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Polymorphism −238 G/A of Tumor Necrosis Factor Alpha Gene Promoter is a Genetic Risk Factor for Ischemic Cerebrovascular Disease

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Dear Sir,

Cerebrovascular disease (CVD) is a common cause of mortality and morbidity in industrialized patients (Sundlow and Warlow 1997). It is related to artherosclerosis, an inflammatory disease where pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) have a role in its pathogenesis. TNF-α is a cytokine with pro-inflammatory and immunoregulatory activity. Its secretion shows a high degree of interindividual variability, which is at least partly genetically determined (Jacob et al. 1990). The −238G/A and −308 G/A polymorphisms of TNF-α have been found to be associated to an altered TNF-α expression in in vitro tests (Braun et al. 1996; De Vries et al. 1995; Wu and Mc Clain 1997).

We have performed an analysis of these polymorphisms on 308 consecutive unrelated patients diagnosed with ischemic CVD. The origin of stroke was classified according to the TOAST criteria (Adams et al. 1993). All included cases of CVD were age (±5 years) and sex matched to a different control without documented history of vascular disease. For all individuals conventional cardiovascular risk factor were recorded. All patients and controls were inhabitants of the same geographic area, so the sample was representative of the Spanish population. Both polymorphisms were detected by PCR using primers containing a single base-pair mismatch adjacent to the polymorphic site in order to introduce a restriction site into the wild-type nucleotide sequences after amplification.

Mean age of both groups was 70.9 ± 0.8 years, (range 23–99 years). The male: female proportion among patients and controls was 47.7 vs 52.3%. The classic risk factors for CVD analyzed were significantly more frequent in patients than controls. Results (Table 1) show a significant higher prevalence of the G/A + A/A genotypes of −238 G/A TNF-α in patients with CVD ($p < 0.01$;OR = 2.16; 95%CI = 1.40–3.34). The prevalence of the A allele was also significantly increased in the group of patients than in the controls (13.6 vs 7.0%; $p < 0.01$; OR = 2.10; 95%CI = 1.40–3.17). The genotypes and allelic frequencies of −308 TNF-α polymorphism was not different between cases and controls (Table 1).When analysis was performed for the two more frequently subtypes of CVD, atherotrombotic subtype showed a higher prevalence of the A allele of −238 G/A polymorphism, as compared with the undetermined etiology (16.5 vs 11.2%; $p = 0.06$; OR = 1.86; 95%CI = 0.91–3.82). In contrast, the variant −308 G/A did not differ from the different subtypes. Logistic regression analysis shows an independent association of A haplotype of −238 G/A TNF-α mutation with ischemic CVD. All of the cardiovascular risk factor recorded, such as hypertension, hypercholesterolemia, diabetes, smoking, age or sex were associated with a significant increased risk of CVD. No statistically significant differences related to cardiovascular risk factors were detected in relationship to both −238 G/A and −308 G/A polymorphisms.

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Table 1 Genotype and allele frequencies of −238 G/A and −308 G/A polymorphisms of TNFα gene in stroke patients and controls

Polymorphism	Patients *n* (%)	Controls *n* (%)	OR (CI95%)	*p*
TNFα −238 G/A				
Genotype:				
G/G	223 (74.1)	259 (86.0)	2.16 (1.40–3.34)[a]	<0.01
G/A	74 (24.6)	42 (14.0)		
A/A	4 (1.3)	0		
Haplotype:				
G	520 (86.4)	560 (93.0)	2.10 (1.40–3.17)[b]	<0.01
A	82 (13.6)	42 (7.0)		
TNFα −308 G/A				
Genotype:				
G/G	248 (84.9)	245 (81.1)	0.76 (0.48–1.20)[a]	0.217
G/A	42 (14.4)	51 (16.9)		
A/A	2 (0.7)	6 (2.0)		
Haplotype:				
G	538 (91.1)	541 (89.6)	0.73 (0.48–1.11)[b]	0.127
A	46 (7.9)	63 (10.4)		

Abbreviations: *OR*, odds ratio; CI95%, Confidence Interval 95%
[a] OR (CI95%) for the comparison between genotypes G/G *vs* G/A + A/A of both polymorphisms of TNFα.
[b] OR (IC95%) for the comparison between alleles G *vs* A of the −238 G/A and −308 G/A polymorphisms.

Most cerebrovascular disease is related to artherosclerosis, an inflammatory disease where inflammatory mediators as several cytokines might play a role in the atheroma formation. TNF-α is a potent immunomodulator and pro-inflammatory cytokine that has been implicated in many pathological processes, as artherosclerosis (Jacob et al. 1990). Because genetic traits could contribute to the global risk of CVD, a number of studies point the hypothesis that variations in the genetics of the inflammatory system may increase the risk of the disease (Sundlow and Warlow 1997). With this background, we conducted a retrospective case-control association study in patients with acute CVD designed to estimate the relevance of theses TNF-α variants in this setting. The results of this study demonstrated an association of −238G/A polymorphism of the TNF-α gene promoter with CVD (OR: 2.16; 95%CI: 1.40–3.34: $p < 0.01$), and show a higher prevalence of the A allele of this polymorphism in atherotrombotic stroke. Since atherosclerosis is a main cause of CVD these results can be expected.

Although there are some evidence that alterations in the genetics of the inflammatory system may modify the risk of ischaemic heart disease, reported data not shown an association between coronary artery disease and the two TNF-α polymorphisms studied (Allen et al. 2001). There are no published data concerning −238G/A TNF-α polymorphism in cerebrovascular disease, and the results derived from our study are therefore encouraging regarding the association of the A-allele with this pathology. However, concerning the −308G/A TNF-α polymorphism the results are ambiguous. Recently, Um et al explored this variant in Koreans and its results suggest that this polymorphism is associated with CVD in this population (Um and Kim 2004). In contrast, we did not find that this polymorphism had any effect on the development of CVD in Spanish population.

Our findings suggest that the −238 G/A TNF-α promoter polymorphism is a genetic risk factor for ischemic CVD in the Spanish population. The relevance of these polymorphisms should be investigated in other populations and with prospective and family studies. Large scale studies should be performed to confirm this preliminar results.

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References

Adams Jr., H. P., Bendixen, B. H., Kappelle, L. J., Biller, J., Love, B. B., Gordon, D. L., et al. (1993). Classification of subtype of acute ischemic stroke: Definitions for use in a multicenter clinical trial: TOAST: Trial of Org 10172 in acute stroke treatment. *Stroke, 24*, 35–41.

Allen, R. A., Lee, E. M., Roberts, D. H., Park, B. K., & Pirmohamed, M. (2001). Polymorphism in the TNF-α and TNF-receptor genes in patients with coronary artery disease. *European Journal of Clinical Investigation, 31*(10), 843–851.

Braun, N., Michel, U., Ernst, B. P., Metzner, R., Bitsch, A., Weber, R., et al. (1996). Gene polymorphism at position −308 of the tumor necrosis factor alpha (TNF-α) in multiple sclerosis and it's influence on the regulation of TNF-alpha production. *Neuroscience Letters, 215*, 75–78.

Jacob, C. O., Fronek, Z., Lewis, C. D., Koo, M., Hansen, J. A., & McDevitt, H. O. (1990). Heritable major histocompatibility complex class II-associated differences in production of tumor necrosis factor alpha: Relevance to genetic predisposition to systemic lupus erythematosus. *Proceedings of the National Academy of Sciences of the United States of America, 87*(3), 1233–1237.

Sundlow, C. L., & Warlow, C. P. (1997). Comparable studies of the incidence of stroke and its pathological types: Results from and international collaboration. International stroke incidence collaboration. *Stroke, 28*, 491–499.

Um, J., & Kim, H. (2004). Tumor necrosis factor alpha gene polymorphism is associated with cerebral infarction. *Molecular Brain Research, 122*, 99–102.

Wilson, A. G., De Vries, N., Van de Putte, L. B., & Duff, G. W. (1995). A tumor necrosis factor alpha polumorphism is not associated with rheumatoid arthritis. *Annals of the Rheumatic Diseases, 54*, 601–603.

Wu, W. S., & Mc Clain, K. L. (1997). DNA polymorphisms and mutation of the tumor necrosis-alpha (TNF-alpha) promoter in Langerhans cell histiocytosis (LCH). *European Journal of Clinical Investigation, 17*, 631–635.