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Research Article

Lack of Association Between Interleukin 6 Gene Promoter Polymorphisms and Aneurysmal Subarachnoid Hemorrhage in Turkish Population

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Summary

Background: The IL6 gene is related to several disease states. Its relation to aneurysm formation and aneurysmal SAH has been studied in the literature, but the reported results have not resulted in definite positive or negative data. In this study we aimed to examine IL6 gene polymorphisms in a Turkish population and further our understanding of the relationship.
Results: We included 120 intracranial aneurysm SAH cases and 120 healthy controls to examine whether there was a genotype difference between these groups in the -174 G > C and -572 G > C promoter polymorphisms located in the IL6 gene. The differences between groups were studied using a chi-squared test. There was no statistical difference between aneurysmal SAH patients and controls for IL6 promoter 174G > C and 572G > C polymorphisms.
Conclusions: IL6 promoter SNPs did not show a correlation with intracranial aneurysm formation and subarachnoid aneurysmal rupture in a Turkish population, but genetic differences between ethnic populations may give different results.

Key words: Intracranial aneurysm, IL6 gene, polymorphism, genotype

Türk Toplumunda İnterlökin 6 Gen Promoter Polimorfizmleri ve Anevrizmal Subaraknoid Kanama Arasındaki İlişki Eksikliği

Özet

Arka plan: IL6 geni bazı hastalık durumları ile ilişkilidir. Literatürde anevrizma oluşumu ve anevrizmal SAK ile ilişkisi çalışılmış fakat bildirilen sonuçlar kesin pozitif veya negative bilgi ile sonuçlanmamıştır. Bu çalışmada IL6 gen polimorfizmlerini bir Türk toplumunda incelemeyi ve ilişki anlayışımızı ilerletmeyi amaçladık.
Sonuçlar: IL6 geninde yerleşmiş -174 G > C ve -572 G > C promotor polimorfizmlerini gruplar arası genotip farklılığı olup olmadığını incelemek için 120 intrakranyal anevrizmal SAK vakası ve 120 sağlıklı kontrolü dahil ettik. Gruplar arası farklılıklar ki-kare testi kullanılarak çalışıldı. Kontroller ile anevrizmal SAK hastaları arasında IL6 promotor 174G > C ve 572G > C polimorfizmleri için istatiksel farklılık yoktu.
Netice: IL6 promotor TNPleri Türk toplumunda intrakranyal anevrizma oluşumu ve subaraknoid anevrizma patlaması ile bağlantı göstermemiştir, fakat etnik toplumlar arasındaki genetik farklılıklar farklı sonuçlar verebilir.

Anahtar Kelimeler: İntrakranyal anevrizma, IL6 geni, polimorfizm, genotip

INTRODUCTION

Subarachnoid hemorrhage (SAH) due to the rupture of an intracranial aneurysm (IA) is a critical neurosurgical emergency and an important public health problem with poor prognosis responsible for 3-11% of all strokes and IA affects 5-10% of the general population[4].

It is known that genetics plays a role in the formation and rupture of IA, with familial aneurysm occurrence as proof of this. To date, however, the literature has not identified any particular gene that causes IA when mutated. Genome-wide association studies and whole genome linkage analysis results have been reported that show chromosomal regions that bear possible causative genes and single nucleotide polymorphisms (SNPs) related to IA formation[4,10,11]. Other studies reported in the literature are mostly based on candidate gene approaches; none have led to any significant findings.

Interleukin 6 (IL6) has important roles in hematopoiesis, immune, and acute phase responses and is produced in response to a number of inflammatory stimuli[9]. Deregulated IL6 production is implicated in the pathology of several disease processes such as multiple myeloma, Kaposi's sarcoma, juvenile chronic arthritis, rheumatoid arthritis, osteoporosis, psoriasis, and atherosclerosis[9].

Studies on the relationship between IL6 gene SNPs changes and aneurysm formation and hemorrhage have been reported, but showed both favorable and unfavorable results in different ethnic populations, in different locations of body vasculature, and different SNPs related to the IL6 gene[1-3,5,6,8].

In this study, we aimed to show an association or lack of association between IL6 SNPs and intracranial aneurysm in a Turkish population.

MATERIAL AND METHODS

Collection of Blood Samples and Isolation of Genomic DNA

This study was approved by the Ondokuz Mayis University Committee of Assessment of Scientific Research (protocol number: 2009/168). Consents were obtained and, blood samples were collected from affected subjects. Total genomic DNA was prepared by isolation of nuclei followed by proteinase sodium dodecyl sulfate lysis and subsequent phenol and chloroform extractions.

Phenotype Assignment of Subjects

All phenotypes were assigned prospectively. Affected status was assigned after confirmation of the presence of an IA based on magnetic resonance angiography (MRA), computed tomography angiography (CTA), or conventional cranial angiogram. In cases where the diagnostic imaging studies were performed at outside institutions, we obtained original images whenever possible and then assigned the phenotype status.

Single Nucleotide Polymorphism Genotyping

The -174 G > C and -572 G > C biallelic polymorphisms in the promoter region of the IL6 gene were assessed according to previously described methods[1,6,9] and then confirmed from genomic data from (http://genome.ucsc.edu/ and http://www.ensembl.org/index.html).
Primers were designed and polymerase chain reaction (PCR) procedures performed according to a previous study[9]. We used BsrBI (MbiI) restriction endonuclease for RFLP analysis.

Statistical analysis

Differences between groups were studied using a chi-squared test (categorical variables). Hardy–Weinberg equilibrium was tested by the chi-squared test in cases and controls separately. The association ofIL6 genotypes with a SAH was tested

using logistic regression analysis under assumption of normal (GC) versus polymorphic (GG + CC). A P value < 0.05 was considered statistically significant.

RESULTS

A total of 120 patients with aneurysmal SAH and 120 control subjects were included in the study. Single IA was found in 103 aneurysmal SAH patients (85.8%). Genotype distribution in both groups was in Hardy–Weinberg equilibrium (P > 0.05). Allele and genotypes frequencies did not differ significantly between the studied groups (Table 1).

Table 1. Distribution of IL6 genotypes

SNP		n (%)			p Value*
174G > C		GG	GC	CC	
	Cases	72 (60.0)	36 (30.0)	12 (10.0)	0.2766
	Controls	66 (55.0)	42 (35.0)	12 (10.0)	
572G > C		GG	GC	CC	
	Cases	94 (78.3)	24 (20.0)	2 (1.6)	0.2502
	Controls	83 (69.1)	33 (27.5)	4 (3.3)	

*Cases vs controls

DISCUSSION

Genomic variability can be present in many forms, including SNPs, and these SNPs could be related with a disease state and influence health[7]. Terry et al.[9] examined the functional effect on gene expression of four SNPs located in the promoter region of the IL6 gene. They showed that different haplotype and alleles of these SNPs affect the expression of the gene cooperatively and they also stated that the function of one variation is determined by the effect of other alleles at distant polymorphic sites.

Jones et al.[2] investigated whether an abdominal aortic aneurysm (AAA) wall is a source of circulating IL6, the relationship between high concentrations of circulating IL-6 and aneurysm growth, and the association of -174 G→C polymorphism in the IL-6 promoter and survival. Their results showed that aortic aneurysm is an important source of circulating IL6 and its concentration is related to genotype. They also stated that the -174 G→C IL6 genotype predicts future cardiovascular mortality. However, they were not able to show any relationship between plasma IL6 concentration and aneurysm growth. Smallwood et al.[6] conducted an association study in an AAA group with age matched group in three SNPs: -174G > C (rs1800795), IL-6-572G > C (rs1800796) and IL-6-597G > A (rs1800797) in the promoter region of the IL6 gene. They concluded that the -572G > C (rs1800796) polymorphism is associated with AAA. These two studies looked at the relationship between IL6 and AAA pathogenesis and reported favorable results.

Morgan et al.[3] investigated whether there was a relationship between the IL6 -572G > C and -174G > C genotypes and intracranial aneurysms in 91 patients harboring an intracranial aneurysm and 2720 healthy controls. Their results showed a significant statistical correlation between IL6 promoter polymorphisms and intracranial aneurysmal disease. Similar results were reported by Sun et al.[8] for -572G > C SNP in 240 Chinese ethnically Han aneurysmal patients and 240 controls. Their results showed a strong statistical association.

In contradistinction to these studies, Fontanella et al.[1] studied the same SNPs in an Italian population and were not able to find any significant difference between intracranial aneurysm cases and controls and concluded that their results did not confirm any association between these SNPs and aneurysmal SAH. Pera et al.[5] studied –174G > C promoter polymorphism in 276 intracranial aneurysmal subarachnoid hemorrhage patients and 581 controls and their results also did not differ significantly between patients and controls as in the Fontanella et al.[1] study. We studied –174G > C and -572G > C IL6 promoter SNPs in a Turkish population and our results were compatible with the results of Fontanella et al.[1] and Pera et al.[5], which did not show a correlation.

In conclusion, our study found that IL6 promoter SNPs did not have a correlation with intracranial aneurysm formation and subarachnoid aneurysmal rupture in a Turkish population. Apart from our results, it must be kept in mind that the genetic basis of diseases may be different in various ethnic populations.

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