Tissue Antigens ISSN 0001-2815

BRIEF COMMUNICATION

OAS1 gene haplotype confers susceptibility to multiple sclerosis

M. Fedetz[1], F. Matesanz[1], A. Caro-Maldonado[1], O. Fernandez[2], J. A. Tamayo[2], M. Guerrero[3], C. Delgado[4], J. A. López-Guerrero[5] & A. Alcina[1]

1 Instituto de Parasitología y Biomedicina López Neyra, CSIC, Granada, Spain
2 Instituto de Neurociencias Clínicas, Hospital Regional Universitario Carlos Haya, Málaga, Spain
3 Hospital Clínico San Cecilio, Granada, Spain
4 Centro Regional de Transfusión Sanguínea, Granada, Spain
5 Centro de Biología Molecular, Universidad Autónoma de Madrid, Madrid, Spain

Key words
2′,5′-oligoadenylate synthetase 1; gene polymorphism; haplotype; multiple sclerosis; restriction fragment length polymorphism

Correspondence
Antonio Alcina
Instituto de Parasitología y Biomedicina
''López Neyra''
CSIC
Av. de Conocimiento
s/n, Parque Tecnológico Ciencias de la Salud
18100 Armilla
Granada
Spain
Tel: 34 958 181668
Fax: 34 958 181632
e-mail: pulgoso@ipb.csic.es

Received 12 May 2006; revised 12 August 2006; re-revised 23 August 2006; Accepted 11 September 2006

doi: 10.1111/j.1399-0039.2006.00694.x

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 antivirus 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 $= 8.8 \times 10^{-5}$). 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 1 IFNs, there is a family of 2′,5′adenylate synthetase enzymes (oligoadenylate synthetase, OAS) that catalyzes the synthesis of oligodadenylates of general structure ppp $(A2'p9)_n$ 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 1 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 *Alu*I; and rs 3741981– forward, GGATCAGGAATGGACCTCAA and reverse, 3719 GGAGAACTCGCCCTCTTTCT, restriction enzyme *Ssi*I. 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 $= 8.8 \times 10^{-5}$) 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)			
AG	164 (49.3)	212 (50.0)	0.36	2	0.84
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)			
AG	180 (54.1)	219 (51.7)	0.48	2	0.79
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	*P*
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.

Acknowledgments

This work was supported by grants FIS PI 020920 and Federación Española de Esclerosis Múltiple (FEDEM) to A. A. and by grants FIS PI041298 and SAS 304-04 to F. M. M. F. is a holder of Fundación IMABIS fellowship.

References

1. Grigoriadis N, Hadjigeorgiou GM. Virus-mediated autoimmunity in multiple sclerosis. *J Autoimmune Dis* 2006: **3**: 1.
2. Kurtzke JF. Epidemiologic evidence for multiple sclerosis as an infection. *Clin Microbiol Rev* 1993: **6**: 382–427.
3. Christensen T. Association of human endogenous retroviruses with multiple sclerosis and possible interactions with herpes viruses. *Rev Med Virol* 2005: **15**: 179–211.
4. Gilden DH. Infectious causes of multiple sclerosis. *Lancet Neurol* 2005: **4**: 195–202.
5. Nguyen MD, Julien JP, Rivest S. Innate immunity: the missing link in neuroprotection and neurodegeneration? *Nat Rev Neurosci* 2002: **3**: 216–27.
6. Kawai T, Akira S. Innate immune recognition of viral infection. *Nat Immunol* 2006: **7**: 131–7.
7. Samuel CE. Antiviral actions of interferons. *Clin Microbiol Rev* 2001: **14**: 778–809.
8 Malathi K, Paranjape JM, Bulanova E et al. A transcriptional signaling pathway in the IFN system mediated by 2′-5′-oligoadenylate activation of RNase L. *Proc Natl Acad Sci U S A* 2005: **102**: 14533–8.
9. Ghosh A, Sarkar SN, Rowe TM, Sen GC. A specific isozyme of 2′-5′ oligoadenylate synthetase is a dual function proapoptotic protein of the Bcl-2 family. *J Biol Chem* 2001: **276**: 25447–55.
10. Perelygin AA, Scherbik SV, Zhulin IB, Stockman BM, Li Y, Brinton MA. Positional cloning of the murine flavivirus resistance gene. *Proc Natl Acad Sci U S A* 2002: **99**: 9322–7.
11. Kajaste-Rudnitski A, Mashimo T, Frenkiel MP, Guenet JL, Lucas M, Despres P. The 2′,5′-oligoadenylate synthetase 1b is a potent inhibitor of West Nile virus replication inside infected cells. *J Biol Chem* 2006: **281**: 4624–37.
12. Sangster MY, Heliams DB, Mac Kenzie JS, Shellam GR. Genetic studies of flavivirus resistance in inbred strains derived from wild mice: evidence for a new resistance allele at the flavivirus resistance locus (Flv). *J Virol* 1993: **67**: 340–7.
13. Bonnevie-Nielsen V, Field LL, Lu S et al. Variation in antiviral 2′,5′-oligoadenylate synthetase (2′5′AS) enzyme activity is controlled by a single-nucleotide polymorphism at a splice-acceptor site in the OAS1 gene. *Am J Hum Genet* 2005: **76**: 623–33.

14. Field LL, Bonnevie-Nielsen V, Pociot F, Lu S, Nielsen TB, Beck-Nielsen H. OAS1 splice site polymorphism controlling antiviral enzyme activity influences susceptibility to type 1 diabetes. *Diabetes* 2005: **54**: 1588–91.
15. Tessier MC, Qu HQ, Frechette R et al. Type 1 diabetes and the OAS gene cluster: association with splicing polymorphism or haplotype? *J Med Genet* 2006: **43**: 129–32.
16. Knapp S, Yee LJ, Frodsham AJ, et al. Polymorphisms in interferon-induced genes and the outcome of hepatitis C virus infection: roles of MxA, OAS-1 and PKR. *Genes Immun* 2003: **4**: 411–9.
17. Poser CM, Paty DW, Scheinberg L et al. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. *Ann Neurol* 1983: **13**: 227–31.
18. Herr M, Dudbridge F, Zavattari P et al. Evaluation of fine mapping strategies for a multifactorial disease locus: systematic linkage and association analysis of IDDM1 in the HLA region on chromosome 6p21. *Hum Mol Genet* 2000: **9**: 1291–301.
19. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005: **21**: 263–5.
20. Becker T, Knapp M. Maximum-likelihood estimation of haplotype frequencies in nuclear families. *Genet Epidemiol* 2004: **27**: 21–32.
21. Tregouet DA, Escolano S, Tiret L, Mallet A, Golmard JL. A new maximum likelihood algorithm for haplotype-based association analysis: the SEM algorithm. *Ann Hum Genet* 2004: **68**: 165–77.
22. Leyva L, Fernandez O, Fedetz M et al. IFNAR1 and IFNAR2 polymorphisms confer susceptibility to multiple sclerosis but not to interferon-beta treatment response. *J Neuroimmunol* 2005: **163**: 165–71.