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Cytotoxic T lymphocyte antigen 4 polymorphism 49 (A>G) and migraine

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Abstract Migraine without aura (MO) and migraine with aura (MA) are disorders involving multiple environmental and genetic factors. The A/G polymorphism located within exon 1 of the gene encoding the cytotoxic T lymphocyte antigen 4 (CTLA-4) is associated with several HLA-associated multifactorial diseases. The CTLA-4 family shows a negative control on T-cell proliferation and cytokine production (TNF- α and IL-10). In the present study we investigated the contribution of the candidate gene CTLA-4 in migraine pathophysiology. Included in the study were 96 MO and 39 MA migraine patients and 106 healthy individuals as control group. The results showed no statistical difference of allele frequencies between patient group and control group. These results would indicate no association between MA and MO migraine and CTLA-4 polymorphism, excluding any possible role of the CTLA-4 gene as a

genetic factor determining susceptibility to migraine.

Key words Candidate gene • Migraine • CTLA-4 • Polymorphism

Introduction

In the past decade increasing attention has been given to the study of migraine. Migraine is a recurring headache disorder manifest in attacks lasting 4–72 h and affecting

about 10% of the general population. Migraine is clinically distinguishable as two main types: migraine with aura (MA) and migraine without aura (MO), i.e., with or without the complex of focal neurological symptoms (scotomas, scintillations, fortification spectra, etc.) that initiates or accompanies pain attacks [1].

The two types of migraine involve multiple environmental and genetic factors. Several studies on familial hemiplegic migraine (FHM), a rare autosomal dominant subtype of MA, identified the responsible genes on chromosome 19 and chromosome 1 [2]. In two previous studies, we hypothesised both a protective role for the HLA-DR2 antigen, providing an additional basis for the proposed genetic heterogeneity between migraine without aura and migraine with aura, and involvement of lymphotoxin a (TNF- β) as a susceptibility gene in migraine without aura [3, 4]. The A/G polymorphism located within exon 1 at position 49 of the gene encoding the cytotoxic T lymphocyte antigen 4 (CTLA-4) is associated with several multifactorial HLA-associated diseases such as type 1 diabetes, inflammatory bowel diseases, etc. The CTLA-4 family shows a negative control on T-cell proliferation and cytokine production (TNF- α and IL-10) [5, 6]. In the present study we searched for an association between migraine and the gene encoding CTLA-4.

Material and methods

Patients and controls

A controlled study was done in 135 migraine patients: 39 with MA (9 males, 30 females, mean age 39.7 ± 7.4) and 96 with MO (21 males, 75 females, mean age 36.7 ± 6.9) diagnosed according to the 2004 International Headache Society (IHS) criteria. One hundred and six unrelated healthy and migraine-free subjects from the same geographic area (Central Italy), randomly selected, were used 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.

Table 1 CTLA-4 allele frequencies in MA and MO patients and controls

CTLA-4 alleles	Controls ($n=106$)		MA ($n=39$)		MO ($n=96$)	
	<i>n</i>	Frequency	<i>n</i>	Frequency	<i>n</i>	Frequency
A	150	0.7075	51	0.6538	121	0.6302
G	62	0.2925	27	0.3462	71	0.3698

Table 2 CTLA-4 allele frequencies in migraine and controls

CTLA-4 alleles	Controls ($n=106$)		Patients ($n=135$)	
	<i>n</i>	Frequency	<i>n</i>	Frequency
A	150	0.7075	172	0.6370
G	62	0.2925	98	0.3630

Methods

Genomic DNA was isolated from proteinase-K-treated peripheral blood leukocytes according to the salting-out method. The PCR-RFLP BstEII polymorphism of the CTLA-4 gene was studied by PCR amplification using specific primers previously described by Marron et al. [5] of a 152-bp fragment in the first exon of the gene, subsequently digested by BstEII restriction enzyme. The presence or absence of the restriction site defines two alleles: CTLA-4 A results in a cleaved fragment of 130 bp and CTLA-4 G allele yields an intact 152-bp fragment. Digested products were separated by electrophoresis on 3.5% agarose gel.

Statistics

CTLA-4 allele frequencies were estimated by direct counting in patients and controls. The frequencies of alleles or genotypes of patients and controls were compared by chi-square contingency table analysis. Differences were considered statistically significant when p was less than 0.05. Hardy-Weinberg equilibrium at CTLA-4 locus was verified in patients and control populations.

Results

Allele distribution of the studied polymorphism is in Hardy-Weinberg equilibrium in both controls and patients (data not shown). No significant CTLA-4 associations either with MO or with MA were found and when we compared the whole group of patients with controls (Tables 1, 2). The distribution of CTLA-4 genotypes in migraine patients and controls are shown in Table 3. No significant differences were observed between patients and controls.

Table 3 Distribution of CTLA-4 genotypes in MA and MO patients and controls

CTLA-4 alleles	Controls (n=106)		MA (n=39)		MO (n=96)	
	n	Frequency	n	Frequency	n	Frequency
A-A	51	0.481	14	0.359	35	0.365
A-G	48	0.453	23	0.590	51	0.531
G-G	7	0.066	2	0.051	10	0.104

Discussion

In previous studies, we observed an involvement of the HLA polymorphism and TNF- β in susceptibility to migraine. In addition we have to look for other susceptibility candidate genes located either in the same region or on other chromosomes. The region 2q33, where the CTLA-4 gene is located, is considered to be associated with several multifactorial diseases.

To the best of our knowledge, this study is the largest ever conducted on the relationship between migraine subjects and CTLA-4 49 A>G polymorphism in the Italian population. It indicates that there is no association between migraine and CTLA-4 polymorphism. This suggests that CTLA-4 polymorphism does not impact on the risk of developing of migraine. As several genetic factors are involved in multifactorial diseases, further studies are needed to identify other genes responsible for genetic susceptibility in migraine.

References

- Baloh RW (2004) Genes and migraine. *Drugs Today* 40(7):577–588
- Montagna P (2004) The physiopathology of migraine: the contribution of genetics. *Neurol Sci* 25[Suppl 3]:S93–S96
- Martelletti P, Lulli P, Morellini M, Mariani B, Pennesi G, Cappellacci S, Brioli G, Giacobazzo M, Trabace S (1999) Chromosome 6p-encoded HLA-DR2 determinant discriminates migraine without aura and migraine with aura. *Hum Immunol* 60:69–74
- Trabace S, Brioli G, Lulli P, Morellini M, Giacobazzo M, Cicciarelli G, Martelletti P (2002) Tumor necrosis factor gene polymorphism in migraine. *Headache* 42:341–345
- Marron MP, Raffel LJ, Garchon HJ et al (1997) Insulin-dependent diabetes mellitus (IDDM) is associated with CTLA4 polymorphisms in multiple ethnic groups. *Hum Mol Genet* 6:1275–1282
- Ueda H, Howson JM, Esposito L et al (2003) Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature* 423:506–511