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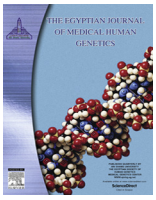

Original article

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 (cardioembolism), 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.