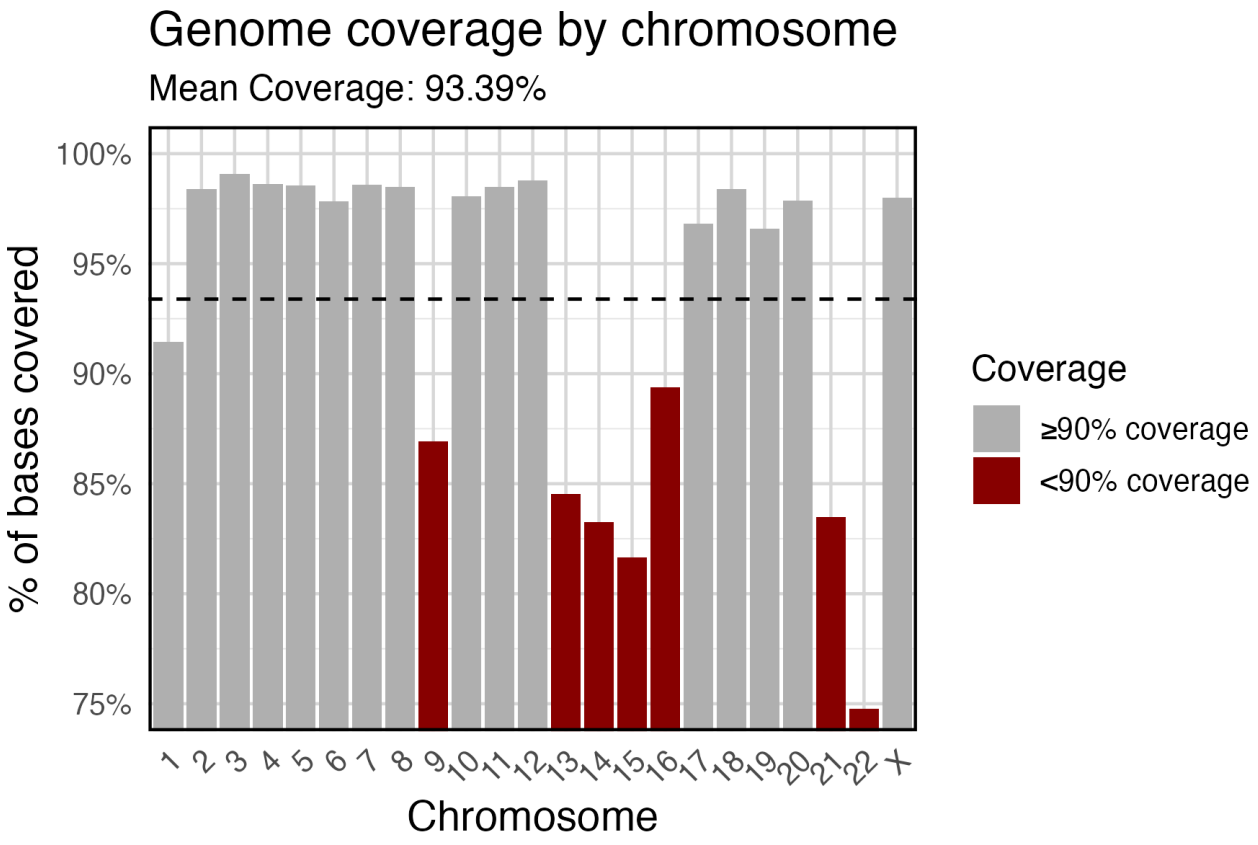

Figure 1. Percentage of bases covered by chromosome. Gray bars indicate chromosomes with ≥ 90% coverage, while red bars indicate chromosomes with < 90% coverage. Although most chromosomes show high coverage, several chromosomes, especially chromosome 22, exhibit reduced coverage.

Initial analysis of variants within known familial hemiplegic migraine (FHM) genes revealed that the majority of variants were classified as having low or modifier impact, with only a few moderate-impact variants (Figure 2A). A small subset of variants were classified as moderate-impact and none as high-impact (Figure 2B). Further filtering based on population frequency showed that no moderate-impact variants fit the rarity threshold (gnomAD population frequency ≤ 0.01) (Figure 3).

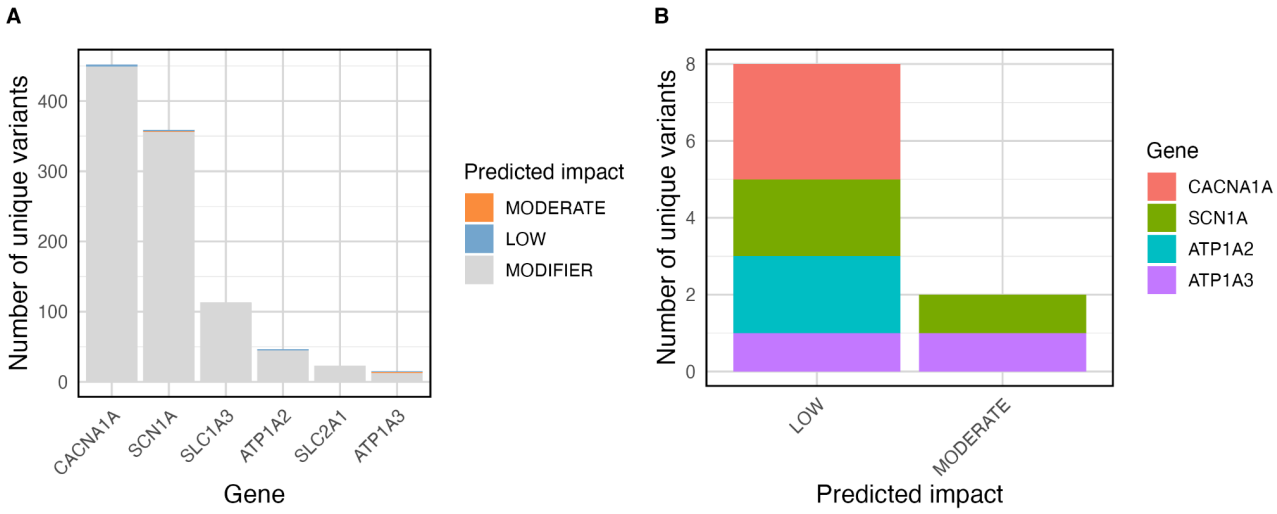

Figure 2. Distribution of variant impact across initial candidate genes. **(A)** Total number of variants categorized by predicted impact (modifier, low, moderate). No high-impact variants were present in these candidate genes. **(B)** Number of variants by impact (low or moderate only). There are 10 low-impact variants and 3 moderate-impact variants in the candidate genes. There are no low or moderate-impact variants in *SLC1A3* or *SLC2A1*.

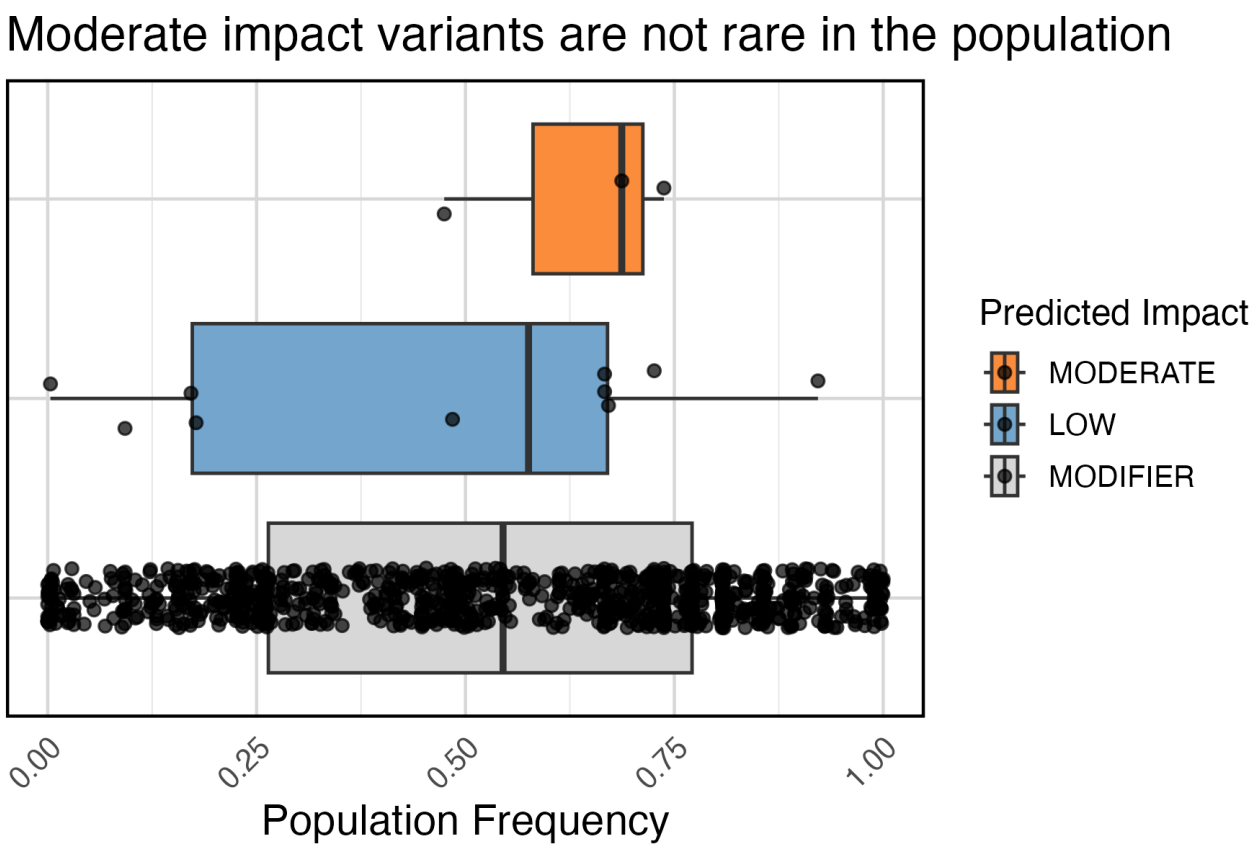

Figure 3. Population frequency of variants by predicted impact. Some low and modifier impact variants are more rare in the population, while all moderate-impact variants in the candidate genes are common in the population.

Variant calling identified a total of 3,567,427 genetic variants across the genome. Restricting the analysis to genes within the extended candidate panel reduced this set to 424 variants, of which 204 variants met the read depth threshold ≥10. Additional filtering based on population allele frequency (≤ 0.01%) and predicted functional impact (high or moderate) resulted in 29 moderate-impact variants and 10 high-impact variants within the candidate gene panel (Table 2). The retained variants were primarily heterozygous. Out of the high-impact variants, one (chr20:46,728,237) was eliminated through manual validation of the population frequency in gnomAD because it is present in 28% of the population.

Gene	Position	Variant	Consequence	Genotype Quality	Read Depth
ATP13A5	chr3:193,289,900	TCA > T	Stop gained & frameshift	45	15
ATP8A1	chr4:42,465,016	T > TCTATCTTTAGA	Frameshift	60	10
ATP8A1	chr4:42,465,019	G > GTTTT	Frameshift	60	10
ATP8A1	chr4:42,465,024	T > TTTTTACCTGGG	Stop gained & frameshift	60	10
ATP8A1	chr4:42,465,026	A > ACTAATAATTTGTCCAACTAATTTTC	Frameshift	60	10
SLC6A18	chr5:1,240,633	TA > T	Frameshift	41	11
SLC25A30	chr13:45,397,266	TC > T	Frameshift	45	10
SLC2A4	chr17:7,283,358	GA > G	Frameshift, splice-region	51	13
KCNJ18	chr17:21,703,469	TGC > T	Frameshift	42	14

Table 2. Candidate variants identified in genes after filtering. Variants shown include predicted high-impact variants detected within candidate genes from the expanded gene panel. Reported information includes the affected gene, genomic position (GRCh38), variant allele change, predicted functional consequence, genotype quality (GQ), and read depth (RD).

After manual review in IGV and assessment of biological relevance, several candidate variants were deprioritized. Variants in *SLC6A18*, *SLC25A30*, and *SLC2A4* were considered less likely to contribute to the phenotype because these genes are primarily expressed in non-neuronal tissues. Despite these exclusions, several variants remained biologically interesting based on known gene function. After filtering and manual review, variants in *ATP13A5*, *ATP8A1*, and *KCNJ18* remained the most biologically relevant candidates identified in the expanded gene panel.

V. Discussion

This study aimed to investigate whether rare variants in genes associated with neuronal excitability and ion channels, particularly those related to hereditary epilepsy, could contribute to familial hemiplegic migraine in the absence of pathogenic variants in the known genes. No rare, high-impact variants were identified in *CACNA1A*, *ATP1A2*, *SCN1A*, or other genes currently associated with FHM. Although several moderate-impact variants were

identified within these genes, none met the rarity threshold expected for a rare monogenic disorder. These findings are consistent with previous studies showing that a subset of clinically diagnosed patients have no pathogenic variants in the canonical genes (Sutherland et al., 2020).

Expansion of the analysis to a broader panel of over 220 genes identified multiple moderate and high-impact variants after filtering. Many retained variants were heterozygous, which is consistent with the autosomal dominant inheritance pattern typical in FHM. However, biological relevance varied considerably across candidate genes. Variants in *SLC6A18*, *SLC25A30*, and *SLC2A4* were deprioritized because these genes are not primarily expressed in neuronal tissues. *SLC6A18* and *SLC25A30* are primarily expressed in the kidneys (Singer et al., 2009; Xu et al., 2026). *SLC2A4* encodes for the GLUT4 protein, which is a glucose-transporter primarily expressed in muscle and fat tissue (Richter et al., 2024). Variants in three genes, *ATP13A5*, *ATP8A1*, and *KCNJ18* remained candidates because of their expression in the central nervous system or phenotypic overlap with FHM.

One particularly plausible candidate gene identified in this study was *ATP13A5*. Recent research has shown that *ATP13A5* is highly specific to central nervous system pericytes in mice (Guo et al., 2024). Pericytes play a role in maintaining the blood-brain barrier and regulating cerebral blood flow. During cortical spreading depression, increases in intracellular calcium can trigger prolonged pericyte contraction, reducing local cerebral perfusion (Khennouf et al., 2018). Dysfunction of *ATP13A5* could therefore contribute to migraine pathophysiology through abnormal vasoconstriction and prolonged hypoperfusion rather than direct ion channel dysfunction. However, interpretation of this variant remains tentative because the variant was supported by only two sequencing reads out of 15

(13.33%). For a heterozygous variant, it is expected that on average 50% of the reads will support the variant. Low read support increases the possibility that the variant may be a false positive induced by sequencing errors.

Variants identified in *ATP8A1* may also be biologically relevant. *ATP8A1* encodes a P-type phospholipid flippase involved in maintaining membrane phospholipid asymmetry, a process known as phospholipid translocation (Pomorski et al. 2003). Disruption of membrane phospholipid organization may alter vesicle formation and synaptic signaling. In mice, both increased and decreased expression of *ATP8A1* is associated with disruption of brain connectivity (Kerr et al., 2016). Manual review of the *ATP8A1* variants in IGV did not show clear visual evidence supporting the predicted insertion(s). However, after enabling visualization of soft-clipped bases, several reads near the variant site showed soft-clipped bases. Soft-clipped bases can indicate inaccurate alignment, but it can also occur near true variants, so the finding could not be definitively excluded.

A single frameshift variant was identified in *KCNJ18*. Mutations in this gene are known to cause thyrotoxic period paralysis (TPP) (Zheng et al., 2016). Given that TPP is a syndrome that causes temporary muscle weakness or paralysis, it has phenotypic overlap with FHM. However, *KCNJ18* is primarily expressed in skeletal muscle, and the weakness in TPP is caused by hyperpolarization of muscle cells which directly affects the neuromuscular junction (Kung, 2006). The pathophysiology of TPP does not align with the mechanism underlying CSD, making this variant an unlikely contributor to FHM.

Despite these biologically plausible connections, it is important to recognize that variants identified in this study are not sufficient to demonstrate causation. Variant prioritization relied heavily on computational prediction tools and existing biological

knowledge, neither of which provides direct evidence of pathogenicity. While VEP annotations and population frequency filtering are useful for prioritizing variants, experimental validation would be required to determine whether any identified variants alter protein function in a biologically meaningful manner.

Several technical limitations further constrain interpretation. First, the mean read depth of approximately 11.01x is substantially lower than the standard 30x coverage recommended for reliable heterozygous variant detection in research and clinical settings. The reported coverage from the laboratory was 25.55x; this discrepancy could be for several reasons, including the exclusion of mitochondrial DNA, alternate or unknown contigs, and unmapped reads in this study. Reduced sequencing depth increases the likelihood of both false positives and false negatives, particularly in regions with uneven coverage. Additionally, several chromosomes demonstrated reduced coverage, potentially limiting variant detection sensitivity in those regions (Figure 1). Second, this study focused exclusively on single-nucleotide variants and small insertions or deletions. Structural variants, copy number variants, and variants in non-coding regions can be pathogenic for some diseases and were not examined here. Third, reliance on population frequency databases such as gnomAD introduces potential bias because these databases do not equally represent all ancestral populations (Popejoy et al., 2018). Finally, this study analyzed only a single affected individual. Without segregation analysis in additional family members, it is not possible to determine whether identified variants co-segregate with disease.

Taken together, these findings suggest that this case of FHM is unlikely to be caused by a single, high-impact mutation in the known genes. Instead, the results suggest mutations in less well-characterized genes, polygenic contributors, or non-coding regulatory regions not

captured in this analysis. A recent study has found that patients with FHM have an increased burden of mutations in *CACNA1H* and *CACNA1I*, suggesting that the cumulative effect of multiple mutations could contribute to the condition (Maksemous et al., 2023). Future work should include higher-depth sequencing, incorporation of structural variant analysis, and family segregation analysis. Functional studies would be essential to determine whether variants in genes such as *ATP13A5* and *ATP8A1* play a role in migraine pathophysiology. In conclusion, while this study does not identify a definitive genetic cause of FHM in this individual, it demonstrates the utility and limitations of exploratory whole-genome analysis in rare disease, and highlights novel candidate genes that warrant further investigation.

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