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Calcium Signaling Related Genes in Migraine

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ABSTRACT

Calcium is essential for the growth of neurons during infancy as well as transmission of signals between neurons; thus, the related signaling pathways might contribute to the pathogenesis of brain disorders. Voltage-gated calcium channels are widely expressed in the trigeminovascular system and are regarded as important contributors to some forms of familial migraines. In fact, these channels participate in the migraine initiation events. We compared the expression of genes coding for ion channels, including *SLC1A1*, *SLC25A12*, *ATP2B2*, and their associated lncRNAs, namely *LINC01231*, *lnc-SLC25A12*, and *lnc-MTR-1*, between migraineurs and healthy controls. All mentioned genes and lncRNAs, except for *ATP2B2* and *LINC01231*, were shown to be up-regulated in total migraineurs compared with controls. Moreover, the expression levels of ATP2B2 were higher in patients with aura compared with those without aura (Expression ratio (95% CI) = 5.83 (2.93–11.59), $p < 0.0001$). Yet, SLC25A12 was down-regulated in patients with aura as compared with those without aura (Expression ratio (95% CI) = 0.21 (0.11–0.42), $p = 0.004$). Notably, we demonstrated high specificity and sensitivity values for some genes in the differentiation of migraineurs from healthy controls. Taken together, we demonstrated significant over-expression of a number of ion channel and transporter genes and their related lncRNAs in migraineurs and suggested these genes as potential markers for this neurological condition.

1 | Introduction

Headache is one of the most common problems faced by clinicians. This disabling and chronic neurovascular headache disorder is estimated to affect about 20% of the population all around the world ("Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015," 2016; Vos et al. 2012). Migraine is a common and complicated brain disorder. It is thought that this disease starts when groups of nerve cells are overactive and send multiple signals to the trigeminal nerve. In addition, brain stem and diencephalic structures are involved in this disorder (Charles 2018). In general, migraine is defined with self-limited severe headaches that are associated with symptoms of the autonomous nervous system. The strength of the pain, the duration of the headache and the frequency of the attacks are different in different people (Neuhauser et al. 2006). More than a third of people with migraine headache experience auras, or premonitory symptoms: a transient visual, sensory, language, or movement disturbance that indicates a headache is about to start (Hui et al. 2023).

Abbreviations: ATP2B2, ATPase Plasma Membrane Ca^{2+} Transporting 2; cDNA, complementary DNA; CGPR, calcitonin gene-related peptide; lncRNA, long noncoding RNA; PCR, Polymerase chain reaction; ROC, Receiver Operating Characteristic; SLC1A1, solute carrier family 1 member 1; SLC25A12, solute carrier family 25 member 12.

Gene expression analyses have been suggested as a tool for obtaining insight into the functional processes contributing to the pathoetiology of migraine (Z. Gerring et al. 2016). Most notably, peripheral blood has been proved to be a biologically effective source for genetic investigations in the field of migraine, particularly with the aim of identification of biomarkers and therapeutic pathways (Ebahimzadeh et al. 2021; Taheri et al. 2024). Pilot blood-based gene expression analyses have revealed different expression profiles between migraine patients and non-migraine controls (Z. Gerring et al. 2016). Another study in this field has shown enrichment of differentially expressed genes in the immune-inflammatory pathways, particularly those expressed in microglial cells. Thus, immune-related pathways contribute to the pathogenesis, clinical features, and evolution of migraine in a number of patients (Z. F. Gerring et al. 2018). A more recent transcriptomic study in the blood samples of migraineurs has identified several disease-specific mediators and pathways in this disorder (Aczél et al. 2021).

Calcium is essential for growth of neurons during infancy as well as transmission of signals between neurons (Congar et al. 1997). Infirmity in transmission of calcium in neurons may have serious adverse effects that cause disease with neurodevelopmental basis. These infirmities may be developed via some mutations or epigenetics elements that affect expression of proteins or non-coding RNAs that have function in calcium signaling (Berridge 2013). Therefore, fluctuation in the expression of these genes may impair calcium homeostasis or signaling which increases risk of diseases such as schizophrenia, autism, and migraine through affecting normal functions of neurons (Baker et al. 2023). Calcium signaling has a pivotal role in the pathogenesis of migraine. Voltage-gated calcium channels have a wide expression in the trigeminovascular system and are regarded as important contributors to some forms of familial migraines. In fact, these channels participate in the migraine initiation events (Chichorro et al. 2024). *SLC25A12* and *ATP2B2* genes code calcium signaling pathway proteins that have a role in storage, regulation, and carriage of calcium in neuron cells. Meanwhile, *lnc-SLC25A12–8:1* and *lnc-MTR-1:1 T* genes encode long non-coding RNAs (lncRNAs) that possibly regulate expression of these genes, as predicted by the LNCipedia (Volders et al. 2013) and RNAcentral ("RNAcentral 2021: secondary structure integration, improved sequence search and new member databases," 2021). *SLC1A1* encodes a glutamate transporter. It is possibly regulated by *LINC01231:1* as predicted by LNCipedia (Volders et al. 2013). It has a role in the homeostatic or cell metabolism (Holmseth et al. 2012) and may indirectly affect calcium levels through effects on glutamate mediated neurotransmission. Based on the importance of glutamatergic system in migraine (Gasparini and Griffiths 2013), we also included this gene and its related lncRNA in the study. SLC25A12 has a role in the process of myelination and neurofilaments (Sakurai et al. 2010). ATP2B2 has a major role in the regulation of calcium signaling and modulation of short-term plasticity in Purkinje cells (A. C. Burette et al. 2010; Empson et al. 2007). However, the functions of lncRNAs that are related with these genes are not fully clarified. These genes have also been suggested to partake in the pathogenesis of autism spectrum disorder (Pourtavakoli et al. 2024) and epilepsy (Taheri et al. 2023). In the present study, we compared expression of these genes between migraineurs and healthy controls.

TABLE 1 | Primer characteristics.

Gene	Transcript type	Locus	Forward primer	Reverse primer	Length of amplicon	Tm (°C)
lnc-MTR-1:1	lncRNA	chr1:236907044–236916931	AGCCTGATGAACCAGTGTGCT	TCCAGCAATCTGCCTCTTTCCA	156	63
ATP2B2	Coding	3p25.3	GCGAGGGCAACGAAGGATGT	CCGTGACCAGGACCACACAGA	123	62
SLC1A1	Coding	9p24.2	CGGCGAGGAAAGGATGCGA	AGAGTTGAGAGGTTGCTGTGTTCT	130	63
SLC25A12	Coding	2q31.1	GCGGTCAAGGTGCAGACAACTA	AACGCTCTCCATCAACCTCAGTA	*94*	63
LINC01231	lncRNA	chr9:3181589–3200500	TTCTGGAGGAAAGGGAAGAGATT	GGAGCCCAAGCACAGGTT	137	60
lnc-SLC25A12–8:1	lncRNA	chr2:171855927–171999859	CAGGTGGGATGGAAGAAGCC	TACTGAGAATGAACTTGGGCAG	80	58

2 | Methods

2.1 | Migraineurs and Controls

The current study was not a pre-registered study. No sample size calculation was performed. Fifty migraineurs with aura (9 males and 41 females) and 49 migraineurs without aura (8 males and 41 females) were considered as the case group. In addition, 71 age-matched healthy individuals were enrolled as the normal control group. Controls were selected from those referred for routine health checkup. The exclusion criteria were a history of systemic or neurodegenerative disorders or chronic headache in themselves or their family. Blood samples were gathered from all cases at the same time during the day, and when the patients were headache-free for at least 24 h. The ethical committee of Shahid Beheshti University of Medical Sciences permitted the study procedure (IR.SBMU.MSP.REC.1402.638). All individuals signed informed written consent forms.

2.2 | Experiments

Five milliliters of total blood were collected in EDTA-containing tubes, snap frozen in liquid nitrogen, and stored at −70°C. RNA was extracted using the High Pure Blood RNA extraction kit (Producer: Kiagene fanavar, catalog number: FPKT032.0100). The purity and yield of RNA were confirmed using the spectrophotometer (Compact Visible Spectrophotometer V1200, Producer: Shanghai Yoke Instrument Co., catalog number: 9027300000). cDNA was synthesized from the extracted total RNA using the SOLIScript RT cDNA synthesis KIT (Producer: SMOBIO Technology Inc., catalog number: 06–35-00050). Expression of *SLC1A1*, *SLC25A12*, *ATP2B2*, *LINC01231:1*, *lnc-SLC25A12–8:1*and *lnc-MTR-1:1 T* genes was measured by StepOne Real-Time PCR Systems from Thermo Scientific (Catalog number: 4376592). Melting curve analyses were done to confirm the specificity of primers. The *B2M* gene was used as a normalizer housekeeping gene. Each PCR run included a negative control sample comprising all PCR reagents except for the cDNA template. All primers were designed in a way that the amplicon covers at least one exon-intron boundary to avoid amplification of possible DNA contamination. In the cases of *ATP2B2* and *SLC25A12*, only transcript variant 1 was amplified by the designed primers. Table 1 shows primers.

Real-time PCR reactions were performed over 40 amplification cycles. Reaction-specific primer efficiencies were determined using LinRegPCR software, which assesses efficiency by analyzing the exponential phase of each amplification curve. Final Ct values were computed by incorporating these primer efficiencies, adjusting for amplification variability.

Samples with undetected target genes (Ct > 40) were assigned a conservative Ct value of 41 (40 cycles +1) to ensure statistical inclusion. Four samples in the migraine without aura group exhibiting outlier *B2M* Ct values (> 99th percentile of the dataset) were excluded from all analyses to mitigate bias. Relative gene expression was quantified using the −ΔCt method, defined as the difference between efficiency-adjusted Ct values of the internal control (*B2M*) and target genes. −ΔCt values were employed

TABLE 2 | Characteristics of cases and controls.

Study groups	Parameters		Values	
Patients	Sex (number)	Males	Aura	9
			Without Aura	8
		Females	Aura	41
			Without Aura	42
	Age (Years, mean ± SD)	Males	Aura	33.28 ± 15.21
			Without Aura	31.83 ± 13.46
		Females	Aura	38.34 ± 10.76
			Without Aura	36.05 ± 11.89
	Age at onset (Years, mean ± SD)	Males	Aura	23.16 ± 11.49
			Without Aura	19.66 ± 6.77
		Females	Aura	26.55 ± 10.14
			Without Aura	27.94 ± 8.71
	Positive family history (number)	Males	Aura	4
			Without Aura	1
		Females	Aura	24
			Without Aura	16
Controls	Sex (number)	Males	41	
		Females	30	

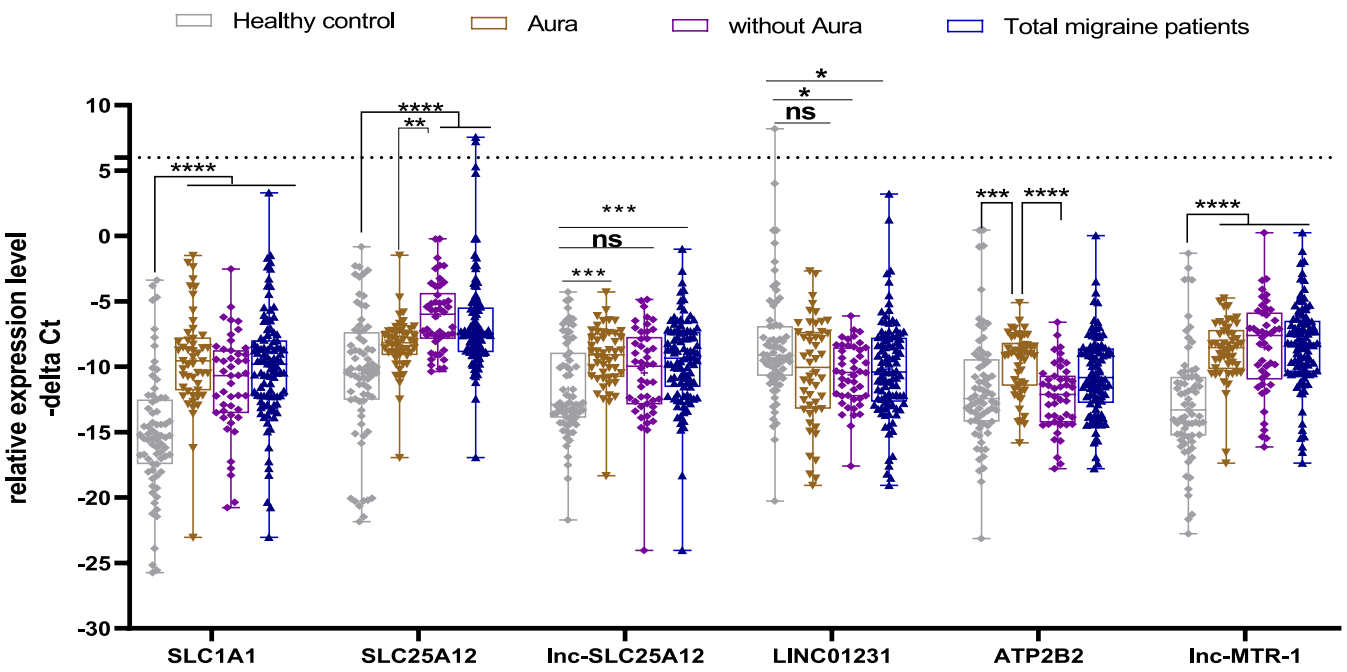

FIGURE 1 | Expression levels of SLC1A1, SLC25A12, ATP2B2, and their related lncRNAs in total migraine patients ($n = 96$), patients with aura ($n = 50$), patients without aura ($n = 46$) and controls ($n = 71$) as measured by –delta Ct values. Kruskal-Wallis test was used to identify differentially expressed genes between study subgroups. Dunn's test was used for multiple comparisons, and adjusted p value was reported (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ and ns; non-significant).

TABLE 3 | Assessment of the effect of Group and Gender (Tests of Between-Subjects Effects) on expression of genes in total migraine patients compared to controls.

	Group effect				Gender effect				Interactions			
Source of Variation	**SS[a]**	***F*[b]**	**DF[css]**	***p***	**SS**	***F***	**DF**	***p***	**SS**	***F***	**DF**	***p***
SLC1A1	801.4	43.86	1	**< 0.0001**	0.16	0.008	1	0.92	10.43	0.57	1	0.45
SLC25A12	310.9	22.08	1	**< 0.0001**	34.75	2.46	1	0.11	25.22	1.79	1	0.18
lnc-SLC25A12	105.1	9.98	1	**0.002**	42.35	4.02	1	**0.04**	10.28	0.97	1	0.32
LINC01231	173.5	12.33	1	**0.0006**	2.75	0.19	1	0.65	0.88	0.06	1	0.8
ATP2B2	27.51	2.22	1	0.13	1.88	0.15	1	0.69	13.58	1.1	1	0.29
lnc-MTR-1	560.3	40.65	1	**< 0.0001**	66.59	4.83	1	**0.03**	4.67	0.33	1	0.56

Note: Two-way ANOVA was used to investigate the effect of main factors and their interactions on gene expression (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ and ****$p < 0.0001$). Bold values represent the statistically significant results.
[a]Sum of squares.
[b]F of variance.
[c]Degrees of freedom.

for statistical analyses, with higher −ΔCt values indicating greater target abundance.

3 | Statistical Methods

Statistical analysis was performed in GraphPad Prism 9.0 (La Jolla, CA, USA). Expression of *SLC1A1*, *SLC25A12*, *ATP2B2*, and their related lncRNAs was measured in the blood samples of migraineurs (50 patients with aura and 49 patients without aura) and 71 healthy subjects. Expression level of each gene was computed using the comparative –delta Ct strategy. Distribution of the values was evaluated using the Shapiro–Wilk test. Since the data was not normally distributed, the Kruskal-Wallis test was used to detect differentially expressed genes between subgroups of patients and healthy control groups. Dunn's post hoc test was used for multiple comparisons, with adjusted p-values reported.

Effect of other factors on expressions was judged using the two-way ANOVA and Tukey post hoc tests. We applied a multiple-test correction to our significance levels. In detail, we used the Tukey test to adjust for false discovery rates across the independent tests conducted. Correlation between expression levels of genes was detected using the Spearman's method.

TABLE 4 | Expression of SLC1A1, SLC25A12, ATP2B2, and their related lncRNAs in the blood of patients with migraine diseases (total) and their subgroups compared with healthy controls.

Genes		Total migraine patients versus healthy controls (96 vs. 71)	migraine patients with aura versus healthy controls (50 vs. 71)	Migraine patients without aura versus healthy controls (46 vs. 71)	Migraine patients with aura versus migraine patients without aura (50 vs. 46)
SLC1A1	Expression ratios (95% CIs)	28.07 (11.3–69.7)	52.7 (17.7–158.5)	14.07 (4.52–43.74)	3.76 (1.29–10.94)
	Adjusted *p* values	**< 0.0001**	**< 0.0001**	**< 0.0001**	0.18
	H statistic	**63.76**			
	df	**3**			
SLC25A12	Expression ratios (95% CIs)	9.4 (4.18–21.14)	4.5 (1.62–12.5)	20.86 (7.02–62.03)	0.21 (0.11–0.42)
	Adjusted *p* values	**< 0.0001**	0.06	**< 0.0001**	**0.004**
	H statistic	**44.46**			
	df	**3**			
lnc-SLC25A12	Expression ratios (95% CIs)	3.65 (1.81–7.37)	5.46 (2.48–12.15)	2.34 (0.92–5.93)	2.33 (1.01–5.41)
	Adjusted *p* values	**0.0006**	**0.0001**	0.17	0.39
	H statistic	22.57			
	df	3			
LINC01231	Expression ratios (95% CIs)	0.24 (0.1–0.54)	0.26 (0.09–0.77)	0.22 (0.08–0.57)	1.2 (0.47–3.06)
	Adjusted *p* values	**0.03**	0.31	0.03	0.99
	H statistic	10.61			
	df	3			
ATP2B2	Expression ratios (95% CIs)	1.66 (0.78–3.52)	3.87 (1.53–9.76)	0.66 (0.25–1.77)	5.83 (2.93–11.59)
	Adjusted *p* values	0.18	**0.002**	**0.99**	**< 0.0001**
	H statistic	24.99			
	df	3			
lnc-MTR-1	Expression ratios (95% CIs)	17.44 (7.97–38.18)	15.88 (6.23–40.44)	19.33 (6.7–55.8)	0.82 (0.35–1.93)
	Adjusted *p* values	**< 0.0001**	**< 0.0001**	**< 0.0001**	0.99
	H statistic	56.44			
	df	3			

TABLE 5 | Correlation coefficients between transcripts among the total migraine patients and healthy controls.

	SLC25A12		lnc-SLC25A12		LINC01231		ATP2B2		lnc-MTR-1	
	All patients	Controls	All patients	Controls	All patients	Controls	All patients	Controls	All patients	Controls
SLC1A1	−0.28*	0.41**	0.53**	0.31*	0.09	0.67**	0.32*	0.68**	0.37**	0.23**
SLC25A12			−0.26*	0.7**	0.13	0.49**	−0.06	0.41**	0.06	0.55**
lnc-SLC25A12					0.16	0.45**	0.53**	0.35**	0.57**	0.52**
LINC01231							0.27*	0.79**	0.19	0.3*
ATP2B2									0.43**	0.28*

*$p < 0.05$.
**$p < 0.001$.

Receiver Operating Characteristic (ROC) curves were constructed to determine the suitability of differentially expressed genes for diagnostic purposes. The Youden index was employed to identify cut-off points that best differentiated migraine patients from healthy controls. Sensitivity, specificity, and area under the curve (AUC) were calculated based on these cut-offs. A multiple logistic regression analysis was conducted to assess the combined diagnostic potential of all analyzed genes (SLC1A1, SLC25A12, ATP2B2, LINC01231, lnc-SLC25A12, and lnc-MTR-1). The discriminatory power of the model was evaluated using the area under the receiver ROC curve (AUC).

4 | Results

4.1 | General Information

Clinical and demographic information about patients and controls are summarized in Table 2.

4.2 | Expression Assays

We detected a significant difference in the expression of SLC1A1, SLC25A12, ATP2B2, and their related lncRNAs between some subgroups of patients and controls (Figure 1). The post hoc study power calculation using mean values of gene expression in each group and alpha = 0.05 revealed that the study power is more than 90%.

Two-way ANOVA was used to study the effect of two independent variables (i.e., disease and gender) on the expression of genes (as a dependent variant). We also examined the interaction between disease and gender and whether this interaction affected the expression of genes. In other words, two-way ANOVA allowed us to determine whether the effect of disease on the expression of genes depends on gender. As demonstrated by the output of the two-way ANOVA test conducted in GraphPad Prism 9.0, the group factor (disease) affected the expression levels of all studied genes. In this method, the sum of squares determined the variation in the data. The higher the sum of squares, the greater the statistical significance of the observed difference. Thus, the effect of the disease factor was most prominent on the expression of SLC25A12. However, the gender factor did not affect the expression levels of genes, except for lnc-SLC25A12. So, we did not use post hoc tests for multiple comparisons (Table 3).

All mentioned genes and lncRNAs except for ATP2B2 and LINC01231 were shown to be up-regulated in total migraineurs compared with controls. Moreover, expression levels of ATP2B2 were higher in patients with aura compared with those without aura (Expression ratio (95% CI) = 5.83 (2.93–11.59), $p < 0.0001$). Yet, SLC25A12 was down-regulated in patients with aura as compared with those without aura (Expression ratio (95% CI) = 0.21 (0.11–0.42), $p = 0.004$). Table 4 shows the detailed statistics of these comparisons.

Expression levels of several mRNAs and lncRNAs were correlated with each other in both patients and controls. The highest correlation coefficient was reported between LINC01231/

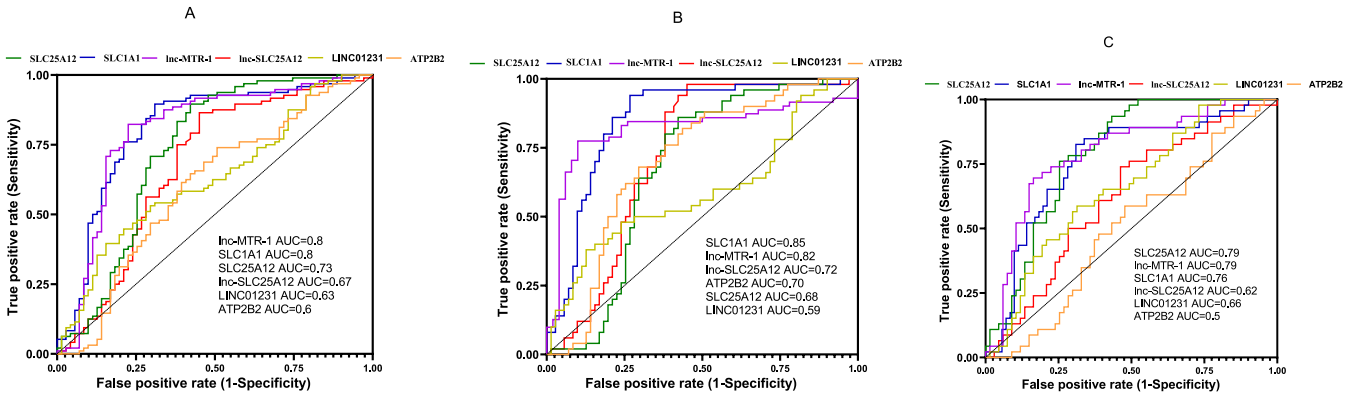

FIGURE 2 | ROC curves of SLC1A1, SLC25A12, ATP2B2 and their related long non-coding RNAs in total migraineurs ($n = 96$) versus healthy controls ($n = 71$) (A), migraineurs with aura ($n = 50$) versus healthy controls ($n = 71$) (B), and migraineurs without aura ($n = 46$) versus healthy controls ($n = 71$) (C).

ATP2B2, SLC25A12/lnc-SLC25A12, and SLC1A1/ATP2B2 pairs, respectively, in controls (Table 5).

SLC1A1 and lnc-MTR-1 could discriminate migraineurs from healthy controls with a suitable AUC value (=0.8) (Figure 2 and Table 6). Moreover, they could separate patients with aura from healthy controls with appropriate AUC, sensitivity, and specificity values.

To further evaluate the diagnostic utility of the genetic markers, we developed a multivariate logistic regression model incorporating five candidate genes (SLC1A1, SLC25A12, lnc-SLC25A12, LINC01231, and ATP2B2). The combined model exhibited strong discriminatory performance, with an AUC of 0.93 (95% CI: 0.89–0.96, $P < 0.0001$) for distinguishing patients from controls (Figure 3). At the optimal cutoff of 0.5, the model achieved an overall accuracy of 88%, with 92.7% sensitivity (correctly classifying 89/96 patients) and 81.7% specificity (correctly identifying 58/71 controls).

Expression of genes was not correlated with any of the clinical or demographic data, such as sex, age, age at onset, disease duration, or family history of migraine (Table 7).

5 | Discussion

The impact of calcium signaling in the pathophysiology of migraine has been discussed previously. For instance, Fila et al. have shown that calcitonin gene-related peptide (CGRP) and its receptor may be a target for migraine therapy (Fila et al. 2022). Similarly, Salvin et al. have reported that CGRP is involved in the inflammatory process of migraine and affects the uptake of calcium. They tried to inhibit the secretion of CGRP by ginger extracts and S-petasin to decrease inflammation and pain (Slavin et al. 2016). Moreover, calcium has a role in the regulation of vascular tone. Dysregulation in these channels may contribute to the pathogenesis of migraine or other similar disorders (Ferrari 1998). Based on the mentioned evidence, we selected three mRNA coding genes and their related lncRNAs for expression assays in migraineurs. Notably, all mentioned genes and lncRNAs except for *ATP2B2* and *LINC01231* were shown to be up-regulated in total migraineurs compared with controls. In a recent study in epileptic patients, we demonstrated up-regulation of *SLC1A1* and *SLC25A12* in female patients compared with healthy females (Taheri et al. 2023). Moreover, *SLC1A1*, *SLC25A12*, *LINC01231*, and *lnc-MTR-1* transcripts were up-regulated in male epileptic patients compared with healthy males (Taheri et al. 2023).

SLC1A1 encodes a glutamate transporter that is expressed in neurons. It has high levels in the hippocampus, cerebellum, and basal ganglia (Conti et al. 1998; Haugeto et al. 1996; Holmseth et al. 2012). Besides, SLC1A1 is mainly expressed in the dendrites and soma, indicating a role for this protein in the homeostatic or cell metabolism instead of direct regulation of synaptic transmission (Holmseth et al. 2012). Up-regulation of this gene in migraineurs might indicate aberrant regulation of cell metabolism in these patients.

Slc25a12 defects have been found to alter myelination and neurofilaments (Sakurai et al. 2010). Moreover, delicate alterations in its expression can influence brain development and increase susceptibility to autism (Sakurai et al. 2010). Meanwhile, polymorphisms in the *ATP2B2* gene have been proposed to partake in the etiology of autism (Yang et al. 2013). The protein encoded by this gene has high expression in the cerebral cortex and hippocampus (A. Burette et al. 2003) and has a major role in the regulation of calcium signaling and modulation of short-term plasticity in Purkinje cells (A. C. Burette et al. 2010; Empson et al. 2007). Dysregulation of calcium signaling in these cells leads to delayed function of these cells (Fakira et al. 2012). Moreover, mutations in this gene result in dystonia, ataxia, intellectual disability, behavioral problems, and seizures, all of them being related to the dysregulation in the metabolism of calcium and abnormal signaling in neurons (Poggio et al. 2023).

In line with our findings about up-regulation of these two genes in migraine, previous studies have reported comorbidity between autism and migraine. Moreover, there is evidence pointing to an association between these two conditions in terms of atypical findings, particularly in the organization of cortical minicolumns, dysfunction in the gut–brain axis, and shared susceptibility *loci* (Vetri 2020).

TABLE 6 | ROC curve analysis in patients with migraine disease (total), migraine patients with aura and without aura.

	SLC25A12				SLC1A1				lnc-MTR-1				lnc-SLC25A12				LINC01231				ATP2B2			
	AUC±SEM	Sensitivity	Specificity	*p* Value	AUC±SEM	Sensitivity	Specificity	*p* Value	AUC±SEM	Sensitivity	Specificity	*p* Value	AUC±SEM	Sensitivity	Specificity	*p* Value	AUC±SEM	Sensitivity	Specificity	*p* Value	AUC±SEM	Sensitivity	Specificity	*p* Value
Total Migraine patients vs. total normal controls	0.73±0.04	0.9	0.57	<0.0001	0.8±0.03	0.9	0.69	<0.0001	0.8±0.03	0.82	0.73	<0.0001	0.67±0.04	0.86	0.55	<0.0001	0.63±0.04	0.39	0.84	0.005	0.6±0.04	0.74	0.49	0.03
Migraine patients with aura vs. normal controls	0.68±0.05	0.86	0.58	0.001	0.85±0.03	0.94	0.73	<0.0001	0.82±0.04	0.77	0.9	<0.0001	0.72±0.04	0.98	0.55	<0.0001	0.59±0.05	0.38	0.84	0.08	0.7±0.04	0.68	0.7	0.0001
Migraine patients without aura vs. total normal controls	0.79±0.04	0.76	0.75	<0.0001	0.76±0.04	0.83	0.69	<0.0001	0.79±0.04	0.7	0.83	<0.0001	0.62±0.05	0.74	0.54	0.03	0.66±0.05	0.59	0.69	0.004	0.5±0.05	0.59	0.52	0.97

FIGURE 3 | Receiver Operating Characteristic (ROC) Curve of the Multivariate Genetic Model. The ROC curve demonstrates the predictive performance of the multivariate logistic regression model incorporating five genetic biomarkers (SLC1A1, SLC25A12, lnc-SLC25A12, LINC01231, ATP2B2) for disease classification. AUC (Area Under the Curve): 0.93 (95% CI: 0.89–0.96; $p < 0.001$), reflecting good discrimination between diseased and non-diseased individuals.

We also reported correlation between expression levels of mentioned mRNA coding genes and lncRNAs in both patients and controls. The highest correlation coefficient was reported between *LINC01231*/*ATP2B2*, *SLC25A12*/*lnc-SLC25A12*, and *SLC1A1*/*ATP2B2* pairs in controls. Besides, correlation coefficients were higher among controls as compared with cases, which suggests alteration in the networks between these genes in the context of migraine.

Notably, we demonstrated high specificity and sensitivity values for *SLC1A1* and *lnc-MTR-1* in the differentiation of migraineurs from healthy controls. This finding might be of value in malingering detection. Our combined analysis of all candidate genes through multivariate logistic regression yielded outstanding diagnostic accuracy (AUC = 0.93), significantly surpassing individual gene performance. This synergistic effect highlights the complex interplay between calcium signaling genes in migraine pathogenesis. While the model's good sensitivity (92.7%) and specificity (81.7%) are clinically promising, the exceptional AUC warrants caution regarding potential overfitting, despite our adequate sample size. These findings parallel our previous observations in epilepsy, suggesting shared neurological pathways. Independent validation across diverse migraine subtypes remains essential for clinical translation.

It is worth mentioning that external validation using an independent cohort would enhance the generalizability and clinical applicability of our findings. In fact, this study was designed as an initial investigation, so we consider these results as preliminary evidence supporting the potential of these genes as biomarkers rather than definitive validation of their diagnostic utility.

In brief, the current study demonstrated significant overexpression of *SLC1A1*, *SLC25A12*, *lnc-SLC25A12*, and *lnc-MTR-1* in the migraineurs and suggested these genes as potential markers for this neurological condition. Future studies are expected

TABLE 7 | Spearman correlation between expression of six studied genes, age, age at onset, disease duration, sex and family history of migraine.

	Age	Age at onset	Disease duration	Family history of migraine	SLC1A1 expression	SLC25A12 expression	lnc-SLC25A12 expression	LINC01231 expression	ATP2B2 expression	lnc-MTR-1 expression
Sex	−0.14	−0.18	0.1	−0.15	0.14	−0.06	0.06	−0.13	0.09	0.002
Age		0.55**	0.51**	0.17	−0.03	−0.06	0.1	0.18	0.06	−0.01
Age at onset			−0.34**	0.05	−0.08	0.02	0.04	0.08	0.03	0.02
Disease duration				0.11	0.008	−0.08	0.07	0.05	0.07	−0.05
Family history of migraine					−0.14	−0.009	0.09	0.05	0.19	−0.03

Note: Disease duration was classified into 3 ranges (1–2, 3–4 and more than 4 years).
**$p < 0.001$.

to find the molecular mechanisms of such dysregulation and the effects of abnormal expression of these transcripts on molecular axes governing the pathoetiology of migraine.

6 | Limitations

The current study was a pilot study. The results should be confirmed in additional cohorts of patients with different types of migraine. In fact, an independent replication of the findings is needed. Moreover, functional analyses are needed to find the underlying mechanism of contribution of these genes to the pathogenesis of migraine.

Author Contributions

Mohammad Taheri: methodology, investigation, writing – original draft. **Ashkan Pourtavakoli:** investigation, methodology. **Solat Eslami:** formal analysis. **Arezou Sayad:** writing – original draft, investigation, supervision. **Soudeh Ghafouri-Fard:** supervision, writing – review and editing, writing – original draft, investigation.

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Ethics Statement

All procedures performed were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments. The study protocol was approved by the ethical committee of Shahid Beheshti University of Medical Sciences.

Consent

Informed consent forms were obtained from all study participants.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All data generated or analyzed during this study are included in this published article [and its Supporting Information—S1].

Peer Review

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/jnc.70112.

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