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Estimation of IncSNHG1 Gene Expression as Potential Biomarkers of Migraine

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Abstract

Neuroinflammatory events and trigeminal nucleus caudalis (TNC) microglia activation play a crucial role in the pathophysiology of migraine, especially chronic migraine. The function of SIRT1 in neural tissues is anti-inflammatory and protective. The expression level of incSNHG1, miR-181a and SIRT1 were compared between migraineur and healthy control in a case-control study, while the protein level of SIRT1 in migraineurs was compared with that of healthy controls. During the study period, patients who visited the neurology outpatient clinic were enlisted. Migraine was diagnosed by a qualified neurologist, using the criteria of the International Classification of Headache Disorders (ICHD-3). The mean relative expression of IncSNHG1 was lower in the migraine group (1.635 ± 0.5900) as compared to the control group (2.106 ± 0.6852). The relative levels of miR-181a expression were measured in patients versus healthy controls. Mean Expression: The patient group's mean relative expression of miR-181a was substantially greater than the control group's (1.499 ± 0.5793 , $p = 0.1496$) (2.014 ± 0.7233 , $p = 0.1321$). Patients' mean expression of SIRT1 was compared with the mean expression in the control group: Mean Expression: The SIRT1 group mean gene expression was 1.506 ± 0.6091 , $p = 0.1112$, compared to the control group mean gene expression 2.106 ± 0.6852 , $p = 0.1769$. The serum SIRT1 protein levels (ng/l) of patients and healthy controls were measured and compared. Mean Serum Levels: Compared to healthy controls (497.2 ± 137.6 ng/l, $p = 39.73$), the patient group's serum levels of SIRT1 protein were considerably lower (316.7 ± 167.6 ng/l, $p = 30.60$). The results indicate that the alterations of IncSNHG1, miR-181a and SIRT1 may be associated with migraine, and may provide molecular markers that will aid in understanding the disease. Specifically, the reduction of SIRT1 expression in the genome and proteins indicates an involvement of impaired inflammatory and anti-inflammatory mechanisms and antioxidant activity in migraine. Larger cohorts of patients, different migraine subtypes and severity parameters, and functional molecular experiments are needed in the future to further validate the clinical relevance and mechanism of these markers.

Keywords

Migraine,
Gene Expression,
miR-181a,
SIRT1,
SIRT1protein,
incSNHG1.



Introduction

Migraine is one of the most common primary headache conditions with more than one billion people worldwide suffering from it, predominantly female. Migraine is one of the most important causes of disability and costs the economy a lot of money [1]! Migraine can be subdivided into several different forms based on the neurology, such as the main types (with/without aura), as well as less common types (ocular, abdominal, vestibular and hemiplegic). Migraine may be episodic or chronic depending on the frequency of the attacks [2].

Genetics seem to play a role in the pathophysiology and susceptibility aspects of migraine [3]. Although the genetic role in migraine has been extensively studied in the past, the involvement of the function of gene regulatory systems in the pathophysiology of migraine has recently also come into the focus. An understanding of the origin and impact of migraine related epigenetic changes can be used to enhance risk stratification, migraine pathogenesis, development, diagnosis and prognosis [4]. This could also be a new potential avenue for studying and developing new therapeutic strategies for migraine, and the current understanding of migraine pathophysiology and therapeutic options is discussed with a particular focus on microRNA, histone acetylation and DNA methylation [5].

A number of genes and their methylation patterns (migraine symptoms and age of migraine onset), (migraine chronification), and microRNA molecules like miR-34a-5p and miR-382-5p (treatment response) seem especially deserving of further investigation in relation to their role in migraine pathogenesis, course,

and therapy [6]. Additionally, the pathophysiology of migraine has been linked to several microRNA molecules, such as lncSNHG1, miR-181a, SIRT1, and SIRT1 protein, and gene changes have been linked to the development of medication overuse headache (MOH) from migraine. By using epigenetic alterations, we may be able to learn more about the pathogenesis of migraines and find new treatment options. To validate these first findings and identify epigenetic targets as therapeutic or disease predictors, however, more studies with larger sample numbers are needed [7].

Aims of study

By determining the expression of the long non-coding RNA lncRNA SNHG1, miR-181a, SIRT1 gene, and SIRT1 protein, a proposed epigenetic axis (SNHG1/miR-181a/SIRT1) that may control neuroinflammation and central sensitization associated with migraine onset is created.

Materials and Methods

1. Study Design and Participants

The expression levels of lncSNHG1, miR-181a, and SIRT1 between migraineurs and healthy controls, as well as the protein level of SIRT1 in migraineurs, were compared in a case-control study. During the study period, patients who visited the neurology outpatient clinic were enlisted. A qualified neurologist used the International Classification of Headache Disorders (ICHD-3) criteria to diagnose migraine.

2. Blood Sample Collection

Using sterile disposable syringes and tubes filled with the proper anticoagulant, five milliliters (mL) of peripheral venous blood

were extracted from each participant. Individuals' blood was collected into simple tubes for serum SIRT1 protein assay and EDTA tubes for RNA analysis. After allowing blood samples to coagulate at room temperature, they were centrifuged at $3,000 \times g$ for around ten minutes in order to prepare serum. For biochemical analysis, separated serum was stored at -80°C in RNase-free tubes. Whole blood samples were either stored according to the manufacturer's recommendations until RNA extraction or processed right away for RNA analysis.

3. RNA Extraction

Following the manufacturer's instructions, total RNA, including small RNA fractions, was extracted from peripheral blood using a commercially available RNA extraction kit. The quantity and purity of the isolated RNA were evaluated by measuring absorbance at 260 and 280 nm using a NanoDrop spectrophotometer. RNA samples of acceptable purity were subjected to reverse transcription and quantitative real-time PCR (qRT-PCR).

All RNA-related tasks were completed using RNase-free materials and equipment in order to minimize RNA contamination and degradation.

4. Reverse Transcription and cDNA Synthesis

For the investigation of incSNHG1 and SIRT1 mRNA, an adequate amount of isolated total RNA was reverse-transcribed into complementary DNA (cDNA) using a commercial reverse-transcription kit in compliance with the manufacturer's instructions. For the investigation of miR-

181a, a specific reverse-transcription technique designed for mature microRNAs was used. The resulting cDNA was stored at -20°C until quantitative real-time PCR analysis.

5. Quantitative Real-Time PCR

SIRT1, miR-181a, and incSNHG1 expression levels were assessed using quantitative real-time polymerase chain reaction (qRT-PCR). The reactions were carried out in a real-time PCR detection instrument using a commercial SYBR Green-based qPCR master mix. Specific primers were designed using the matched human nucleotide sequences of incSNHG1 and SIRT1. For miR-181a, either a commercially certified miRNA assay or a specific mature miRNA primer was used. An appropriate endogenous reference gene was used to normalize each target.

The internal control for the mRNA/lncRNA expression analysis was GAPDH or another validated housekeeping gene. The endogenous control for miR-181a studies was either U6 snRNA or another validated small-RNA reference. The amplification procedure included an initial denaturation phase and subsequent cycles of denaturation, annealing, and extension in compliance with the manufacturer's recommendations. A melting-curve analysis was used to verify the amplification's specificity. Relative gene expression was calculated using the $2^{-\Delta\Delta\text{Ct}}$ technique, where ΔCt is the difference between the Ct value of the target gene and the matching reference gene.

6. Determination of SIRT1 Protein

Serum SIRT1 protein levels were measured using a commercial human SIRT1 enzyme-linked immunosorbent assay (ELISA) kit in compliance with the manufacturer's

instructions. In conclusion, serum samples, standards, and appropriate controls were added to the wells of an antibody-coated microplate. Following incubation and washing, the appropriate enzyme-conjugated detection antibody was applied. After further incubation and cleaning steps, the substrate solution was added and the enzymatic reaction was allowed to continue. After stopping the reaction with the stop solution, the absorbance was measured using a microplate reader at the wavelength suggested by the kit manufacturer. The IRT1 concentrations were calculated using the standard calibration curve and expressed in the manufacturer's specified units.

7. Statistical Analysis

For statistical analysis, SPSS software was utilized. Continuous variables were checked for normality before statistical comparison. Regularly distributed data were expressed as mean $\pm$ standard deviation (SD), while non-normally distributed data were shown as median and interquartile range (IQR). A P-value of less than 0.05 was considered statistically significant.

Results

Assessment of Migraine-Related Clinical Characteristics

Both the age and sex of the migraine patients, the type of migraine and the duration of illness were recorded. If appropriate, a validated assessment tool to measure the severity of headache, including the Visual Analog assessment (VAS) and the Numerical Rating Scale (NRS), was used.

The possible associations between these clinical characteristics and incSNHG1, miR-

181a, SIRT1 mRNA expression and SIRT1 protein levels were then evaluated.

The clinical features of migraine patients and healthy control groups are shown in Table 1. Fifty healthy controls and one hundred migraineurs participated in the study.

As for age, the highest proportion of people who suffer from migraine is over 35 (40%), followed closely by the 26-35 age group (35%) and the 15-25 age group (25%). Approximately, 50% of the participants in the control group were older than 35 years, 30% were aged between 26 and 35 years and 20% were aged between 15 and 25 years. The distribution of age groups was statistically significantly different between the two groups ($P = 0.01$).

In terms of gender, 45% of migraineurs were female and 55% of migraineurs were male. The control group consisted of 50% males and 50% females. Sex distribution between the two groups was similar with a difference not statistically significant ($P = 0.20$).

Among the patients with migraine, chronic migraine with continuous pain (40%) was the most common type of migraine, followed by migraine with aura (20%), migraine without aura (10%) and usual aura without headache (30%). The migraine types were statistically significantly different ($P = 0.01$).

When it came to the duration of their illness, most migraineurs (40%) had suffered from migraine for more than 10 years, 35% for 6 to 10 years and 25% for less than 5 years. A statistically significant difference was found between the disease-length groups ($P = 0.01$) with the migraine patients under study having a longer disease duration when compared with the other groups.

In general, the results indicate significant differences between the migraine patients and the controls with respect to age

distribution, type of migraine, and duration of disease, but not with respect to sex.

Table 1: displays the clinical features of both healthy control groups and migraine patients.

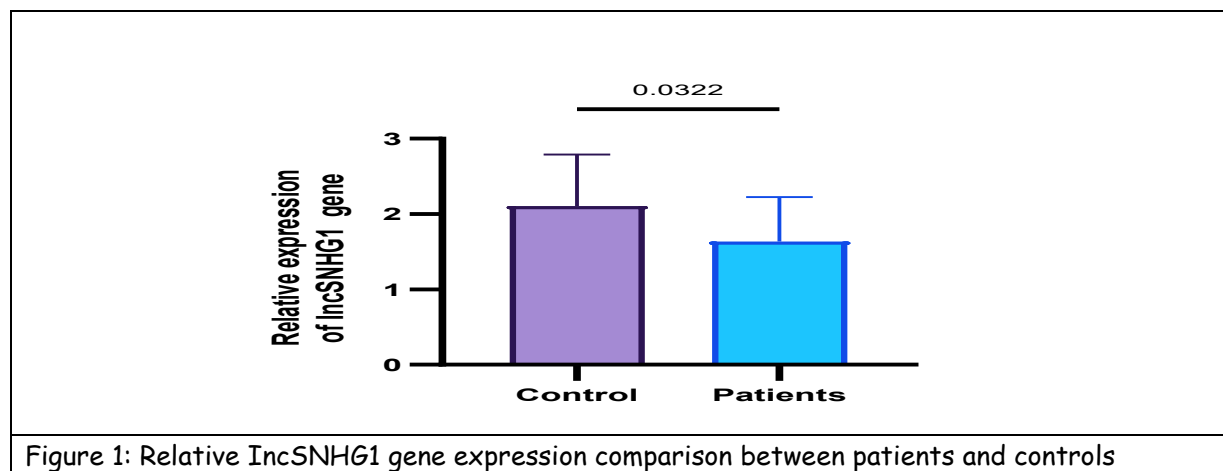
Clinical Characteristics		F (%)		P- Value
		Migraine No. 100	Control No. 50	
age	15-25	25(25%)	10(20%)	0.01
	26-35	35(35%)	15(30%)	
	>35	40(40%)	25(50%)	
sex	Male	55(55%)	25(25%)	0.2
	Female	45(45%)	25(25%)	
migraine type	Migraine without aura	10(10%)	----	0.01
	Migraine with aura	20(20%)	-----	
	Typical aura without headache	30(30%)	-----	
	Chronic migraine with continuous pain	40(40%)	-----	
disease duration	1-5 years	25(25%)	-----	0.01
	6-10 years	35(35%)	-----	
	➤ 10 years	40(40%)	-----	

The expression level of lncSNHG1 was assessed between migraine patients and healthy controls. Gene expression levels: The mean relative expression of the lncSNHG1 was found to be reduced in migraine patients (1.635 ± 0.5900) as compared with the control group (2.106 ± 0.6852). Median and Distribution: The median expression value for migraine patients was 1.696 (IQR: 1.096 - 2.791), while for controls, the expression value was 2.039 (IQR: 1.545 - 2.640). Overall

Trend: The present results suggest that the expression of the gene for lncSNHG1 is down-regulated in migraine patients than healthy ones. These results showed a statistically significant decrease in the expression of lncSNHG1 gene in migraine patients compared to the healthy controls (Mean: 1.635 vs. 2.106, respectively), indicating that there might be a relationship between the down-regulation of lncSNHG1 and the pathogenesis of migraine.

Table2: Relative IncSNHG1 gene expression comparison between migraine patients and controls

IncSNHG1	Control	Patients Migraine
Minimum	1.009	0.5010
25% Percentile	1.545	1.096
Median	2.039	1.696
75% Percentile	2.640	2.004
Maximum	3.052	2.791
Mean	2.106	1.635
Std. Deviation	0.6852	0.5900
Std. Error of Mean	0.1769	0.1077

**Figure 1: Relative IncSNHG1 gene expression comparison between patients and controls**

The expression of miR-181a in patients was compared to healthy controls. Mean Expression: The mean relative expression of miR-181a was significantly higher in the patient group (2.014 $\pm$ 0.7233, $p = 0.1321$) compared to the control group (1.499 $\pm$ 0.5793, $p = 0.1496$). The median expression value for patients was 2.038 (IQR: 1.329 - 2.617), while the control group had a median value of 1.329 (IQR: 1.009 - 1.990). Range: Values ranged from 1.009 to 3.440 in patients, compared to 0.7000 to 2.870 in

controls. Overall Trend: When compared to healthy control subjects, patients have a clear up-regulation of the gene expression of miR-181a ($p = 0.0144$). The data show a statistically significant up-regulation of miR-181a gene expression in patients in comparison with healthy controls (2.014 vs. 1.499, $p = 0.0144$), suggesting that high levels of miR-181a might be used as a potential biomarker or as part of the disease pathogenesis.

Table 3: Relative miR-181a gene expression comparison between patients and controls

miR-181a	Control	Patients
Minimum	0.7000	1.009
25% Percentile	1.009	1.329
Median	1.329	2.038
75% Percentile	1.990	2.617
Maximum	2.870	3.440
Range	2.170	2.431
Mean	1.499	2.014
Std. Deviation	0.5793	0.7233
Std. Error of Mean	0.1496	0.1321

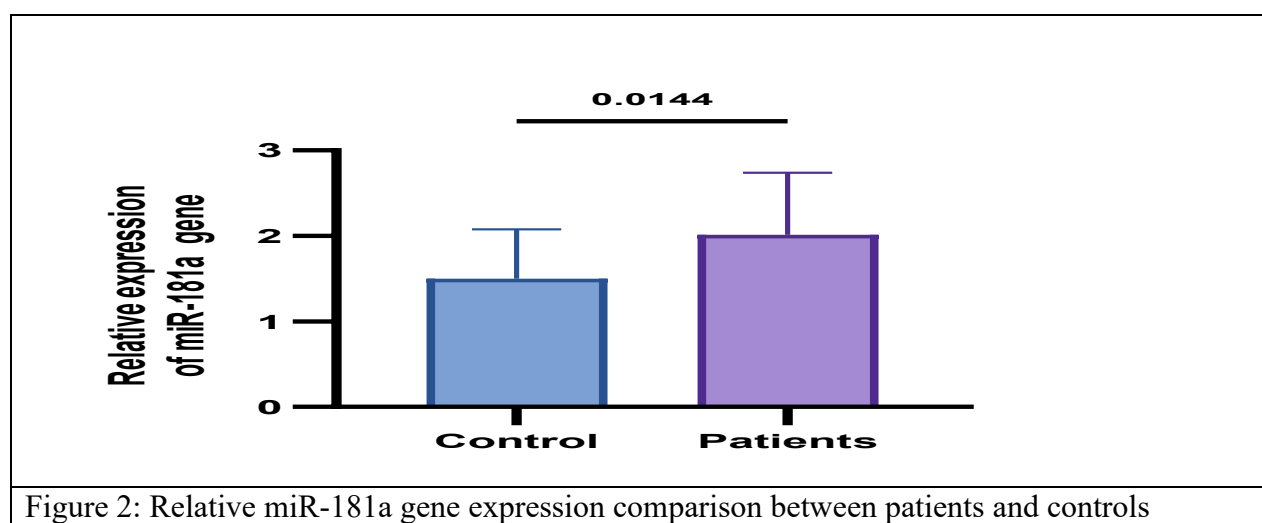


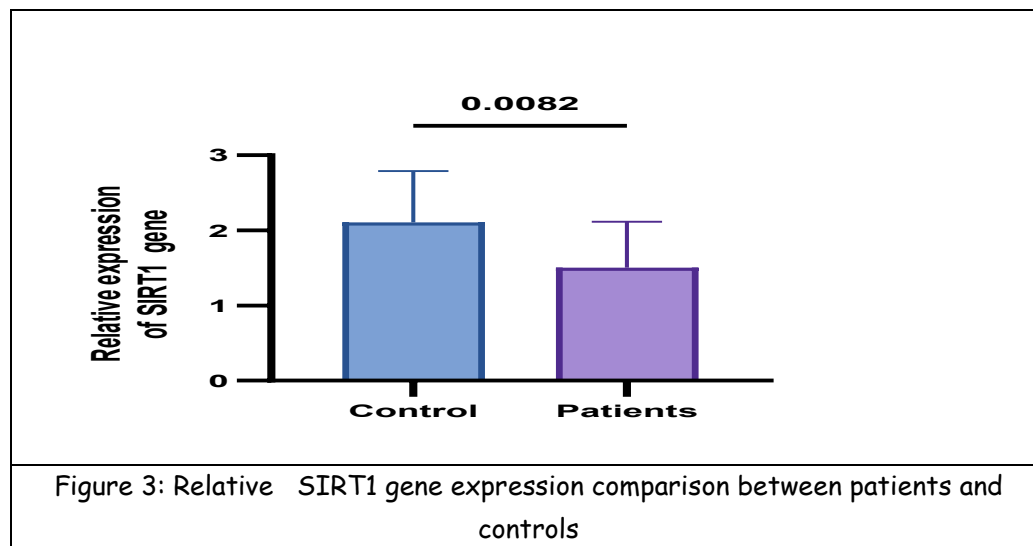
Figure 2: Relative miR-181a gene expression comparison between patients and controls

The mean expression of SIRT1 in patients was 1.506 ± 0.6091 ($p = 0.1112$) and it was significantly less than the expression of SIRT1 in healthy controls (2.106 ± 0.6852 , $p = 0.1769$). Median (IQR) for the patient group expression was 1.560 (0.9875 - 2.000) while the control group showed a median of 2.039 (1.545 - 2.640). Range: Values ranged from 0.4632 to 2.423 in patients, compared to 1.009 to 3.052 in controls. Results: Overall,

gene expression of SIRT1 was found to be down-regulated in patients compared to healthy controls ($p = 0.0082$). The results of these findings indicate that SIRT1 gene expression is significantly down-regulated in the patients compared to healthy controls (1.506 vs 2.106, $p = 0.0082$), which implies that down-regulation of SIRT1 may be a part of the molecular mechanisms of the disease.

Table 4: Relative SIRT1 gene expression comparison between patients and controls

SIRT1	Control	Patients
Minimum	1.009	0.4532
25% Percentile	1.545	0.9875
Median	2.039	1.560
75% Percentile	2.640	2.000
Maximum	3.052	2.423
Mean	2.106	1.506
Std. Deviation	0.6852	0.6091
Std. Error of Mean	0.1769	0.1112



The serum level of SIRT1 protein (ng/l) was measured and compared between patients and healthy controls: Mean Serum Levels: Serum levels of SIRT1 protein were significantly decreased in the patient group (316.7 $\pm$ 167.6 ng/l = 30.60) compared to healthy controls (497.2 $\pm$ 137.6 ng/l, = 39.73). Median and Percentiles: The median serum concentration found in patients was 299.5 ng/l (IQR: 195.0 - 466.0) compared to the higher median serum concentration of 499.5 ng/l (IQR: 399.3 - 639.3) found in the controls. Patients ranged from 85.00 to

650.0 ng/l of protein concentration while controls had a range from 209.0 to 670.0 ng/l. Statistically Significant: Statistically significant reduction in SIRT1 protein level was confirmed in the patients compared to the controls, $p = 0.0014$. It was found that patients had significantly less amounts of SIRT1 protein in the blood than healthy controls (316.7 ng/l vs. 497.2 ng/l, $p = 0.0014$), which suggests that decreased circulating SIRT1 protein levels correlate with the disease state and might be applied as a marker.

Table 5: Relative SIRT1protein expression comparison between patients and controls

SIRT1protein	Control	Patients
Minimum	209.0	85.00
25% Percentile	399.3	195.0
Median	499.5	299.5
75% Percentile	639.3	466.0
Maximum	670.0	650.0
Mean	497.2	316.7
Std. Deviation	137.6	167.6
Std. Error of Mean	39.73	30.60

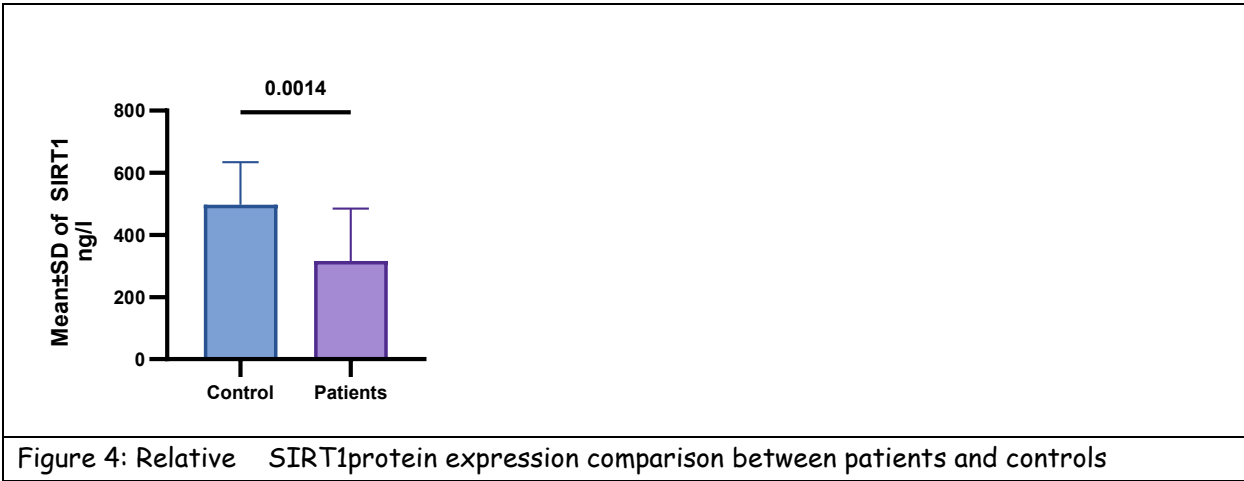


Figure 4: Relative SIRT1protein expression comparison between patients and controls

Discussion

In this study the 100 patients diagnosed with migraine headache (according to the criteria used), 55(55%) male and 45(45%) female, were analyzed, which is consistent with the results of large-scale studies. This imbalance in sex ratio, which is largely attributable to hormonal changes that occur during menstruation and pregnancy¹, and genetic susceptibility. The median age at onset for this study was range 40(40%) in >35years. The most prevalent subtype (40%) was Continuous pain - Migraine Chronic migraine. Prevalence of migraine in 15-25 years age group was reported as 0.8% by Almohammadawi et al., (8) episodic

syndromes/childhood periodic syndromes with 6-10 years duration and migraine with aura. The Glasgow Coma Scale (GCS) score in this sub-group was between the ranges 3 and 14 but this result was not significant.

Approximately 9% of participants experienced chronic migraine with continual pain and chronic migraine (alternative criteria) and both conditions impacted negatively on their quality of life and on their socioeconomic functioning. It usually develops from an episode of migraine, with or without aura, which turns into a continuum, with an undetermined annual conversion rate (9). Medicationoveruse headache (MOH) was

another large group of migraine patients that represented about 2.6% of the total sample (10). Migraine is a complex neurological disorder with a genetic component; therefore, it is important to understand the role that genetic factors play in unravelling the mechanisms involved in the disease. In this study, the authors combined the techniques of lncSNHG1, miR-181a and SIRT1 to obtain novel information regarding the biological basis of migraine (11). Microglia are important immune cells within the CNS which are highly responsive to changes in microenvironment and responses rapidly. They are crucial for the development, maintenance and repair of the CNS, and are the most common regulators of neuroinflammation.

In cases of abnormal activation of microglia however, migraine headaches (12) may occur. The gene expression results revealed that microglia gene expression changes post inflammatory stimulation after expression of genes including integrates lncSNHG1, miR-181a, SIRT1. This suggests that activation of the immune system can cause changes in the gene expression of microglia, which might contribute to the development of migraine. In this study, the expression of the long non-coding RNA (lncRNA) SNHG1, the microRNA (miRNA) miR-181a, and the sirtuins (SIRT) SIRT1 have been investigated in patients with migraine and healthy controls in terms of their gene-expression and protein-level. The results revealed differences in these molecular markers, suggesting that non-coding RNA dysregulation and the SIRT1 signaling pathway are interrelated and may be associated with pathophysiological mechanisms of migraine. The average relative expression of lncSNHG1 was found to be lower in the migraine group (1.635 ± 0.5900)

than in the control group (2.106 ± 0.6852) in the present study. The decreased expression might indicate the possible implication of lncSNHG1 in the molecular mechanisms of migraine.

The lncRNAs can regulate the expression of genes through binding to microRNA, transcription factor and signaling pathway. Therefore, the expression of these lncRNAs can influence the expression of the downstream genes that are involved in inflammation and oxidative stress and maintaining neuronal functions (13). Although little is known about the biological role of lncSNHG1 in migraine, further studies are required to determine if a loss of lncSNHG1 is a primary or secondary event in migraine (14).

The expression of miR-181a was found to be higher in the migraine patients than in the healthy. Mean relative expression levels for miR-181a were 2.014 ± 0.7233 and 1.499 ± 0.5793 respectively. While the statistical significance of this difference needs to be interpreted based on the precise P value obtained by statistical analysis, the observed up-regulation of miR-181a could suggest that post-transcriptional regulatory mechanisms play a role in migraine. MicroRNAs are known to be regulators of pathways involved in inflammation, oxidative stress, apoptosis and neuronal signaling and have a critical role in regulating gene expression. The expression of microRNAs has been linked to many neurological diseases, including migraine (15).

SIRT1 is an important regulator of inflammatory responses and experimental SIRT1 inhibition has been linked to an increase in neuroinflammation and central sensitization in migraine models. In the

present results, also, the expression level of SIRT1 gene was found to be decreased in the migraine group (1.506 ± 0.6091) compared to healthy control group (2.106 ± 0.6852). This discovery has biological relevance because the SIRT1 protein is an NAD⁺-dependent deacetylase, involved in cellular stress responses, mitochondrial functioning, antioxidant defense and regulation of inflammatory signaling. Animal studies have shown that decreased SIRT1 activity can lead to neuroinflammation and oxidative stress, which are both known to play a role in migraine pathogenesis.

The gene expression decreases of SIRT1 observed in the present study is in accordance with the recent experimental data on the connection between SIRT1 and migraine (16). In 2024, a research study found that SIRT1 activation decreased migraine-like behaviors and neuroinflammation and oxidative damage by blocking inflammatory signaling in microglia. Similarly, in a recently created chronic migraine model, there was a reduction in the expression of SIRT1, an increase in central sensitization and associated molecular changes, and these changes were reversed by pharmacological activation of SIRT1. The findings indicate that the observed reduction in the expression level of SIRT1 in the migraine patients might be a sign of defective endogenous mechanisms involved in the regulation of inflammation and oxidative stress (17).

Besides, the present study revealed a considerably lowered level of SIRT1 protein in the blood of patients with migraine, whose mean blood level of SIRT1 was 316.7 ± 167.6 ng/L as compared to 497.2 ± 137.6 ng/L in healthy controls. There is a possibility of multiple molecular levels of alteration in the

SIRT1 pathway, as indicated by the simultaneous decrease in SIRT1 gene expression and circulating SIRT1 protein. But the expression of genes and the amount of proteins in the blood does not always match up with the expression of a protein as post-transcriptional and post-translational events may influence protein stability and secretion.

Increased expression of miR-181a and decreased expression of SIRT1 reported in this study could indicate a regulatory relationship between the two molecules. MicroRNA can target genes by binding to a complementary sequence in the target transcript leading to the inhibition of translation or degradation of mRNA (18). Thus, the upregulation of miR-181a may lead to SIRT1 downregulation, if this is shown to be a direct target of miR-181a in tissue relevant for migraine. This correlation is not causal, however, due to the expression data that was observed during this time period (19). Additional experiments (Bioinformatic prediction, Correlation analysis, Luciferase reporter assays, Gain and loss of function) would help establish a direct regulatory interaction by miR-181a and SIRT1.

The molecular signature observed may be more relevant in particular to the neuroinflammatory mechanisms of migraine. The current evidence suggests that migraine is a result of interactions between neurons, glial cells, vascular components and immune mediators. Microglia and other glial cells can be activated to play a role in inflammatory signaling and central sensitization, both of which are important mechanisms in migraine (20).

Conclusions:

According to this research, men are more likely than women to experience migraine-related headaches that cause brief periods of functional impairment. The distribution was considerably altered by a family history of migraines, and the average age at first attack was young. Migraine without aura was the most common type of migraine. Since experienced aura was the most prevalent kind of migraine with aura, although aura without a headache and aura with migraine were also common, it is important to distinguish between migraine subtypes. Chronic migraine with constant discomfort was the most commonly reported consequence.

When considered collectively, the current findings point to a potential lncSNHG1-miR-181a-SIRT1 regulation axis in migraine. Increased miR-181a and decreased SIRT1 expression and protein levels, along with decreased lncSNHG1 expression, may indicate disruption of molecular pathways related to neuroinflammation and neuronal stress. However, the suggested axis is still a theory that has to be verified through experimentation. The interpretation of the results would be strengthened by correlation analysis between lncSNHG1, miR-181a, SIRT1 mRNA, and SIRT1 protein, which could help ascertain whether these markers are functionally related.

References

- 1- Tzankova, V.; Becker, W.J.; Chan, T.L.H. Diagnosis and Acute Management of Migraine. *CMAJ* 2023, 195, E153-E158.
- 2- Zobdeh, F.; Kraiem, A.; Attwood, M.M.; Chubarev, V.N.; Tarasov, V.V.; Schiöth, H.B.; Mwinyi, J. Pharmacological Treatment of Migraine: Drug Classes, Mechanisms of Action, Clinical Trials and New Treatments. *Br. J. Pharmacol.* 2021, 178, 4588-4607.
- 3- SUTHERLAND, Heidi G.; JENKINS, Bronwyn; GRIFFITHS, Lyn R. Genetics of migraine: complexity, implications, and potential clinical applications. *The Lancet Neurology*, 2024, 23.4: 429-446.
- 4- GOSALIA, Helin; KARSAN, Nazia; GOADSBY, Peter J. Genetic mechanisms of migraine: insights from monogenic migraine mutations. *International Journal of Molecular Sciences*, 2023, 24.16: 12697.
- 5- ZOBDEH, Farzin, et al. The epigenetics of migraine. *International Journal of Molecular Sciences*, 2023, 24.11: 9127.
- 6- GRODZKA, Olga; SŁYK, Stanisław; DOMITRZ, Izabela. The role of MicroRNA in migraine: A systemic literature review. *Cellular and Molecular Neurobiology*, 2023, 43.7: 3315-3327.
- 7- GRODZKA, Olga; SŁYK, Stanisław; DOMITRZ, Izabela. The role of MicroRNA in migraine: A systemic literature review. *Cellular and Molecular Neurobiology*, 2023, 43.7: 3315-3327.
- 8- Almohammadawi, Khalid Obiad Mohsin; ALHILFI, Haider Saadoon Qasim; ALKHALIDY, Rafid Adil Abood. Clinical characteristics of migraine: A prospective cross-sectional study over nine years. *F1000Research*, 2018, 7: 1973.
- 9- STEINER, Timothy J., et al. Migraine-attributed burden, impact and disability, and migraine-impacted quality of life: expert consensus on definitions from a Delphi process. *Cephalalgia*, 2022, 42.13: 1387-1396.

- 10- LÓPEZ-MEDINA, Diana Carolina, et al. Cognition, mental health, and quality of life in patients with chronic and episodic migraine during the interictal period. *BMC neurology*, 2025, 25.1: 108.
- 11- TAHERI, Mohammad, et al. Alteration in the expression of long non-coding RNAs in the circulation of migraineurs. *Acta Neurologica Belgica*, 2024, 124.4: 1295-1301.
- 12- TAFURI, Emmanuele, et al. MicroRNA profiling in migraine without aura: pilot study. *Annals of medicine*, 2015, 47.6: 468-473..
- 13- Zhou B, et al. Downregulation of miR-181a upregulates sirtuin-1 (SIRT1) and improves hepatic insulin sensitivity. *Diabetologia*. 2012;55(8):2212-2220.
- 14- CHEN, Yu, et al. SIRT1 activation by Ligustrazine ameliorates migraine via the paracrine interaction of microglia and neurons. *Phytomedicine*, 2024, 135: 156069..
- 15- TAFURI, Emmanuele, et al. MicroRNA profiling in migraine without aura: pilot study. *Annals of medicine*, 2015, 47.6: 468-473.
- 16- ZHANG, Xiaoyan, et al. SIRT1-regulated ROS generation activates NMDAR2B phosphorylation to promote central sensitization and allodynia in a male chronic migraine rat model. *Frontiers in Molecular Neuroscience*, 2024, 17: 1387481..
- 17- Jiménez-Jiménez FJ, Alonso-Navarro H, García-Martín E, Espada-Rubio S, Agúndez JAG. Oxidative stress and migraine. *Mol Neurobiol*. 2024;61(10):8344-8360. doi:10.1007/s12035-024-04114-7.
- 18- CHEN, Qiao-Wen; MENG, Run-Tian; KO, Chih-Yuan. Modulating oxidative stress and neurogenic inflammation: the role of topiramate in migraine treatment. *Frontiers in aging neuroscience*, 2024, 16: 1455858..
- 19- KREKORA, Jan, et al. Sirtuins Contribute to the Migraine-Stroke Connection. *International Journal of Molecular Sciences*, 2025, 26.14: 6634.
- 20- ZHANG, Li, et al. The role of non-coding RNAs in the pathogenesis of migraine: from molecular insights to potential biomarkers. *Frontiers in Neurology*, 2026, 17: 1838515.