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CTLA-4 gene polymorphism is not associated with conventional multiple sclerosis in Japanese

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Abstract

We investigated the polymorphisms of exon 1 (+49A/G) and promoter (−318C/T and −651C/T) regions of the CTLA-4 gene in 133 Japanese patients with conventional/classical multiple sclerosis (MS) and 156 healthy controls. Patients with optico-spinal MS (OSMS) or atypical clinical attacks were excluded from the study. There was no significant difference in the distribution of polymorphisms between patients and controls. Furthermore, there were no associations between polymorphisms and clinical characteristics, such as age at onset, disease prognosis, and HLA profiles. Our results suggest that CTLA-4 gene polymorphisms are neither conclusively related to susceptibility nor to the clinical characteristics of MS, especially in Japanese patients with conventional/classical form and clinical features identical to those of their counterparts in Western countries.

Keywords: CTLA-4 gene polymorphism; HLA-DRB1*1501; Multiple sclerosis; Disease susceptibility; Disease prognosis; Japanese

1. Introduction

Multiple sclerosis (MS) is considered as an autoimmune disease, and susceptibility to this condition is controlled by multiple genes and environmental factors (Vyse and Todd, 1996). Despite evidence for a strong genetic influence, a weak major histocompatibility complex (MHC) association is the only consistently observed genetic feature in MS (Hillert, 1994; Compston et al., 1995), and recent genome wide linkage studies demonstrated that MS follows a polygenetic trait with multiple loci (Ebers et al., 1996). The genes involved in polygenic diseases like MS are not easily identified because clinical manifestation requires several disease-associated alleles of several genes rather than one specific mutation. The analysis of multifactorial diseases like MS is further complicated by the fact that functional differences of known polymorphisms have not yet been identified.

CTLA-4 gene is a strong candidate gene for involvement in autoimmune diseases because it plays an important role in the termination of T cell activation (Waterhouse et al., 1995; Ueda et al., 2003). The CTLA-4 gene is located on chromosome 2q33 region, a region recognized as a candidate locus by linkage genome scan (Ebers et al., 1996). Several polymorphisms in the CTLA-4 locus have been reported, and several studies have addressed the potential role of single nucleotide polymorphism (SNP) in exon 1 (+49A/G), a microsatellite (AT)n marker at position 642 of exon 4, and SNPs in the promoter regions (−318C/T and −651C/T) of the CTLA-4 gene in susceptibility to MS with different results in different ethnic groups (Harbo et al., 1999; Ligers et al., 1999; Andreevskii et al., 2002; Rasmussen et al., 2001; Dyment et al., 2002; Maurer et al., 2002; van Veen Tineke et al., 2003; Kantarci et al.,

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2003). Interactions between CTLA-4 gene and HLA DR2 in the development of MS were also reported (Rasmussen et al., 2001; Alizadeh et al., 2003). In contrast, we previously found no association between exon 1 (+49A/G) SNP and MS in 74 Japanese patients and 93 controls, although the polymorphism was suggested to modulate the disease (Fukazawa et al., 1999). In our previous study, however, only exon 1 (+49A/G) SNP was investigated, and the subjects of the study included patients with clinically or paraclinically atypical attacks, as described previously (Fukazawa et al., 1999; 2004).

The aims of the present study were to analyze the relationships of three CTLA-4 gene polymorphisms [exon 1 (+49A/G) and promoter regions (−318C/T and −651C/T)] with disease onset and disease prognosis in an expanded data set of 133 Japanese patients with MS. We also investigated whether the CTLA-4 gene polymorphism interacts with HLA-DRB1*1501 in the development of MS. Patients with optico-spinal MS (OSMS) were excluded from the present study. Patients with atypical clinical or paraclinical findings (Fukazawa et al., 2004) were also excluded, and thus, the clinical features of the selected patients were identical to those in Western countries. All subjects studied were residents of Hokkaido, the northernmost island of Japan.

2. Patients and methods

2.1. Subjects

The study subjects were 133 unrelated Japanese patients with conventional/classical MS (CMS) who met the inclusion and exclusion criteria described below. All patients exhibited two or more clinical attacks and had objective clinical evidence of multiple lesions without any evidence of other disorders. They also fulfilled the diagnostic criteria for MS (Poser et al., 1983; McDonald et al., 2001). All patients showed a relapsing–remitting or secondary progressive course. Patients with neuromyelitis optica (NMO) or optico-spinal MS (OSMS) were excluded. Patients with clinically or paraclinically atypical attacks were also excluded because they have been reported to be a clinically and immunogenetically distinct subtype among patients with diagnosis of MS (Fukazawa et al., 2003; 2004). The definitions of OSMS and atypical attacks were described previously (Yamasaki et al., 1999; Fukazawa et al., 2000; 2003; 2004). Therefore, in the current study, all patients studied were classified as having "conventional/classical MS (CMS)" with involvement of multiple sites in the CNS, including the cerebrum, cerebellum, or brainstem, with clinical features similar to those observed in Western countries (Fukazawa et al., 2000; 2004; Weinshenker, 2003). Among 133 patients studied, 61 patients had participated in our previous study and were analyzed for exon 1 A/G polymorphisms (+49; Fukazawa et al., 1999). The control group comprised 156 healthy Japanese volunteers. All study participants were Japanese and were resident of Hokkaido, the northernmost island of Japan. Their ancestors were from various parts of Japan, since Hokkaido was first reclaimed around 1870. The native inhabitants of Hokkaido are said to be the Ainu tribe, but this remains a controversial issue partly due to lack of information on the origin of this tribe. Informed consent was obtained from each individual in writing at the time of blood sampling.

2.2. Analysis of CTLA-4 polymorphism and HLA-typing

A blood sample was obtained, and high molecular weight DNA was extracted from peripheral blood cells. Exon 1 A/G polymorphisms (+49) were determined using the method described previously (Fukazawa et al., 1999). Genotypes at polymorphic sites −318 and −651 in the promoter region of the CTLA-4 gene were determined by polymerase chain reaction (PCR)–restriction fragment length polymorphism (RFLP). The genotype at position −318 in the promoter region was identified as described previously (Rasmussen et al., 2001). The −651 SNP was detected using mismatch primers with sequences 5′-ttttatggacggctctaatctc-3′ and 5′-agaaaaaaatcacaagaaataaactgaaaatagc-3′. The amplified products were digested with *Msp*I (Boehringer Mannheim, Mannheim, Germany) and analyzed on 3% agarose gel. The C allele corresponds to the presence of two 42- and 144-bp fragments generated by *Msp*I digestion, and the T allele corresponds to the 186-bp uncleaved fragment with no *Msp*I site. Exon 1 (+49) and the promoter (318) dimorphisms were determined by using the protocol described previously (Harbo et al., 1999). We used primers with sequences 5′-TCTTTTCCGCCTATTTTCAGTT-3′ and 5′-CCCTGGAATACAGAGCCAGC-3′, and the amplified products were treated with the restriction enzymes *Tse*I and *Mse*I. On agarose gel electrophoresis, the haplotype combination of the polymorphic positions at +49 and 318 was identified by the 626-bp fragment (corresponding to the 318C, +49 A haplotype), 530-bp fragment (corresponding to the 318 T, +49 A haplotype), 460-bp fragment (corresponding to the 318 C, +49 G haplotype), and 365-bp fragment (corresponding to the 318 T, +49 G haplotype).

DNA typing of DRB1 alleles was analyzed by the nonisotopic oligotyping method using reverse dot blot hybridization. When the discrimination was not clear by the reverse dot blot hybridization method, we used the standard PCR-specific oligonucleotide probe (PCR-SSOP) method.

2.3. Disease prognosis

MS severity was defined according to the expanded disability status scale of Kurtzke (EDSS), progression index (PI), and ranked severity score (RSS). PI was calculated as a