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Machine learning-driven identification of critical gene programs and key transcription factors in migraine

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Abstract

Background Migraine is a complex neurological disorder characterized by recurrent episodes of severe headaches. Although genetic factors have been implicated, the precise molecular mechanisms, particularly gene expression patterns in migraine-associated brain regions, remain unclear. This study applies machine learning techniques to explore region-specific gene expression profiles and identify critical gene programs and transcription factors linked to migraine pathogenesis.

Methods We utilized single-nucleus RNA sequencing (snRNA-seq) data from 43 brain regions, along with genome-wide association study (GWAS) data, to investigate susceptibility to migraine. The cell-type-specific expression (CELLEX) algorithm was employed to calculate specific expression profiles for each region, while non-negative matrix factorization (NMF) was applied to decompose gene programs within the single-cell data from these regions. Following the annotation of brain region expression profiles and gene programs to the genome, we employed stratified linkage disequilibrium score regression (S-LDSC) to assess the associations between brain regions, gene programs, and migraine-related SNPs. Key transcription factors regulating critical gene programs were identified using a random forest model based on regulatory networks derived from the GTEx consortium.

Results Our analysis revealed significant enrichment of migraine-associated single nucleotide polymorphisms (SNPs) in the posterior nuclear complex-medial geniculate nuclei (PoN_MG) of the thalamus, highlighting this region's crucial role in migraine pathogenesis. Gene program 1, identified through NMF, was enriched in the calcium signaling pathway, a known contributor to migraine pathophysiology. Random forest analysis predicted ARID3A as the top transcription factor regulating gene program 1, suggesting its potential role in modulating calcium-related genes involved in migraine.

Conclusion This study provides new insights into the molecular mechanisms underlying migraine, emphasizing the importance of the PoN_MG thalamic region, calcium signaling pathways, and key transcription factors like ARID3A. These findings offer potential avenues for developing targeted therapeutic strategies for migraine treatment.

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Introduction

Migraine is a prevalent and debilitating neurological disorder affecting approximately 15% of the global population, imposing a significant disease burden worldwide [1]. Characterized by recurrent episodes of severe headache often accompanied by nausea, photophobia, and phonophobia, migraine substantially impairs quality of life and productivity [2]. The World Health Organization ranks migraine as one of the leading causes of disability among individuals under 50 years of age, underscoring its public health significance [3]. Despite extensive research, the precise mechanisms underlying migraine pathogenesis remain incompletely understood.

Current evidence suggests that migraine is a complex disorder involving brain dysfunction, genetic, environmental, and neurovascular factors [4]. Functional and structural neuroimaging studies have implicated several brain regions in migraine pathophysiology, including the cortex, brainstem, thalamus, hypothalamus, and cerebellum [5, 6]. However, the specific gene expression patterns within these migraine-associated brain regions are not well characterized. Understanding these patterns is crucial, as they may reveal molecular pathways contributing to the initiation and propagation of migraine attacks [7–9]. Previous genetic studies have identified multiple susceptibility loci associated with migraine, indicating a polygenic inheritance pattern [10–13]. Genome-wide association studies (GWAS) have uncovered genes related to ion channels, neurotransmitter systems, and inflammatory processes [14]. Nevertheless, GWAS typically do not provide information about tissue-specific gene expression, leaving a gap in our knowledge regarding regional brain activity in migraine. Exploring gene expression profiles in specific brain regions may bridge this gap and enhance our understanding of migraine pathophysiology. Moreover, the heterogeneity of migraine phenotypes suggests that different biological mechanisms may be involved in different individuals [15, 16]. Analyzing gene expression in targeted brain regions could uncover specific biomarkers and therapeutic targets, potentially leading to personalized medicine approaches. For instance, alterations in the expression of genes involved in the calcitonin gene-related peptide (CGRP) pathway have been linked to migraine, leading to the development of novel CGRP antagonists [17]. Similarly, dysregulation of serotonergic and dopaminergic systems has been implicated in migraine pathogenesis [18].

Currently, several studies have used transcriptome or single-cell transcriptome sequencing methods on specific brain regions to explore gene expression associated with migraine in these regions [19–21]. Their findings provide insights into the potential mechanisms underlying the pathogenesis of migraine. However, these studies have certain limitations. For example, Angeliki Vgontzas and colleagues collected single-cell level expression profiles from both central and peripheral tissues and assessed the expression levels of putative migraine-associated genes across various cell types [19]. However, their research lacked a comprehensive understanding of gene programs. In this context, a gene program refers to a series of processes during the cell life cycle, including signatures that define cell identity, cell processes that are either specific to certain cell types or shared among different cell types, and disease-related signatures that are dependent on particular cell types [22]. Although the studies [20, 21] by Andreas H. Rasmussen and Else Eising utilized single-nucleus sequencing data from mouse brain tissue to explore gene networks associated with specific cell types in migraine, this approach inevitably introduces species differences. In contrast, the present study incorporated single-nucleus sequencing data derived from human tissue [8, 23], specifically focusing on brain regions previously implicated in migraine. By analyzing the single-nucleus expression profiles across different brain regions and integrating migraine-associated SNPs, we identified brain regions closely related to migraine. Additionally, previous studies investigating the expression profiles of migraine-related brain regions have similarly lacked a comprehensive understanding of how molecular changes influence gene regulatory networks, which is crucial for identifying key regulatory events in the pathogenesis of migraine. In this study, we not only analyzed snRNA-seq data from migraine-related brain regions to construct and explore potential gene programs associated with migraine, but also employed a machine learning approach—random forest—to investigate the transcription factors that regulate these key gene programs involved in migraine. Random forest algorithms construct multiple decision trees during training and output the class that is the mode of the classes or mean prediction of the individual trees, making them robust to overfitting and capable of modeling complex nonlinear relationships. Understanding the key gene programs and their transcriptional regulators in migraine-related brain regions is crucial for elucidating the pathophysiological mechanisms of migraine. This knowledge not only enhances our understanding of the underlying processes such as migraine aura, cortical spreading depression, and central sensitization, but also opens up potential avenues for identifying novel therapeutic targets for migraine treatment. For example, changes in the expression of

genes related to excitatory and inhibitory neurotransmission may influence neuronal excitability and trigger migraine attacks [24]. Furthermore, inflammatory mediators within specific brain regions may play a role in migraine pathophysiology [25, 26].

Given these considerations, the present study aims to investigate the specific gene programs in brain regions associated with migraine pathogenesis, as well as their transcriptional regulators, using random forest machine learning methods. This study may contribute to a deeper understanding of the molecular mechanisms of migraine and facilitate the development of targeted therapies.

Methods

Data collection

For this study, we obtained single-nucleus RNA sequencing (snRNA-seq) data from 43 distinct regions of the human brain, including the trigeminal ganglion. Transcriptomic data for 42 brain regions were obtained from the work of Siletti et al. (2023) [23] and through the CELLxGENE repository (https://cellxgene.cziscience.com). The snRNA-seq data for the trigeminal ganglion were acquired from Yang et al. (2022) [8] and retrieved from the Gene Expression Omnibus (GEO) under accession number GSE197289. For the genome-wide association study (GWAS) data on migraine, we used the dataset 'ukb-b-13190' from the Open GWAS project (https://gwas.mrcieu.ac.uk). The regulatory network constructed using PANDA (Passing Attributes between Networks for Data Assimilation) based on the Genotype-Tissue Expression (GTEx) consortium data [27] was obtained from the Zenodo database (https://zenodo.org/record/838734).

Single-cell data processing

To process the single-nucleus RNA sequencing (snRNA-seq) data, we first merged all brain region datasets into a single unified dataset. To ensure a balanced representation of nuclei from each region, we applied the sample function to randomly sample 1/4 of the total nuclei from each sample. Subsequently, quality control (QC) was performed on the merged dataset, and nuclei with unique molecular identifier (UMI) counts less than 800 were excluded. To correct for batch effects arising from different studies, we applied the harmony package (version 1.0; https://github.com/immunogenomics/harmony) for batch correction. Harmony aligns datasets by integrating them into a shared low-dimensional space, effectively mitigating batch effects while preserving the underlying biological variation. Dimensionality reduction, clustering, and cell-type annotation were then performed using the Seurat R package (version 4.0.0; https://satijalab.org/seurat/) [28]. Cell types were annotated based on previously published canonical marker genes [8, 23]. For example, astrocytes were defined by AQP4, GFAP, and GJA1; Schwann cells by MPZ and S100B; oligodendrocytes by MOG and MOBP; and OPCs by VCAN. Other cell types, including CNS macrophages, leukocytes, fibroblasts, and neurons, were similarly annotated using established markers. Neurons were further categorized into excitatory and inhibitory neurons based on CAMK2A and GAD1, followed by additional dimensionality reduction, clustering, and subgrouping based on highly expressed genes.

Calculation of region-specific expression profiles

We extracted the single-cell count matrix covering all regions, along with the metadata containing the brain region information. The cellex module (https://github.com/perslab/CELLEX) on the Python platform was used to calculate the region-specific expression profiles [29]. Cell-type-specific expression (CELLEX) integrates multiple metrics, including Differential Expression T-statistics, Gene Enrichment Scores, and Expression Proportion, to quantify differential gene expression across regions and identify region-specific genes. The region-specific expression profiles were uploaded to the Zenodo database (https://doi.org/10.5281/zenodo.13894995) [30].

Identification of gene programs

To identify gene programs, we applied non-negative matrix factorization (NMF) to the batch-effect-corrected and standardized single-cell expression matrix. NMF is a dimensionality reduction technique commonly used in transcriptomics for identifying latent factors or gene programs within high-dimensional gene expression data. We employed the NMF R package (version 0.23.0) [31]. The gene x gene program matrix represents the contribution of each gene to the identified gene programs. From the resulting basis matrix, we identified the top-ranked genes for each gene program by sorting genes according to their contribution weights.

Discovery of migraine-critical regions and gene programs

We employed a three-step process to identify critical brain regions and gene programs associated with migraine. First, we created annotations for each region and gene program. For regional annotations, the expression specificity values of each region were assigned to genetic variants. For gene programs, the gene weights derived from non-negative matrix factorization were assigned to genetic variants. Given that most enhancers are located within 100 kb of their target promoters [32], we set the annotation window size to 100 kb. This relatively large window captures the effects of nearby regulatory variants, as the majority of trait-associated variants are located in non-coding regions [33]. While constructing the annotations, we utilized the same set of SNPs

from the 1000 Genomes Project [34] as in the default baseline model of S-LDSC.

Next, we calculated linkage disequilibrium (LD) scores for HapMap3 SNPs [35] for each annotation using the recommended settings. HapMap3 (The International HapMap Project Phase 3) is a comprehensive reference dataset cataloging genetic variations across 11 globally diverse populations, including African, European, Asian, and Native American groups. It provides high-resolution data on allele frequencies and linkage disequilibrium (LD) patterns, making it a robust resource for genetic studies. In this study, HapMap3 was selected for its broad population representation and reliability in LD score regression analyses. LD scores for SNPs in HapMap3 were computed using the recommended parameters to ensure precise estimation of SNP-heritability relationships.

Finally, to evaluate the association between migraine-related genomic data and our annotations, we integrated migraine GWAS summary statistics and ran stratified linkage disequilibrium score regression (S-LDSC) following the standard workflow recommended by the authors [36]. This analysis yielded *p*-values reflecting the positive association between heritability and the regional expression specificity and gene program weights.

To address potential biases due to varying SNP counts across brain regions, we further refined the heritability enrichment estimates. First, we calculated the proportion of SNPs in each brain region relative to the total number of SNPs across all regions. Next, we normalized the h^2 enrichment for each brain region by dividing its raw h^2 enrichment value by the corresponding SNP proportion. This adjustment accounts for differences in SNP representation across regions, ensuring that the normalized h^2 enrichment values reflect heritability contributions independent of SNP abundance.

Functional analysis of gene programs, cell subtypes, and brain region-specific differences

To further clarify the function of each gene program, genes within each program were ranked, and the top 200 genes were used for Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis [37]. Additionally, to further validate the function of the Neuron_c3 cell subtype, the FindMarker function in the Seurat package [28] was used to compare differentially expressed genes (DEGs) between Neuron_c3 and other cell subtypes. Gene set enrichment analysis (GSEA) was then performed on the KEGG-enriched pathways to identify pathways significantly enriched in Neuron_c3. To further investigate the regional functional differences between brain regions, the FindMarker function was also applied to identify DEGs across different brain regions. The identified DEGs were then analyzed for Gene Ontology (GO) enrichment [38], focusing on biological processes, to elucidate region-specific functional roles.

Identification of key transcriptional regulators using random forest

To identify key transcriptional regulators for migraine-critical gene programs, we employed a random forest approach, integrating data from the GTEx-PANDA transcriptional regulatory network [27] and gene weight information from migraine- critical gene programs to construct the feature matrix. The dataset was split into a training set and a test set with a ratio of 8:2, ensuring balanced distribution of the target variable across both sets by applying stratified sampling using the createDataPartition function from the R caret package. For feature selection, we utilized the glmnet package to fit a generalized linear model with L1 regularization (lasso), which resulted in the selection of 23 important variables. Subsequently, parameter tuning for the random forest model was performed using the caret package, with the optimal parameters identified as ntree = 500 and mtry = 2. The random forest model was then trained on the training set using the selected features and tuned parameters, and its performance was evaluated on the test set.

To further evaluate the statistical significance of the identified transcription factors, we conducted permutation testing using the **rfPermute** package (version 2.5.2) in R (version 4.0.2). Permutation testing involves random shuffling of the values of each variable and measuring its impact on the model's predictive performance [39]. This process was repeated multiple times to generate a distribution of importance scores for each transcription factor. Observed importance scores were compared against this distribution to calculate *p*-values, which helped determine the statistical significance of the identified transcription factors.

Results

Linking PoN_MG nuclei in the thalamus to migraine susceptibility

To investigate the genetic basis of migraine, we utilized single-nucleus transcriptomic data from 42 brain regions and the trigeminal ganglion (Fig. 1). The dataset included 315,450 nuclei, categorized into distinct cell types and brain regions. Figure 2A displays the two-dimensional visualization of the nuclei using t-distributed stochastic neighbor embedding (t-SNE), a dimensionality reduction technique commonly used for visualizing high-dimensional data [40]. This projection highlights the largest populations of neurons, followed by oligodendrocytes, astrocytes, and other glial cells. Smaller populations, such as pericytes and fibroblasts, were also present in the dataset. Figure 2B illustrates the nuclei distribution by brain region, showing distinct clustering that reflects the

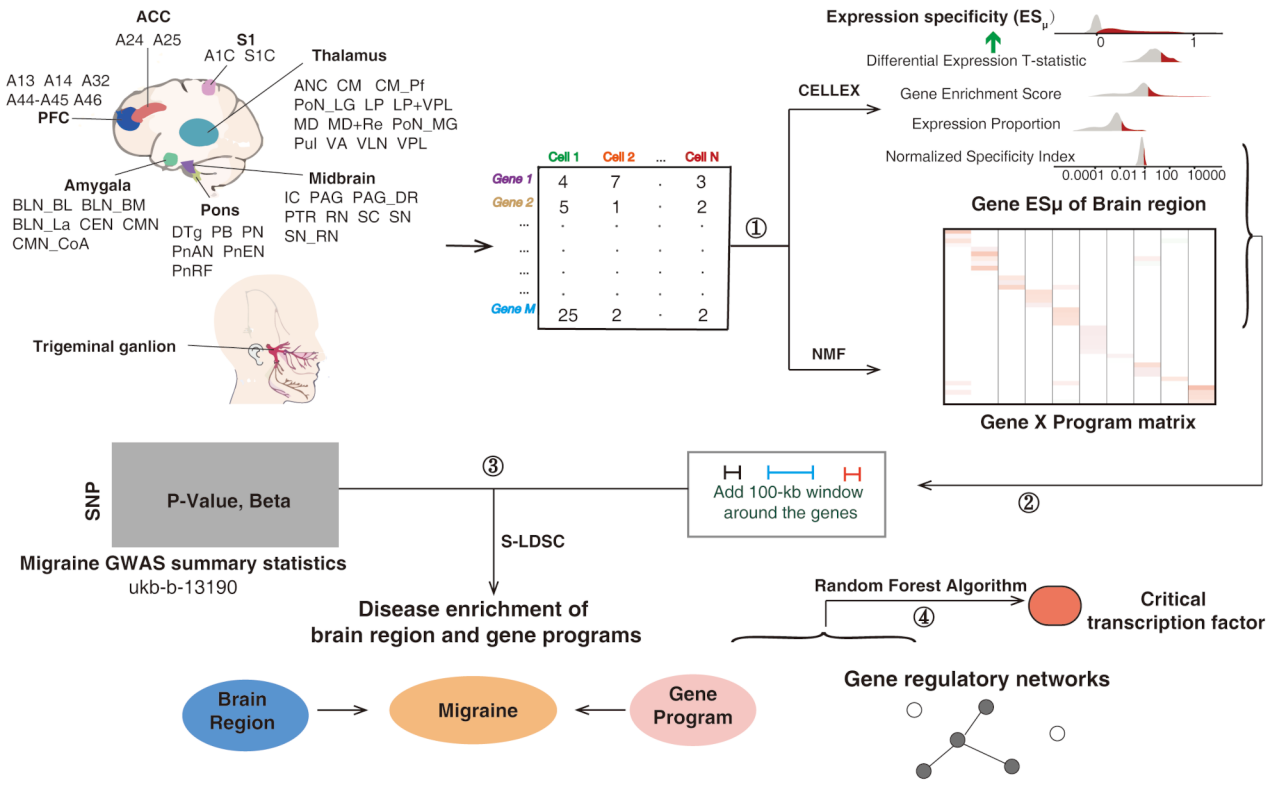

Fig. 1 A flowchart for identifying migraine-critical regions and gene programs by integrating single-cell expression profiles and human genetics. The overall process of this study is divided into four parts. The first step involves calculating the expression specificity of regions using the CELLEX algorithm, while the NMF algorithm is employed to compute the gene x program matrix from single-cell data in brain regions. In the second step, genes from both the region and gene program are annotated within a 100 kb range of the genome. The third step utilizes S-LDSC to identify regions and gene programs that are significantly enriched in migraine-related SNPs. Finally, the random forest algorithm, in conjunction with the gene regulatory network from GTEx-PANDA, is used to identify key transcription factors regulating the migraine-critical gene program. The abbreviations for brain regions used in the diagram can be found in full in Table S1

transcriptional diversity across migraine-related brain areas. Table S1 provides comprehensive annotations for the brain regions analyzed in this study, detailing the specific genes expressed in each region. This distribution reinforces the region- and cell-type specificity captured in the dataset, providing a comprehensive landscape of gene expression profiles across critical migraine-related brain areas.

As illustrated in Fig. 2C, SNPs annotated to the genes specifically expressed in the PoN_MG region of the thalamus are significantly enriched in migraine-associated SNPs, indicating that PoN_MG is a critical region involved in migraine pathogenesis. While other regions did not exhibit significant SNP enrichment, the heritability (h^2) scores for multiple regions are noteworthy. For instance, thalamic regions such as PoN_MG, LP, and VPL demonstrate relatively high normalized heritability enrichment scores, as highlighted by the color gradient in the heatmap. These findings underscore the potential involvement of specific thalamic subregions in the onset and progression of migraine and emphasize the importance of considering heritability metrics in understanding the genetic architecture of migraine.

Discovery of critical gene programs associated with migraine

Identifying disease-associated gene programs is crucial for elucidating disease mechanisms and discovering potential therapeutic targets. Based on the results in Fig. 2C, which highlight the important roles of the thalamus and trigeminal ganglion in migraine, we further extracted single-nucleus expression profiles from these regions. Figure 3A presents the distribution of various cell subtypes in the Uniform Manifold Approximation and Projection (UMAP) space, where the gene names displayed in different colors represent the marker genes of specific neuronal subtypes. The marker genes for each subtype are further detailed in Fig. 3B.

Using non-negative matrix factorization (NMF), we decomposed these single-nucleus expression profiles into 10 distinct gene programs (Fig. 3C, Table S2). The high-weight genes from each of these gene programs

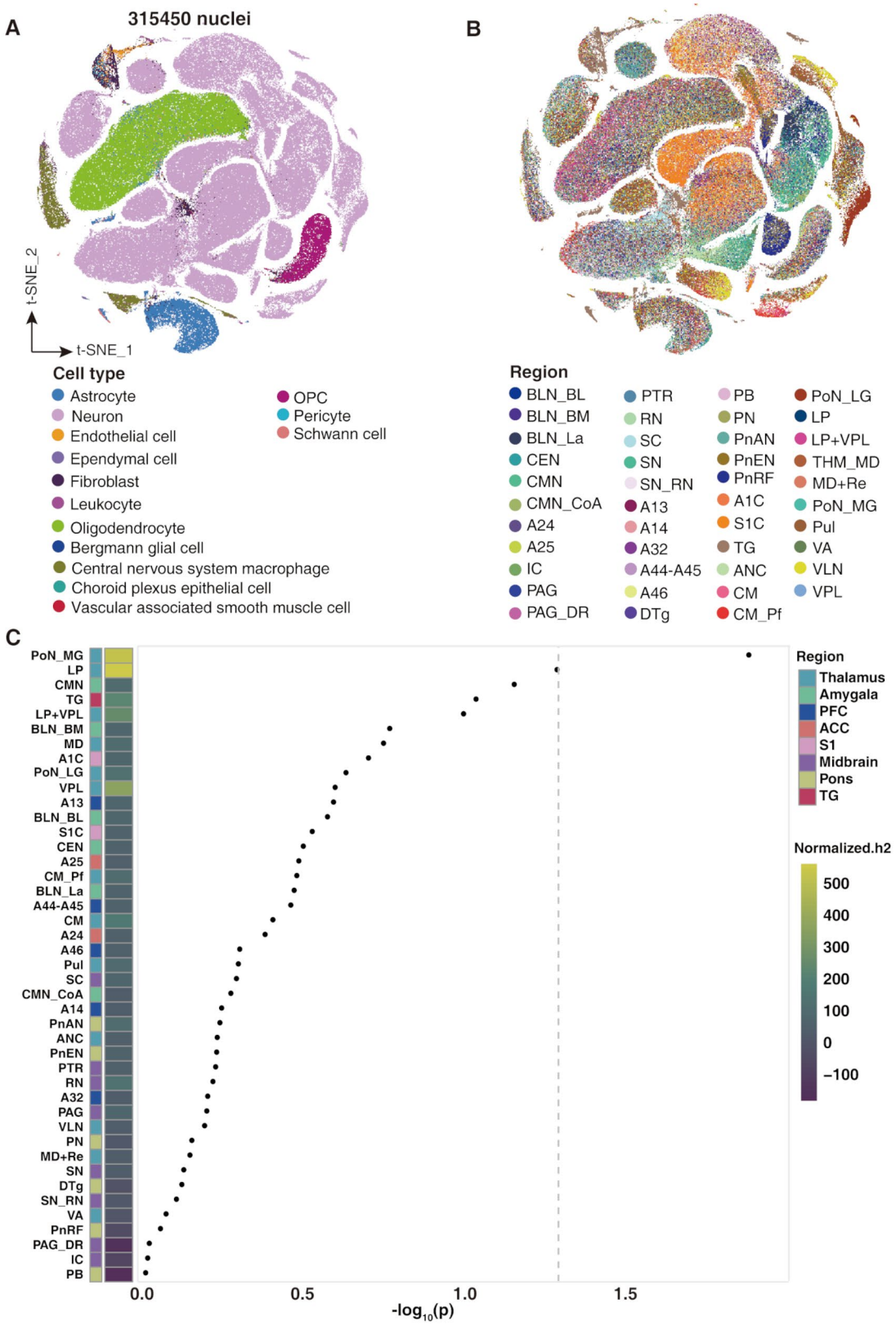

Fig. 2 (See legend on next page.)

(See figure on previous page.)

Fig. 2 Neuclei distribution and genetic enrichment analysis of migraine-related brain regions. (**A**) This panel illustrates the coordinate distribution of nuclei across all brain regions in the first two dimensions of the t-Distributed Stochastic Neighbor Embedding (t-SNE) space, with each point representing a nucleus and the color mapping indicating different cell types. (**B**) This panel displays the same nuclei as in panel A, along with their distribution in the t-SNE space; however, the color of the points is mapped to specific regions. (**C**) This figure shows the enrichment of the specific expression profiles of different regions within a 100 kb genomic window in migraine-related SNPs. The color of each cell in the heatmap represents the proportion of normalized heritability enrichment for that region, while the scatter plot displays the significance levels for different regions. the x-axis values correspond to -$\log_{10}$(*p* value of S-LDSC), with the dashed line indicating the position of -$\log_{10}$(0.05) on the x-axis. The small boxes on the far left annotate the larger anatomical regions to which the different regions belong. Oligodendrocyte Precursor Cells

were annotated with SNPs, and S-LDSC was employed to assess their enrichment in migraine-associated SNPs. Notably, gene program 1 was significantly enriched in migraine-related SNPs, indicating its potential role in migraine pathogenesis (Fig. 3D). The functions of some high-weight genes in Program 1 may explain their association with migraine. For example, IL1RAPL2 is involved in the regulation of immune responses through its interaction with interleukin-1 receptors [41]. Given the established link between inflammation and migraine [42], IL1RAPL2 may play a role in the neuroimmune response during migraine episodes. Furthermore, RGS16 is a regulator of G-protein signaling, which is crucial for modulating neurotransmitter pathways [43]. As dysregulation of neurotransmission is a known factor in migraine [44], RGS16 may contribute to this process. These findings collectively suggest the reliability of the migraine-associated gene programs identified through our methodology.

Enrichment of gene program 1 highlights migraine-associated pathways

To further clarify the pathways involved in the previously identified migraine-related gene program 1, we conducted KEGG enrichment analysis to explore the functions of gene programs derived from NMF. The results revealed significant enrichment of multiple pathways in gene program 1, including the calcium signaling pathway and long-term depression (Fig. 4A, Table S3). Both the calcium signaling pathway and neuroactive receptor interactions have been reported to play roles in the mechanisms of migraine [45, 46], which supports the reliability of our findings to some extent. Furthermore, we examined the expression levels of gene program 1 across different neuronal subpopulations, these results indicated that the Neuron_c3 subtype exhibited the highest expression of gene program 1 (Fig. 4B). Given that the expression level of gene program 1 is significantly higher in the Neuron_c3 subgroup compared to other subgroups, we further conducted gene set enrichment analysis to evaluate the enrichment of relative gene expression levels between Neuron_c3 and other cell subgroups in KEGG pathways. The results revealed significant enrichment in the calcium signaling pathway and neuroactive receptor interaction pathway (Fig. 4CD, Table S4), suggesting that these two pathways may play important roles in migraine.

In addition to the KEGG enrichment analysis of gene program 1, we investigated the differentially expressed genes in the PoN_MG region compared to other thalamic subregions, given its significant enrichment in migraine-associated SNPs. GO enrichment analysis revealed several pathways prominently associated with these genes(Figure S1). Notably, the pathways "associative learning" and "long-term synaptic potentiation" identified in the GO analysis align closely with the previously highlighted KEGG pathways, such as the calcium signaling pathway and neuroactive receptor interaction pathway [47, 48]. Both processes are critical in neuronal activity modulation, synaptic plasticity, and signal transduction, which are believed to be central to migraine pathogenesis.

Prediction of key transcription factors regulating gene program 1

Identifying the key transcription factors regulating gene program 1 is crucial for developing interventions for migraine. In this study, we utilized the transcriptional regulatory network inferred from the Genotype-Tissue Expression (GTEx) project using the passing attributes between networks for data assimilation algorithm [27]. We applied a random forest algorithm, combined with gene weight information from gene program 1, to predict the importance of transcription factors in regulating gene program 1.

The results indicated that ARID3A, SOX3, NR1D1, PLAG1, and NFATC3 were the top five transcription factors in terms of regulatory importance for gene program 1 (Fig. 5AB). Notably, the permutation test revealed that ARID3A had the most statistically significant impact on regulating gene program 1, as reflected by its highest significance score. This finding highlights the pivotal role of ARID3A in modulating the genetic architecture of migraine. We further constructed a network depicting these five transcription factors, KEGG pathways, and the genes enriched in the pathways. The results showed that ARID3A regulated the majority of targets within these pathways (Fig. 5C), suggesting that it may play a critical role in the pathogenesis of migraine.

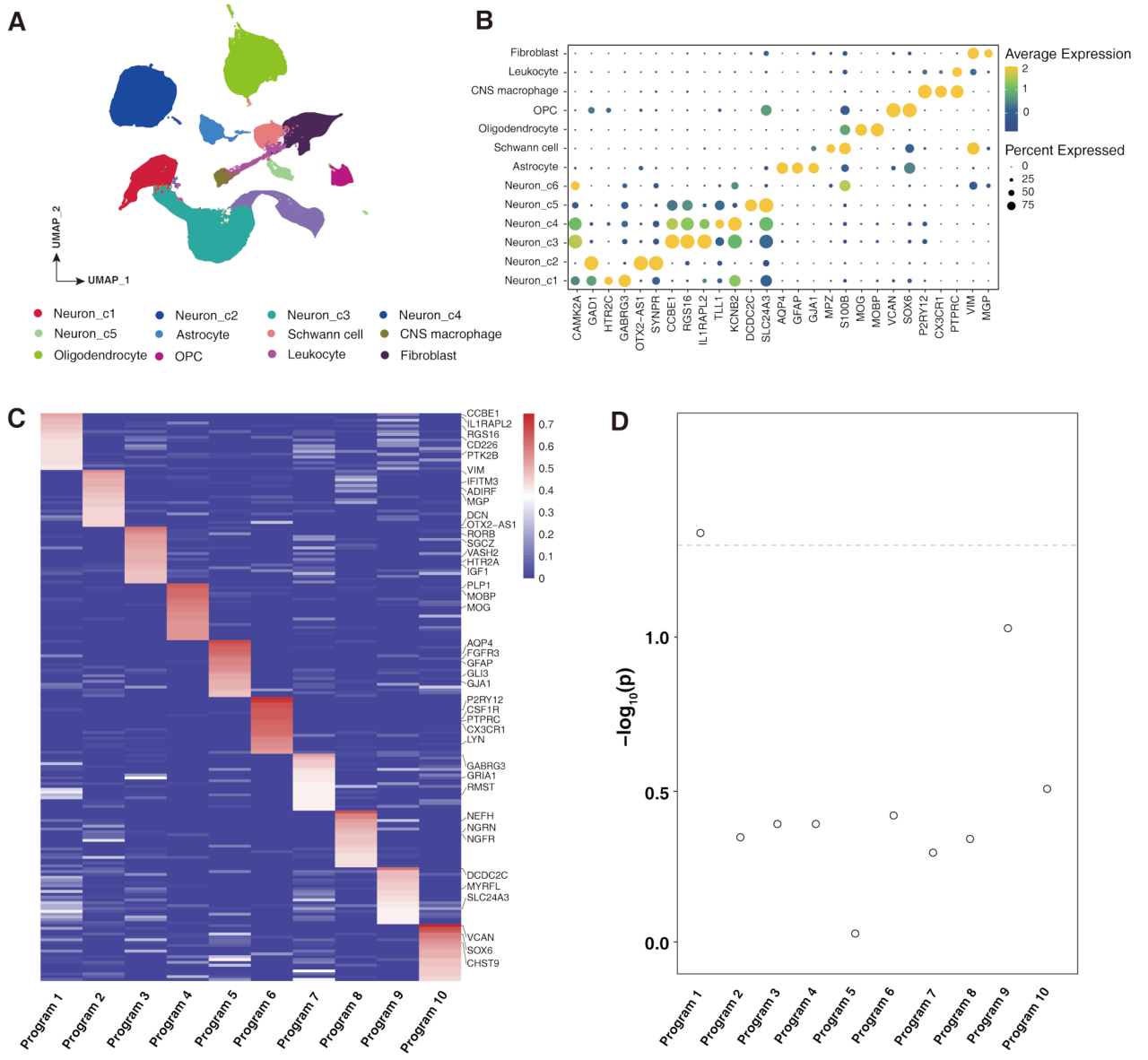

Fig. 3 Cell Nucleus Distribution and Gene Program Analysis in the Thalamus and Trigeminal Ganglion. (**A**) Distribution of nuclei in the thalamus and trigeminal ganglion in Uniform Manifold Approximation and Projection (UMAP) reduced-dimensional space, with the color of the nuclei mapped to different cell types. (**B**) A dot plot showing the expression levels of marker genes for each cell type, where the size of the dots represents the proportion of marker gene expression in different subpopulations, and the color of the dots indicates the expression levels. (**C**) A heatmap displaying the weight levels of representative genes within each gene program, with the right side highlighting key representative genes from each gene program; the colors in the heatmap represent the magnitude of the weights. (**D**) A scatter plot illustrating the enrichment of gene weight annotations within 100 kb for each gene program in migraine-related SNPs, where the x-axis values correspond to -$\log_{10}$(*p* value of S-LDSC), with the dashed line indicating the position of -$\log_{10}$(0.05) on the x-axis

Discussion

In this study, we integrated single-nucleus transcriptomic data, migraine GWAS data, and machine learning techniques to uncover key components of migraine pathogenesis. Our results identified the PoN_MG region of the thalamus as a critical area linked to migraine, with significant enrichment of migraine-associated SNPs in genes specifically expressed in this region. Using NMF and S-LDSC, we also identified gene program 1, which is enriched in migraine-related pathways, including calcium signaling. Moreover, we predicted key transcription factors regulating gene program 1, with ARID3A emerging as the top regulator. This transcription factor controls a broad range of targets within migraine-associated pathways, positioning it as a potential therapeutic target. Taken together, these findings provide new insights

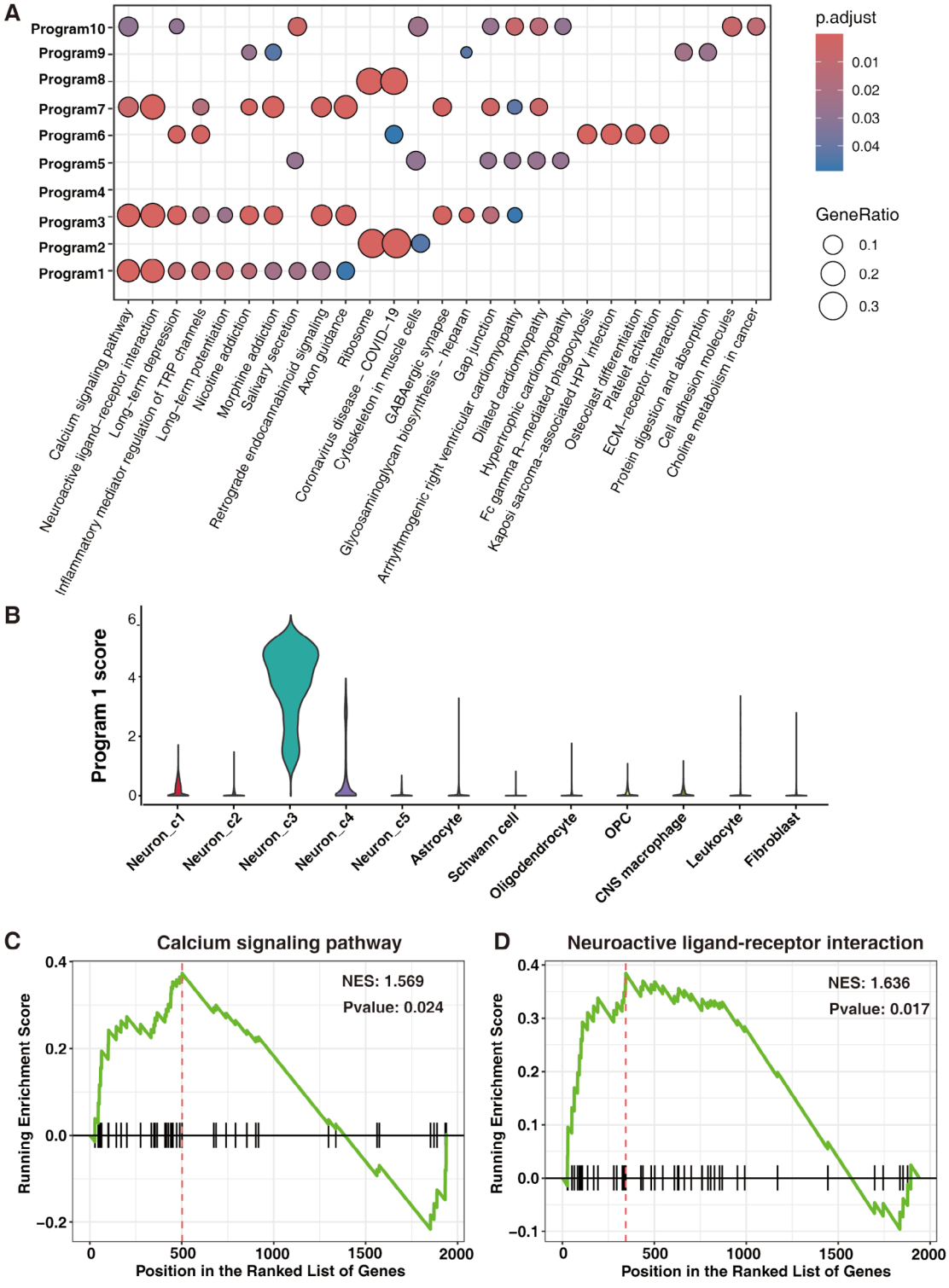

Fig. 4 Enrichment Analysis of Key Gene Programs and Pathways. (**A**) The dot plot displays the enrichment of the top 200 weighted genes from each gene program in KEGG pathways, where the size of the dots represents the gene ratio and the color of the dots indicates the adjusted *p*-value. (**B**) The violin plot illustrates the score values of gene program 1 across different cell types, with the color of the violin corresponding to the cell type. CD. The GSEA results for the Calcium signaling pathway (**C**) and Neuroactive ligand-receptor interaction (**D**) in the Neuron_c3 subpopulation, showing the enrichment score on the y-axis. The vertical black line represents the position of the ranked list of genes. NES. Normalized Enrichment Score

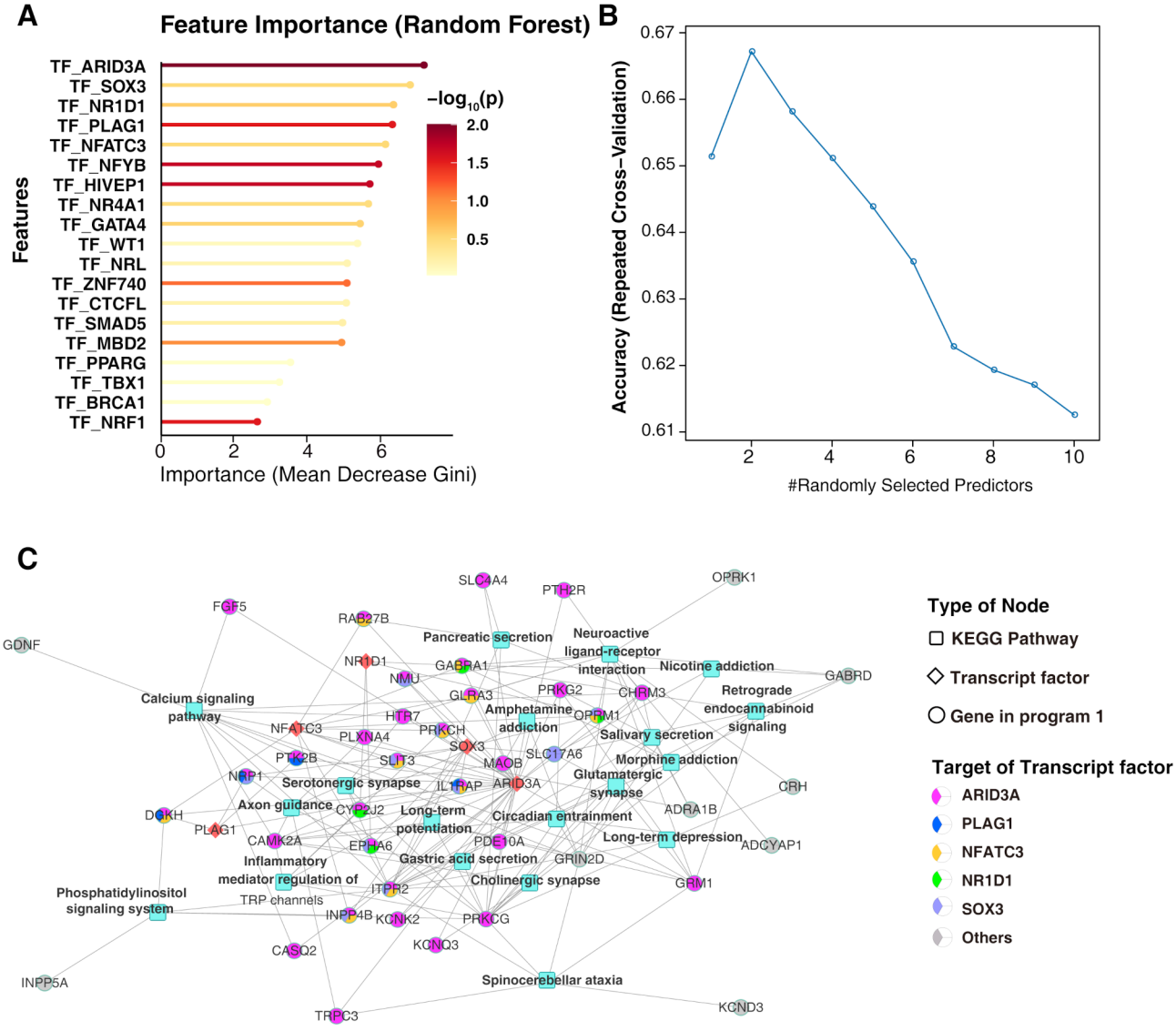

Fig. 5 Regulatory Importance of Transcription Factors in Gene Program 1. **A**. The bar plot displays the regulatory importance of various transcription factors predicted for gene program 1 using the random forest algorithm. The length of each bar represents the feature importance (mean decrease Gini), while the color gradient corresponds to the -log10(*p*-value) obtained from permutation tests, with darker red indicating higher statistical significance. **B**. The line graph represents the accuracy of the random forest model assessed through cross-validation. **C**. The network diagram illustrates the regulatory interactions of the top five transcription factors identified in panel A with the genes of various pathways obtained from KEGG enrichment for gene program 1. In this diagram, the shape of each node corresponds to different categories: rectangles represent KEGG pathways, diamonds represent transcription factors, and circles represent genes within the pathways. Each circular node is color-coded in segments, reflecting the corresponding upstream transcription factors

into the molecular mechanisms underlying migraine, highlighting specific brain regions, gene programs, and transcription factors that may be crucial for developing future therapeutic strategies.

Thalamus in migraine pathophysiology

Within migraine pathophysiology, the thalamus—especially the posterior nuclear complex (PoN), medial geniculate nuclei (MG), ventral posterior lateral nucleus (VPL)—is essential for sensory processing and pain modulation. These structures significantly influence the altered sensory perception observed in migraine patients. As key components of the pain network, they are involved in relaying and regulating nociceptive signals from the peripheral nervous system to higher brain regions [49–52]. The PoN and VPL of the thalamus is a key relay station for nociceptive and non-nociceptive sensory information, linking peripheral pain signals to cortical areas responsible for pain perception, such as the somatosensory cortex and the insula [53–55].

In alignment with these roles, our results provide strong evidence supporting the critical role of the PoN_MG region in migraine susceptibility. As illustrated in Fig. 2C, SNPs annotated to genes specifically expressed in the PoN_MG region of the thalamus are significantly enriched in migraine-associated SNPs, highlighting the importance of this subregion in migraine pathogenesis. Although other thalamic regions did not exhibit significant SNP enrichment, several regions, including LP, LP + VPL, and MD, ranked highly in terms of heritability, indicating their potential role in the onset and progression of migraine. This finding underscores the broader involvement of the thalamus in migraine beyond the PoN_MG region.

The PoN receives direct input from the trigeminocervical complex (TCC), a primary relay for craniofacial nociception, which processes inputs from trigeminal afferents involved in headache pain. This nociceptive information is then transmitted to higher-order cortical areas, contributing to the complex and often debilitating sensory symptoms observed in migraine, including allodynia, photophobia, and phonophobia [56, 57]. The PoN is particularly involved in processing and modulating somatosensory information, and its role in migraine may involve the amplification of pain signals, leading to hypersensitivity to normally innocuous stimuli (allodynia), a common feature in chronic migraine [58]. Additionally, studies have shown that cortical spreading depression (CSD), a key mechanism in migraine aura, can lead to the activation of thalamic structures, including the VPL, suggesting that it contributes to the central processing of headache pain. This activation might modulate the perception of pain intensity and contribute to the development of hyperalgesia or allodynia in chronic conditions [55]. The MG, part of the auditory thalamus, are involved in processing auditory information and are implicated in phonophobia, one of the sensory amplifications commonly associated with migraine [59]. The MG relays auditory information to the auditory cortex but also interacts with other thalamic nuclei and cortical regions that modulate sensory integration and emotional processing. During a migraine attack, there is likely increased excitability in the MG, contributing to the heightened sensitivity to sound that characterizes phonophobia [58, 60]. This heightened sensory responsiveness may be driven by abnormal thalamocortical oscillations and increased network excitability, a hallmark of the migraine brain [61].

From a neural circuit perspective, the thalamus functions as a hub that integrates and modulates sensory inputs from the trigeminovascular system (TGV) [52]. The TGV system is activated by the trigeminal ganglion and its projections to the TCC, which then communicate with the thalamus. The thalamus, particularly the PoN and MG, relays this nociceptive information to cortical areas involved in pain perception, sensory discrimination, and affective responses to pain [52, 56]. Additionally, there is evidence suggesting that thalamic nuclei, including the PoN, undergo maladaptive plasticity in chronic migraine, contributing to persistent changes in sensory processing and pain perception [62, 63]. The plasticity involved may lead to changes in synaptic strength, receptor function, and irregular neuronal firing patterns, all of which can intensify and increase the frequency of migraine attacks. In summary, the posterior nuclear complex of the thalamus and the medial geniculate nuclei play a crucial role in the pain network associated with migraines. They are involved in the disrupted sensory processing typical of migraines, such as pain amplification and increased sensitivity to sensory input. These thalamic nuclei are part of a larger network that integrates both nociceptive and non-nociceptive signals, influencing pain perception and contributing to the chronic and severe nature of migraines through maladaptive plasticity and altered sensory processing mechanisms.

Calcium signaling pathway in migraine pathophysiology

Calcium signaling plays a central role in the pathophysiology of various headache disorders, including migraines, cluster headaches, and neuropathic pain, by regulating neuronal excitability, neurotransmitter release, and synaptic plasticity, which are crucial for pain transmission and modulation [64, 65]. In migraines, dysregulated calcium channels contribute to excessive release of calcitonin gene-related peptide (CGRP), a neuropeptide implicated in the onset and propagation of migraine attacks. Elevated CGRP levels lead to enhanced pain transmission and exacerbation of migraine symptoms. In this study, through NMF and KEGG enrichment analysis, we found that gene program 1 is significantly enriched for migraine-associated SNPs (Fig. 3CD), with its genes showing prominent enrichment in the calcium signaling pathway (Fig. 4A). his suggests a link between calcium dysregulation and migraine pathogenesis, further emphasizing the importance of targeting calcium signaling in therapeutic strategies.

Blocking calcium channels has been shown to reduce CGRP release, providing significant relief from migraine pain. For instance, in chronic migraine models, inhibiting calcium signaling in the insular cortex was shown to lower CGRP levels and alleviate both pain and associated anxiety behaviors [65]. Similarly, in cluster headaches, calcium signaling is involved through calcium-activated ion channels like Anoctamin 3 (ANO3), which have been genetically linked to the condition. Abnormal calcium ion influx via these channels likely contributes to the intense pain experienced during cluster headache attacks [66]. Moreover, calcium channel blockers like verapamil are commonly used as prophylactic treatments for cluster

headaches, demonstrating their effectiveness in preventing attacks by reducing neuronal excitability through the inhibition of calcium influx [66].

Calcium signaling also plays a significant role in neuropathic pain, where calcium-permeable ion channels, including voltage-gated calcium channels (VGCCs) and transient receptor potential (TRP) channels, are key drivers of persistent pain and heightened sensitivity [67]. Beyond its immediate effects, calcium influences long-term neuronal changes that underlie chronic pain, particularly through NMDA receptors, which mediate central sensitization—a mechanism by which acute pain transitions to chronic pain [68]. Upon activation by glutamate, calcium influx through NMDA receptors triggers intracellular signaling cascades that enhance synaptic plasticity, increasing pain sensitivity [69]. This prolonged activation of calcium-dependent pathways is crucial in conditions like chronic migraine and neuropathic pain, where persistent sensitization leads to ongoing pain.

At the synaptic level, calcium signaling also modulates neurotransmitter release, influencing pain transmission. Voltage-gated calcium channels at presynaptic terminals control the release of neurotransmitters, and inhibiting calcium influx can reduce neurotransmitter release, thereby dampening the transmission of pain signals to higher brain regions [69]. This modulation plays an essential role in current pain therapies aimed at controlling abnormal pain processing without affecting normal sensory function. In conclusion, calcium channel blockers and other treatments that modulate calcium signaling have shown promise in mitigating pain symptoms, though further research is necessary to fully understand its role in pain modulation and to develop more effective treatments for chronic pain conditions.

The role of ARID3A in migraine

ARID3A is a transcription factor known for its role in regulating immune responses and cellular differentiation. While its involvement in various diseases has been reported, its role in the nervous system and pain pathways remains less understood. In this study, ARID3A was predicted to be the top regulator of gene program 1 (Fig. 5), which is significantly enriched in the migraine-associated calcium signaling pathway. Previous research has shown that ARID3A can block calcium flux, suggesting that it may influence migraine pathogenesis by modulating key genes involved in calcium signaling pathways [70]. ARID3A represents a potential regulator in migraine pathogenesis through its influence on calcium signaling pathways; however, given its broad range of functions, further studies are necessary to elucidate its specific role in migraine and to evaluate its potential as a therapeutic target.

Limitations

Despite these promising findings, several limitations must be acknowledged. First, the GWAS dataset used in this study included a relatively small number of migraine cases, which may limit the ability to detect genetic variants with smaller effects. Expanding the sample size in future GWAS studies will enhance statistical power and help uncover additional loci associated with migraine. Second, while our integrative approach identified key molecular players, validation using biological specimens from migraine patients, such as brain tissues, cerebrospinal fluid, or blood samples, is needed to confirm the clinical relevance of these findings. Third, the functional role of key molecules like ARID3A remains speculative and requires further experimental validation. This includes designing targeted in vitro and in vivo experiments to elucidate their precise functions in migraine-related pathways, particularly in relation to calcium signaling. Addressing these limitations in future studies will strengthen the evidence base and facilitate the translation of these findings into therapeutic strategies.

Overall, our study provides novel insights into the molecular underpinnings of migraine, identifying key regions, gene programs, and transcription factors involved in its pathogenesis. By leveraging single-nucleus transcriptomic data, GWAS data, and machine learning, we offer a comprehensive framework for understanding migraine susceptibility. However, further research is warranted to explore the functional roles of the identified genes and transcription factors. Additionally, the interplay between neuroinflammation and calcium signaling in migraine pathogenesis should be investigated in greater detail to fully elucidate the underlying mechanisms and identify viable therapeutic strategies.

Supplementary Information

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Supplementary Material 1

Author contributions

GG and WW conceived and designed the study. LZ and YL were responsible for data collection. LZ and YX performed the data analyses. GG and LZ interpreted the data analysis results. GG and WW drafted the manuscript. All authors read, reviewed, and approved the final version of the manuscript.

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Data availability

Data is provided within the manuscript or supplementary information files. The reproducible code has been uploaded to the Zenodo database and is available under https://doi.org/10.5281/zenodo.1389499530.

Declarations

Ethics approval and consent to participate
Not applicable.

Informed consent
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Competing interests
The authors declare no competing interests.

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References

1. GBD 2019 Diseases and Injuries Collaborators (2020) Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet (London, England) 396(10258):1204–1222
2. Headache Classification Committee of the International Headache Society (IHS) (2018) The International Classification of Headache Disorders, 3rd edition. Cephalalgia: an International Journal of Headache 38(1):1–211
3. Steiner TJ, Stovner LJ, Jensen R, Uluduz D, Katsarava Z (2020) Migraine remains second among the world's causes of disability, and first among young women: findings from GBD2019. J Headache Pain 21(1):137
4. Ornello R, Andreou AP, De Matteis E, Jürgens TP, Minen MT, Sacco S (2024) Resistant and refractory migraine: clinical presentation, pathophysiology, and management. EBioMedicine 99:104943
5. Schulte LH, May A (2016) The migraine generator revisited: continuous scanning of the migraine cycle over 30 days and three spontaneous attacks. Brain 139(Pt 7):1987–1993
6. Messina R, Rocca MA, Goadsby PJ, Filippi M (2023) Insights into migraine attacks from neuroimaging. Lancet Neurol 22(9):834–846
7. Sutherland HG, Griffiths LR (2017) Genetics of migraine: insights into the molecular basis of migraine disorders. Headache 57(4):537–569
8. Yang L, Xu M, Bhuiyan SA, Li J, Zhao J, Cohrs RJ, Susterich JT, Signorelli S, Green U, Stone JR et al (2022) Human and mouse trigeminal ganglia cell atlas implicates multiple cell types in migraine. Neuron 110(11):1806–1821e1808
9. Hershey A, Horn P, Kabbouche M, O'Brien H, Powers S (2012) Genomic expression patterns in menstrual-related migraine in adolescents. Headache 52(1):68–79
10. Anttila V, Winsvold BS, Gormley P, Kurth T, Bettella F, McMahon G, Kallela M, Malik R, de Vries B, Terwindt G et al (2013) Genome-wide meta-analysis identifies new susceptibility loci for migraine. Nat Genet 45(8):912–917
11. Hautakangas H, Winsvold BS, Ruotsalainen SE, Bjornsdottir G, Harder AVE, Kogelman LJA, Thomas LF, Noordam R, Benner C, Gormley P et al (2022) Genome-wide analysis of 102,084 migraine cases identifies 123 risk loci and subtype-specific risk alleles. Nat Genet 54(2):152–160
12. Gormley P, Anttila V, Winsvold BS, Palta P, Esko T, Pers TH, Farh KH, Cuenca-Leon E, Muona M, Furlotte NA et al (2016) Meta-analysis of 375,000 individuals identifies 38 susceptibility loci for migraine. Nat Genet 48(8):856–866
13. Choquet H, Yin J, Jacobson AS, Horton BH, Hoffmann TJ, Jorgenson E, Avins AL, Pressman AR (2021) New and sex-specific migraine susceptibility loci identified from a multiethnic genome-wide meta-analysis. Commun Biology 4(1):864
14. Sutherland HG, Albury CL, Griffiths LR (2019) Advances in genetics of migraine. J Headache Pain 20(1):72
15. Ashina M, Terwindt GM, Al-Karagholi MA, de Boer I, Lee MJ, Hay DL, Schulte LH, Hadjikhani N, Sinclair AJ, Ashina H et al (2021) Migraine: disease characterisation, biomarkers, and precision medicine. Lancet (London England) 397(10283):1496–1504
16. Juhasz G, Gecse K, Baksa D (2023) Towards precision medicine in migraine: recent therapeutic advances and potential biomarkers to understand heterogeneity and treatment response. Pharmacol Ther 250:108523
17. Ho TW, Connor KM, Zhang Y, Pearlman E, Koppenhaver J, Fan X, Lines C, Edvinsson L, Goadsby PJ, Michelson D (2014) Randomized controlled trial of the CGRP receptor antagonist telcagepant for migraine prevention. Neurology 83(11):958–966
18. Gasparini CF, Smith RA, Griffiths LR (2017) Genetic and biochemical changes of the serotonergic system in migraine pathobiology. J Headache Pain 18(1):20
19. Vgontzas A, Renthal W (2020) Migraine-associated gene expression in cell types of the central and peripheral nervous system. Cephalalgia: Int J Headache 40(5):517–523
20. Rasmussen AH, Kogelman LJA, Kristensen DM, Chalmer MA, Olesen J, Hansen TF (2020) Functional gene networks reveal distinct mechanisms segregating in migraine families. Brain 143(10):2945–2956
21. Eising E, Huisman SMH, Mahfouz A, Vijfhuizen LS, Anttila V, Winsvold BS, Kurth T, Ikram MA, Freilinger T, Kaprio J et al (2016) Gene co-expression analysis identifies brain regions and cell types involved in migraine pathophysiology: a GWAS-based study using the Allen Human Brain Atlas. Hum Genet 135(4):425–439
22. Jagadeesh KA, Dey KK, Montoro DT, Mohan R, Gazal S, Engreitz JM, Xavier RJ, Price AL, Regev A (2022) Identifying disease-critical cell types and cellular processes by integrating single-cell RNA-sequencing and human genetics. Nat Genet 54(10):1479–1492
23. Siletti K, Hodge R, Mossi Albiach A, Lee KW, Ding SL, Hu L, Lönnerberg P, Bakken T, Casper T, Clark M et al (2023) Transcriptomic diversity of cell types across the adult human brain. Sci (New York NY) 382(6667):eadd7046
24. Vitale M, Tottene A, Zarin Zadeh M, Brennan KC, Pietrobon D (2023) Mechanisms of initiation of cortical spreading depression. J Headache Pain 24(1):105
25. Karatas H, Erdener SE, Gursoy-Ozdemir Y, Lule S, Eren-Koçak E, Sen ZD, Dalkara T (2013) Spreading depression triggers headache by activating neuronal Panx1 channels. Sci (New York NY) 339(6123):1092–1095
26. Cropper HC, Conway CM, Wyche W, Pradhan AA (2024) Glial activation in pain and emotional processing regions in the nitroglycerin mouse model of chronic migraine. Headache 64(8):973–982
27. Sonawane AR, Platig J, Fagny M, Chen CY, Paulson JN, Lopes-Ramos CM, DeMeo DL, Quackenbush J, Glass K, Kuijjer ML (2017) Understanding tissue-specific gene regulation. Cell Rep 21(4):1077–1088
28. Stuart T, Butler A, Hoffman P, Hafemeister C, Papalexi E, Mauck WM 3rd, Hao Y, Stoeckius M, Smibert P, Satija R (2019) Comprehensive integration of single-cell data. Cell 177(7):1888–1902e1821
29. Timshel PN, Thompson JJ, Pers TH (2020) Genetic mapping of etiologic brain cell types for obesity. eLife 9
30. Zhang L, Guo GY (2024) Identify of Critical Gene Programs and Key Transcription Factors in Migraine Using Machine Learning. *Zenodo*
31. Gaujoux R, Seoighe C (2010) A flexible R package for nonnegative matrix factorization. BMC Bioinformatics 11:367
32. Fulco CP, Nasser J, Jones TR, Munson G, Bergman DT, Subramanian V, Grossman SR, Anyoha R, Doughty BR, Patwardhan TA et al (2019) Activity-by-contact model of enhancer-promoter regulation from thousands of CRISPR perturbations. Nat Genet 51(12):1664–1669
33. Gusev A, Lee SH, Trynka G, Finucane H, Vilhjálmsson BJ, Xu H, Zang C, Ripke S, Bulik-Sullivan B, Stahl E et al (2014) Partitioning heritability of regulatory and cell-type-specific variants across 11 common diseases. Am J Hum Genet 95(5):535–552
34. Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, Kang HM, Marth GT, McVean GA (2012) An integrated map of genetic variation from 1,092 human genomes. Nature 491(7422):56–65
35. Altshuler DM, Gibbs RA, Peltonen L, Altshuler DM, Gibbs RA, Peltonen L, Dermitzakis E, Schaffner SF, Yu F, Peltonen L et al (2010) Integrating common and rare genetic variation in diverse human populations. Nature 467(7311):52–58
36. Finucane HK, Bulik-Sullivan B, Gusev A, Trynka G, Reshef Y, Loh PR, Anttila V, Xu H, Zang C, Farh K et al (2015) Partitioning heritability by functional annotation using genome-wide association summary statistics. Nat Genet 47(11):1228–1235
37. Kanehisa M, Goto S (2000) KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res 28(1):27–30

38. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT et al (2000) Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet 25(1):25–29
39. Li J, Malley JD, Andrew AS, Karagas MR, Moore JH (2016) Detecting gene-gene interactions using a permutation-based random forest method. BioData Min 9:14
40. Kobak D, Berens P (2019) The art of using t-SNE for single-cell transcriptomics. Nat Commun 10(1):5416
41. Peters VA, Joesting JJ, Freund GG (2013) IL-1 receptor 2 (IL-1R2) and its role in immune regulation. Brain Behav Immun 32:1–8
42. Erdener ŞE, Kaya Z, Dalkara T (2021) Parenchymal neuroinflammatory signaling and dural neurogenic inflammation in migraine. J Headache Pain 22(1):138
43. O'Brien JB, Wilkinson JC, Roman DL (2019) Regulator of G-protein signaling (RGS) proteins as drug targets: progress and future potentials. J Biol Chem 294(49):18571–18585
44. Hamel E (2007) Serotonin and migraine: biology and clinical implications. Cephalalgia: Int J Headache 27(11):1293–1300
45. Kowalska M, Prendecki M, Piekut T, Kozubski W, Dorszewska J (2021) Migraine: Calcium channels and Glia. Int J Mol Sci 22(5)
46. Thanh CD, Men CV, Kim HM, Kang JS (2022) Network pharmacology-based investigation on therapeutic mechanisms of the angelica dahurica radix and ligusticum chuanxiong rhizoma herb pair for anti-migraine effect. Plants (Basel, Switzerland) 11(17)
47. Berridge MJ (2012) Calcium signalling remodelling and disease. Biochem Soc Trans 40(2):297–309
48. Kandel ER, Dudai Y, Mayford MR (2014) The molecular and systems biology of memory. Cell 157(1):163–186
49. Blomqvist A, Zhang ET, Craig AD (2000) Cytoarchitectonic and immunohistochemical characterization of a specific pain and temperature relay, the posterior portion of the ventral medial nucleus, in the human thalamus. Brain: a journal of neurology 123 Pt 3:601–619
50. Sgourdou P, Schaffler M, Choi K, McCall NM, Burdge J, Williams J, Corder G, Fuccillo MV, Abdus-Saboor I, Epstein DJ (2024) Impaired pain in mice lacking first-order posterior medial thalamic neurons. Pain 166(1):130–143. https://doi.org/10.1097/j.pain.0000000000003325
51. Wang W, Yuan Z, Zhang X, Bai X, Tang H, Mei Y, Qiu D, Zhang Y, Zhang P, Zhang X et al (2023) Mapping the aberrant brain functional connectivity in new daily persistent headache: a resting-state functional magnetic resonance imaging study. J Headache Pain 24(1):46
52. Vila-Pueyo M, Hoffmann J, Romero-Reyes M, Akerman S (2019) Brain structure and function related to headache: brainstem structure and function in headache. Cephalalgia: Int J Headache 39(13):1635–1660
53. Ab Aziz CB, Ahmad AH (2006) The role of the thalamus in modulating pain. Malaysian J Med Sciences: MJMS 13(2):11–18
54. Park A, Uddin O, Li Y, Masri R, Keller A (2018) Pain after spinal cord injury is associated with abnormal presynaptic inhibition in the posterior nucleus of the thalamus. J pain 19(7):727e721–727e715
55. Fu X, Chen M, Lu J, Li P (2022) Cortical spreading depression induces propagating activation of the thalamus ventral posteromedial nucleus in awake mice. J Headache Pain 23(1):15
56. Brennan KC, Pietrobon D (2018) A systems neuroscience approach to migraine. Neuron 97(5):1004–1021
57. Hoffmann J, Baca SM, Akerman S (2019) Neurovascular mechanisms of migraine and cluster headache. J Cereb Blood flow Metabolism: Official J Int Soc Cereb Blood Flow Metabolism 39(4):573–594
58. Goadsby PJ, Holland PR, Martins-Oliveira M, Hoffmann J, Schankin C, Akerman S (2017) Pathophysiology of migraine: a disorder of sensory processing. Physiol Rev 97(2):553–622
59. Winer JA (1984) The human medial geniculate body. Hear Res 15(3):225–247
60. Kuner R, Kuner T (2021) Cellular circuits in the brain and their modulation in acute and chronic pain. Physiol Rev 101(1):213–258
61. Hodkinson DJ, Wilcox SL, Veggeberg R, Noseda R, Burstein R, Borsook D, Becerra L (2016) Increased amplitude of thalamocortical low-frequency oscillations in patients with migraine. J Neuroscience: Official J Soc Neurosci 36(30):8026–8036
62. Liang SH, Zhao WJ, Yin JB, Chen YB, Li JN, Feng B, Lu YC, Wang J, Dong YL, Li YQ (2020) A neural circuit from thalamic paraventricular nucleus to central amygdala for the facilitation of neuropathic pain. J Neuroscience: Official J Soc Neurosci 40(41):7837–7854
63. Wang H, Dong P, He C, Feng XY, Huang Y, Yang WW, Gao HJ, Shen XF, Lin S, Cao SX et al (2020) Incerta-thalamic circuit controls nocifensive behavior via cannabinoid type 1 receptors. Neuron 107(3):538–551e537
64. Al-Khazali HM, Christensen RH, Chaudhry BA, Melchior AG, Ashina M, Burstein R, Ashina H (2024) Effects of PDE-3 inhibition in persistent post-traumatic headache: evidence of cAMP-dependent signaling. J Headache Pain 25(1):56
65. Li Y, Li C, Chen QY, Hao S, Mao J, Zhang W, Han X, Dong Z, Liu R, Tang W et al (2024) Alleviation of migraine related pain and anxiety by inhibiting calcium-stimulating AC1-dependent CGRP in the insula of adult rats. J Headache Pain 25(1):81
66. Ran C, Fourier C, Arafa D, Liesecke F, Sjöstrand C, Waldenlind E, Steinberg A, Belin AC (2019) Anoctamin 3: a possible link between cluster headache and ca(2+) signaling. Brain Sci 9(8)
67. Chen YY, Feng LM, Xu DQ, Yue SJ, Fu RJ, Zhang MM, Tang YP (2022) Combination of paeoniflorin and liquiritin alleviates neuropathic pain by lipid metabolism and calcium signaling coordination. Front Pharmacol 13:944386
68. Chen P, Lin D, Wang C, Song C, Wang W, Qu J, Wu Z (2021) Proteomic analysis of emodin treatment in neuropathic pain reveals dysfunction of the calcium signaling pathway. J pain Res 14:613–622
69. Bourinet E, Altier C, Hildebrand ME, Trang T, Salter MW, Zamponi GW (2014) Calcium-permeable ion channels in pain signaling. Physiol Rev 94(1):81–140
70. Guo CC, Xu HE, Ma X (2023) ARID3a from the ARID family: structure, role in autoimmune diseases and drug discovery. Acta Pharmacol Sin 44(11):2139–2150

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