VERSITA

Acta Medica Marisiensis 2013;59(2):75-77 DOI: 10.2478/amma-2013-0016

RESEARCH ARTICLE

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

Petrișor Felicia[1], Popp RA[1], Cătană Andreea[1], Porojan M[2], Pop IV[1]

[1] Department of Medical Genetics, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania.
[2] Department of Internal Medicine II, Cluj-Napoca, Romania

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 TNFα -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 TNFα -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. TNFα -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 TNFα -308G>A polymorphism between ischemic stroke patients and controls.

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

Received: 27 April 2012

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 TNFα 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-1β 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 TNFα 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 etiophatogenesis [17,18].

The aim of our study was to investigate the association between TNFα -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 Commitee of the „Iuliu Haţieganu" 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 µl peripheral blood samples using Wizard Genomic DNA Purification Kit (Wizard® Genomic DNA Purification Kit, Promega, MA, USA).

Correspondence to: Felicia Petrișor
E-mail: genetica.umfcj@gmail.com

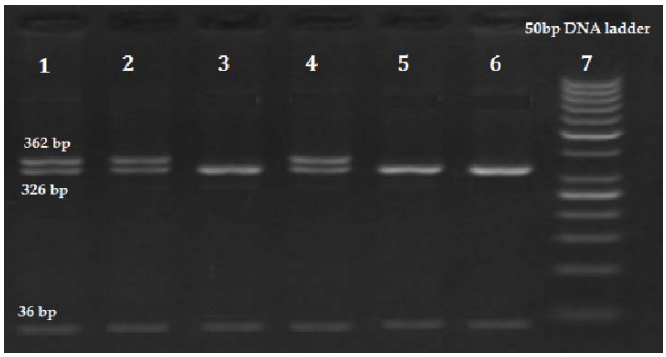

Fig. 1. Electroforetic analysis for -308A/G polymorphism of TNFα gene. Lane1, 2, 4 – heterozygous genotype A1A2; lane 3, 5, 6 – homozygous wild type A1A1, lane 7 – 50 bp reference marker.

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

Forward primer 5`-TCCCCAAAAGAAATGGAGGCAATA- 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 µl reaction mixture (Thermo Fischer Scientific Inc., MA, USA) containing reaction buffer of 1.5 nM $MgCl_2$, 20 pmol of each primer, 200 µm 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 TNFα -308G>A polymorphism in patients and controls

TNFα -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 TNFα -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 (TNFα) is a cytokine with diverse proinflammatory actions, including endothelial leukocyte adhesion molecule expression; the neuronal expression of TNFα 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 TNFα -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 TNFα and stroke [23,24,25]. Other studies contradict the results mentioned above, revealing a connection between TNFα polymorphisms and stroke. One of these studies highlights the protective role of TNFα 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 proinflamatory cytokines such as TNFα 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.

TNFα 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 TNFα 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 TNFα -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.

References

1. World Health Organization. Reducing risks, promoting healthy life. The World Health Report. 2002, Geneva
2. Ohman EM, Bhatt DL, Steg PG, et al. The Reduction of Atherothrombosis for Continued Health (REACH) Registry: an international, prospective, observational investigation in subjects at risk for atherothrombotic events-study design. Am Heart J 2006; 151:786-796
3. Cinteza M, Pana B, Cochino M, et al. Prevalence and control of cardiovascular risk factors in Romania cardio-zone national study. Mædica A Journal of Clinical Medicine. 2007;2(4).
4. Kiely DK, Wolf PA, Cupples LA, Beiser AS, Myers RH. Familial aggregation of stroke. The Framingham Study. Stroke. 1993;24:1366-1371.
5. Liao D, Myers R, Hunt S, Shahar E, Paton C, et al. Familial history of stroke and stroke risk. The Family Heart Study. Stroke. 1997;28:1908-1912.
6. Alberts MJ. Genetic aspects of cerebrovascular disease. Stroke. 1991;22(2):276-80.
7. Ariyaratnam R, Casas JP, Whittaker J, et al. Genetics of ischaemic stroke among persons of non-European descent: a meta-analysis of eight genes involving approximately 32,500 individuals. PLoS Med. 2007;4(4):e131.
8. Matarin M, Brown WM, Dena H, et al. Candidate gene polymorphisms for ischemic stroke, Stroke. 2009;40(11):3436-42. Epub 2009 Sep 3.
9. Carr FJ, McBride MW, Carswell HVO, et al. Genetic Aspects of Stroke: Human and Experimental Studies. Journal of Cerebral Blood Flow & Metabolism. 2002;22:767-773.
10. Cvetkovic JT, Wiklund PG, Ahmed E, et al. Polymorphisms of IL-1β, IL-1Ra, and TNF-α genes: A nested case-control study of their association with risk for stroke. Journal of Stroke & Cerebrovascular Diseases. 2005;14(1):29-35.
11. Clark WM. Cytokines and reperfusion injury. Neurology. 1997;Suppl.4:10-4
12. Revilla M, Castillo J, Davalos A, Chamorro A. A-174G/C polymorphism of the interleukin 6 gene in patients with lacular infarction. Neurosci Lett. 2002;324:29-32.
13. Hoppe C, Klitz W, D'Harlingue K, et al. Confirmation of an association between the TNF(–308) promoter polymorphism and stroke risk in children with sickle cell anemia. Stroke. 2007;38:2241-2246.
14. Um JY, Lee JH, Joo JC, et al. Association between tumor necrosis factor-alpha gene polymorphism and Sasang constitution in cerebral infarction. Am J Chin Med. 2005;33:547-557.
15. Hallenbeck JM. Cytokines, macrophages and leukocytes in brain ischemia. Neurology. 1997;24:445-451.
16. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med. 2002;21:1539-1558.
17. Welton NJ, Caldwell DM, Adamopoulos E, Vedhara K. Mixed treatment comparison meta-analysis of complex interventions: psychological interventions in coronary heart disease. Am J Epidemiol. 2009;169:1158-1165.
18. Guanglin Cui et al. Polymorphism of tumor necrosis factor alpha (TNF-alpha) gene promoter, circulating TNF-alpha level, and cardiovascular risk factor for ischemic stroke. Journal of Neuroinflammation. 2012;9:235
19. Ishii T, Matsuse T, Teramoto S, et al. Neither IL-1beta, IL-1 receptor antagonist, nor TNF-alpha polymorphisms are associated with susceptibility to COPD. Respir Med. 2000;94(9):847-51.
20. Zhang LF, Yang J, Hong Z, et al. Collaborative Group of China Multicenter Study of Cardiovascular Epidemiology. Proportion of different subtypes of stroke in China. Stroke. 2003;34:2091-2096.
21. Dichgans M, Markus HS. Genetic association studies in stroke: methodological issues and proposed standard criteria. Stroke. 2005;36: 2027-203.
22. Liu T, Clark RK, McDonnell PC, et al. Tumor necrosis factor-alpha expression in ischemic neurons. Stroke. 1994;25:1481-1488
23. Achrol AS, Pawlikowska L, McCulloch CE, et al. Tumor necrosis factor-α –238G > A promoter polymorphism is associated with increased risk of new hemorrhage in the natural course of patients with brain arteriovenous malformations. Stroke. 2006;37:231-234.
24. Bis JC, Heckbert SR, Smith NL, et al. Variation in inflammation-related genes and risk of incident nonfatal myocardial infarction or ischemic stroke. Atherosclerosis. 2008;198:166-173.
25. Banerjee I, Gupta V, Ahmed T, et al. Inflammatory system gene polymorphism and the risk of stroke: a case-control study in an Indian population. Brain Res Bull. 2008;75:158-165.
26. Tong Y, Geng Y, Xu J, et al. The role of functional polymorphisms of the TNF-alpha gene promoter in the risk of ischemic stroke in Chinese Han and Uyghur populations: two case-control studies. Clin. Chim. Acta. 2010;411:1291-1295.
27. Hoppe C, Klitz W, D'Harlingue K, et al. Confirmation of an association between the TNF(-308) promoter polymorphism and stroke risk in children with sickle cell anemia. Stroke. 2007;38:2241-2246.
28. Khan SA, Aslam M, Owais M, Zaheer MS. Correlation between tumour necrosis factor-alpha and other co-variates and different grades of blood pressure in essential hypertensive patients. Ind medica Biomedical Research. 2010;21(2):184-188.
29. Zhao Y, Xia S, Zou L. The association between polymorphism of TNF-alpha gene and hypertensive disorder complicating pregnancy. J Huazhong Univ Sci Technolog Med Sci. 2007;27(6):729-32.
30. Yan-yan Li. Tumor Necrosis Factor-Alpha G308α Gene Polymorphism and Essential Hypertension: A Meta-Analysis Involving 2244 Participants. PLoS ONE. 2012;7(4):e35408.