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TNFA gene: the -308 promoter polymorphism in migraine

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Abstract Molecular analysis of TNFA alleles in Italian subjects affected by migraine was performed in order to define the involvement of HLA region genes in migraine. No statistically significant differences in TNFA allele distribution between patients and controls were observed.

Key words HLA • TNF • Migraine • Susceptibility

Introduction

In the past decade, increasing attention has been given to the study of molecular genetics of migraine on the basis of the evidence of familial clustering [1]. Our work is an association study of the -308 TNFA polymorphism with migraine.

Migraine without aura (MwoA) and migraine with aura (MwA) are disorders in which multiple factors including environmental and genetic factors are involved. The pathophysiology of migraine is still controversial, although sterile inflammation has been demonstrated to play a key role at the cranial vascular epithelial level.

The association between HLA genes and different multifactorial diseases presenting an inflammation-based pathophysiology, has been described [2]. In a previous study we hypothesized a protective role of HLA-DR2 antigen, providing additional basis for the proposed genetic heterogeneity between MwoA and MwA [3].

The HLA system is located on the short arm of chromosome 6. Class I and class II molecules are arm controlled by two different HLA, highly complex and polymorphic, including multiple loci and different alleles at each known locus. The third class of MHC includes genes that control the synthesis of proteins that have different functions.

Tumor necrosis factor (TNF) cytokines are polypeptide effectors of inflammatory reaction and endothelial function. TNF genes are located in the HLA class III region [4]. Circulating TNFA has been described to act as a crucial mediator in a migraine-like headache syndrome, cervicogenic headache.

Thus, an association study of the -308 TNFA polymor-

phism with MwA and MwoA was performed in order to better define the involvement of HLA region genes in migraine.

Materials and methods

Patients and controls

A controlled study was carried out in 79 migraine patients including 32 with MwA (5 males and 27 females) and 47 with MwoA (12 males and 35 females). The diagnoses were made in accordance with the International Headache Society (IHS) criteria. The control group consisted of 101 randomly selected unrelated healthy subjects from the same geographic area of patients (central Italy). The study protocol was approved by our institutional ethics board and informed consent was obtained from all patients and from controls. During this clinical research study, the Declaration of Helsinki's recommended principles were closely observed.

Methods

The study was performed by analyzing the PCR/RFLP TNFA polymorphism by PCR amplification of a 107-bp fragment [5]. The PCR product was digested by the NcoI restriction enzyme. It is possible to obtain a simple characterization of the two alleles by separation based on dimensions in gel electrophoresis.

A G to A transition at position -308 produces the presence or absence respectively of the restriction site that defines two alleles: TNFA1 and TNFA2. Therefore, the possible genotypes are: homozygous 1–1, heterozygous 1–2 and homozygous 2–2.

Statistics

Statistical analysis was performed by Fisher's exact test and the probability was corrected (p_c) multiplying p by the number of comparisons. Differences were considered statistically significant when p_c was less than 0.05.

Results

In Table 1, allele frequencies for TNFA1 and TNFA2 in patients and controls are reported. No significant TNFA associations either with MwoA or with MwA were found. In Table 2, the distribution of TNFA genotypes in migraine patients and controls is shown. Also in this case, no statistically significant differences were observed between patients and controls.

Discussion

We report for the first time the molecular analysis of TNFA alleles in Italian subjects affected by migraine. No significant TNFA associations either with MwA or with MwoA were found. No statistically significant differences in TNFA allele distribution between patients and controls were observed.

The results of previous studies of the polymorphisms in TNFA promoter region are conflicting. Although studies have generally not been able to show any association between TNFA polymorphism, susceptibility and autoim-

Table 1 TNFA allele frequencies in migraine without aura (MwoA) and migraine with aura (MwA) patients and healthy controls

	Controls (n = 101)		MwA (n = 32)		MwoA (n = 47)	
	n	(%)	n	(%)	n	(%)
1	189	(93.57)	57	(89.06)	9	(94.68)
2	13	(6.43)	7	(10.94)	5	(5.32)

Table 2 Distribution of TNFA genotypes in migraine without aura (MwoA) and migraine with aura (MwA) patients and healthy controls

	Controls (n = 101)		MwA (n = 32)		MwoA (n = 47)	
	n	(%)	n	(%)	n	(%)
1–1	90	(89.11)	25	(78.13)	42	(89.36)
1–2	9	(8.91)	7	(21.87)	5	(10.64)
2–2	2	(1.98)	0	–	0	–

mune diseases, more interesting results have been reported in relation to infectious disease.

In a previous study we hypothesized a protective role of HLA–DR2 antigen, providing additional basis for the proposed genetic heterogeneity between MwoA and MwA.

Since DR genes are involved, we are looking for other genes in the region responsible for genetic susceptibility to migraine. As the HLA region seems to be involved in migraine pathogenesis, it is our intention to begin investigating the TNFB gene as soon as possible in order to attain a better definition of the relationship between MHC loci and migraine.

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