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Original article

Association between Interleukin-18 promoter polymorphisms and risk of ischemic stroke: A case-control study

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ABSTRACT

Background: Ischemic stroke (IS) is one of the main causes of death worldwide. It is worthy to attempt to identify genes that acts as risk factors for IS for early prediction and primary prevention that may reduce its incidence. Aim of the study was to determine the relation between interleukin-18 (IL-18) (−607 C/A) and (−137 G/C) polymorphisms and the risk of IS in Egyptian patients. In parallel, to analyze these polymorphisms as risk factors for large-vessel versus small-vessel diseases.

Patients and methods: A total of 120 subjects (60 IS patients and 60 healthy controls) were recruited to the study. Genotypic analysis of IL-18 promoter polymorphisms were performed using sequence-specific primers- polymerase chain reaction (SSP-PCR) method.

Results: For IL-18 (−607 C/A) polymorphism, a significant higher risk of IS was related to the AA genotype (odd ratio (OR) = 5.38, $P = 0.004$,) and A allele (OR = 2.07, $P = 0.006$), than in controls. Whereas, for IL-18 (−137 G/C) polymorphism, a significant lower risk of IS was related to the GC genotype (OR = 0.17, $P < 0.001$) and C allele (OR = 0.37, $P < 0.001$) than in controls. Moreover, both polymorphisms did not exhibit any significant differences between large vessel (LV) and small vessel (SV) disease of IS ($P > 0.05$). In addition, the haplotype analysis showed non-significant differences between IS patients and controls ($P > 0.05$).

Conclusion: This study concludes that IL-18 −607AA genotype and A allele may be risk factors to IS, whereas IL-18 −137 GC genotype and C allele may be protective factors against IS in Egyptian population.

1. Introduction

Stroke is the third main cause of death after ischemic heart disease and cancer worldwide, about 5.7 million deaths that mostly occur in middle and low-income countries [1]. In Egypt, there is no accurate survey for the incidence of stroke, but only little studies in Upper Egypt, which demonstrated that it accounts for about 150,000–210,000 per year [2–4]. The American Heart Association reported that about 87% of stroke patients are categorized as ischemic stroke (IS) [5].

IS refers to an episode of neurological dysfunction due to focal, cerebral, spinal or retinal infarction [6]. The etiology of IS is regulated by interaction between multiple risk factors including lifestyle, environmental factors, genetic factors and inflammation [7,8].

Neuroinflammation is a general process of numerous neurodegenerative diseases and may play a key role in the pathogenesis of IS [9]. In a setting of cerebral injury, numerous proinflammatory cytokines are secreted in ischemic region, which stimulate the synthesis of inflammatory molecules that recruit more circulating leukocyte and infiltrate the ischemic region causing the acceleration of inflammatory processes and enlarging the cerebral infarct area [10,11].

Interleukin-18 (IL-18), an IL-1 cytokine superfamily with proinflammatory properties, acts as a stimulator for interferon γ (IFNγ) production [12]. Numerous cells such as Kupffer cells, monocytes, macrophages and dendritic cells can produce IL-18. It acts as a modifier for the immune response through inducing cytokine gene expression and T helper cell differentiation, activating natural killer cells, and serving as a major proinflammatory cytokine in inflammatory and autoimmune diseases [13]. In the central

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nervous system (CNS), microglia, astrocytes and neurons can produce receptors for IL-18, which participated in local inflammation and related with many neurological diseases such as meningitis, Alzheimer's disease and stroke [14].

IL-18 gene is found on chromosome 11q22.2–q22.23, including two promoter single nucleotide polymorphisms (SNPs), −607 C/A (rs1946518) and −137 G/C (rs187238) polymorphisms, which could have effect on the binding of transcription factors and thus modulate IL-18 mRNA expression [15,16]. Previous studies demonstrated that IL-18 genetic variants was related with multiple diseases that express abnormal immune response [15,17–21].

Few epidemiological literatures have assessed the relationship between the IL-18 genetic polymorphisms in the promoter region and the risk of IS, nevertheless, the results were controversial [22–25].

To the greatest of our knowledge, this is the first Egyptian literature investigating the association between the IL-18 promoter polymorphisms and the risk of ischemic stroke in addition to different subtypes. Therefore, the present study was aimed to determine the association between the genetic polymorphisms (−607 C/A) and (−137 G/C) of IL-18 and the risk of IS in an Egyptian patients. Furthermore, to elucidate their role as a possible risk factors for large vessel (LV) against a small vessel (SV) diseases.

2. Material and methods

2.1. Subjects

This was a case-control study consisted of 60 cases with IS recruited from Zagazig University Hospitals, Intensive Care Units (ICUs), Egypt, and were classified into two stroke subtypes (30 patients with large vessel (LV) disease and 30 patients with small vessel (SV) disease) according to the modified Trial of Org 10172 Acute Stroke Treatment (TOAST) criteria [26]. A control group was also recruited comprising 60 clinically healthy subjects without a history of stroke, matched for sex and age with cases, and came to the hospital for regular health checkup during the same time of the study.

Full history was taken from all subjects including hypertension, diabetes mellitus, heart disease, previous stroke, cigarette smoking. Included patients were undergone detailed medical and neurological examination, neuroimaging evidence using electro cardiogram (ECHO), computed tomography (CT), and brain magnetic resonance imaging (MRI) to confirm the diagnosis, and laboratory investigations such as liver function tests, renal function tests, fasting and post-prandial blood sugar levels, lipid profiles, and assessment of body mass index (BMI).

Exclusion criteria were brain tumor, chronic hepatic or renal disease, recurrent strokes, persons who are less than 50 years old, cardiac disease (cardioemobilism), and autoimmune diseases.

The study was approved by the Ethics Committee at the Faculty of Medicine, Zagazig University, Egypt (Code number: ZU-IRB#3316). This work has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in humans.

All participants gave their written informed consent before participation in this study.

2.2. Methods

2.2.1. Sampling

Blood samples (2 mL of venous blood) were collected under aseptic conditions from both patients and healthy controls into EDTA containing tubes, and then stored at −80 °C until DNA extraction. The quality and quantity of DNA was evaluated by 0.7% agarose gel electrophoresis and by UV spectrophotometer, respectively.

2.2.2. Determination of IL-18 genetic polymorphisms

Genomic DNA was extracted from whole blood samples using a QIAamp DNA blood mini kit (QIAGEN GmbH, Hilden, Germany).

The IL-18 (−607 C/A) and (−137 G/C) SNPs were genotyped by sequence-specific primers PCR (SSP-PCR) method [27].

The PCR primer sequences for the IL-18 (−607 C/A) SNP were a common reverse primer (R): 5′-TAA CCT CAT TCA GGA CTT CC-3′, two sequence-specific forward primers (F1): 5′-GTT GCA GAA AGTGTA AAA ATT ATT AC-3′ and (F2): 5′-GTT GCA GAA AGTGTA AAA ATT ATT AA-3′ with a fragment size 196-bp. A control forward primer (CTRL): 5′-CTT TGC TAT CAT TCC AGG AA-3′with a fragment size 301-bp and was used as an internal positive control.

For the IL-18 (−137 G/C) SNP were a common reverse primer (R): 5′-AGGAGG GCA AAATGC ACT GG-3′, two sequence-specific forward primers (F1): 5′-CCC CAA CTT TTA CGG AAG AAAAG-3′and (F2): 5′-CCC CAA CTT TTA CGG AAG AAA AC-3′with a fragment size 261-bp. A control forward primer (CTRL): 5′-CCA ATA GGA CTG ATT ATT CCG CA-3′with a fragment size 446-bp and was used as an internal positive control.

The PCR reaction mixture (20 μL) contains 10 μL master mix (Thermo Scientific, Fermentas), 1 μL of each primer (10 pmol/μL), 4.5 μL deionized water, and 2.5 μL DNA template. Amplification was performed in a thermal cycler (Biometra, Germany) with the following conditions: 94 °C for 3 min; followed by 40 cycles of 20 sec at 94 °C, 20 sec at 50 °C for (−607 C/A) and, 54 °C for (−137 G/C) respectively, 20 sec at 72 °C; and a final extension of 5 min at 72 °C.

The PCR products were screened by 1.5% agarose gel electrophoresis in 1 × TAE buffer and were visualized in a gel documentation system with transilluminator. The size of the amplified product was compared with the 100-bp ladder DNA marker.

2.3. Statistical analysis

All the statistical tests were done using SPSS v. 20 (IBM SPSS Inc., Chicago, IL, USA). Qualitative data were presented as number (N) and percentage (%). Chi-square test ($\chi 2$) test was used to compare between groups. The association between a particular genotype and allele and the IS risk was assessed by odds ratios (ORs) and their 95% confidence intervals (95% CIs). The haplotype analysis was assessed using the Phase program [28]. All tests were two-sided and $P < 0.05$ was considered statistically significant.

3. Results

3.1. General characteristics of the studied population

The demographic profile and clinical data of IS patients and controls are presented in Table 1. A total of 120 subjects were enrolled in this case-control study, including 60 IS Egyptian patients (32 males and 28 females) with the mean age 64.3 ± 8.9 years, and 60 healthy controls (32 males and 28 females) with a mean age 64 ± 7.97 years. These results proposing that the cases features were matched with those of controls ($P > 0.05$).

3.2. Genotypes and alleles frequencies of IL-18 (−607 C/A) and (−137 G/C) polymorphisms in the studied groups

The genotypes and alleles frequencies of the IL-18 (−607 C/A) and (−137 G/C) promoter polymorphisms in IS patients and healthy controls were shown in. Table 2.

Table 1
Demographic data and clinical features of IS patients and controls.

Variable	IS patients (N = 60) N (%)	Controls (N = 60) N (%)	χ^2	*P*
Age, Years				
<65 y	36 (60%)	39 (65%)	0.32	0.57
≥65 y	24 (40%)	21 (35%)		
Sex				
Male	32 (53.3%)	32 (53.3%)	0.0	1.0
Female	28 (46.7%)	28 (46.7%)		
Smoking				
Yes	28 (46.7%)	22 (36.7%)	1.23	0.27
No	32 (53.3%)	38 (63.3%)		
Hypertension				
Yes	38 (63.3%)	30 (50%)	2.17	0.14
No	22 (36.7%)	30 (50%)		
Diabetes				
Yes	31 (51.7%)	32 (53.3%)	0.03	0.85
No	29 (48.3%)	28 (46.7%)		
Dyslipidemia				
Yes	31 (51.7%)	27 (45%)	0.53	0.46
No	29 (48.3%)	33 (55%)		
BMI (kg/m^2)				
<25	21 (35%)	20 (33.3%)	0.04	0.84
≥25	39 (65%)	40 (66.7%)		

BMI: body mass index, kg: kilogram, m: meter.
Data were expressed as number (N) and percentage (%).
P values calculated using the chi-square (χ2) test, *P > 0.05* is considered non-significant.

Table 2
Genotypes and alleles frequencies of IL-18 promoter polymorphisms in IS patients and controls.

	IS patient (N = 60) N (%)	Controls (N = 60) N (%)	OR (95% CI)	*P*
IL-18 −607 C/A genotypes				
CC	5 (8.3%)	14 (23.3%)	1 (Ref.)	
CA	30 (50%)	33 (55%)	2.55 (0.73–9.2)	0.09
AA	25 (41.7%)	13 (21.7%)	5.38 (1.38–22.1)	0.004 *
IL-18 −607 C/A alleles				
C	40 (33.3%)	61 (50.8%)	1 (Ref.)	
A	80 (66.7%)	59 (49.2%)	2.07 (1.19–3.61)	0.006*
IL-18 −137 G/C genotypes				
GG	42 (70%)	19 (31.7%)	1 (Ref.)	
GC	13 (21.7%)	35 (58.3%)	0.17 (0.07–0.42)	<0.001*
CC	5 (8.3%)	6 (10%)	0.38 (0.09–1.63)	0.24
IL-18 −137 G/C alleles				
G	97 (80.8%)	73 (60.8%)	1 (Ref.)	
C	23 (19.2%)	47 (39.2%)	0.37 (0.21–0.66)	<0.001*

Data were expressed as number (N) and percentage (%).
OR: Odds ratio, CI: Confidence interval, Ref.: reference.
* statistically significant P value, *P < 0.05*.

For IL-18 (−607 C/A) SNP, a significant higher in the frequencies of the mutated AA genotype and A allele was observed in IS patients than in controls (OR = 5.38, 95% CI 1.38–22.1, *P* = 0.004, and OR = 2.07, 95% CI 1.19–3.61, *P* = 0.006, respectively) (Table 2; Fig. 1). These results concluded that the IL-18 −607AA genotype and A allele were significantly associated with increased risk of IS in our Egyptian population.

Whereas, for IL-18 (−137 G/C) SNP, a significant lower in the frequencies of the heterozygous GC genotype and C allele was observed in IS patients than in controls (OR = 0.17, 95% CI 0.07–0.42, *P* < 0.001, and OR = 0.37, 95% CI 0.21–0.66, *P* < 0.001, respectively) (Table 2; Fig. 2). These results concluded that the IL-18 −607 GC genotype and C allele were significantly associated with decreased risk of IS in our population.

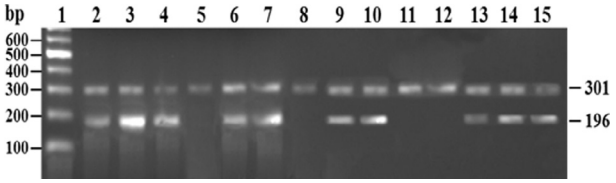

Fig. 1. PCR amplification of IL-18 −607 C/A SNP of Egyptian IS patients and healthy controls with agarose gel electrophoresis. Lane 1: 100 bp DNA ladder; Lanes 2, 3, 6, 7, 14, 15: heterozygous CA genotype; Lanes 4, 5, 10, 11: homozygous wild type CC genotype and Lanes 8, 9, 12, 13: homozygous variant AA genotype.

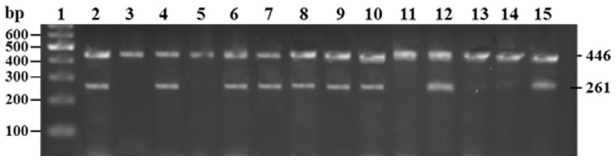

Fig. 2. PCR amplification of IL18 −137 G/C SNP of Egyptian IS patients and healthy controls with agarose gel electrophoresis. Lane 1: 100 bp DNA ladder; Lanes 2, 3, 4, 5, 10, 11, 12, 13: homozygous GG genotype; Lanes 6, 7, 8, 9: heterozygous GC genotype and lanes 14, 15: homozygous CC genotype.

3.3. Genotypes and alleles frequencies of IL-18 (−607 C/A) and (−137 G/C) polymorphisms in IS subtypes

Table 3 showed the genotypes distribution and allele frequencies of both polymorphisms in different IS subtypes (large vessel (LV) and small vessel (SV) patients). Both polymorphisms did not exhibit any significant differences between both patient groups (*P* > 0.05).

3.4. The relationship between the IL18 (−607 C/A) and (−137 G/C) genotypes and the demographical data and clinical features of IS patients

The relation between IL-18 (−607 C/A) and (−137 G/C) polymorphisms and demographic and clinical characteristics of IS patients were listed in Tables 4 and 5, respectively. No statistically significant differences were observed between the different variables and the distribution of the three genotypes of both polymorphisms in IS patients (*P* > 0.05).Table 5.

Table 3
Genotypes and alleles frequencies of IL-18 promoter gene polymorphisms in ischemic stroke subtypes.

	Large vessels IS patients (N = 30) N (%)	Small vessels IS patients (N = 30) N (%)	OR (95% CI)	*P*
IL-18 −607 C/A genotypes				
CC	2 (6.7%)	3 (10%)	1 (Ref.)	
CA	21 (70%)	9 (30%)	3.5 (0.37–37.5)	0.42
AA	7 (23.3%)	18 (60%)	0.58 (0.06–6.4)	0.47
IL-18 −607 C/A alleles				
C	25 (41.7%)	15 (25%)	1 (Ref.)	
A	35 (58.3%)	45 (75%)	1.4 (0.6–3.38)	0.5
IL-18 −137 G/C genotypes				
GG	22 (73.3%)	20 (66.7%)	1 (Ref.)	
GC	6 (20%)	7 (23.3%)	0.78 (0.22–2.71)	0.7
CC	2 (6.7%)	3 (10%)	0.61 (0.09–4.01)	0.6
IL-18 −137 G/C alleles				
G	50 (83.3%)	47 (78.3%)	1 (Ref.)	
C	10 (16.7%)	13 (21.7%)	0.72 (0.29–1.81)	0.5

Data were expressed as number (N) and percentage (%).
OR: Odds ratio, CI: Confidence interval, Ref.: reference.
P > 0.05 is considered non-significant.

Table 4
The association between demographical data and clinical features, and IL-18 (−607 C/A) in IS patients.

Variable	IL-18 −607 C/A Genotypes			P
	CC (n = 5)	CA (n = 30)	AA (n = 25)	
Sex				
Male	2 (40%)	17 (56.7%)	13 (52%)	0.77
Female	3 (60%)	13 (43.3%)	12 (48%)	
Age (years)				
≤65	2 (40%)	16 (53.3%)	18 (72%)	0.23
>65	3 (60%)	14 (46.7%)	7 (28%)	
Hypertension				
No	0	13 (43.3%)	9 (36%)	0.17
yes	5 (100%)	17 (56.7%)	16 (64%)	
Diabetes				
No	3 (60%)	13 (43.3%)	13 (52%)	0.7
yes	2 (40%)	17 (56.7%)	12 (48%)	
Smoker				
No	3 (60%)	12 (40%)	17 (68%)	0.11
yes	2 (40%)	18 (60%)	8 (32%)	
Dyslipidemia				
No	2 (40%)	13 (43.3%)	14 (56%)	0.59
yes	3 (60%)	17 (56.7%)	11 (44%)	
BMI				0.1
≤25	5 (100%)	17 (56.7%)	17 (68%)	
>25	0	13 (43.3%)	8 (32%)	

N: number, BMI: Body mass index.
Data were expressed as number (N) and percentage (%).
P > 0.05 is considered non-significant.

Table 5
The association between demographical, clinical features, and IL-18 (−137 G/C) in IS patients.

Variables	IL-18 −137 G/C Genotypes			P
	GG (n = 42)	GC (n = 13)	CC (n = 5)	
Sex				
male	22 (52.4%)	7 (53.8%)	3 (60%)	0.94
female	20 (47.6%)	6 (46.2%)	2 (40%)	
Age (years)				
≤65	22 (52.4%)	10 (76.9%)	4 (80%)	0.18
>65	20 (47.6%)	3 (23.1%)	1 (20%)	
Hypertension				
No	16 (38.1%)	5 (38.5%)	1 (20%)	0.72
yes	26 (61.9%)	8 (61.5%)	4 (80%)	
Diabetes				
No	20 (47.6%)	6 (46.2%)	3 (60%)	0.85
yes	22 (52.4%)	7 (53.8%)	2 (40%)	
Smoker				
No	23 (54.8%)	6 (46.2%)	3 (60%)	0.82
yes	19 (45.2%)	7 (53.8%)	2 (40%)	
Dyslipidemia				
No	21 (50%)	5 (38.5%)	3 (60%)	0.66
yes	21 (50%)	8 (61.5%)	2 (40%)	
BMI				
≤25	28 (66.7%)	7 (53.8%)	4 (80%)	0.53
>25	14 (33.3%)	6 (46.2%)	1 (20%)	

N: number, BMI: Body mass index.
Data were expressed as number (N) and percentage (%).
P > 0.05 is considered non-significant.

3.5. Haplotype analysis of IL-18 (−607 C/A) and (−137 G/C) polymorphisms

Four haplotypes were detected from these SNPs. The haplotype frequencies were not significantly different between IS patients and controls ($P > 0.05$) (Table 6).

Table 6
Haplotype frequencies of IL-18 promoter polymorphisms in IS patients and controls.

Haplotypes	IS patients	Controls	OR	95% CI	P
$A^{-607}C^{-137}$	27 (22.5%)	19 (15.8%)	1.54	0.77–3.11	0.1
$A^{-607}G^{-137}$	48 (40%)	37 (30.8%)	1.5	0.85–2.62	0.13
$C^{-607}C^{-137}$	8 (6.7%)	15 (12.5%)	0.5	0.19–1.32	0.12
$C^{-607}G^{-137}$	37 (30.8%)	49 (40.8%)	0.65	0.37–1.14	0.1

OR: Odds ratio, CI: Confidence interval, Ref.: reference.
P > 0.05 is considered non-significant.

4. Discussion

IS is a complex vascular process causing death of neuronal cells and ischemic tissue which lead to death of that area of tissue. Its mechanism is complex and poorly defined [29]. Cytokines may play a pivotal role in immune response and regulates the normal homeostatic environment of the CNS [30]. Accumulating evidence suggested that the inflammatory cytokine gene polymorphisms have a major role in the development of the IS [31,32].

IL-18 (−607 C/A) and (−137 G/C) polymorphisms are the most common SNPs in the promoter region, which have effect on the transcription activity of IL-18 gene and modify its expression and production by disrupting the nuclear factor binding sites for the cAMP responsive element binding protein and histone H4 transcription factor (H4TF-1), respectively [33].

In the present study, for IL-18 (−607 C/A), the AA genotype was statistically significantly higher in IS patients than in healthy controls with OR of 5.38. In addition, a risk of having A allele in ischemic stroke patients was 2.07 times more than controls. These results suggested that the mutated AA genotype and A allele may be risk factors for developing IS in our Egyptian population.

These results may be elucidated by Jin et al. [34] who indicated that inflammatory cytokines play an important role in the pathogenesis of tissue injury and cerebral lesion following stroke. IL-18 can stimulate synthesis of inflammatory molecules such as IFN-γ and tumor necrosis factor-α (TNF-α) which may lead to neuroinflammation, and stimulate the atherosclerosis progression through inducing carotid artery sclerosis plaques propagation and instability. Moreover, it is involved in hypoxic ischemic brain damage indirectly by initiating inflammation in the brain or directly through enhancing the cytotoxic activity of immune cells [18,35].

In contrast to our results, several studies demonstrated that IL-18 (−607 CC) wild type and C allele were associated with increased risk of IS when compared with the (AA) genotype in Chinese population [22–24]. However, the results from the studies of Li et al. [36] demonstrated that there was no significant association between IL-18 (−607 C/A) polymorphism and the risk of acute cerebral infarction (ACI), and Zhang et al. [25] who inferred that IL-18 (−607 C/A) polymorphism was significantly associated with the decreased risk of IS.

Regarding IL-18 (−137 G/C) SNP, we demonstrated that a higher frequency of C allele was associated with decreased risk of IS (OR = 0.37, $P < 0.001$). Moreover, the heterozygous GC genotype was significantly decreased in IS patients than in controls (OR = 0.17, $P < 0.001$). These findings revealed that −137 GC genotype besides C allele may be protective factor against IS in our population.

These findings can be clarified by Arimitsu et al. [37] who demonstrated that IL-18 −137 G/G genotype was significantly higher than IL-18 −137 G/C genotype in the group with high lipopolysaccharide (LPS) stimulated IL-18 production by monocytes, and by Giedraitis et al. [15] who showed that the C allele might be associated with relatively lower expression of IL-18. Both results support the hypothesis that lower production of IL-18 may cause decreased inflammation in IS and the susceptibility to the disease.

Consistent with our findings, Li et al. [36] found that the GC genotype was less frequent in ACI patients than in controls, and C allele might be a protective factor against ACI. Conversely, Zhang et al. [22] and Wei et al. [38] found that the GC genotype was associated with increased risk of cerebral infarction. Shi et al. [24] and Zhang et al. [25] also reported that there was no significant association between IL-18 (−137 G/C) gene polymorphism and the risk of IS in Chinese population.

The results of the present study showed that both polymorphisms did not exhibit any significant differences between both patient groups. However, Zhang et al. [22] and Zhang et al. [2] revealed that both IL-18 genetic polymorphisms were significantly correlated with the risk of IS subtypes.

The present study showed no association between both polymorphisms and age, sex, diabetes mellitus, hypertension, smoking, dyslipidemia and BMI in IS risk, which is in agreement with previous studies in a Chinese population [24,39].

Contrarily, Mallat et al. [40], de Nooijer et al. [41] and Bouki et al. [42] observed that the effect of −607A polymorphism was stronger in male subjects and subjects younger than age 65, and weaker in females and individuals older than age 65. The accumulation of environmental factors and phenocopies in elders dilute the effect of gene polymorphism in the pathogenic cascade [25].

In our study, the haplotype investigation revealed that there was non significant difference between IS patients and controls in the haplotype frequencies. Nevertheless, Zhang et al. [22] revealed that patients with the −607 C/−137 G haplotype had significantly increased risk of IS compared to controls.

The differences between our study and previous studies may result from the difference in lifestyle and habits between different populations, the variance in genetic background, sample sizes, and the presence of other inflammatory pathways in a combination with the risk factors affecting the disease.

In conclusion, the current study proposes that the mutant variant AA and A allele of IL-18 (−607 C/A) polymorphism may be risk factors for developing IS, whereas the heterozygous GC genotype and C allele of IL-18 (−137 G/C) polymorphism may be protective factors against IS in our Egyptian population. However, these polymorphisms were not correlated with large and small vessel diseases of IS. These results encourage the hypothesis that genetic markers, which are involved in the inflammatory response, may be related to the pathogenesis of stroke. Our results should be confirmed by further studies with large sample size in different population. Moreover, it is crucial to recognize new genetic and inflammatory biomarkers to predict this disease and guide the treatment at its early onset.

Conflicts of interest

The authors declare that they have no conflict of interest.

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Study limitations

This is a single center study with small sample size due to cost limitations.

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