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RESEARCH

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Altered expression of long non-coding RNAs *NRON* and *SNHG11* in patients with ischemic stroke

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Abstract

Background Long non-coding RNAs, known as LncRNAs, have demonstrated a robust association with the pathogenesis of stroke. NRON and SNHG are among the most extensively studied lncRNAs in the context of atherosclerosis and inflammatory conditions. Given the absence of a current pathophysiological hypothesis regarding the potential relevance of the SNHG family and NRON lncRNAs in ischemic stroke (IS), this study aimed to investigate the altered expression of NRON and SNHG11 following atherosclerotic ischemic stroke (AIS) and their potential association with the risk of AIS.

Methods Blood samples were collected from 65 AIS patients (with large artery atherosclerosis or small vessel disease) and 65 controls. The expression levels of NRON and SNHG11 were assessed within the first 24 h following the stroke using quantitative real-time PCR.

Results NRON expression exhibited a significant decrease in patients compared to controls, while no substantial difference was observed in the expression level of SNHG11 between the two groups. Furthermore, logistic regression analysis revealed a significant negative association between NRON expression and the risk of AIS (adjusted odds ratio = 0.70; 95% confidence interval 0.55–0.89, $P = 0.004$).

Conclusions These findings suggest that NRON may play a role in the pathogenesis of AIS and could potentially serve as a biomarker for the disease. To fully comprehend the mechanism underlying the association between NRON and AIS and to explore its potential therapeutic implications, further investigation is warranted.

Keywords Gene expression, Ischemic stroke, Long non-coding RNA, *NRON*, *SNHG11*

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Introduction

Ischemic stroke (IS) is an acute and prevalent neurological condition characterized by a sudden interruption of blood flow to a specific area of the brain, leading to high mortality rates [1]. The genetic factors of individuals have been identified as playing crucial roles in various pathophysiological processes associated with stroke [2].

Long non-coding RNAs (lncRNAs), comprising transcripts of more than 200 nucleotides, constitute a significant portion of the human genome and are recognized as non-protein-coding RNAs [3]. Approximately 3000 lncRNAs exhibit dynamic changes in their expression profiles

within the initial hours following a stroke, and this alteration may persist for up to seven days after the occurrence of IS [4]. Recent studies have increasingly focused on delineating the diverse roles of lncRNAs in the pathogenesis of IS [5]. As a pivotal endogenous regulatory mechanism, lncRNAs are anticipated to emerge as promising targets for the treatment of ischemic stroke [6].

Among these lncRNAs, NRON (non-coding repressor of NFAT, nuclear factor of activated T cells) stands out by its ability to inhibit NFAT [7]. NFAT proteins, a family of transcription factors, play a role in regulating the expression of genes involved in classic inflammatory mediators and cytokines [8]. The activation of NFAT is commonly observed in neurodegenerative disorders, such as Alzheimer's disease [9]. NRON, acting as an NFAT suppressor, has shown a negative correlation with the risk of multiple sclerosis (MS) [10] and a positive correlation with the risk of heart failure [11].

Another group of lncRNAs, the long non-coding small nucleolar RNA host genes (SNHGs), is overexpressed in various tissue cancers [12]. Although altered expression of specific SNHG family members, such as SNHG15 [13], has been reported in cerebral ischemic models, changes in the expression of the broader SNHG family following IS remain unknown. NRON and SNHG lncRNAs have been extensively studied in the context of atherosclerosis and inflammatory diseases [14, 15]. However, there is a paucity of research on the potential roles of SNHG family and NRON lncRNAs in the pathophysiology of AIS.

The present study, for the first time, aimed to focus on investigating the altered expression levels of NRON and SNHG11 after AIS and explore their potential association with the risk of AIS. NRON and SNHG11 are not extensively studied in the context of IS, and this study highlights the absence of a current pathophysiological hypothesis regarding their potential relevance to IS. Therefore, this research contributes to filling the existing gap in understanding the roles of these lncRNAs in the context of ischemic stroke pathogenesis, providing new insights into the involvement of these lncRNAs in stroke.

Methods

Study subjects

This case–control study was conducted at Namazi Hospital in Shiraz, Iran, spanning from May 2020 to 2021. Ischemic stroke diagnoses were made by two neurologists using the Recognition of Stroke in the Emergency Room (ROSIER) scale, defining it as an acute focal neurological disorder lasting more than 24 h [16]. Confirmation of IS diagnosis was achieved through non-contrast computed tomography (CT) or diffusion-weighted magnetic resonance imaging (MRI) of the brain [17]. Stroke types were classified based on the TOAST classification [18]. Exclusion criteria encompassed patients with a transient ischemic attack, those treated with immunosuppressants, severe inflammation, or malignancy. Sixty-five AIS patients with large artery atherosclerosis (LAA) or small vessel disease (SVD) were admitted within 24 h of symptom onset. Sixty-five age and sex-matched controls from the population of Shiraz, with no history of stroke or transient ischemic attack, were also enrolled. Ages for both cases and controls ranged from 32 to 90 years. Hypertension and diabetes were diagnosed according to predefined criteria [19].

The severity of stroke was assessed upon admission using the National Institutes of Health Stroke Scale (NIHSS) score, with higher scores indicating greater severity [20]. Functional outcomes were evaluated based on the modified Rankin scale (mRS) three and six months after admission, with assessments conducted in a blinded manner with respect to lncRNA levels [21]. Following informed consent from patients or their proxy respondents, blood samples were collected. Ethical approval for this research, with the reference number IR.IAU.A.REC.1399.019, was obtained from the local ethics committee of the Arsenjan Branch, Islamic Azad University, Iran.

RNA extraction and cDNA synthesis

Blood samples (5 ml) were collected from peripheral venous sources for RNA extraction. Total RNA was extracted using a TRIzol kit (GeneAll, South Korea) following the manufacturer's protocol. RNA concentration was determined using a Nanodrop Spectrophotometer (ND-1000, Thermo Fisher, MA, USA), and all samples exhibited high-quality RNA (OD260/280 = 1.8–2.1). Prior to cDNA synthesis (Thermo Fisher, Germany), DNase I treatment was applied to all RNAs. The cDNA was synthesized using the AddBio cDNA Synthesis Kit (AddBio, Korea) with a mixture of oligo-dT and random hexamer primers.

Quantitative real-time polymerase chain reaction

Specific primers for NRON and SNHG11 were designed using AlleleID 7.5 (Premier Biosoft International, Palo Alto, CA) and Generunner, targeting specific exon-exon junctions of the genes of interest (Table 1). The ABI QuantStudio-3 instrument was employed for quantitative real-time PCR reactions (Applied Biosystems, USA). The thermal cycling protocol included an initial denaturation phase at 95 ℃ for 10 min, followed by 40 cycles of denaturation at 95 ℃ for 15 s and annealing/extension at 60 °C for 1 min. A melting phase preceded each amplification phase, with steps at 95 °C for 15 s, 60 °C for 1 min, and 95 °C for 15 s. Each sample was analyzed in duplicate. The ACTβ gene was selected as the reference gene

Table 1 Primer sequences of two selected lncRNAs and *ACTβ*

Genes	Gene ID	Sequences (5′ → 3′)	Product length (bp)
NRON	641373	Forward CGGCAGCTCGCCCTTAAATA	184
		Reverse GAACCCCCAAACCTTCCGAT	
SNHG11	128439	Forward GCCTCCTCATGATTGTTTG	189
		Reverse AGGGTCTTCAACTCTGGATC	
ACTβ	60	Forward GCCTCGCCTTTGCCTATCC	236
		Reverse TCTCTTGCTCTGGTCCTCGTC	

for normalizing the expression of the target genes. The relative expression levels of NRON and SNHG11 for each individual were determined using the $2^{-\Delta CT}$ method.

Statistical analysis

All data were presented as mean ± SEM or as *n* (%). The normal distribution of the data was assessed using the Kolmogorov–Smirnov test. The Chi-square test was employed to compare two groups of independent categorical variables, while the independent samples *t* test was used to compare two groups of numeric variables. Subgroup analysis was conducted in AIS patients to explore the association between NRON and SNHG11 with clinical parameters. A binary logistic regression model was utilized to assess the association of NRON expression levels with the risk of AIS, adjusting for variables such as hypertension, diabetes, ischemic heart disease, and HDL. A significance level of $P<0.05$ was considered statistically significant for each test.

Result

Main characteristics of study subjects

A total of 130 subjects, comprising 65 patients with AIS and 65 healthy controls, were included in our study. Research flowchart is provided in Fig. 1. As depicted in Table 2, traditional vascular risk factors, namely hypertension, diabetes, and ischemic heart diseases (IHD), were significantly more prevalent among AIS patients compared to controls ($P<0.01$). No statistically significant differences were observed between the case and control groups in terms of serum levels of total cholesterol, triglycerides, LDL, BUN, and Cr. However, AIS patients exhibited significantly lower levels of HDL than controls (Table 2).

Relative expression of NRON and SNHG11

The relative expression of NRON significantly decreased in AIS patients compared to controls (0.83 ± 0.21 vs. 1.89 ± 0.25, $P=0.002$). However, there were no notable differences between the case and control groups in the expression level of SNHG11 within the first 24 h after IS (0.14 ± 0.03 vs. 0.18 ± 0.05, $P=0.58$) (Fig. 2A and B).

Association of NRON and SNHG11 levels with clinical parameters in IS patients

Subgroup analyses were conducted to explore potential associations between the levels of NRON and SNHG11 with demographic and clinical parameters in AIS patients. As depicted in Table 3, no statistically significant differences were observed in the expression levels of NRON and SNHG11 across various parameters.

Associations between expression level of NRON and risk of atherosclerotic IS

After adjusting for various variables, including hypertension, diabetes, ischemic heart disease, and HDL, logistic regression analysis revealed that the uncontrolled diabetes mellitus (DM) group had a 5.9-fold higher risk of AIS compared to the controlled DM group (adjusted OR 5.97; 95% CI 1.81–19.8, $P=0.003$). Additionally, for every one-unit increase in HDL level, there was an 11% decrease in AIS risk (adjusted OR 0.89; 95% CI 0.85–0.94, $P=0.000$). Furthermore, NRON expression exhibited a negative correlation with AIS risk, indicating that each increment of one unit in the relative circulating NRON was associated with a 30% decrease in AIS risk (adjusted OR 0.70; 95% CI 0.55–0.89, $P=0.004$) (Table 4).

Discussion

This study revealed a significant decrease in circulating NRON lncRNA within 24 h after stroke in AIS patients compared to controls. Logistic regression analysis further confirmed a significant negative association between NRON levels and the risk of AIS. However, no significant difference in SNHG11 expression levels was detected between cases and controls.

Ischemic stroke is associated with high mortality rates. Understanding the genetic factors and molecular mechanisms underlying stroke pathophysiology is crucial for developing effective diagnostic and therapeutic strategies. LncRNAs have emerged as potential regulators of gene expression and key players in various diseases, including stroke. The study suggests that NRON may play a role in the pathogenesis of ischemic stroke and could potentially serve as a biomarker for the disease. The findings contribute to expanding our knowledge of the molecular mechanisms involved in stroke and may have implications for the development of novel therapeutic approaches targeting lncRNAs.

NRON expression has been studied in various neurodegenerative diseases, including schizophrenia and multiple sclerosis [22]. Previous research has reported NRON downregulation in multiple sclerosis patients

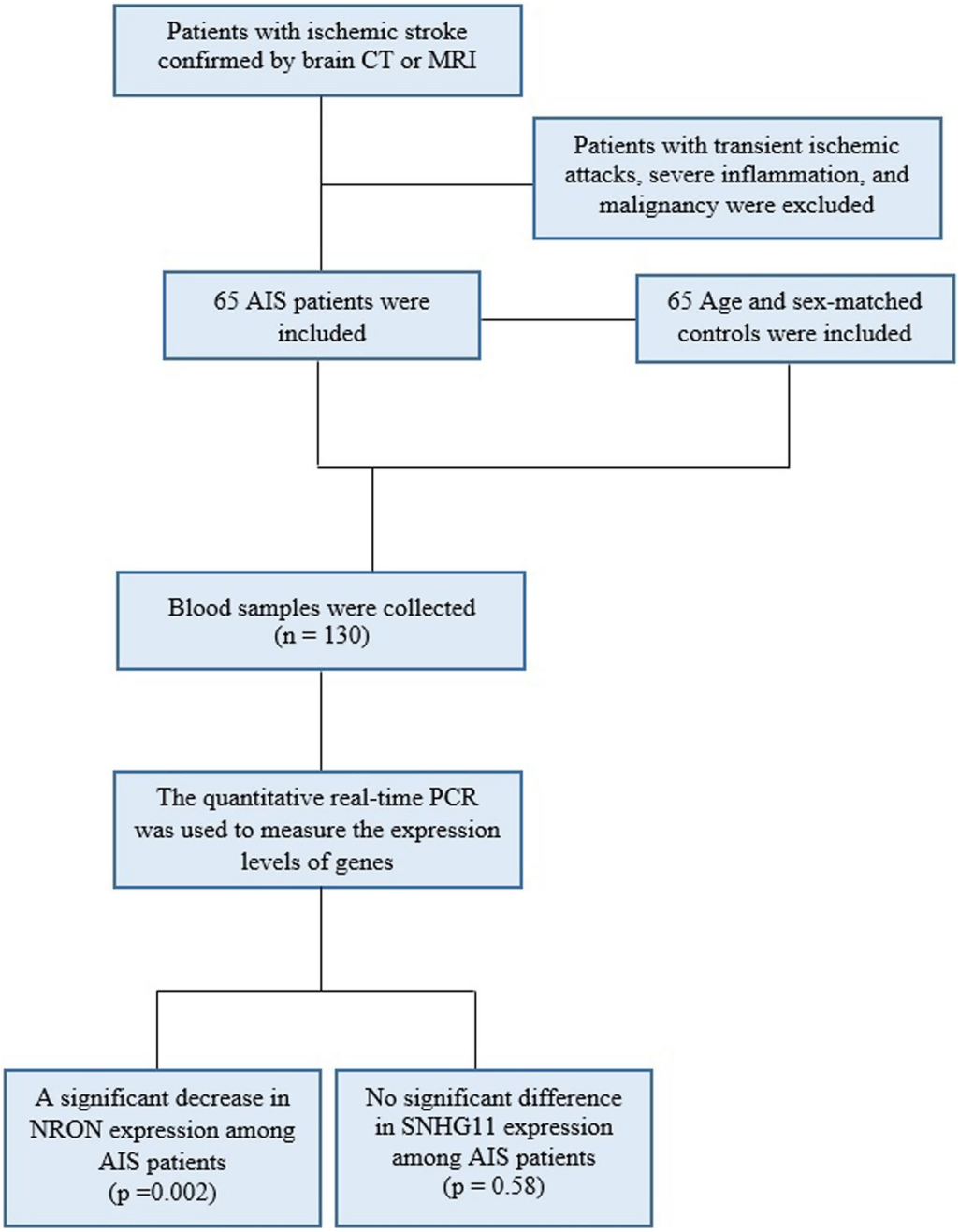

Fig. 1 Flowchart for patient and control enrollment

compared to controls [10]. Interestingly, no considerable difference in circulating NRON levels was observed between patients with schizophrenia and controls [23].

The downregulation of NRON has been reported in hepatocellular carcinoma (HCC) [24] and breast cancer tissues [25], where its overexpression demonstrated inhibitory effects on tumor growth and metastasis. Conversely, some studies have suggested that NRON inhibition could be a potential therapeutic target for diseases such as heart failure [11].

NRON acts as a lncRNA repressor of NFAT, a family of transcription factors with crucial roles in the immune and inflammatory responses [8]. The NFAT/Ca^{2+} calmodulin pathway has been implicated in various neurodegenerative diseases and injuries to the central nervous system [26]. The hyperactivation of calcineurin/NFAT4

Table 2 Main characteristics of study subjects

Demographic and clinical variables	Cases (n = 65)	Controls (n = 65)	*P*-value
Age (years)	63.83 ± 1.8	64.45 ± 1.7	0.806[a]
Sex (Male/Female)	44/21	44/21	1.000
BMI (kg/m^2)	26.42 ± 0.64	25.91 ± 0.48	0.528[a]
Vascular risk factors, *n* (%)			
Smoking	10 (15.4)	8 (12.3)	0.613[b]
Hypertension	35 (53.8)	19 (29.2)	0.005[b]
Diabetes	23 (35.4)	8 (12.3)	0.003[b]
IHD	21 (32.3)	11 (16.9)	0.045[b]
Hyperlipidemia	21 (32.3)	18 (27.7)	0.566[b]
Laboratory findings			
TG, mg/dL	124.63 ± 6.6	134.26 ± 9.3	0.401[a]
Total cholesterol, mg/dL	161.12 ± 5.2	161.43 ± 3.9	0.936[a]
LDL, mg/dL	97.53 ± 4.3	90.18 ± 3.5	0.193[a]
HDL, mg/dL	33.84 ± 0.8	43.55 ± 1.5	0.001[a]
BUN, mg/dL	16.47 ± 0.59	16.12 ± 0.66	0.697[a]
Creatinine, mg/dL	1.21 ± 0.03	1.19 ± 0.04	0.776[a]
Relative *NRON* expression	0.83 ± 0.21	1.89 ± 0.25	0.002[a]
Relative *SNHG11* expression	0.14 ± 0.03	0.18 ± 0.05	0.58[a]
Main characteristics of IS patients			
Types of strokes, *n* (%)			
Large-artery atherosclerosis	33 (50.80)		
Small-vessel occlusion	32 (49.20)		
NIHSS at admission, *n* (%)			
≤ 6	25 (38.5)		
≥ 7	40 (61.5)		
mRS at admission, *n* (%)			
0–2	17 (26.2)		
3–6	48 (73.8)		
mRS at 6 months, *n* (%)			
0–2	31 (47.7)		
3–6	34 (52.3)		
mRS at 3 months, *n* (%)			
0–2	29 (44.6)		
3–6	36 (55.4)		

Data were shown as mean ± SEM or as *n* (%). a Independent two-sample *T* test, b Chi-square Test. $P < 0.05$ was considered statistically significant for all tests

BMI body mass index; *IHD* Ischemic heart disease; *TG* triglyceride; *LDL* low-density lipoprotein; *HDL* high-density lipoprotein; *BUN* blood urea nitrogen; *NIHSS* National Institutes of Health Stroke Scale; *mRS* modified Rankin scale.

signaling in astrocytes leads to neuroinflammation, synapse dysfunction, and excitotoxicity. In stroke pathophysiology, involving neuroinflammation, glutamate toxicity, synaptic plasticity impairment, and astrocyte activation, NRON downregulation may contribute to neuroinflammation through NFAT activation [27, 28]. While our results suggested a possible protective role of NRON expression against AIS in the Iranian population, the correlation between NRON levels and stroke severity was not significant, which is most likely due to a small sample size. Additional research with larger sample sizes is needed to confirm these findings and elucidate the specific downstream targets and signaling pathways involved in NRON-mediated regulation of neuroinflammation in stroke. Understanding these mechanisms could potentially open new avenues for therapeutic interventions targeting NRON-NFAT signaling to modulate neuroinflammatory responses and mitigate the detrimental effects of stroke.

No significant differences in NRON expression were found between different types of atherosclerotic ischemic stroke (LAA vs. SVD). Additionally, SNHG11 expression

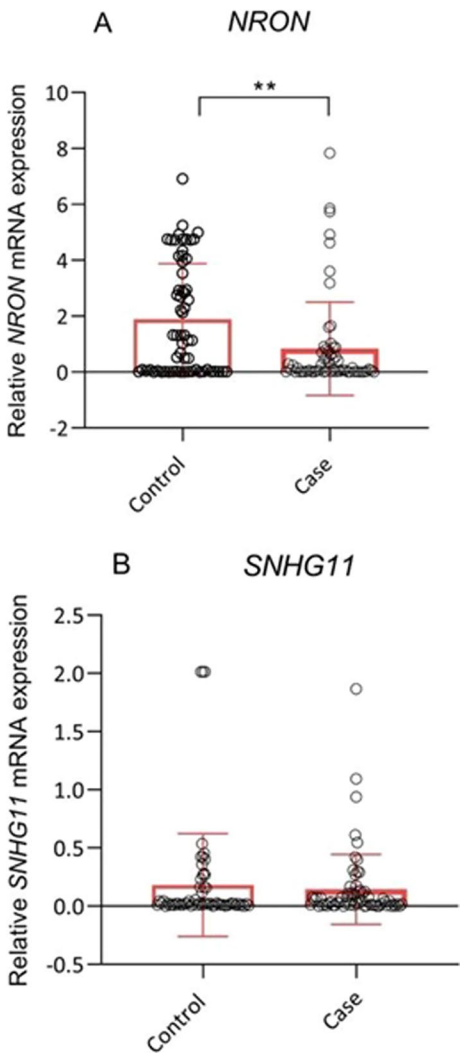

Fig. 2 The expression levels of *NRON* (**A**) and *SNHG11* **B** in peripheral blood

Table 3 Association of *NRON* and *SNHG11* Levels with Clinical Parameters in IS Patients

Variables	AIS patients	Relative *NRON* expression		Relative *SNHG11* expression	
	n = 65	Mean ± SD	*P* value	Mean ± SD	*P* value
Sex					
Male	44	0.63 ± 0.2	0.16	0.09 ± 0.03	0.06
Female	21	1.27 ± 0.51		0.24 ± 0.09	
BMI (kg/m²)					
< 24	17	0.72 ± 1.24	0.53	1.46 ± 0.78	0.90
≥ 24	48	0.97 ± 1.45		1.49 ± 0.88	
Smoking					
Yes	10	0.29 ± 1.47	0.80	0.08 ± 0.04	0.50
No	55	0.96 ± 0.57		0.15 ± 0.04	
Hypertension					
Yes	35	1.12 ± 0.36	0.14	0.15 ± 0.05	0.67
No	30	0.49 ± 0.17		0.12 ± 0.04	
Diabetes					
Yes	23	1.01 ± 0.45	0.54	0.13 ± 0.03	0.80
No	42	0.73 ± 0.22		0.15 ± 0.05	
Hyperlipidemia					
Yes	21	1.34 ± 0.52	0.10	0.12 ± 0.03	0.67
No	44	0.59 ± 0.19		0.15 ± 0.05	
Ischemic heart disease					
Yes	21	0.65 ± 0.30	0.54	0.14 ± 0.05	0.99
No	44	0.92 ± 0.28		0.14 ± 0.04	
NIHSS (admission)					
≤ 6	25	0.77 ± 0.26	0.82	0.17 ± 0.05	0.52
≥ 7	40	0.87 ± 0.31		0.12 ± 0.04	
mRS (admission)					
0–2	17	0.83 ± 0.34	0.97	0.11 ± 0.04	0.65
3–6	48	0.84 ± 0.27		0.15 ± 0.04	
mRS [3 months]					
0–2	29	0.82 ± 0.27	0.97	0.13 ± 0.04	0.86
3–6	36	0.83 ± 0.32		0.15 ± 0.05	
mRS (6 months)					
0–2	31	0.97 ± 0.30	0.52	0.13 ± 0.03	0.76
3–6	34	0.69 ± 0.30		0.15 ± 0.06	
Type of AIS					
LAA	33	0.75 ± 0.29	0.71	0.10 ± 0.02	0.26
SVD	32	0.91 ± 0.31		0.18 ± 0.06	

Data are mean ± SEM. The data were analyzed using Student's *t*-test

BMI body mass index; *NIHSS* National Institutes of Health Stroke Scale; *mRS* modified Rankin Scale; *AIS* atherosclerotic ischemic strokes; *LAA* large artery atherosclerosis; *SVD* small-vessel diseases.

did not show significant differences between AIS patients and controls, and its expression in neurodegenerative disorders remains unexplored. The in vitro study revealed a significant upregulation of SNHG11 in response to the oxygen–glucose deprivation/re-oxygenation (OGD/R) model, as outlined in reference [29]. This experimental model, simulating ischemic conditions, underscores the potential involvement of SNHG11 in cellular stress responses. Despite these findings, an evaluation of lncRNA SNHG11 expression in patients with neurodegenerative disorders is notably absent from existing literature. Investigating the expression patterns of SNHG11 in patients is imperative for elucidating its role in the context of neurological health and potential implications for neurodegenerative diseases.

Moreover, the existing body of research highlights alterations in circulating SNHG11 in several cancer types, including colorectal cancer [30]. This underscores the multifaceted nature of SNHG11 and its potential relevance across diverse pathological conditions. While its

Table 4 Associations between Expression Level of *NRON* and Risk of AIS

Variables	OR	*p*	AD(OR)	95% CI lower	Upper
Hypertension	0.731	0.131	2.077	0.804	5.36
NRON	−0.355	0.004	0.701	0.552	0.891
Diabetes	1.788	0.003	5.976	1.802	19.812
HDL	−0.108	0.000	0.898	0.852	0.946

After adjusting for different variables such as hypertension, diabetes, ischemic heart disease, and HDL, the logistic regression analysis showed that *NRON* expression was correlated negatively with AIS risk

HDL high density lipoprotein; *AIS* atherosclerotic IS

role in cancers has been explored, its involvement in neurodegenerative disorders remains a critical knowledge gap that warrants thorough investigation.

Future studies should focus on elucidating the specific mechanisms through which SNHG11 operates in neurodegenerative contexts. Additionally, exploring its potential utility as a diagnostic marker or therapeutic target in neurological conditions is crucial. Bridging this gap in understanding may contribute not only to the fundamental knowledge of SNHG11 but also to the development of novel strategies for diagnosis and treatment in neurodegenerative disorders.

In summary, the in vitro findings in the OGD/R model prompt further exploration of SNHG11 in neurodegenerative disorders, positioning it as a potential player in the intricate landscape of cellular stress responses and disease pathogenesis. The study suggests that SNHG11 expression changes locally in brain tissue within the first hours after ischemic stroke, possibly requiring more than 24 h to manifest in peripheral blood.

Conclusion

This pioneering study on NRON and SNHG11 expression levels in IS patients indicates a potential protective role of NRON against AIS in the Iranian population. Larger studies are recommended for robust confirmation of these findings and in-depth exploration of the underlying mechanisms, highlighting the potential of circulating NRON as a biomarker for ischemic stroke.

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Author contributions
NG contributed to study concept and design, acquisition of data, and drafting of manuscript. HM contributed to study concept and design, acquisition of data, analysis and interpretation of data, study supervision, and drafting/revising the manuscript for content. EH contributed to study concept and design, acquisition of data, and drafting/revising the manuscript for content. MR involved in acquisition of data and analysis and interpretation of data. MB contributed to study concept and design and revising the manuscript for content. NK involved in acquisition of data. SSH involved in acquisition of data. ZZ involved in acquisition of data. RT involved in analysis and interpretation of data. AB contributed to study concept and design, acquisition of data, study supervision, and drafting/revising the manuscript for content. All authors read and approved the final manuscript.

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Availability of data and materials
All data are available on reasonable request.

Declarations

Ethics approval and consent to participate
Ethics approval for this study was obtained by the local ethics committee of the Arsenjan Branch, Islamic Azad University, Iran, with the number IR.IAU.A.REC.1399.019. This study follows the ethical standards of the institutional and national research committee and with the Helsinki Declaration or comparable ethical standards. The statement confirming consent was obtained from all participant samples.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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