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RAPID COMMUNICATION

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Tumor necrosis factor B gene polymorphism contributes to susceptibility to migraine without aura

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Abstract Migraine without aura (MWOA) and migraine with aura (MWA) are disorders in which multiple factors, including environmental and genetic factors, are involved. 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. The cytokines TNFA and TNFB are polypeptide effectors of inflammatory reaction and endothelial function. To better define the involvement of HLA region genes in migraine, we performed an association study of the tumor necrosis factor (TNF) genes, located in the HLA class III region, with MWOA and MWA. TNFB alleles 1 and 2 were analyzed by PCR-RFLP in 30 MWOA patients, in 47 MWA patients and in 101 random controls. The frequency of TNFB*2 was significantly increased in MWOA patients as compared with controls (78.72% vs. 61.4%, $p_c = 0.004$), while no significant differences were found between MWA patients and controls. The distribution of TNFB genotypic frequencies showed a significant decrease of TNFB 1,1 homozygotes in MWOA patients ($p_c = 0.0201$). The observed increase of TNFB*2 in MWOA is distributed in TNFB 2,2 and TNFB 1,2 genotypes, meaning that the susceptibility allele could act as "dominant": people with TNFB 1, 1 genotype are less predisposed to the disease. While more studies are needed in larger migraine samples to reinforce the statistical power of the reported data, the present study supports the hypothesis that TNFB is a susceptibility gene in MWOA.

Key words Migraine, genetics • Tumor necrosis factor genes • Heredity • Susceptibility

Introduction

In the past decade, increasing attention has been given to the study of molecular genetics of migraine on the basis of previous evidence for a familial hereditary influence [1, 2]. More studies mainly focused on familial hemiplegic migraine (FHM), a rare autosomal dominant subtype of migraine with aura (MWA) associated with transient ictal hemiparesis and, often, progressive cerebellar atrophy. FHM is linked to chromosomes 1 and 19 [3–5]. However, despite the scientific resonance of these discoveries, the rarity of FHM (compared to the incidence of migraine) leads us to continue genetic studies on the role of human leukocyte antigens (HLA) in migraine. The pioneering HLA report in migraine was published by Lee Kudrow more than 20 years ago [6]. We previously demonstrated, in 8 households with more than one family member affected by

migraine witout aura (MWOA), an increase of shared HLA haplotypes, suggesting that migraine heredity was HLA-linked [7]. More recently, we demonstrated that HLA class II DR2 antigen shows a decreased frequency in migraine with aura (MWA) when compared with both MWOA and controls; these results support the hypothesis of a protective role of DR2 antigen in MWA and provide additional basis for the proposed difference within MWOA and MWA [8].

Tumor necrosis factor (TNF)-A and TNFB have similar biologic activities and are 30% identical at the amino acid level. Each of the genes is about 3 kb long and contains 3 introns. They are closely linked and are situated on chromosome 6 according to studies performed in human-mouse somatic cell hybrids [9]. Studies of hybrid cells made with rearranged human chromosome 6 showed that both TNFA and TNFB map to the 6p23-q12 segment. Only the last exons of these genes, which code for more than 80% of the secreted protein, are homologous (56% identical) [10].

Structural or regulatory defect of HLA genes may contribute to the pathogenesis of MHC-associated disease, especially with inflammatory and autoimmune components. TNF gene polymorphism is associated with susceptibility to Behçet's diseases [11], rheumatoid arthritis [12, 13], systemic lupus erythematosus [14], multiple sclerosis [15, 16], celiac disease [17], and narcolepsy [18].

The pathophysiology of migraine is still controversial, although sterile inflammation plays a key role at the cranial vascular endothelial level [19, 20]. The cytokines TNFA and TNFB are polypeptide effectors of the inflammatory reaction and of endothelial function [21]. Serum TNFA also acts as a crucial mediator in another form of headache, the cervicogenic headache [22, 23].

To better define the involvement of HLA region genes in migraine, we performed an association study of the TNF genes, located in the HLA class III region, with MWOA and MWA.

Materials and methods

We studied 77 migraine patients, including 30 patients with MWA (5 males and 25 females; mean age, 39.7±7.4) and 47 patients with MWOA (12 males and 35 females; mean age, 36.7±6.9 years), diagnosed according to the International Headache Society (IHS) criteria [24]. Additionally, 101 unrelated healthy subjects from the same geographic area (central Italy), randomly selected, served as controls. The study protocol was approved by our institutional ethics board and informed consent was obtained from all patients and from controls. The recommended principles of the Declaration of Helsinki, September 1989, were closely observed during this clinical research study.

The PCR/RFLP NcoI polymorphism of the TNFB gene was studied by PCR amplification of a 740-bp fragment, subsequently digested by NcoI restriction enzyme. The two alleles (TNFB*1 and TNFB*2) were characterized for the presence or absence of the NcoI restriction site of the first TNF gene intron [25]. Digestion was verified by 2% agarose gel electrophoresis and ethidium bromide staining.

The significance of associations was evaluated by Fisher's exact test from 2x2 contingency tables. Corrected p (p_c) value was calculated as p x number of comparisons [26]. Differences were considered statistically significant when p_c was less than 0.05.

Results

The frequency of TNFB*2 was significantly increased in MWOA patients (78.72%) as compared with that in controls (61.4%) (p_c = 0,004), while no significant differences were found between patients with MWA and controls (Table 1).

The TNFB genotypic frequencies are shown in Table 2. There was a significant decrease of TNFB 1/1 homozygotes in MWOA patients (p_c = 0.0201). When the frequencies of TNFB genotypes were compared in MWA patients and controls, no differences were found.

Table 1 TNFB allele frequencies in patients with migraine without aura (MWOA) or migraine with aura (MWA), and in healthy controls

	Controls (n = 101)		WMWA (n = 30)		MWOA (n = 47)	
Allele	n	(%)	n	(%)	n	(%)
1	78	38.6	19	31.67	20	21.28
2	124	61.4	41	68.33	74	78.72*

* MWOA vs. controls, p = 0.002 (p_c = 0.004)

Table 2 Distribution of TNFB genotypes in patients with migraine without aura (MWOA) or migraine with aura (MWA), and in healthy controls

	Controls (n = 101)		MWA (n = 30)		MWOA (n = 47)	
Genotype	n	(%)	n	(%)	n	(%)
1, 1	17	16.8	5	16.67	1	2.13*
1, 2	44	43.6	9	30.00	18	38.30
2, 2	40	39.6	16	53.33	28	59.57**

* MWOA vs. controls, p = 0.0067 (p_c = 0.0201)
** MWOA vs. controls, p = 0.0182 (p_c = ns)

Discussion

The probabilty of developing a disease is due to the sum of genetic and environmental influences. Since migraine does not fit a simple mendelian pattern, the liability to this disease, which must be considered to be normally distributed in the population, might depend on genetic factors and on environmental influences which contribute to the manifestion of such a "multifactorial disease".

We report for the first time the molecular analysis of TNFB alleles in Italian subjects affected by migraine. The observed increase of TNFB*2 in MWOA is distributed in TNFB 2,2 and TNFB 1,2 genotypes, meaning that the susceptibility allele could act as "dominant". People with TNFB 1,1 genotype are less predisposed to the disease.

The observed significant increase of TNFB*2 in MWOA suggests that this gene may influence the strength, effectiveness and duration of local inflammation (perivascular brain plasma extravasation via nitroxidergic endothelial activation), namely "sterile inflammation" as *primum movens* – although of migraine pain [27–29]. Otherwise, the structural or regulatory defective TNFB genes in migraine may contribute to reach the threshold brain excitability and to the subsequent propagation of its neuronal hyperexcitability (via increased TNF expression, leading to cell-to-cell signaling) that is now considered among the prevailing hypotheses for migraine, especially for MWA [30].

Furthermore, a comparison of genetic data for MWOA with new studies on the genes encoding cytokines may contribute to understanding the biological implications of TNFB*2 expression and may confirm that this gene has some functional effect that acts both in *linkage disequilibrium* with different potential HLA candidates and in cooperation with different risk factors in migraine [31, 32]. Finally, as advances in gene mapping technology reveal new genes associated with migraine, more studies are needed to fully investigate inflammatory candidate genes in a larger migraine population in order to corroborate the statistical power of the evidence that we have provided here.

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