

BRIEF COMMUNICATION

***OAS1* gene haplotype confers susceptibility to multiple sclerosis**M. Fedetz¹, F. Matesanz¹, A. Caro-Maldonado¹, O. Fernandez², J. A. Tamayo², M. Guerrero³, C. Delgado⁴, J. A. López-Guerrero⁵ & A. Alcina¹

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Abstract

Multiple sclerosis (MS) is associated with genetic susceptibility and unknown environmental triggers, possible viral infections, but the specific etiological mechanism that subsequently develops into an inflammatory/autoimmune cascade of events is poorly understood. Recently, genetic variants of 2',5'-oligoadenylate synthetase 1 (*OAS1*) gene, a critical enzyme involved in innate antiviral response, have been associated with differential enzyme activity and type 1 diabetes in both case-control and family studies. We hypothesized that polymorphisms in the *OAS1* gene could influence the susceptibility to MS. To test this hypothesis, we conducted a case-control study of 333 patients with MS and 424 healthy controls and genotyped two *OAS1* single nucleotide polymorphisms (SNPs) by restriction fragment length polymorphism method: rs 10774671, A/G SNP altering the splicing site at the seventh exon, and rs 3741981, a nonsynonymous (Ser162Gly) A/G SNP in the third exon. Haplotype but not single-marker analysis revealed an association of the haplotype created by the G allele at rs 10774671 and the A allele at rs 3741981 with the susceptibility to MS (P value = 8.8×10^{-5}). Subjects carrying this haplotype had an increased risk of MS comparing with those not carrying it (odds ratio = 4.7, 95% confidence interval 2.1–10.9). Our findings indicate that the *OAS1* gene polymorphisms may confer susceptibility to MS or serve as markers of functional variants and suggest that *OAS1* activity is involved in the etiology of the disease. Future studies in a larger sample and association analysis with functional variants will clarify the role of the *OAS1* gene in the susceptibility to MS.

Multiple sclerosis (MS) is a chronic neurodegenerative disease of the central nervous system that is characterized by inflammatory demyelination and, to a variable degree, axonal damage. Epidemiological and genetic findings suggest that MS is an acquired autoimmune disease triggered by unknown environmental factor in genetically susceptible individuals (1). Several cases of virus-induced demyelinating encephalomyelitis in human beings and in experimental models as well as the presence of immunoglobulin G oligoclonal bands in the cerebrospinal fluid

indicate that the causative factor may be viral (2, 3). The virus may remain dormant in the central nervous system but then becomes activated in adulthood and provoke direct lysis of oligodendrocytes or could initiate immunopathology (4). The possible causative agents were studied, but conclusions are not definite. However, the absence of identifiable infectious agent, especially viral, does not rule out its presence at a certain time-point and the concomitant long-term triggering of an autoimmune cascade of events thereafter (1).

The central nervous system mounts an organized innate immune response during systemic bacterial or viral infection (5). Two different classes of pattern-recognition receptors (PRRs), including multiple Toll-like receptors and several RNA helicases, are responsible for the recognition of various viral conserved molecular motifs in different cellular compartments and induce antiviral responses through different signaling pathways. However, signaling through these PRRs converges on type I interferon (IFN) induction and leads to the elimination of viruses (6).

Among the critical proteins with antiviral activity induced by type I IFNs, there is a family of 2',5'-adenylate synthetase enzymes (oligoadenylate synthetase, OAS) that catalyzes the synthesis of oligoadenylates of general structure ppp (A2'p)_n A (2-5A). OAS enzymes require double-stranded RNA structures, such as viral genomes or single-stranded transcripts that possess significant double-stranded character, to become activated (7). The functional 2-5A oligomers bind to and activate latent endoribonuclease, RNA degrading enzyme, which was also shown to produce a strong stimulation of transcription for genes that suppress virus replication and promote cellular apoptosis (8, 9). Studies on inbred strains of laboratory mice demonstrate that mutation in exon 4 of their *Oas1b* gene plays an important role in susceptibility to West Nile Virus (10, 11), meanwhile wild-type mice are resistant to this virus (12).

In humans, the activity of the OAS1 enzyme is found to be controlled by a single nucleotide polymorphism (SNP) at the splice-acceptor site of exon 7 in the *OAS1* gene (13). The G allele at acceptor site is associated with high enzyme activity. It conserves the splice site and generates a p46 enzyme isoform, while A allele ablates the splice site that results in the generation of p48 and p52 isoforms. This SNP (rs 10774671) is reported to influence susceptibility to type I diabetes in a case-control study (14). The association of the *OAS1* gene with type I diabetes (T1D) in a family-based study (15) is attributed to rs 3741981 (*OAS1* third exon) as a more likely functional candidate than rs 10774671. SNP in the seventh exon of *OAS1* gene (rs 2660), which is in almost complete linkage disequilibrium with SNP at the splice-acceptor site of the exon 7 (13), is associated with the outcome of hepatitis C viral infection (16).

The aim of this study was to assess the influence of *OAS1* gene polymorphisms in susceptibility to MS. For this purpose, we chose two SNPs: one, A/G polymorphism at the splice-acceptor site of exon 7 (rs 10774671) and another, non-synonymous A/G SNP in the third exon of gene (rs 3741981), which leads to amino acid substitution (Ser162Gly).

Our study sample consisted of 333 unrelated cases with clinically defined MS according to Poser's criteria (17) and 424 ethnically matched unrelated Caucasian controls of similar age range (donors of the regional blood bank). The study was approved by the Ethics Committee of Hospital, and written informed consent was obtained from all the

participants. The restriction fragment length polymorphism method was used for genotyping of these SNPs. Primer sequences used were as follows: rs 10774671 – forward, TCCAGATGGCATGTCACAGT and reverse, TAGAAGGCCAGGAGTCAGGA, restriction enzyme *AfuI*; and rs 3741981 – forward, GGATCAGGAATGGACCTCAA and reverse, 3719 GGAGAACTCGCCCTCTTCT, restriction enzyme *SsiI*. Amplification conditions included initial denaturation at 94°C for 2 min, followed by 28 cycles of 94°C for 20 s, 62°C for 40 s and 72°C for 30 s, with a final extension for 7 min at 72°C.

In both panels, the genotype frequencies of both SNP were distributed according to Hardy-Weinberg equilibrium. Single-marker results were analyzed by χ^2 statistics. No significant difference was observed between patient and control genotype or allelic frequencies (Table 1). Stratification of patient group by clinical course [relapsing-remitting (RR) or secondary progressive (SP)], age at the onset of disease and gender did not reveal any association with *OAS1* genotypes. Since linkage disequilibrium with a disease locus may be stronger for haplotypes than for single alleles (18), we examined whether haplotypes of *OAS1*, determined by studied SNPs, could reveal an association with the disease. Haplotype analysis was performed using HAPLOVIEW software (19). Linkage disequilibrium characteristics between two markers in our sample set were as follows: $D' = 0.898$ and $r^2 = 0.588$. We found that the haplotype GA created by the G allele at rs 10774671 and by the A allele at rs 3741981 (hap 4) was 4.6-fold more frequent in unstratified patient group with MS (P value = 8.8×10^{-5}) as shown in Table 2. Global $\chi^2 = 23.53$, d.f. was 3 and P value

Table 1 Genotype and allelic frequencies of *OAS1* polymorphisms in patients with multiple sclerosis and healthy controls^{a,b}

	Patients (n = 333)	Controls (n = 424)	χ^2	d.f.	P
rs 10774671					
Genotype					
AA	120 (36.0)	156 (36.8)	0.36	2	0.84
AG	164 (49.3)	212 (50.0)			
GG	49 (14.7)	56 (13.2)			
Allele					
A	404 (60.7)	524 (61.8)	0.20	1	0.65
rs 3741981					
Genotype					
AA	87 (26.1)	119 (28.0)	0.48	2	0.79
AG	180 (54.1)	219 (51.7)			
GG	66 (19.8)	86 (20.3)			
Allele					
A	354 (53.2)	457 (53.9)	0.08	1	0.78

n, number of the subjects genotyped.

^a The values given in parenthesis are in percentages.

^b Statistical analysis of genotype and allelic frequencies between the groups was carried out using the contingency table 3×2 (genotypes) and 2×2 (alleles) χ^2 statistics (<http://faculty.vassar.edu/lowry/VassarStats.html>).

Table 2 The haplotype frequency estimation for the patients with multiple sclerosis and control group^a

Haplotype (rs 10774671–rs 3741981)		Patients	Control	χ^2	<i>P</i>
hap1	AA	0.493	0.534	2.54	0.1113
hap2	GG	0.356	0.372	0.44	0.5061
hap3	AG	0.114	0.085	3.43	0.0640
hap4	GA	0.037	0.008	15.38	8.8105E-5

^a Analyzed by HAPLOVIEW software package (19); global $\chi^2 = 23.53$, d.f. = 3, $P < 0.0001$ in the analysis by FAMHAP software (20).

was less than 0.0001 in analysis by FAMHAP (version 12) software (20). Odds ratio (OR) was calculated for hap 4 vs three other haplotypes using two-way contingency table analysis. Subjects carrying this haplotype had a 4.7-fold increased risk of MS [95% confidence interval (CI) 2.1–10.9]. Haplotype frequencies were also estimated by THESIAS (21) software, and similar results were obtained. Our results demonstrated that hap 4 was associated with RR [P value = 6.04E-5, OR 5.1 (95% CI 2.2–12.2)] and SP [$P = 0.003$, OR 4.8 (95% CI 1.6–14.4)], the clinical courses of MS.

We have assessed the association of GA haplotype with IFN-beta therapy response in a group of patients that were classified as responders and non-responders with IFN-beta treatment, as described by Leyva et al. (22). The estimated haplotype frequencies were not different between IFN-beta responders ($n = 139$) and non-responders ($n = 56$).

Report by Field et al. (14) presents data suggesting that polymorphism G/A at the splice site of exon 7 in *OAS1* gene could influence susceptibility to T1D, the frequencies of genotypes GG and GA are found to be significantly increased in patient with T1D. These investigators also show that the minor G allele at this SNP has a higher frequency in individuals with high enzyme activity than in those with low enzyme activity (13).

In the study of Tessier et al. (15), significant increased risk of T1D is related only with homozygosity for the minor allele of each of the *OAS1* SNPs (rs 10774671, rs 3741981 and rs 3177979), a recessive effect of these polymorphisms is shown by FBAT analysis. Results of transmission disequilibrium test performed by these authors suggest that rs 3741981 may be the sole functional variant accounting for the genetic effect in T1D in contrast to the hypothesis of Field et al. *OAS* locus is characterized by a long range linkage disequilibrium between polymorphisms (15). Due to this fact, we are in agreement with the opinion of Tessier et al. (15) that the studied SNPs could be merely markers for a haplotype containing a functional variant that determines the susceptibility to disease.

In conclusion, we found an association of a haplotype consisting of the SNPs in exons 3 and 7 (splice-acceptor site)

of the *OAS1* gene, which is involved in innate host antiviral response, with MS. Because these two markers did not show any association with MS in single-marker analysis, the causative variant may be located in an adjacent region. Future studies in a large case-control or family-based sample will clarify the role of *OAS1* gene in susceptibility to MS.

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