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Research article

An examination of the Apo-1/Fas promoter Mva I polymorphism in Japanese patients with multiple sclerosis

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Abstract

Background: The Apo-1/Fas (CD95) molecule is an apoptosis-signaling cell surface receptor belonging to the tumor necrosis factor (TNF) receptor family. Both Fas and Fas ligand (FasL) are expressed in activated mature T cells, and prolonged cell activation induces susceptibility to Fas-mediated apoptosis. The Apo-1/Fas gene is located in a chromosomal region that shows linkage in multiple sclerosis (MS) genome screens, and studies indicate that there is aberrant expression of the Apo-1/Fas molecule in MS.

Methods: Mva I polymorphism on the Apo-1/Fas promoter gene was detected by PCR-RFLP from the DNA of 114 Japanese patients with conventional MS and 121 healthy controls. We investigated the association of the Mva I polymorphism in Japanese MS patients using a case-control association study design.

Results: We found no evidence that the polymorphism contributes to susceptibility to MS. Furthermore, there was no association between Apo-1/Fas gene polymorphisms and clinical course (relapsing-remitting course or secondary-progressive course). No significant association was observed between Apo-1/Fas gene polymorphisms and the age at disease onset.

Conclusions: Overall, our findings suggest that Apo-1/Fas promoter gene polymorphisms are not conclusively related to susceptibility to MS or the clinical characteristics of Japanese patients with MS.

Background

Apoptosis is a physiologic process that regulates normal homeostasis and is likely to contribute to the pathogenesis of autoimmune diseases by impairing elimination of autoreactive T and B cells [1]. Physiologic regulation of cell death is essential for removal of potentially autoreactive lymphocytes during development and excess cells after the completion of an immune response. Apo-1/Fas, also known as CD95, is a 36-kDa transmembrane glycoprotein expressed on the surface of many cell types, such as lymphocytes, epithelial cells, fibroblasts, and certain endothelial cells. Apo-1/Fas mediates apoptosis of these

cells [2], whereas Fas ligand (FasL) expression is tightly regulated and restricted to activated T cells, NK cells, and at sites of immune privilege [3]. Apo-1/Fas is considered to have an important role in the regulation of the immune system by deleting autoreactive lymphocytes. Inadequate co-stimulation of T cells by antigen-presenting glial cells might render T cells susceptible to activation-induced apoptosis. T cells expressing Apo-1/Fas might also die in an antigen-nonspecific manner after interacting with glial cells expressing Apo-1/FasL. T-cell apoptosis contributes to resolution of the central nervous system (CNS) inflammation and clinical recovery from attacks of experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS) [4]. Apoptosis of autoreactive T cells in the CNS is likely to be important in preventing the development of MS [5]. On the other hand, Apo-1/Fas mRNA and protein are both induced after UV light exposure [6], indicating that the regulation of this gene is important in the cellular response to UV light. The geographic distribution of MS might be related to the degree of sunlight exposure, and exposure to UV light might have a protective effect in MS. Taken together, Apo-1/Fas is a candidate proapoptotic gene in MS.

Apo-1/Fas is a type I transmembrane protein that belongs to the tumor necrosis factor receptor superfamily (TNFRSF), and maps to the long arm of chromosome 10q23/10q24.1 in humans [7,8]. Positive lod scores with microsatellite markers near this region were identified in the United States [9] and Canadian genome screens [10]. The gene encoding Apo-1/Fas, TNFRSF6, contains a single nucleotide polymorphism (SNP) in the promoter at position -670 that disrupts a gamma-activated sequence (GAS) transcription factor binding sequence [11]. This polymorphism creates an Mva I restriction fragment length polymorphism (RFLP), and abolishes the binding site of the nuclear transcription element, GAS. The function of the polymorphism remains unclear, but its location, on a consensus sequence of the GAS, implies that it might be associated with altered Fas gene transcription.

In the present study, we examined the association of the promoter -670 polymorphism in the Apo-1/Fas gene in Japanese patients with MS.

Patients and Methods

Patients and healthy individuals

Unrelated patients (n = 114) with relapsing-remitting type MS (RRMS) or secondary-progressive type MS (SPMS) who, after having been observed for at least 1 year, were diagnosed as MS according to the criteria of McDonald [12] (Table 1). The patients were considered "conventional" MS patients, which were quite similar clinically to Western MS patients, and "optic-spinal form" MS patients were excluded from this study [13]. The control group was composed of 29 unrelated healthy men and 92 unrelated healthy women ranging from 20 to 58 years old (mean ± SD; 33.1 ± 9.2). All of the patients and controls were Japanese and were residents of Hokkaido, the northernmost island of Japan. There were no significant differences in the sex ratio and age between the patients and controls. This study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Table 1: Clinical profiles of MS patients

	Total (n = 114)
Female : Male	84 : 30
Age (mean years ± SD)	35.0 ± 10.7
Age at onset (mean years ± SD)	26.2 ± 9.0
Course	
relapsing-remitting type	79 (69.3%)
secondary-progressive type	35 (30.7%)
Duration (mean years ± SD)	8.9 ± 8.4
EDSS (mean ± SD)	3.0 ± 2.5
HLA-DRB1*1501 allele positive	34/107 (31.8%)

EDSS; Expanded Disability Status Scale of Kurtzke

Genotyping of the polymorphism at position -670

After obtaining the informed consent of each subject, high molecular weight DNA was extracted from their peripheral blood cells. Apo-1/Fas promoter Mva I polymorphisms were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using DNA, according to previously described methods [11]. The Mva I restriction enzyme detected a dimorphism with either a band at 189 bp (allele G) or a band at 233 bp (allele A) from PCR products.

Genomic HLA-DRB1 typing

The genomic HLA typing methods were previously described [14]. In this study, DRB1 alleles were determined in 107 MS patients and 103 controls.

Statistical analysis

Comparisons between the various alleles of patients with MS and controls were made using the Chi-square test for two-by-two or two-by-three comparisons. The statistical analysis between the genotypes of Mva I polymorphism and the onset age of MS patients was tested by analysis of variance (ANOVA) followed by Fisher's protected least significant difference (PLSD).

Table 2: Apo-1/Fas promoter polymorphism at position -670 in the enhancer region: Case-control analysis of genotype and alleles

	MS patients			Controls
	Total	R-R	S-P	
	N = 114 (%)	N = 79 (%)	N = 35 (%)	N = 121 (%)
Genotype frequency				
G/G	23 (20.2%)	16 (20.3%)	7 (20.0%)	25 (20.7%)
G/A	65 (57.0%)	44 (55.7%)	21 (60.0%)	63 (52.1%)
A/A	26 (22.8%)	19 (24.1%)	7 (20.0%)	33 (27.3%)
Allele frequency				
G	111 (48.7%)	76 (48.1%)	35 (50.0%)	113 (46.7%)
A	117 (51.3%)	82 (51.9%)	35 (50.0%)	129 (53.3%)

There was no significant difference in the distributions of -670 polymorphisms in Apo-1/Fas gene between MS patients and control subjects. Furthermore, there were no significant differences in the distributions of -670 polymorphisms in the Apo-1/Fas gene between patients with a relapsing-remitting course and those with a secondary-progressive course or between patients with either course and controls ($P > 0.05$).

Results

Mva I genotype and allele frequencies

The proportions of Mva I genotypes in the Apo-1/Fas promoter gene in the MS patients and controls are shown in Table 2. In the control subjects, the genotype frequencies conformed to Hardy-Weinberg expectations. There was no significant difference in the distributions of Mva I polymorphisms between MS patients and the control group. Among the 114 MS patients, there was no association between the Mva I gene polymorphisms and the clinical course (relapsing-remitting course or secondary progressive course).

The association between Mva I polymorphism and age at disease onset

In the relation between Mva I polymorphism and age at onset of MS, the mean onset ages of the patients with G/G, G/A, and A/A genotypes were (age ± SD) : 25.0 ± 9.5, 25.4 ± 7.7, and 29.1 ± 11.2, respectively (Table 3). Patients with the A/A genotype tended to have a later onset than those with the G/G or G/A genotype, but this difference was not statistically significant ($P = 0.168$).

*Mva I polymorphism in DRB1*1501 positive and negative individuals*

The positive rates of HLA-DRB1*1501 in MS patients and controls were 34/107 (31.8%) and 15/103 (14.6%), respectively. The positive rate of DRB1*1501 in MS patients was significantly higher than that in controls ($P < 0.005$). The Mva I genotype and allele frequencies in the MS patients and in controls positive and negative for the DRB1*1501 allele are shown in Table 4. There were no significant differences in the Mva I allele and genotype frequencies in DRB1*1501 positive or negative patients.

Table 3: Age at disease onset in Apo-1/Fas promoter polymorphism at position -670

Genotype	No. of patients	Age at onset (mean age ± S.D.)
G/G	23	25.0 ± 9.5
G/A	65	25.4 ± 7.7
A/A	26	29.1 ± 11.2

Statistical analysis was performed using ANOVA. There was a trend for patients with the A/A genotype to have a later onset than patients with the G/G or G/A genotype, but the difference was not statistically significant ($P = 0.168$).

Discussion

We analyzed a new genetic marker, Mva I polymorphism on the Fas promoter gene, in patients with MS. There were no differences in genotype or allele frequencies among MS patients and controls. Furthermore, our results indicated that Mva I polymorphisms are not associated with the clinical course (relapsing-remitting course or secondary progressive course). Patients with the A/A genotype tended to have a later onset than those with the G/G or G/A genotype but this difference was not statistically significant. To date, there has been one report regarding this Mva I polymorphism and MS [15]. In that report in Australian population, there were increases in the A allele in MS individuals overall, and in HLA-DRB1*1501 negative MS patients [15]. This tendency was not detected in the Japanese MS individuals in the present study.

Table 4: Genotype and allele frequencies of the Apo-1/Fas Mva I polymorphism in thepresence and absence of the DRB1*1501

	DRB1*1501 positive		DRB1*1501 negative	
	MS patients N = 34 (%)	Controls N = 15 (%)	MS patients N = 73 (%)	Controls N = 88 (%)
Genotype frequency				
G/G	13 (38.2%)	3 (20.0%)	15 (20.5%)	20 (22.7%)
G/A	14 (41.2%)	8 (53.3%)	47 (64.4%)	48 (54.5%)
A/A	7 (20.6%)	4 (26.7%)	11 (15.1%)	20 (22.7%)
Allele frequency				
G	40 (58.8%)	14 (46.7%)	77 (52.7%)	88 (50.0%)
A	28 (41.2%)	16 (53.3%)	69 (47.3%)	88 (50.0%)

The positive rates of HLA-DRB1*1501 in MS patients and controls were 34/107 (31.8%) and 15/103 (14.6%), respectively. The positive rate of DRB1*1501 in MS patients was significantly higher than that in controls ($P < 0.005$). There was no significant difference in the distributions of -670 polymorphisms in the Apo-1/Fas gene in DRB1*1501 positive or negative MS patients. ($P > 0.05$).

Some studies reported an association between the Fas promoter -670 polymorphism and autoimmune diseases or degenerative diseases. Huang et al. demonstrated a skewed distribution of Mva I genotypes in the first cohort of 103 Australian patients with rheumatoid arthritis (RA), but this association was not confirmed in a second cohort [16]. The -670 polymorphism is associated with development of anti-RNP antibodies in systemic lupus erythematosus (SLE) [17]. The -670 polymorphism is also associated with Alzheimer's disease and interacts with the APO-E variant [18], indicating that it has potential biologic significance.

MS is considered to be an autoimmune disorder of unknown etiology. The pathogenesis of the disease remains obscure, but genetic factors are considered to be contributory. In the HLA, there was an association between DRB1*1501 and MS in our study as well as in Caucasian. With respect to the genetics of MS, it is possible that non-HLA genes have a pathogenetic role. Because a case-control study design might provide spurious results attributable to population stratification, including negative findings such as those reported in the present study, additional studies are necessary to exclude the possibility of type 2 errors. Population association studies that compare the frequency of variants between cases and controls might be particularly effective and might be a powerful assay for common genetic variants of weak effect [19]. While our results are not entirely consistent with those of another report examining other ethnicities [15], this inconsistency might be due, in part, to differences in the polymorphism background of the different ethnic group. Further research in gene polymorphisms present in other ethnic groups is needed, as well as further research in the Japanese patient group. In addition, susceptibility genes might provide a small individual contribution in MS, thus their identification might be important for providing clues to the pathogenesis of the different clinical forms of the disease and for designing the most effective therapy for each individual patient.

Conclusions

We investigated Mva I polymorphism on the Apo-1/Fas promoter gene from the DNA of 114 Japanese patients with conventional MS and 121 healthy controls. We found no evidence that the polymorphism contributes to susceptibility to MS. Furthermore, there was no association between Apo-1/Fas gene polymorphisms and clinical course (relapsing-remitting course or secondary-progressive course). No significant association was observed between Apo-1/Fas gene polymorphisms and the age at disease onset.

Competing interests

None declared.

Authors' contributions

MN carried out and coordinated this studies, performed the data and statistical analysis and drafted the manuscript. TF contributed to collecting materials and participated in its design and coordination together with drafting the manuscript. RM contributed to examining HLA typing. IY contributed to examining Mva I polymorphism. SK and KT conceived of the study and participated in its design and coordination together with drafting the manuscript.

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