J Neurol (2009) 256:194–197
DOI 10.1007/s00415-009-0961-8

ORIGINAL COMMUNICATION

Carlo Asuni
Maria Erminia Stochino
Alessandra Cherchi
Mirko Manchia
Donatella Congiu
Francesca Manconi
Alessio Squassina
Maria Paola Piccardi
Maria Del Zompo

Migraine and tumour necrosis factor gene polymorphism

An association study in a Sardinian sample

Received: 6 September 2007
Received in revised form: 9 January 2008
Accepted: 3 April 2008
Published online: 1 March 2009

M. Del Zompo (✉) · C. Asuni · M. E. Stochino · A. Cherchi · M. Manchia · D. Congiu · F. Manconi · A. Squassina · M. P. Piccardi
Headache Centre "Franco Tocco"
Section of Clinical Pharmacology
Dept. of Neurosciences "B.B. Brodie"
University of Cagliari
P.O. San Giovanni di Dio
Via Ospedale 46
09124 Cagliari, Italy
Tel.: +39-070/6092438
Fax: +39-070/653584
E-Mail: delzompo@unica.it

Abstract To assess the possibility of an association between TNF gene polymorphisms and migraine without aura, a case-control study was performed in a Sardinian sample.

Migraine without aura is a complex genetic disease in which susceptibility and environmental factors contribute towards its development. Several studies suggest that tumour necrosis factors (TNF) (TNF-α and lymphotoxin-alpha or TNF-ß) may be involved in the pathophysiology of migraine. The TNF-α and TNF-ß genes are located on chromosome 6p21.3 in the human leukocyte antigene (HLA) class III region. We evaluated 299 patients affected by migraine without aura (I.H.S. criteria 2004) and 278 migraine-free controls. The polymorphisms G308A of the TNF-α gene, and G252A of TNF-β gene were determined by NcoI restriction fragment length polymorphism analysis.

We found a statistically significant difference in allele ($p = 0.018$; OR = 1.46 95 % CI: 1.066 to 2.023) and genotype (trend $\chi^2 = 5.46$, $df = 1$, $p = 0.019$) frequencies of TNF-β gene, between cases and controls. Allele and genotype frequencies of TNF-α polymorphism did not differ significantly between the two groups.

These data suggest that subjects with the TNFB2 allele have a low risk of developing migraine without aura and/or that the polymorphism of the TNF-β gene is in linkage disequilibrium with other migraine responsible genes in the HLA region.

Key words migraine · tumour necrosis factor · polymorphism · HLA · gene

Introduction

Migraine is a common neurovascular disorder and the two main clinical forms are migraine with aura and migraine without aura. Migraine without aura is a complex disease in which susceptibility and environmental factors contribute towards its development. The pathophysiology of this primary headache is still unknown, although "sterile inflammation" or "neurogenic inflammation" seems to play a key role [8]. Tumour necrosis factors (TNF) (TNF-α and lymphotoxin-alpha or TNF-ß) are cytokines implicated in inflammatory reactions and endothelial function. Several studies suggest that TNF may be involved in migraine [4, 9, 12]. The TNF-α and TNF-ß genes are located on chromosome 6p21.3 in the human leukocyte antigene (HLA) class III region [3]. TNF-α and TNF-ß genes show a biallelic polymorphism (TNF-308A and TNF-308G alleles for TNF-α gene; TNFB1 and TNFB2 alleles for TNF-β gene). The TNF polymorphisms are located in a region characterised by wide polymorphic variation and are in linkage disequilibrium both with the HLA genes and with each other [3].

JON 2961

The Sardinian population is a genetic isolate resulting from a small number of founders ("founder effect"). Genetic studies suggest that genetic isolates play an important role in identifying candidate genes predisposing to complex disorders [1]. To assess the possibility of an association between TNF-α and TNF-ß gene polymorphisms and migraine without aura, a case control study was performed in a Sardinian sample.

Methods

Patients and controls

Two hundred and ninety-nine Sardinian patients (38 men, 261 women; mean age ± DS: 35.28 ± 10.22) affected by migraine without aura, attending the "Franco Tocco" Headache Centre of the University of Cagliari (Italy) took part in the study. Diagnosis of migraine without aura was made according to the International Classification of Headache Disorders (IHCD-II) criteria [13]. A group of 278 healthy Sardinian subjects (134 men, 144 women; mean age ± DS: 40.11 ± 11.65) served as controls. The controls were blood donors and were screened by general practitioners of local transfusion centres, in order to exclude a clinical history of headache. The characteristics of cases and controls are shown in Table 1. The study protocol was approved by the ethical committee of the local health unit and informed consent was obtained from all subjects.

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes [6]. The biallelic polymorphisms G308A, in the promoter region of the TNF-α gene, and G252A in the first intron of TNF-ß gene, were assessed according to described methods [5].

TNF-α

The assay mix contained in a volume of 50 µl 150 ng of genomic DNA, 0.2 mM of each dNTP, 0.25 µM of each primer (5'AGGCAATAGGTTTTGAGGGCCAT-3' and 5' TCCTCCCTGCTCCGATTCCG-3'), 2 U Amplitaq DNA polymerase (Perkin-Elmer), 1X PCR buffer. After an initial denaturation at 95 °C for 3 min, 33 cycles of 94 °C for 30 s, 61 °C for 30 s and 72 °C for 45 s were performed, followed by a final extension period of 72 °C for 10 min. The 107 bp amplification product was subjected to NcoI digestion (> 12 h). The digestion products were separated on 3 % agarose gel supplemented with ethidium bromide allowing differentiation of the G (107 bp) and A (87 and 20 bp) variant.

Table 1 Summary of the characteristics of patients with migraine without aura (MWA) and controls (C)

Characteristics	MWA	C
No. total	299	278
No. men	38	134
No. women	261	144
Age (mean ± SD)	35.28 ± 10.22	40.11 ± 11.65
Mean age at onset of the disease ± SD (years)	16.06 ± 7.00	
Mean duration of the disease ± SD (years)	19.11 ± 10.16	

Lymphotoxin α (TNF-β)

TNFB genotypes were determined using primers 5'GGTTTCCTTCTCTGTCTCTGACTCTCC-3' and 5'GAGAGAGATCGACAGAGAAGGGGAC-3'. A 173-bp fragment was amplified and PCR conditions were the same as for TNFα genotyping with the exception of extension time of 60 s and annealing temperature of 64 °C. Restriction enzyme NcoI (BioLab) was again used. Digested TNFB products were examined with electrophoresis in 3 % agarose gel. Ethidium bromide staining of the gel revealed the original 173-bp fragment for the allele TNFB2 and two fragments of 71 and 102 bp for the allele TNFB1.

Statistical analysis

The Hardy–Weinberg Equilibrium was performed using the HWE program in the Linkage Utility Program. The Chi-square or Monte Carlo test was used to compare allele and genotype frequency between cases and controls. One-way analysis of variance performed via the Kruskal-Wallis test was used for evaluating the possible influence of G308A TNF-α and G252A TNF-β polymorphisms on the age at onset. The sample was stratified for genotypes at each locus. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS, version 11.0). Power analysis was calculated using the "Power and Sample-Size Calculation" programme, version 2.1.30 [2]. The sample size was calculated presuming a frequency of 9 % for the risk allele and a α value of 0.05, with the 80 % power to detect association for a susceptibility marker with an OR of 2.1.

Results

At the polymorphic loci considered, the genotype counts were in Hardy-Weinberg equilibrium in both controls and patients. The genotype distributions and allele frequencies of G308A TNF-α gene polymorphism are shown in Table 2. No significant differences were found in the frequency of the genotype or allele between patients and controls. We found a statistically significant difference in allele ($\chi^2 = 5.55$, $p = 0.018$; OR = 1.46 95 % CI, 1.066–2.023) but not in genotype ($\chi^2 = 5.69$, $df = 2$, $p = 0.05$) frequencies of TNF-ß, between cases and controls. Moreover, a significant result was afforded by the Armitage Linear Trend test when applied to our genotype data (trend: $\chi^2 = 5.46$, $df = 1$, $p = 0.019$) (Table 3). The non-parametric analysis of variance performed via Kruskal-Wallis test showed no significant differences in the age at onset medians between genotypes groups of the G308A TNF-α ($p = 0.7$) and G252A TNF-β ($p = 0.4$) polymorphisms.

Table 2 Genotype distribution and allele frequencies of the G308A TNFα gene polymorphism

	Genotype*			Allele*	
	GG	GA	AA	G	A
Control #278	249	28	1	526 (94.6)	30 (5.4)
Case #299	272	26	1	570 (95.3)	28 (4.7)
$X^2 = 0.32$; $p = 0.83$ Monte Carlo test				$X^2 = 0.17$; $p = 0.67$	

* Values are numbers (percentage).

Table 3 Genotype distribution and allele frequencies of the G252A lymphotoxin α (TNF-β) gene polymorphism

	Genotype*			Allele*	
	B1B1	B1B2	B2B2	B1	B2
Control #278	6	62	210	74 (13.3)	482 (86.7)
Case #299	10	90	199	110 (18.4)	488 (81.6)
$\chi^2 = 5.69$; df = 2; p = 0.05 Trend $\chi^2 = 5.46$; df = 1; p = 0.019				$\chi^2 = 5.55$; df = 1; p = 0.018 OR = 1.46 95 % CI; 1.066–2.023	

* Values are numbers (percentage)

Discussion

Our study demonstrates that the G252A TNF-ß gene polymorphism is associated with migraine without aura. No significant differences in the genotype distributions and allele frequencies of G308A TNF-α gene polymorphism were found between patients with migraine without aura and healthy controls. When taking into consideration homozygous (TNFB1/TNFB1) and heterozygous (TNFB1/TNFB2) patients with migraine without aura, the observed increase of TNFB1 allele suggests that this allele could be involved in predisposing towards development of the disease in our sample. Our data are in discordance with the finding of two recent association studies showing an increased risk of migraine without aura associated with the TNF-α G308A polymorphism. In one of these studies, homozygosity for the G allele was associated with an increased risk of migraine in a group of 261 Caucasian patients [11]; in the other, performed with 221 Iranian patients, the frequency of the -308A variant allele proved to be significantly higher in patients than in controls [7]. These discordant results can be explained considering the differences among the populations studied and hypothesizing the same linkage disequilibrium with the susceptibility genes of migraine without aura.

On the contrary, in the first published work on tumour necrosis factor (TNF) gene polymorphism in migraine [14], the G252A TNF-ß gene polymorphism was associated with migraine without aura, but the carriage of the TNFB2 allele of TNF-ß gene in 47 patients with migraine without aura was associated to a high risk of developing the disease, while no significant association was found at the TNF-308 polymorphism. However, the small number of migraineurs examined in the study does not enhance the detection of a significant difference in a complex genetic disease such as migraine.

Our results, extrapolated from the Sardinian sample, exploit an important genetic potency associated to the existence of a "founder effect" and geographical isolation. Theoretically, these features result in an increase of linkage disequilibrium [1]. The G252A polymorphism of the TNF-ß gene is a silent point mutation, which could however prove capable of modifying gene expression.

A possible explanation for our results may lie in the fact that the polymorphism of the TNF genes are in a linkage disequilibrium with other migraine susceptibility genes. The TNF genes are located on chromosome 6p21.3, within the HLA Class III region. More than 200 genes are contained in the HLA region, which is characterized by a strong linkage disequilibrium. A recent study found a significant association between the *16 allele of DRB1 gene, located in the HLA Class II region, and migraine without aura [10].

In conclusion, our results support the theory of a presence of migraine susceptibility genes located on or linked with the HLA region. Further studies in other populations are needed to confirm our data and to identify susceptibility genes within this chromosomic region.

Acknowledgments The authors would like to thank Dr. Elia Casanova and Dr. Roberto Casanova, general practitioners of the AVIS Transfusion Centre of Cagliari for the collaboration to identify blood donors with no clinical history of headache.

References

1. Arcos-Burgos M, Muenke M (2002) Genetics of population isolates. Clin Genet 61:233–247 (Review)
2. Dupont WD, Plummer WD (1997) PS power and sample size program available for free on the Internet. Controlled Clin Trials 18:274
3. Hajeer AH, Hutchinson IV (2001) Influence of TNF-alpha gene polymorphisms on TNF-alpha and disease. Human Immunology 62:1191–1199
4. Kemper RH, Meijler WJ, Korf J, Ter Horst GJ (2001) Migraine and function of the immune system: a meta-analysis of clinical literature published between 1966 and 1999. Cephalalgia 21:549–557
5. Keso T, Perola M, Laippala P, et al. (2001) Polymorphisms within the tumor necrosis factor locus and prevalence of coronary artery disease in middle-aged men. Atherosclerosis 154: 691–697
6. Lahiri DK, Nurnberger JI Jr. (1991) A rapid non-enzymatic method for preparation of HMW DNA from blood for RLFP studies. Nucleic Acid Res 19: 44–54
7. Mazaheri S, Hajilooi M, Rafiei A (2006) The G-308A promoter variant of the tumor necrosis factor-alpha gene is associated with migraine without aura. J Neurol 253:1589–1593
8. Moskowitz. MA, Macfarlane R (1993) Neurovascular and molecular mechanisms in migraine headaches Cerebrovascular and Brain Metabolism Reviews 5:159–157
9. Perini F, D'Andrea G, Galloni E, et al. (2005) Plasma cytokine levels in migraineurs and controls. Headache 45:926–931
10. Rainero I, Dall'Omo AM, Rubino E, et al. (2006) HLA-DRB1 genotyping in Italian migraine patients. Neurosci Lett 393:90–93

11. Rainero I, Grimaldi LM, Salani G, et al. (2004) Association between the tumor necrosis factor-alpha -308 G/A gene polymorphism and migraine. Neurology 13:141–143
12. Sarchielli P, Alberti A, Baldi A, et al. (2006) Proinflammatory cytokines, adhesion molecules, and lymphocyte integrin expression in the internal jugular blood of migraine patients without aura assessed ictally. Headache 46: 200–207
13. Headache Classification Committee of The International Headache Society. Classification and diagnostic criteria for headache disorders, cranial neuralgias and facial pain, 2nd Edition (2004) Cephalalgia 24(Suppl 1):1–160
14. Trabace S, Brioli G, Lulli P, et al (2002) Tumor necrosis factor gene polymorphism in migraine. Headache 42: 341–345