RESEARCH ARTICLE

Long noncoding RNA GAS5 and miR-137 and two of their genetic polymorphisms contribute to acute ischaemic stroke risk in an Egyptian population

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Abstract

Objectives: To assess the serum expression levels of long non-coding ribonucleic acid growth arrest specific-5 and micro-ribonucleic acid-137, and the different genotypes of long non-coding ribonucleic acid growth arrest specific-5 rs2067079 (C>T) and micro-ribonucleic acid-137 rs1625579 (T>G) in acute ischaemic stroke patients.

Methods: The case-control study was conducted at Cairo University, Cairo, Egypt, from January to August 2020, and comprised adult acute ischaemic stroke patients of either gender selected from the stroke unit of the Neurology Department at Kasr Alainy Hospital of Cairo University. Healthy individuals matched for age and gender were enrolled as controls. Quantitative real-time polymerase chain reaction was used to quantify serum expression levels of long non-coding ribonucleic acid growth arrest specific-5 and micro-ribonucleic acid-137, and to genotype long non-coding ribonucleic acid lncRNA growth arrest specific-5 rs2067079 and micro-ribonucleic acid-137 rs1625579 using TaqMan allelic discrimination. Data was analysed using SPSS 22.

Results: Of the 100 subjects, 50(50%) were patients; 34(68%) males and 16(32%) females with mean age 60.4±10.0 years. The remaining 50(50%) were controls; 28(56%) males and 22(44%) females with mean age 56.9±12.2 years ($p>0.05$). The patients had more smokers, more hypertensives and more diabetics than the controls ($p<0.05$). Long non-coding ribonucleic acid growth arrest specific-5 expression levels were significantly increased, while micro-ribonucleic acid-137 expression levels were significantly reduced among the patients ($p<0.05$). Acute ischaemic stroke risk was significantly higher in patients with growth arrest specific-5 rs2067079 (C>T) recessive model (homozygous minor TT genotype), while micro-ribonucleic acid-137 rs1625579 (T>G) was protective against acute ischaemic stroke in allelic (G minor allele), codominant (GT genotype), dominant (GT+GG), and over-dominant (GT genotype) models ($p<0.05$).

Conclusion: Long non-coding ribonucleic acid growth arrest specific-5 rs2067079 and micro-ribonucleic acid-137 rs1625579 may act as novel genetic markers of acute ischaemic stroke risk.

Keywords: RNA, Polymorphism, Nucleotide, Alleles, Genetic, Ischemic stroke, Transcriptase polymerase, MicroRNAs, Genotype. **DOI: 10.47391/JPMA.EGY-S4-37**

Introduction

Acute ischaemic stroke (AIS) contributes a significant proportion to global mortality and long-term disability.[1] It is believed to be a complex illness that results from both hereditary and environmental factors.[2] Identifying the genetic variants that predispose one to AIS would enable early screening of high-risk individuals and a better understanding of the disease's pathophysiology.

Long non-coding ribonucleic acid (lncRNAs), which are RNA transcripts that do not code for proteins and have a length >200 nucleotides, were observed to be dysregulated in several human disorders, including cancers, neurodegenerative diseases and cardiovascular diseases.[3-5]

During AIS, lncRNA growth arrest specific-5 (GAS5) has been linked to neuronal cell death.[6] Also, lncRNA GAS5 neuronal expression was found to be increased in AIS experimental models in rats.[7] As a result, lncRNA GAS5 is believed to contribute to the pathophysiology of AIS.

Micro-ribonucleic acid (miRNAs) are defined as short non-coding, single-stranded RNAs having length of around 22 nucleotides, regulating post-transcriptional gene expression. MiRNAs have been correlated with the pathophysiology of multiple human illnesses.[8]

One miRNA that is abundantly expressed in the brain is miR-137, and dysregulation in its expression has been linked to the development of AIS. During AIS and through inhibiting the Proto-oncogene c-Src (Src) -mediated mitogen-activated protein kinase (MAPK) signalling

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pathway, miRNA-137 has been shown to protect neurons from inflammation, oxidative stress and apoptosis.[9]

In addition, miR-137 has been proposed as a downstream target of lncRNA GAS5 in AIS, where the latter has been demonstrated to compete with miR-137 for the control of Notch1 signalling pathway.[10]

Single nucleotide polymorphisms (SNPs) are the most common deoxyribonucleic acid (DNA) sequence variations in the human genome that can genotypically distinguish between individuals. In genetic association studies, SNPs are often employed as markers to evaluate their effect on the risk of development and pathogenesis of complex diseases.[11]

SNP rs2067079 in the lncRNA GAS5 gene has been linked to lncRNA GAS5 transcription and secondary structure stability, which suggest a possible role of this SNP in AIS lncRNA GAS5 expression dysregulation.[12] Furthermore, the miR-137 gene's SNP rs1625579 has been demonstrated to influence miR-137 production levels.[13]

The current study was planned to assess the serum expression of the two non-coding RNAs (ncRNAs), miR-137 and lncRNA GAS5, and to genotype two of their SNPs, the lncRNA GAS5 rs2067079 and the miR-137 rs1625579, in AIS patients to evaluate their potential role in AIS risk and pathogenesis.

Patients and Methods

The case-control study was conducted at Cairo University, Cairo, Egypt, from January to August 2020. After approval from the institutional ethics review committee, the sample size was calculated using Epi-Info 3.01[14] with confidence level 95%, power of study 80% and based on previous findings[15]. The sample was raised from among adult AIS patients of either gender selected from the stroke unit of the Neurology Department at Kasr Alainy Hospital of Cairo University through non-probability convenient sampling technique. A computed tomography (CT) brain scan was used for AIS diagnosis. Pregnant or nursing women, cancer patients, those with concomitant infection, and those on long-term steroid treatment were excluded. Healthy individuals matched for age and gender without clinical indications or a family history of AIS were enrolled as controls from the same hospital. Written informed consent was obtained from all the participants.

SNPs were selected based on the following criteria: global minor allele frequency (MAF) >0.1; SNPs previously reported to have a functional relationship to their gene products; and SNPs with previously reported correlation with neurological diseases.

Using the QIAamp DNA MiniKit (Qiagen, Valencia, CA, United States), according to the manufacturer's protocol, genomic DNA was extracted from whole ethylenediaminetetraacetic acid (EDTA) blood samples from each participant. The NanoDrop® (ND)-1000 spectrophotometer (NanoDrop Technologies, Inc. Wilmington, USA) was utilised to evaluate DNA quantitation and purity.

Genotyping was done using real-time polymerase chain reaction (RT-PCR) with TaqMan allelic discrimination assay (Applied Biosystems, USA) with a predesigned primer/probe sets for the 6 genotypes (Applied Biosystems, USA) as previously explained.[16] DNA amplification was done in a total volume of 25µl containing 12.5µl TaqMan master mix, 1.25 primer/probe, 1µl DNA (~100ng), and 10.25 water by a Rotor Gene Q RT-PCR system (Qiagen, Valencia, CA, USA). Following a denaturation period of 10m at 95°C, 45 cycles at 92°C for 15s then at 60°C for 90s for annealing and extension were performed and fluorescence was assessed at the end of every cycle and at the endpoint.

RT-PCR was used to quantify serum lncRNA GAS5 and miR-137 expression levels. First, total RNA was extracted from haemolysis-free serum utilising miRNeasy mini-kit (Qiagen, Valencia, CA, USA) complying with the manufacturer's guidelines. Then, by using the mi Script II RT kit (Qiagen, Valencia, CA, USA), reverse transcription of total RNA into complementary DNA was carried out in a final volume of 20µl RT reaction as per the manufacturer's protocols. The expression levels of lncRNA GAS5 and miR-137 were determined by RT-PCR using mi Script SYBR® Green PCR kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocols. RT-PCR was performed using a 20µL final volume reaction mixture as previously explained.[17] A Rotor Gene Q System was used to perform RT-PCR. The reaction conditions were 95°C for 15m then a total of 40 cycles of 95°C for 15m, 55°C for 30s and 70°C for 30s for miR-137, and 95°C for 15m then a total of 45 cycles of 95°C for 15m, and 60°C for 60s for lncRNA GAS5.

Melting curve analyses were done after the completion of the PCR cycles to confirm the precise production of the anticipated PCR product. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the internal reference of lncRNA GAS5 and the housekeeping miScript PCR control and miRNA small nucleolar RNA, C/D Box 68 (SNORD68) were used as the internal reference of miR-137. Gene expression relative to the internal control ($2^{-\Delta Ct}$) was estimated, and then the fold change in lncRNA GAS5 or miR-137 expression was estimated using the equation $2^{-\Delta\Delta Ct}$.[18]

Data was analysed using SPSS 22. Quantitative data was

expressed as mean and standard deviation, or median and range, as appropriate. Categorical data was expressed as frequencies and percentages. Categorical data was compared using chi-square test. GAS5 and miR-137 data did not follow a normal distribution, so Mann-Whitney U tests or Kruskal-Wallis tests were used to compare them, as needed. Logistic regression analysis was done to identify the best predictors of AIS risk. Receiver operating characteristic (ROC) curve was generated with the area under curve (AUC) analysis to detect the best cut-off value of identification markers. P<0.05 was considered statistically significant.

Results

Of the 100 subjects, 50(50%) were patients; 34(68%) males and 16(32%) females with mean age 60.4±10.0 years. The remaining 50(50%) were controls; 28(56%) males and 22(44%) females with mean age 56.9±12.2 years (p>0.05). The patients had more smokers, more hypertensives and more diabetics than the controls (p<0.05) (Table 1).

The lncRNA GAS5 expression levels were significantly increased, while miR-137 expression levels were significantly reduced among the patients (Table 2). At a cut-off value of >2.11 (fold), ROC curve analysis showed that lncRNA GAS5 distinguished AIS patients from healthy controls (AUC=0.92, p<0.001) with a sensitivity 92% and specificity 100%. MiR-137 additionally distinguished AIS cases from healthy controls (AUC=0.833, p<0.001) with sensitivity 82% and specificity 100% at a cut-off value of <0.87 (fold) (Figure 1, Table 3).

The distribution of rs2067079 (C/T) and rs1625579 (G/T) genotypes in both the patients and the controls differed from in terms of Hardy-Weinberg equilibrium (HWE), but the difference was not significant (Table 4).

The MAF of both SNPs in the control group was >0.1 (G=0.23 for rs1625579 and T=0.26 for rs2067079).

Regarding rs2067079, the major T and minor G allele frequencies non-significantly differed between the groups (p>0.05). The minor homozygous TT genotype was associated with a 16.5-fold higher risk of AIS in the recessive model (TT vs CC+CT; p=0.033) with adjustments for age, hypertension (HTN), diabetes mellitus (DM) and smoking. No significant change was found among cases and controls in the codominant, dominant, and over-dominant models (Table 5).

As regards rs1625579, the frequencies of major T and minor G alleles differed significantly between the cases and the controls, with a lower AIS risk in the minor G allele (G vs T; p=0.043). In the codominant model, the heterozygous GT

Table-1: Demographic and clinical data of the study groups.

Parameters		AIS Patients (n=50)	Control group (n=50)	Test	*p-value*
Gender	Male	34(68.0%)	28(56.0%)	1.52	0.216
	Female	16(32.0%)	22(44.0%)		
Age (years)		60.4 ± 10.0	56.9 ± 12.2	1.53	0.127
Smoking	yes	22(44.0%)	8(16.0%)	9.33	0.002*
	No	28(56.0%)	42(84.0%)		
HTN	yes	32(64.0%)	9(18.0%)	21.87	0.000*
	No	18(36.0%)	41(82.0%)		
DM	yes	16(32.0%)	6(12.0%)	5.82	0.016*
	No	34(68.0%)	44(88.0%)		

AIS: Acute ischaemic stroke, HTN: Hypertension, DM: Diabetes mellitus.

Table-2: Serum expression levels of lncRNA GAS5 and miR-137 in AIS patients.

	Fold change (FC)	*p-value*
lncRNA GAS5	11.31(5.65-23.64)	0.000*
miR-137	0.21(0.05-0.89)	0.000*

lncRNA: Long non-coding ribonucleic acid, GAS5: Growth-arrest specific-5, miR-137: MicroRNA-137, AIS: Acute ischaemic stroke.
*Significant. Values are presented as median (25%–75% percentiles) and analyzed using Mann–Whitney U test. Expression levels in control group were taken as 1 and changes in AIS patients were expressed as fold-changes.

Table-3: ROC curve analysis of serum lncRNA GAS5 and miR-137 to discriminate AIS group from healthy controls:

	LncRNA GAS5	miR-137
AUC (95% CI)	0.920(0.845-0.995)	0.833(0.733-.0933)
Cut off point	2.11	0.87
Sensitivity	92.0%	82.0%
Specificity	100.0%	100%
PPV	100.0%	100.0%
NPV	92.6%	84.7%
P value	< 0.001*	< 0.001*

*Significant. ROC: Receiver operating characteristic, lncRNA: Long non-coding ribonucleic acid, GAS5: Growth-arrest specific-5, miR-137: MicroRNA-137, AIS: Acute ischaemic stroke, AUC: Area under the curve, CI: Confidence interval, PPV: Ppositive predictive value, NPV: Negative predictive value).

Table-4: Hardy-Weinberg equilibrium for different genotypes of rs2067079 and rs1625579 in control group and AIS groups.

	rs2067079					
	CC	CT	TT	C	T	P value
Control	28	18	4	74	26	0.096
AIS	22	27	1	71	29	0.165
	rs1625579					
	TT	GT	GG	T	G	P value
Control	29	19	2	77	23	0.816
AIS	18	28	4	64	36	0.270

AIS: Acute ischaemic stroke.

Table-5: Genotype and allele frequencies of rs2067079 (C/T) in AIS patients and and controls.

		Controls (n=50)	AIS Patients (n=50)	Crude OR (95% CI)	*p-value*	Adjusted OR (95% CI)	*p-value*
Codominant	**-CC**	28(56.0%)	22(44.0%)	1.00	-	1.00	-
	-CT	18(36.0%)	27(54.0%)	0.52(0.23-1.18)	0.121	0.48(0.16-1.40)	0.180
	-TT	4(8.0%)	1(2.0%)	3.14(0.33-30.16)	0.321	11.4(0.82-158.35)	0.070
CC vs CT vs TT							0.115
Dominant	**CC**	28(56.0%)	22(44.0%)	1.00	-	1.00	-
	CT+TT	22(44.0%)	28(56.0%)	0.61(0.28-1.36)	0.231	0.65(0.24-1.78)	0.407
Recessive	**CC+CT**	46(92.0%)	49(98.0%)	1.00	-	1.00	-
	TT	4(8.0%)	1(2.0%)	4.26(0.46-39.54)	0.202	16.50(1.25-218.49)	0.033*
Over dominant	**CC+ TT**	32(64.0%)	23(46.0%)	1.00	-	1.00	-
	CT	18(36.0%)	27(54.0%)	0.48(0.22-1.07)	0.072	0.38(0.14-1.09)	0.073
Alleles	**C**	74(0.74)	71(0.71)	1.00	0.63		
	T	26(0.26)	29(0.29)	1.16(0.62-2.16)			

*Significant. AIS: Acute ischaemic stroke, CI: Confidence interval, OR: Odds ratio, AOR: Adjusted OR.

Table-6: Genotype and allele frequencies of rs1625579 (G/T) in AIS patients and controls.

		Controls (n=50)	AIS Patients (n=50)	Crude OR (95% CI)	*p-value*	Adjusted OR (95% CI)	*p-value*
Codominant	**-TT**	29(58.0%)	18(36.0%)	1.00	-	1.00	-
	-GT	19(38.0%)	28(56.0%)	0.42(0.18-0.96)	0.041*	0.19(0.05-0.61)	0.006*
	-GG	2(4.0%)	4(8.0%)	0.31(0.51-1.87)	0.202	0.61(0.06-5.67)	0.661
TT vs GT vs GG							0.083
Dominant	**TT**	29(58.0%)	18(36.0%)	1.00	-	1.00	-
	GT + GG	21(42.0%)	32(64.0%)	0.41(0.18-0.91)	0.029*	0.22(0.07-0.68)	0.008*
Recessive	**TT+ GT**	48(96.0%)	46(92.0%)	1.00	-	1.00	-
	GG	2(4.0%)	4(88.0%)	0.48(0.08-2.74)	0.409	1.19(0.14-10.18)	0.869
Over dominant	**TT+ GG**	31(62.0%)	22(44.0%)	-	1.00	1.00	-
	GT	19(38.0%)	28(56.0%)	0.48(0.22-1.07)	0.073	0.19(0.06-0.63)	0.006*
Alleles	**T**	77(0.77)	64(0.64)	1.00			
	G	23(0.23)	36(0.36)	0.53(0.28-0.98)	0.043*		

*Significant. AIS: Acute ischaemic stroke, CI: Confidence interval, OR: Odds ratio, AOR: Adjusted OR.

Table-7: Univariate logistic regression analysis for identifying risk factors of AIS.

Variables	B	SE	OR	(95% CI)	*p-value*
miR-137	-1.08	0.53	0.34	(0.12-0.96)	0.041*
lncRNA GAS5	1.41	0.47	4.09	(1.61-10.38)	0.003*
rs1625579					
GT+GG vs TT	-1.37	51	0.25	(0.09-0.69)	0.008*
rs2067079					
TT vs CC+CT	2.08	1.18	7.99	(0.78-80.91)	0.078

Adjusted by age, hypertension (HTN) and diabetes mellitus (DM).
AIS: Acute ischaemic stroke, B: Logistic regression coefficient, SE: Standard error, OR: Odds ratio (exponential B), CI: Confidence interval.

Table-8: Multivariate logistic regression analysis for identifying risk factors of AIS.

Variables	B	SE	OR	(95% CI)	*p-value*
miR-137	-23.55	11.45	0.00	(0.12-0.96)	0.040*
GAS5	22.36	10.64	4.09	(4.46-59.6)	0.036*
rs1625579					
GT+GG vs TT	0.64	2.87	1.90	(0.01-531.81)	0.823

Adjusted by age, hypertension (HTN) and diabetes mellitus (DM).
AIS: Acute ischaemic stroke, B: Logistic regression coefficient, SE: Standard error, OR: Odds ratio (exponential B), CI: Confidence interval, GAS5: Growth-arrest specific-5.

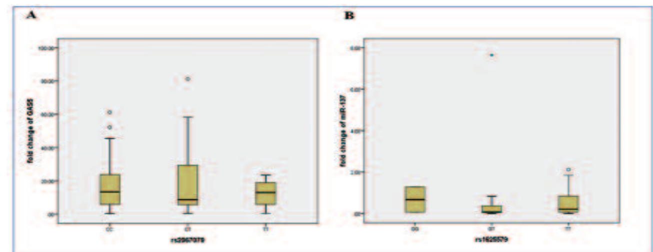

Figure 1: ROC curve analysis of serum miR-137 and lncRNA GAS5 to discriminate between AIS group healthy controls. (A) ROC curve analysis of serum lncRNA GAS5. (B) ROC curve analysis of miR-137.
ROC: ROC: Receiver operating characteristic, lncRNA: Long non-coding ribonucleic acid, GAS5: Growth-arrest specific-5, miR-137: MicroRNA-137, AIS: Acute ischaemic stroke,

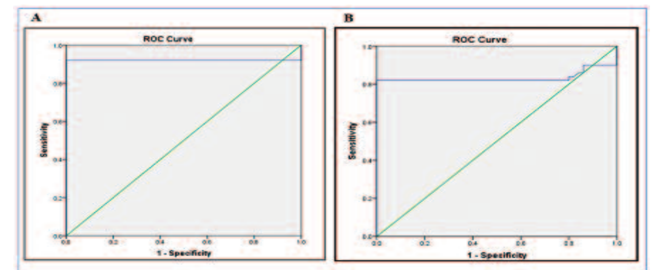

Figure 2: (A) Serum lncRNA GAS5 expression levels in AIS patients with different rs2067079 genotypes. (B) Serum miR-137 expression levels in AIS patients with different rs1625579 genotypes.
lncRNA: Long non-coding ribonucleic acid, GAS5: Growth-arrest specific-5, AIS: Acute ischaemic stroke, miR-137: MicroRNA-137,

genotype significantly reduced AIS risk (GT vs TT; p=0.006), adjusted for age, HTN, DM and smoking. In addition, the dominant model showed a decreased AIS risk in the minor genotypes (GT+GG) compared to the major homozygous genotype (TT) (GT+TT vs TT; p=0.008) after adjustments for age, HTN, DM and smoking. In the over-dominant model, the GT heterozygous genotype was associated with a significant lower AIS risk (GT vs TT+GG; p=0.006) adjusted for age, HTN, DM and smoking (Table 6).

Serum lncRNA GAS5 expression levels in AIS patients did not significantly differ in various rs2067079 genotypes (p=0.954). Also, the expression levels of miR-137 in AIS cases' serum non-significantly differed among different rs1625579 genotypes (p=0.503) (Figure 2).

Univariate analysis for each predictor alone showed that serum miR-137 levels and rs1625579 GT+GG (dominant model) were significant negative predictors of AIS risk (p=0.041 and p=0.008, respectively). Serum GAS5 levels were significant positive predictors of AIS (p=0.003) (Table 7).

In multivariate analysis, serum miR-137 and lncRNA GAS5 levels were confirmed to be significant negative and positive predictors of AIS risk, respectively, (Table 8).

Discussion

Globally, AIS is considered a major cause of mortality and irreversible disability.[1] Despite extensive research to uncover the risk factors and pathophysiological mechanisms of AIS, the precise genetic components remain incompletely understood.[19]

Uncovering the genetic predisposition to AIS may help to identify high-risk individuals and discover novel therapeutic targets, providing a better prognosis and promoting recovery after AIS.[20]

The ncRNAs are emerging as vital regulators of central nervous system (CNS) biological functions and pathological events, like apoptosis and inflammation.[21] Therefore, the current study investigated two ncRNAs, lncRNA GAS5 and miR-137, and two of their genetic variants (rs2067079 for GAS5 and rs1625579 for miR-137) for a possible correlation with AIS.

LncRNA GAS5 is recognised to control cell survival negatively, causing a variety of cell types to arrest and/or undergo apoptosis.[22] The current results showed significantly higher serum expression levels of lncRNA GAS5 in AIS cases than in the control group.

Similar results were reported by Wang et al. in 2019 as lncRNA GAS5 was highly expressed and contributed to apoptosis and inflammatory injury in mice who underwent middle cerebral artery occlusion (MCAO), and in cells treated with oxygen glucose deprivation (OGD) in vitro.[23]

Despite the fact that the precise molecular pathways by which lncRNA GAS5 participates in the pathophysiology of AIS are still not fully understood, prior studies have suggested that lncRNA GAS5 may function in AIS as a competitive endogenous RNA (ceRNA) for certain miRNAs, like miRNA sponge. Zhou et al. in 2020 suggested that in hypoxia conditions, lncRNA GAS5 promotes neuronal apoptosis via sponging miR-221 which causes up-regulation of p53 up-regulated modulator of apoptosis/ c-jun N-terminal kinase/ histone family member X (PUMA/JNK/H2AX) signalling, a pathway linked to brain ischaemic injury.[24] In addition, lncRNA GAS5 served as a ceRNA for miR-9, increasing the forkhead box O (FOXO3) expression, a transcription factor linked to cerebral infarction and cell death in AIS.[23]

Recently, miR-137 has been suggested to protect neurons in AIS. Zhang et al. in 2020 reported that miR-137 over-expression decreased apoptosis and alleviated inflammation in MCAO rats, which showed a lower infarct size and an enhanced neurological function score. They suggested that miR-137 improved AIS neuronal injury through blocking the Janus protein-tyrosine kinases/Signal transducer and activator of transcription 1 (JAK1/STAT1) pathway.[25] Moreover, miR-137 may reduce AIS damage via inhibition of the Src/MAPK pathway which lowers oxidative stress and inflammation.[9]

Some studies have suggested that miR-137 may act as a downstream target of lncRNA GAS5. [10, 26]

In the present study, both lncRNA GAS5 and miR-137 serum expression levels significantly discriminated AIS cases from controls with high accuracy. Additionally, the study demonstrated that the serum lncRNA GAS5 and miR-137 levels may be used as positive and negative indicators, respectively, of AIS susceptibility.

Currently, much of the research is aimed at finding genetic variations that affect the incidence and pathogenesis of prevalent multifactorial polygenic disorders.[27] SNPs are at the forefront of such studies because they are the most prevalent genetic variations in humans.[28]

According to Guo et al. in 2017, SNP rs2067079 (C>T) in the lncRNA GAS5 gene may affect the stability as well as the secondary structure of lncRNA GAS5.[29] In addition, Li et al. in 2020 demonstrated that the same SNP may be linked to a higher risk of ischaemic heart disease (IHD).[30] Both AIS and IHD have many common risk factors and underlying pathophysiological events.[31] Moreover, miR-137 SNP rs1625579 (T>G) has been found to influence the miR-137

expression in the brain.[13] Together, these findings suggest that rs2067079 (C>T) and rs1625579 (T>G) may contribute to AIS risk and pathogenesis.

Interestingly, the current study revealed a significant correlation between rs2067079 (C>T) and a higher AIS risk in the recessive model (TT genotype). Moreover, there was a significant association between rs1625579 (T>G) and a lower AIS risk in allelic (G minor allele), codominant (GT genotype), dominant (GT+GG), and over-dominant (GT genotype) models.

To the best of the researchers' knowledge, no previous study has investigated GAS5 rs2067079 and miR-137 rs1625579 in AIS patients. Therefore, the current study is the first to introduce rs2067079 and rs1625579 as potential biomarkers for genetic susceptibility to AIS and as possible contributors to disease pathogenesis.

Other SNPs in GAS5 have been previously linked to AIS risk, like a correlation between rs145204276 del allele and increased AIS risk among a Chinese population.[32] Similarly, rs2067079 has been reported to influence lncRNA GAS5 expression level in multiple sclerosis (MS) patients with rs2067079 TT genotype.[15] However, the current study showed no similar effect of rs2067079 on lncRNA GAS5 expression which could be attributed to the relatively small sample size and the different clinical subtypes of AIS that the subjects had.

The current study also investigated whether any of the rs1625579 genotypes was associated with dysregulation in miR-137 expression in the serum of AIS patients. However, the results were statistically non-significant, which was in line with literature.[15] In contrast, Guella et al. in 2013 suggested that rs1625579 can modulate miR-137 expression.[13]

The current study also has several limitations, like the subjects having different clinical AIS subtypes, which might render the statistical power insufficient. Besides, the molecular mechanisms by which rs2067079 and rs1625579 may affect AIS pathogenesis were not examined. Also, the study was limited to Egyptian population, and the findings need to be validated in other populations to warrant their generalisation.

Conclusions

The serum levels of lncRNA GAS5 and miR-137 could act as diagnostic biomarkers of AIS, and serum levels of both ncRNAs could positively and negatively predict AIS risk, respectively. The two polymorphisms, lncRNA GAS5 rs2067079 and miR-137 rs1625579, could act as new genetic markers of AIS risk.

Acknowledgement: We are grateful to all the subjects who participated in the study.

Disclaimer: None.

Conflict of Interest: None.

Source of Funding: None.

References

1. Gorelick PB. The global burden of stroke: persistent and disabling. Lancet Neurol 2019;18:417-18. doi: 10.1016/S1474-4422(19)30030-4.
2. Boehme AK, Esenwa C, Elkind MS. Stroke Risk Factors, Genetics, and Prevention. Circ Res 2017;120:472-95. doi: 10.1161/CIRCRESAHA.116.308398.
3. Jiang MC, Ni JJ, Cui WY, Wang BY, Zhuo W. Emerging roles of lncRNA in cancer and therapeutic opportunities. Am J Cancer Res 2019;9:1354-66.
4. Wan P, Su W, Zhuo Y. The Role of Long Noncoding RNAs in Neurodegenerative Diseases. Mol Neurobiol 2017;54:2012-21. doi: 10.1007/s12035-016-9793-6.
5. Farsangi SJ, Rostamzadeh F, Sheikholeslami M, Jafari E, Karimzadeh M. Modulation of the Expression of Long Non-Coding RNAs H19, GAS5, and MIAT by Endurance Exercise in the Hearts of Rats with Myocardial Infarction. Cardiovasc Toxicol 2021;21:162-8. doi: 10.1007/s12012-020-09607-0.
6. Deng Y, Chen D, Gao F, Lv H, Zhang G, Sun X, et al. Silencing of Long Non-coding RNA GAS5 Suppresses Neuron Cell Apoptosis and Nerve Injury in Ischemic Stroke Through Inhibiting DNMT3B-Dependent MAP4K4 Methylation. Transl Stroke Res 2020;11:950-66. doi: 10.1007/s12975-019-00770-3.
7. Li J, Lv H, Che YQ. Long non-coding RNA Gas5 potentiates the effects of microRNA-21 downregulation in response to ischaemic brain injury. Neuroscience 2020;437:87-97. doi: 10.1016/j.neuroscience.2020.01.014.
8. Paul P, Chakraborty A, Sarkar D, Langthasa M, Rahman M, Bari M, et al. Interplay between miRNAs and human diseases. J Cell Physiol 2018;233:2007-18. doi: 10.1002/jcp.25854.
9. Tian R, Wu B, Fu C, Guo K. miR-137 prevents inflammatory response, oxidative stress, neuronal injury and cognitive impairment via blockade of Src-mediated MAPK signaling pathway in ischemic stroke. Aging (Albany NY) 2020;12:10873-95. doi: 10.18632/aging.103301.
10. Chen F, Zhang L, Wang E, Zhang C, Li X. LncRNA GAS5 regulates ischemic stroke as a competing endogenous RNA for miR-137 to regulate the Notch1 signaling pathway. Biochem Biophys Res Commun 2018;496:184-90. doi: 10.1016/j.bbrc.2018.01.022.
11. Vohra M, Sharma AR, Prabhu B N, Rai PS. SNPs in Sites for DNA Methylation, Transcription Factor Binding, and miRNA Targets Leading to Allele-Specific Gene Expression and Contributing to Complex Disease Risk: A Systematic Review. Public Health Genomics 2020;23:155-70. doi: 10.1159/000510253.
12. Rakhshan A, Esmaeili MH, Kahaei MS, Taheri M, Omrani MD, Noroozi R, et al. A Single Nucleotide Polymorphism in GAS5 lncRNA is Associated with Risk of Bladder Cancer in Iranian Population. Pathol Oncol Res 2020;26:1251-4. doi: 10.1007/s12253-019-00693-2.
13. Guella I, Sequeira A, Rollins B, Morgan L, Torri F, van Erp TG, et al. Analysis of miR-137 expression and rs1625579 in dorsolateral prefrontal cortex. J Psychiatr Res 2013;47:1215-21. doi: 10.1016/j.jpsychires.2013.05.021.
14. Dean AG, Sullivan KM, Soe MM. OpenEpi: Open Source Epidemiologic Statistics for Public Health, Version 3.01. [Online] 2013

[Cited 2023 March 09]. Available from URL: https://www.openepi.com/Menu/OE_Menu.htm

15. Mahmoud RH, Hefzy EM, Shaker OG, Ahmed TI, Abdelghaffar NK, Hassan EA, et al. GAS5 rs2067079 and miR-137 rs1625579 functional SNPs and risk of chronic hepatitis B virus infection among Egyptian patients. Sci Rep 2021;11:20014. doi: 10.1038/s41598-021-99345-2.
16. Senousy MA, Shaker OG, Sayed NH, Fathy N, Kortam MA. LncRNA GAS5 and miR-137 Polymorphisms and Expression are Associated with Multiple Sclerosis Risk: Mechanistic Insights and Potential Clinical Impact. ACS Chem Neurosci 2020;11:1651-60. doi: 10.1021/acschemneuro.0c00150.
17. Fathy N, Kortam MA, Shaker OG, Sayed NH. Long Noncoding RNAs MALAT1 and ANRIL Gene Variants and the Risk of Cerebral Ischemic Stroke: An Association Study. ACS Chem Neurosci 2021;12:1351-62. doi: 10.1021/acschemneuro.0c00822
18. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001;25:402-8. doi: 10.1006/meth.2001.1262.
19. Hachiya T, Hata J, Hirakawa Y, Yoshida D, Furuta Y, Kitazono T, et al. Genome-Wide Polygenic Score and the Risk of Ischemic Stroke in a Prospective Cohort: The Hisayama Study. Stroke 2020;51:759-65. doi: 10.1161/STROKEAHA.119.027520.
20. Kumar A, Misra S, Nair P, Algahtany M. Epigenetics Mechanisms in Ischemic Stroke: A Promising Avenue? J Stroke Cerebrovasc Dis 2021;30:105690. doi: 10.1016/j.jstrokecerebrovasdis.2021.105690.
21. Mehta SL, Chokkalla AK, Vemuganti R. Noncoding RNA crosstalk in brain health and diseases. Neurochem Int 2021;149:105139. doi: 10.1016/j.neuint.2021.105139.
22. Zhou Y, Chen B. GAS5mediated regulation of cell signaling (Review). Mol Med Rep 2020;22:3049-56. doi: 10.3892/mmr.2020.11435.
23. Wang L, Niu Y, He G, Wang J. Down-regulation of lncRNA GAS5 attenuates neuronal cell injury through regulating miR-9/FOXO3 axis in cerebral ischemic stroke. RSC Adv 2019;9:16158-66. doi: 10.1039/c9ra01544b.
24. Zhou XB, Lai LF, Xie GB, Ding C, Xu X, Wang Y. LncRNAGAS5 sponges miRNA-221 to promote neurons apoptosis by up-regulated PUMA under hypoxia condition. Neurol Res 2020;42:8-16. doi: 10.1080/01616412.2019.1672382.
25. Zhang M, Ge DJ, Su Z, Qi B. miR-137 alleviates focal cerebral ischemic injury in rats by regulating JAK1/STAT1 signaling pathway. Hum Exp Toxicol 2020;39:816-27. doi: 10.1177/0960327119897103.
26. Zhang L, Cai Q, Lin S, Chen B, Jia B, Ye R, et al. Qingda granule exerts neuroprotective effects against ischemia/reperfusion-induced cerebral injury via lncRNA GAS5/miR-137 signaling pathway. Int J Med Sci 2021;18:1687-98. doi: 10.7150/ijms.53603.
27. Zou H, Wu LX, Tan L, Shang FF, Zhou HH. Significance of Single-Nucleotide Variants in Long Intergenic Non-protein Coding RNAs. Front Cell Dev Biol 2020;8:e347. doi: 10.3389/fcell.2020.00347.
28. Liu Y, Huang K, Wang Y, Hu E, Wei B, Song Z, et al. Integration of SNP Disease Association, eQTL, and Enrichment Analyses to Identify Risk SNPs and Susceptibility Genes in Chronic Obstructive Pulmonary Disease. Biomed Res Int 2020;2020:e3854196. doi: 10.1155/2020/3854196.
29. Guo Z, Wang Y, Zhao Y, Jin Y, An L, Wu B, et al. Genetic polymorphisms of long non-coding RNA GAS5 predict platinum-based concurrent chemoradiotherapy response in nasopharyngeal carcinoma patients. Oncotarget 2017;8:62286-97. doi: 10.18632/oncotarget.19725.
30. Li H, Liu Y, Huang J, Liu Y, Zhu Y. Association of genetic variants in lncRNA GAS5/miR-21/mTOR axis with risk and prognosis of coronary artery disease among a Chinese population. J Clin Lab Anal 2020;34:e23430. doi: 10.1002/jcla.23430.
31. Dichgans M, Malik R, König IR, Rosand J, Clarke R, Gretarsdottir S, et al. Shared genetic susceptibility to ischemic stroke and coronary artery disease: a genome-wide analysis of common variants. Stroke 2014;45:24-36. doi: 10.1161/STROKEAHA.113.002707.
32. Deng F, Zhu P, Liao C, Li S, Hu X, Wang Y, et al. Genetic Variants of lncRNA GAS5 Contribute to Susceptibility of Ischemic Stroke among Southern Chinese Population. Biomed Res Int 2021;2021:e6634253. doi: 10.1155/2021/6634253.