Myelin Oligodendrocyte Gene Polymorphisms and Childhood Multiple Sclerosis

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ABSTRACT

Myelin oligodendrocyte glycoprotein (MOG) is a quantitatively minor glycoprotein of the CNS localized preferentially on the outermost myelin lamellae and the oligodendrocyte plasma membrane. In several animal models, MOG displays highly immunogenic properties by inducing a severe multiple sclerosis-like disease, characterized by inflammatory demyelinating lesions. Immunologic findings implicate MOG as a target autoantigen in multiple sclerosis. We have performed a molecular study on the MOG gene by sequencing the promotor and the entire coding region, as well as the exon-intron boundaries, in 75 children with multiple sclerosis. A total of five unknown polymorphic sites in the promotor region not affecting any of the putative cis-acting transcriptional regulation motifs as well as nine additional base changes in four different exons each with

similar distribution in patients and controls ($n = 100$) were detected. Exon 2 coding for the Ig-like domain revealed two rare heterozygous missense mutations, possibly altering favorable conformational epitopes (P43H; R66P). P43 is part of the encephalitogenic epitope MOG_{35–55}. A putative C1q binding site in the C'-D loop of the Ig superfamily motif encompasses R66. In conclusion, the polymorphisms observed do not provide evidence to support a significant role for MOG in multiple sclerosis susceptibility. (*Pediatr Res* 52: 175–179, 2002)

Abbreviations

EAE, experimental autoimmune encephalomyelitis

MOG, myelin oligodendrocyte protein

MS, multiple sclerosis

MOG is a CNS-specific integral membrane protein of 26–28 kD, preferentially expressed on the outermost lamellae of myelin and on the surface of oligodendrocytes (1). It is a minor component of myelin, constituting 0.01–0.05% of total myelin proteins (2, 3). The sequences of MOG cDNA from rat, mouse, bovine, and human show that the mature MOG protein is a 218-amino acid member of the Ig superfamily (4–7), with its single glycosylated Ig-like domain located to the extracellular surface of the plasma membrane and thus directly accessible to a humoral immune response (8).

The expression of MOG is late in oligodendrocyte differentiation and shows a caudal–rostral gradient throughout development (5, 9, 10). However, its biologic function remains unknown. Given its biochemical properties, MOG might be actively involved in the completion of the myelinating process or might be part of the network to maintain the compaction of myelin by modulating the dynamics of microtubule stabilization in oligodendrocytes [reviewed in Johns and Bernard (11)].

A predominant T-cell response to MOG and the presence of anti-MOG antibodies in patients with MS implicate MOG as an

attractive target autoantigen in MS (12–17). EAE, the most widely used animal model of MS, is a CD4⁺ T cell-mediated disease. Depending on the species, EAE can be induced either actively with purified or recombinant MOG or MOG-derived peptides or transferred by T cells and antibodies reactive against MOG [(18–22), reviewed in 't Hart *et al.* (23)]. Furthermore, comparative observations of acute lesions in MS patients and in the nonhuman primate models of EAE underscore the potential role of anti-MOG autoantibodies in the destruction of myelin in both MS plaques and EAE lesions (24, 25).

MS is presumed to be a multifactorial autoimmune disease. However, despite extensive efforts to identify a single locus determining susceptibility to MS, a consistent albeit weak association or linkage has been reported only for the HLA region on 6p21 (26–29). Reports demonstrating positive results for other candidate genes have been difficult to replicate beyond the population studied and provided contradictory results.

The human MOG locus has been mapped to the HLA region on chromosome 6p21 and has been shown to be less than 100 kb telomeric to HLA-F in the class 1b region in a head-to-head orientation (30). In line with other myelin genes (31), the *MOG* gene is composed of 11 exons demonstrating a complex array of alternatively spliced isoforms (32, 33). However, apart from the original MOG transcript, none of the 12 splice variants

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known at the mRNA level have been detected at the protein level. Inclusion of exon 3 encoding a short hydrophilic domain would result in a truncated form of MOG (33).

Considering the immunologic and genetic implications, we have performed a comprehensive molecular analysis on the *MOG* gene in childhood MS. In the present report, we describe an efficient long-range PCR method for amplifying the entire *MOG* gene as two single amplicons for mutational analysis. Sequencing was performed for the entire coding region and the exon-intron splice junctions, as well as for parts of the intervening intronic sequences. The analysis included also 1200 bp of the 5'-flanking region sufficient for high level activity in the mouse model of promotor efficiency (34).

METHODS

Patients and control subjects. A total of 75 unrelated children of German origin (46 girls aged 8 to 19 y with a median of 13 y and 29 boys aged 7 to 18 y with a median of 14 y) were selected from a study on inflammatory demyelination (35, 36). All patients fulfilled the established criteria for clinically defined and laboratory supported MS (37). It was established during these studies that MS in adults and childhood is basically the same disease. The control group ($n = 100$) matched with the MS patients for ethnic origin, age, and sex to reduce the risk of introducing population stratification artifacts. The study was approved by the local ethics committee, and informed consent by the parents was obtained for each patient.

DNA isolation and long-range PCR. Total genomic DNA was extracted from peripheral blood by standard techniques. With the exception of the 3'-untranslated region, the entire *MOG* gene was amplified by two overlapping PCR fragments with the Expand Long Template PCR System (Roche Molecular Products, Mannheim, Germany) according to the manufacturer's recommendations with some modifications. Primers were designed according to the published human genomic DNA sequence (38). For fragment A (MOGA, Table 1) samples were initially denatured at 94°C for 2 min, before undergoing 10 cycles of 94°C for 10 s, 66°C for 30 s, and 68°C for 9.5 min. For the subsequent 19 cycles, elongation increased

20 s each cycle, with a final extension of 7 min at 68°C. For fragment B (MOGB) thermocycler conditions (Personal Cycler, Biometra, Göttingen, Germany) were 94°C for 2 min, followed by 10 cycles at 94°C for 10 s, 65°C for 30 s, and 68°C for 3.75 min. For the subsequent 17 cycles annealing and extension were at 68°C for 4.25 min with a cycle elongation of 20 s for each cycle. A final prolonged extension step was performed for 7 min at 68°C. Reactions contained 50 mM Tris-HCl (pH 9.2), 16 mM (NH₄)SO₂, 32.5 mM (for MOGA), and 27.5 mM (for MOGB) MgCl₂, 500 μM each dNTP, 0.4 μM (for MOGA) and 0.3 μM (for MOGB) each primer, and 500 ng of template DNA. PCR products were subsequently purified by Ultrafree-MC 30,000 NMWL Filter Units (Millipore Corporation, Bedford, MA, U.S.A.). The strategy to minimize errors caused by PCR reactions involved the pooling of three independent PCR reactions for each fragment to be sequenced.

DNA sequencing. For direct sequencing each region of interest was PCR-cycled with the indicated primers (Table 1). The BigDye terminator cycle sequencing program consisted of 25 cycles of 94°C for 10 s and 60°C for 4 min. Data were selected using a model 377 sequencer (Applied Biosystems, Foster City, CA, U.S.A.) with model 48E-1200. Automated sequence comparison for mutation detection was performed with the Sequence Navigator Software (Applied Biosystems). In cases of ambiguities, a second independent PCR approach on the genomic DNA level or antisense sequencing was applied to confirm the observed mutation.

Statistical analysis. Allelic distributions for *MOG* were compared between normal and MS individuals by applying the χ^2 or Fisher's exact test. Bonferroni correction for multiple testing was done when necessary. Five percent was chosen as the level of significance ($p < 0.05$).

RESULTS

The *MOG* gene was amplified to facilitate mutational analysis by direct automated fluorescent DNA sequencing of all 11 exons and the exon-intron junctions, as well as of 1200 bp of the promotor region, from 75 unrelated children with MS and

Table 1. PCR primers for long-range amplification and sequencing reactions

Primer Type		Product Size
Long-range PCR		
Fragment	5'-GTCTGATGAGATAACCACTAAGTTGAGTGC-3'	11.8 kb
MOG-A	5'-GGAAGTTACCAAAAGTCCGGTGGAG-3'	
Fragment	5'-CTGCCTTTCAGATCCTTTCTACTGGGTG-3'	5.3 kb
MOG-B	5'-TGTCCTTAGCCAGAGAGGGCTTCCAGG-3'	
Sequencing		Expected Read Length Ranges
MOGA		
Promotor	5'-GTCTGATGAGATAACCACTAAGTTGAGTGC-3'	570
Promotor, Exon 1	5'-ACAGGGCAGAGTTGGATTGCGGGTAG-3'	750
Exon 2	5'-GGCGACAGAGAGTAAGACTGTCTC-3'	820
Exon 3	5'-TTCACGTCACATTAGAAAAGTCACAG-3'	420
Exon 4	5'-GGCTGACCAATACACAATCTTGAC-3'	820
MOGB		
Exons 5, 6	5'-GAGAAAGAGCCGCAACCATTTGGC-3'	750
Exons 7, 8, 9	5'-CAGGCATGAGCCACCACACCTGGC-3'	750
Exons 10A, 10B	5'-CAGTGCCTCACCTCTCAGATCAACC-3'	400

100 healthy control subjects. We used the long expand technique to generate two overlapping PCR fragments covering 15.3 kb of the *MOG* gene. Screening for sequence variants did not reveal any base changes within the consensus sequences of the exon-intron junctions or in exons 3 (soluble MOG), 5, 6, 7, 9, 10A, and 10B (cytoplasmic regions). Larger rearrangements caused by deletions or insertions were not detected.

Variability of the human *MOG* gene. We identified a total of 16 intronic polymorphisms each with similar allele frequencies in patients and controls and with a range varying between 0.9% and 16.6% (data not shown). Base changes within the promotor region and in expressed sequences are summarized in Table 2. Of a total of 75 patients examined, five base substitutions were found in the 5'-flanking region of the *MOG* gene. The sequence coding for the signal peptide (exon 1) revealed a G to A transition at nucleotide position 15, which is predicted to cause a silent mutation and has also been found by reverse transcription-PCR analysis of normal human CNS RNA samples (33). The heterozygous deletion of codon 22 within the signal peptide (Δ CTC) was always accompanied by polymorphisms within the 5'-flanking region at nt(-910), nt(-875), and nt(-93) both in MS patients and in controls. The haplotype [-910T/C, -875A/C, -93T/C, 64-66CTC/ Δ CTC] was under-represented in the MS population, but this difference was not significant. Three distinct silent transitions were identified in exons 2 and 8 (A306G, C336T, and G672A). Two heterozygous missense mutations were detected in exon 2 coding for the Ig-like domain of the protein. The Pro to His exchange at amino acid position 43 was found both in MS patients and in one control subject's chromosome. The heterozygous G to C transversion at nucleotide 284 resulting in Arg to Pro at residue 66 was found in a 16-year-old patient with no positive family history of MS. This mutation was inherited through her unaffected mother as revealed by genomic DNA analysis and was subsequently not found in her 14-year-old healthy sister. In addition to these rare substitutions, two missense mutations within exon 4 were found to be particularly common (Val142Leu, Val145Ile).

In summary, the distribution of alleles was similar in patients and in control subjects for all substitutions identified ($p > 0.05$). No statistically significant association or haplotype with predisposition for MS was observed, even after stratification of data for HLA status as well as of patients for severity or temporal course of disease and combinations thereof.

DISCUSSION

The substantial genetic component in the etiology of MS has been established by studies in twins, adoptees, and half siblings and by the occurrence of familial clusterings of MS cases (39-41). With the exception of the MHC region, the lack of a major MS gene is highlighted by independent systematic genome-wide searches for linkage in multiplex families (27-29, 42-46).

We have performed a detailed mutational analysis of the *MOG* gene within a patient group of moderate size ($n = 75$). We found a total of nine polymorphisms in four different exons (Table 2), each with similar frequencies in patients and control subjects. None of the substitutions observed confer obviously major deleterious effects on the function of the protein. A trend exists toward the clustering of base substitutions as four of them occurred within exon 2 coding for the Ig-like domain of the protein.

MOG is the focus of both a T cell- and an antibody-mediated immune attack in MS (14-17). Polymorphisms (such as P43H or R66P; Table 2) may influence the state of tolerance or may modulate the generation of anti-MOG antibody responses in a subset of patients with the appropriate genetic (MHC) background or may even be involved in determinant spreading as a result of ongoing demyelination. By considering the disease heterogeneity (47), anti-MOG antibodies have been described in lesions of acute MS and the marmoset model of EAE (25). T-cell autoreactivity to MOG in rhesus monkeys immunologically closely related to humans was directed against MOG₃₅₋₅₀, which was also an immunodominant epitope for the T-cell response to MOG in patients with MS (48). Furthermore, recent studies suggest that the immunodominant demyelinating autoantibody response to MOG is confor-

Table 2. *MOG*-specific polymorphisms in MS ($n = 75$) and control subjects ($n = 100$)

Nucleotide*	Location	Aa Variant	Allele		MS			Controls			MS		Controls	
					Genotypes (%)			Genotypes (%)			Frequencies (%)		Frequencies (%)	
			1	2	1/1	1/2	2/2	1/1	1/2	2/2	1	2	1	2
-1077	5'-flanking	—	T	C	96.0	4.0	0	97.0	3.0	0	98.0	2.0	98.5	1.5
-918	5'-flanking	—	G	A	96.0	4.0	0	98.0	2.0	0	98.0	2.0	99.0	1.0
-910	5'-flanking	—	T	C	90.7	9.3	0	88.0	12.0	0	95.3	4.7	94.0	6.0
-875	5'-flanking	—	A	C	90.7	9.3	0	88.0	12.0	0	95.3	4.7	94.0	6.0
-93	5'-flanking	—	T	C	90.7	9.3	0	88.0	12.0	0	95.3	4.7	94.0	6.0
15	Exon 1	S5S†	G	A	78.7	17.3	4.0	76.0	19.0	5.0	87.4	12.6	85.5	14.5
64-66	Exon 1	del22L†	CTC	Δ CTC	90.7	9.3	0	88.0	12.0	0	95.3	4.7	94.0	6.0
215	Exon 2	P43H	C	A	97.3	2.7	0	99.0	1.0	0	98.7	1.3	99.5	0.5
284	Exon 2	R66P	G	C	98.7	1.3	0	100	0	0	99.3	0.7	100	0
306	Exon 2	K73K	A	G	94.7	5.3	0	98.0	2.0	0	97.3	2.7	99.0	1.0
336	Exon 2	L83L	C	T	98.7	1.3	0	99.0	1.0	0	99.3	0.7	99.5	0.5
511	Exon 4	V142L	G	C	54.7	41.3	4.0	57.0	36.0	7.0	75.3	24.7	75.0	25.0
520	Exon 4	V145I	G	A	82.7	17.3	0	88.0	9.0	3.0	91.3	8.7	92.5	7.5
672	Exon 8	L195L	G	A	98.7	1.3	0	98.0	2.0	0	99.3	0.7	99.0	1.0

* Nucleotide positions (7, 32, 38).

† Numbering of the signal peptide.

mation dependent [reviewed in Stefferl *et al.* (49) and Iglesias *et al.* (50)] and may mediate demyelination only in a small subset of MS cases (16).

In addition, Arg⁶⁶ is part of the complement-binding motif Glu⁶⁴-X-Arg⁶⁶-X-Arg⁶⁸ similar to the C1q binding site Glu³¹⁸-X-Lys³²⁰-X-Lys³²² within the CH2 domain on IgG molecules (51) and might directly activate the classic complement pathway. It has been shown that purified native MOG and the recombinant extracellular Ig-like domain of MOG both bind C1q in a dose-dependent manner (52).

The encephalitogenicity as well as the T- and B-cell responses to MOG peptides harboring the identified mutations might be assessed in the EAE model of MS. In addition, synthetic peptides overlapping amino acid positions 43 and 66 might be constructed to identify the effects for the autoimmune T-cell response in MS patients by establishing a PBL proliferation assay.

The concept of molecular mimicry is still an attractive but unresolved issue in MS to explain immune-mediated injury to normal tissue. The possibility of stimulating MBP-specific T-cell clones established from MS patients by structurally related viral and bacterial peptides (not necessarily the sequence) has been demonstrated (53). The possible involvement of environmental pathogens in the induction of MS has been shown by molecular mimicry between Semliki Forest Virus (SFV) peptide E2₁₁₅₋₁₂₉ and MOG₁₈₋₃₂. Both of them were capable of inducing a later-onset chronic EAE-like disease in C57B16/J mice that correlated with histopathologic signs of vacuolation in the CNS white matter (54). Amino acid substitutions (Table 2) may have an influence on cross-reactivity if the appropriate immunologic background is available. Further studies should provide more information about the spectrum of epitopes from self- and pathogen-derived antigens.

There has been no functional analysis of the human MOG promotor up to now. A comparable study of the mouse MOG 5'-flanking region suggests that potential regulatory elements in the proximal -657 region were sufficient for high-level activity (34). We have identified five previously unknown base substitutions within the 5'-flanking region (Table 2), which are not part of the suggested protein-DNA interfaces (38). Given that three purine-rich *cis*-regulatory motifs, which are thought to be important for the expression of other myelin genes, including MBP, PLP, 2',3'-cyclic nucleotide 3'-phosphodiesterase, MAG, and P₀ [reviewed in Hudson *et al.* (55)], were not included in the -657-bp region, novel and as yet uncharacterized positive regulatory motifs may account for the maximal expression in the mouse model of the MOG promotor efficiency (34).

The substitution of isoleucine for valine at amino acid position 145 (exon 4) has also been used for a case-control association study from France (56). In line with our results (Table 2), this variant is not associated with an increased MS susceptibility. Conflicting results have been obtained by analyzing microsatellite markers. MOGc (38), located within the *MOG* gene, revealed significant differences in allele frequencies between white MS patients and control subjects (57). These findings could in part be explained assuming the disease-associated, extended HLA haplotype and the apparent linkage disequilibrium in this region. However, one of the observed allelic associations was independent of the DRB1*1501 effect. No significantly different MOG haplo-

types were detected in an association study from France using three microsatellites—MOGc and two located upstream of the MOG transcription start site (58, 59). Linkage analysis to four markers performed in multiplex MS families revealed a potential role of MOG (60).

Assessing association with the case-control method can be prone to biases owing to population stratification, admixture artifacts, or limited statistical power (61, 62). The genetic dissection of MS also is hampered by several basic unsolved problems, *e.g.* incomplete penetrance, locus heterogeneity, or polygenic inheritance. The importance of ethnic and clinically homogeneous and comparable study groups was illustrated by analyzing an *MOG* gene restriction fragment length polymorphism, revealing inconsistent results (7, 63).

In conclusion, the variations in the coding sequences and the upstream region of the *MOG* gene do not play a significant role in the pathology of MS and probably of EAE as well. Evidence for an association with a certain MOG haplotype was not provided after data stratification for severity or temporal course of disease or for the HLA status and combinations thereof ($p > 0.05$). However, to verify the significance of variations, a comprehensive analysis of a larger group of patients with MS is necessary to create HLA/MOG haplotypes, which may correlate with B- and T-cell responses to MOG. Further investigations may also provide insight into the 3' untranslated region of the *MOG* gene, which was not included in this study.

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