

RESEARCH ARTICLE

Genetic Polymorphism TNF α -308G>A and Ischemic Stroke in Northern Romania

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Introduction: Stroke is one of the leading causes of death in Romania. Evidence in supporting the role of the pro-inflammatory cytokine TNF-alpha in ischemic pathogenesis is now well established. The aim of the present study is to evaluate the relationship between TNF α -308G>A polymorphism and ischemic stroke in a Northern Romanian population group and to determine whether it has an influence on the risk of cerebral events. This is a cross-sectional, randomized, case-control study for the evaluation of TNF α -308G>A polymorphism alleles frequency among patients with ischemic stroke.

Material and method: The study included 108 patients diagnosed with ischemic stroke (neurological and CT scan examination), and 118 healthy unrelated controls. TNF α -308G>A genotyping was carried out using PCR-RFLP technique. The amplification of the relevant gene fragment was subjected to restriction enzyme digestion, followed by gel electrophoresis.

Results: Molecular analysis did not reveal an increased frequency of GA mutant genotype in the study group compared to the control group ($p = 0.879$, OR = 0.928, CI = 0.512–1.682).

Conclusions: We found no significant differences in distribution of the TNF α -308G>A polymorphism between ischemic stroke patients and controls.

Keywords: ischemic stroke, tumour necrosis factor- α , -308 polymorphism

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Introduction

Ischemic stroke is one of the major public health issues, accounting for one in four causes of death in Europe [1,2]. The CARDIO-Zone study showed that general prevalence of stroke and its risk factors in Romania is high, with no significant differences regarding risk factors compared to EU countries [3].

Ischemic stroke has a genetic basis and it is considered a multifactorial disorder [4,5]. Stroke is difficult to study from a molecular point of view, because of its gene-environment interactions, genetic and nongenetic heterogeneity, polygenic inheritance with variable penetrance and not least, late age of onset [6]. Candidate genes, stroke susceptibility alleles and their association with stroke pathogeny have been intensely studied in the last few years [7,8,9]. Immunity and inflammation are key elements of the pathobiology of ischemic stroke [10], several studies have shown that the serum of patients with stroke highlights increased levels of proinflammatory cytokines, and therefore support the hypothesis promoting their involvement in the etiopathogenesis of the disease [11,12]. The TNF α gene is located on chromosome 6 (p21.3) and is one of the most potent proinflammatory cytokines with both beneficial and negative properties for the central nervous system [13,14]. Tumour necrosis factor alpha (TNF- α) and its receptors are normally expressed in the

brain, animal studies have already shown that proinflammatory cytokines like IL-1 β and TNF- α are related with the Shwartzman phenomenon (induced cytokine vulnerability of blood vessels) and therefore stroke [15]. Functional polymorphisms in the promoter region of the TNF α gene (G to A substitution in the -308 position) is associated with increased plasma levels of this cytokine [16], therefore the potential role of this genetic variant in stroke etiopathogenesis [17,18].

The aim of our study was to investigate the association between TNF α -308G>A gene polymorphism and the risk of ischemic stroke in a Romanian population group.

Material and method

This cross-sectional, randomized case-control study involved 108 patients diagnosed with ischemic stroke and 118 unrelated controls.

The study was approved by the Ethics Committee of the „Iuliu Hațieganu” University of Medicine and Pharmacy of Cluj Napoca, and all participants were provided a written informed consent. Diagnosis was confirmed following neurological assessment and CT scan evaluation in all cases. A sample of 3 ml EDTA venous blood was collected from all patients and controls.

Genotyping (adapted after Ishii T et al. 2000) [19]

DNA was extracted from 300 μ l peripheral blood samples using Wizard Genomic DNA Purification Kit (Wizard® Genomic DNA Purification Kit, Promega, MA, USA).

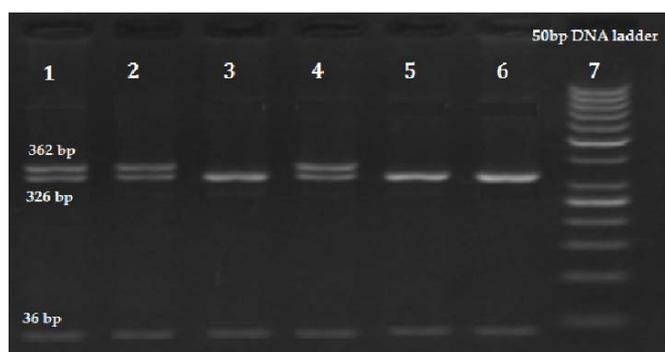


Fig. 1. Electroforetic analysis for -308A/G polymorphism of TNF α gene. Lane 1, 2, 4 – heterozygous genotype A1A2; lane 3, 5, 6 – homozygous wild type A1A1, lane 7 – 50 bp reference marker.

The TNF α -308G>A polymorphism was detected using a PCR-RFLP method. The PCR primers (Eurogentec, Belgium) are described as follows:

Forward primer 5'-TCCCCAAAAGAAATGGAG-GCAATA-3'

Reverse primer 5'-GGTTTTGAGGGCCATGAGACGTCTGCTGGCTGGGTG-3'

For the specific genetic analysis, a total amount of 100 ng of genomic DNA was amplified in a total volume of 25 μ l reaction mixture (Thermo Fischer Scientific Inc., MA, USA) containing reaction buffer of 1.5 nM MgCl₂, 20 pmol of each primer, 200 μ m of each dNTPs and 0.5 units of Taq polymerase.

PCR was carried out using a commercial thermal cycler (Eppendorf Mastercycler Thermal Cycler). The amplification steps consisted of an initial 12 minutes denaturation at 95 °C, followed by 35 cycles of denaturation at 95 °C for 30 seconds each, primer annealing at 60 °C for 30 seconds, primer elongation at 72 °C for 1 minute and a 5 minutes final elongation at 72 °C.

The PCR amplification products were digested overnight with 5 units of NcoI enzyme (Thermo Fischer Scientific Inc., MA, USA); the resulted fragments were then separated on a 3 % agarose gel (MetaPhor® Agarose, Cambrex Bio Science Inc.) and transilluminated with UV light. Electrophoretic analysis defines 3 distinct banding patterns, each corresponding for 3 possible genotypes: A1A1 wild type homozygous genotype (326 and 36 bp fragments), A1A2 heterozygous genotype (362, 326 and 36 bp fragments) and A2A2 mutant homozygous genotype (362 bp undigested fragment).

Statistical analysis

For statistical analysis we used SPSS 18.0 for Windows. (SPSS, Inc., Chicago, IL). P value and Odds ratio (OR) assessment with 95% confidence limits were calculated by logistic regression.

Results

There was no statistically significant difference in mean age and gender distribution between the cases and controls.

Table I. Comparative analysis for TNF α -308G>A polymorphism in patients and controls

TNF α -308G>A Polymorphism	p	Odds ratio	95% CI	
			Inferior limit	Superior limit
Patients vs. controls	0.879	0.928	0.512	1.682
Stroke women vs. male	0.837	1.155	0.514	2.592
Stroke women vs. control women	1.000	0.900	0.368	2.196
Stroke men vs. control men	0.168	0.561	0.259	1.215
Stroke and SHT* vs. control SHT*	0.270	0.580	0.243	1.384

Statistically significant for p < 0.05
*SHT – systemic hypertension

Molecular analysis of TNF α -308G>A polymorphism did not reveal a statistically increased frequency of mutant allele in the study group compared to the control group (p = 0.879, OR = 0.928, CI = 0.512–1.682). No statistically significant associations were observed in the sex of the patients, controls and heterozygous status (p = 0.837, OR = 1.155, CI = 0.514–2.592); no statistically significant associations were observed among patients and controls with a positive history of hypertension (p = 0.270, OR = 0.580, CI = 0.243–1.384). Comparative analysis for stroke diagnosed women versus healthy women and stroke diagnosed men versus healthy men, did not reveal any statistical significant associations (p = 1.000, OR = 0.9000, CI = 0.368–2.592, and p = 0.168, OR = 0.561, CI = 0.259–1.215, respectively) (Table I).

The AA genotype was not detected in any of the subjects, probably because of the decreased number of AA carriers in the population, since the A allele is very rare, therefore a more accurate statistical analysis using the Fisher comparative analysis according dominant and recessive model could not be performed.

Discussions

Ischemic stroke is a multifactorial disorder with a strong genetic component. The pathophysiology of stroke implies several different pathways, like lipid metabolism, coagulation, systemic chronic inflammation, blood pressure regulation, and cellular adhesion, therefore candidate gene polymorphisms in these pathways have been proposed as genetic risk factors and consequently studied in few medical studies [20,21].

Tumor necrosis factor-alpha (TNF α) is a cytokine with diverse proinflammatory actions, including endothelial leukocyte adhesion molecule expression; the neuronal expression of TNF α seems to facilitate the infiltration of inflammatory cells in cerebral ischemia and might contribute to increased sensitivity and risk in ischemic stroke [22].

Comparative analysis of TNF α -308G>A polymorphism did not reveal a statistically increased frequency of mutant allele in the study group compared to the controls. Our results come in agreement with relevant data highlighted in other studies that indicated no correlations be-

tween TNF α and stroke [23,24,25]. Other studies contradict the results mentioned above, revealing a connection between TNF α polymorphisms and stroke. One of these studies highlights the protective role of TNF α in ischemic stroke [26], while another study performed in the USA, places the same polymorphisms in the group of genetic risk factors for stroke [27].

Although there is increased evidence about the role of proinflammatory cytokines such as TNF α and stroke etiopathogenesis, the results of our study failed to demonstrate this hypothesis. We found no association between the distribution of variant -308G>A alleles among patients diagnosed with stroke compared to controls.

TNF α levels are elevated in essential hypertension, supporting the fact that hypertension is in part an inflammatory disorder [28]. Several studies [29,30] revealed a link between TNF α polymorphisms and pregnancy associated hypertension, placing the characteristic female hormonal profile among the risk factors for stroke, nevertheless no statistically significant associations were observed among mutant carrier patients and controls regarding the sex and associated pathology (systemic hypertension), probably because of the more complex etiopathogenesis of this multifactorial disease.

Conclusions

The results of our study reveals no association between TNF α -308G> genetic polymorphism and ischemic stroke. The complex etiology of stroke suggests that individual genetic polymorphisms have modest effects that are difficult to detect, as has been observed to date, therefore larger studies are needed to assess these genetic polymorphisms as risk factors.

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