Polymorphisms in the genes encoding interferon-γ and interferon-γ receptors in multiple sclerosis

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Summary

Genome screens suggest that several genes, each contributing to a small extent, are involved in multiple sclerosis (MS) susceptibility. Simultaneous analysis of related genes may improve the power to detect such small effects. Interferon-γ (IFN-γ), mediating its effects through the IFN-γ receptor, is a pleiotropic, pro-inflammatory cytokine for which a detrimental effect on the course of MS has been reported. The role of IFN-γ receptor 1 (*IFNGR1*) and IFN-γ receptor 2 (*IFNGR2*) gene polymorphisms has not been studied in MS, and, for the *IFNG* gene polymorphism there is only one previous study, which incorporates clinical, but not imaging, data. The aim of this study was to investigate whether polymorphisms in the *IFNG* and *IFNGR1* and *IFNGR2* genes are associated with susceptibility to MS, or disease characteristics, as defined by clinical and imaging criteria. Genotypes for *IFNG*, *IFNGR1* and *IFNGR2* were determined in 509 patients with MS and in 193 healthy controls. Patient files were reviewed for disease course, age at onset of disease, and rate of progression. Serial magnetic resonance imaging (MRI) data were available for 107 patients. No significant differences in the distribution of *IFNG*, *IFNGR1* and *IFNGR2* genotype and allele frequencies were found between patients and controls. A progressive, as opposed to a relapsing, onset was significantly more frequent in carriers of the *IFNGR2* allele Arg64 ($P = 0.028$). Moreover, *IFNGR2* allele Arg64 carriers had a lower black hole ratio than non-carriers ($P = 0.016$). No other associations with clinical parameters, such as age at onset or rate of progression, or with imaging parameters, were observed. The *IFNG* intron 1 gene polymorphism studied is unlikely to play a major role in MS susceptibility or disease course. The *IFNGR1* and *IFNGR2* gene polymorphisms studied do not exert an important influence on MS susceptibility, but allele *IFNGR2**Arg64 may be associated with a progressive disease onset.

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Introduction

Interferon-γ

Interferon-γ (IFN)-γ is a pleiotropic cytokine with potent immunomodulatory capacity, having positive or negative effects on the expression of various genes and proteins. Originally discovered as antiviral factor (Wheelock, 1965), it has become clear that IFN-γ has a range of activities, including enhancement of major histocompatibility complex (MHC) expression on antigen-presenting cells, regulatory effects on T cells and increased expression of intercellular adhesion molecule (ICAM)-1 on endothelial cells (Billiau, 1996). The major IFN-γ-producing cells are natural killer (NK) cells and T cells, although macrophages also produce a significant amount of IFN-γ (Gessani & Belardelli, 1998). IFN-γ receptors (IFNGRs) are present on virtually all cells, and consist of two subunits (transmembrane chains), both of which are required for activation. The IFN-γ R1 chain binds the IFN-γ ligand, whereas the IFN-γ R2 chain is required for signal transduction (Pestka, 1997).

Role of IFN-γ in disease

The role of IFN-γ in disease is ambiguous. Beneficial effects have been noted in infectious disease, where IFN-γ strengthens cellular defence mechanisms and favours the generation of specific immunity. In non-infectious diseases, however, most of the described effects are disease-promoting (Billiau, 1996). Furthermore, controversy exists between multiple sclerosis (MS), where treatment with IFN-γ was found to trigger exacerbations (Panitch *et al*., 1987), and its animal model, experimental allergic encephalomyelitis (EAE), where a number of studies support a disease-limiting role of IFN-γ (Billiau *et al*., 1988; Krakowski & Owens, 1996). Summarizing the available data, IFN-γ can be characterized a janus-headed cytokine.

Role of IFN-γ in MS

A body of evidence implicates IFN-γ in the pathophysiology of MS. Increased levels of IFN-γ mRNA have been detected in the peripheral blood and cerebrospinal fluid of MS patients (Link, 1998) and IFN-γ has been shown in and around MS lesions (Woodroofe & Cuzner, 1993; Brosnan *et al*., 1995). In relapsing-remitting MS, an increase in IFN-γ production was observed prior to a relapse (Dettke *et al*., 1997), and a moderate positive correlation between IFN-γ production and disability has been reported (Petereit *et al*., 2000). In the Northern American MS genome screen, a region of the *IFNG* gene showed linkage (Haines *et al*., 1996). Logically, the genes for IFN-γ and its receptors are interesting candidates in relation to MS susceptibility or phenotype.

Polymorphisms and associations

The *IFNG* gene is located on chromosome 12q24, contains four exons and three introns, and codes for a protein of 166 amino acids (Gray & Goeddel, 1982). A number of single nucleotide polymorphisms (SNPs) have been described (Giedraitis *et al*., 1999; Bream *et al*., 2000; Pravica *et al*., 2000; Henri *et al*., 2002), but a microsatellite polymorphism consisting of a dinucleotide (CA)-repeat in the first intron is the one most extensively studied (Ruiz-Linares, 1993). At this polymorphism, allele 2 (12 repeats) correlates with high IFN-γ production (Pravica *et al*., 1999). Associations with susceptibility or disease course in rheumatoid arthritis (Khani-Hanjani *et al*., 2000) and MS have been reported (Vandenbroeck *et al*., 1998; Weinshenker *et al*., 2002), as well as observations that provide little support for a role of *IFNG* gene polymorphisms (Pociot *et al*., 1997; Wansen *et al*., 1997; He *et al*., 1998; Goris *et al*., 1999; Reboul *et al*., 2000; Constantin *et al*., 2001; Dai *et al*., 2001). The *IFNGR1* and *IFNGR2* genes are located on chromosomes 6q23 and 21q22, respectively (Pestka, 1997). Evidence that polymorphisms in the *IFNGR1* and *IFNGR2* genes play a role in autoimmune disease is provided by two Japanese studies. First, an association of the *IFNGR1* Met14/Val14 genotype with systemic lupus erythematosus (SLE) susceptibility was observed (Tanaka *et al*., 1999), and subsequently it was demonstrated that patients who had the combination of this *IFNGR1* genotype with the *IFNGR2* Gln64/Gln64 genotype, had a 9.6-times increased risk of developing SLE (Nakashima *et al*., 1999). These findings suggest interaction between the two polymorphisms, although only eight patients had the specific combination of genotypes, and the confidence interval (CI) was wide (1.1–85.7).

Study rationale

To date, only one study has investigated the role of the *IFNG* gene polymorphism in the disease course of MS, using only clinical data (Dai *et al*., 2001), and no studies have addressed the role of *IFNGR1* and *IFNGR2* gene polymorphisms. Furthermore, IFN-γ and its receptors form a functional complex and it is conceivable that the biological effects of IFN-γ are influenced by the combination of polymorphisms in the *IFNG*, *IFNGR1* and *IFNGR2* genes. Therefore, we analysed whether *IFNG* or *IFNGR1* and *IFNGR2* gene polymorphisms influence MS susceptibility or phenotype. In addition, associations with magnetic resonance imaging (MRI) data were investigated.

Materials and methods

Subjects

A total of 509 patients with clinically definite MS (Poser *et al*., 1983) and 193 healthy controls, all unrelated Dutch caucasians, participated in the study. The study was carried out with the approval of the Medical Ethics Committee of the VU medical center, and informed consent was obtained from all subjects.

Clinical data

Patient files were reviewed for age at onset, disease type and progression rate [defined as time from disease onset to reach an Expanded Disability Status Scale (EDSS) score of 6]. With regard to disease onset, relapsing-remitting (RR) and secondary progressive (SP) patients were pooled as relapse onset, as opposed to progressive onset (primary progressive patients, PP), as most of the SP patients commence with a relapsing-remitting course.

MRI

Serial T1- and T2-weighted lesion volume (T1LV; T2LV) data were available for 107 patients; and for a second, partially overlapping group of 99 patients, serial data on parenchymal and ventricular volumes were available. MRI examinations were performed as described previously (Kalkers *et al*., 2001). To assess the rate of lesion development, ΔT1LV and ΔT2LV were calculated by dividing the difference in lesion volume by the time between the scans. Furthermore, severity of the lesions was assessed by the black hole ratio (BHR), a baseline descriptive defined as T1LV ÷ T2LV. In the second group of patients, parenchymal and ventricular volumes were measured on T1-weighted images, and intracranial volume was measured on the corresponding slices of the heavily T2-weighted images. Two ratios were calculated: the parenchymal fraction (PF), defined as whole brain parenchymal volume ÷ intracranial volume, as a measure of global brain atrophy; and the ventricular fraction (VF), defined as ventricular volume ÷ intracranial volume, to assess central atrophy. The progression of atrophy was assessed by dividing the difference in PF and VF by the time between the scans.

Genotyping

DNA was extracted from peripheral blood by using standard proteinase K digestion and phenol/chloroform extraction. For the *IFNG* intron 1 CA-repeat polymorphism,

polymerase chain reaction (PCR) was performed using the fluorescence-labelled primers 5′-TTATTCTTACAACAC-AAAATCAAATC-3′ and 5′-ATACAAAAACAAAAAA-CAGCAAAGC-3′. Cycling conditions were as follows: an initial 10 min at 94 °C followed by 33 cycles of 30 s at 94 °C, 30 s at 55 °C and 30 s at 72 °C, and a final 30 s at 72 °C. Products of 190–200 bp were separated on a 3700 DNA sequencer (PE Applied Biosystems, Foster City, CA, USA) and analysed by GENESCAN 3.5 and GENOTYPER 2.0 software (PE Applied Biosystems). The nomenclature of the alleles is adapted from the report of Pravica *et al.* (1999), in which five alleles of this polymorphism are described (alleles 1–5 correspond to 11–15 CA repeats, respectively). In our sample, allele 1 was not present, and alleles 6 and 7 (16 and 17 CA repeats) had frequencies of ≤ 0.5% and were not included in the analysis. All genotypes were read independently by two investigators. A polymorphism in the first intron of the *IFNGR1* gene, previously assigned to the second intron, was typed by PCR–restriction fragment length polymorphism (PCR–RFLP), as described previously (Gao *et al.*, 1999). A 5-min denaturation at 95 °C preceded 40 cycles of 95 °C for 30 s and 60 °C for 30 s. The primers used were IFNGR1-F (5′-CGGGGTTGGAGCCAGCGAC-3′) and IFNGR1-R (5′-CCTCCCTCCCTCTCGT-3′). The 170-bp PCR products were digested with *Cac*8I. When allele *IFNGR1**A is present, this results in an invariant fragment of 13 bp (recognition site within the primer IFNGR1-F) and a 157-bp fragment. Allele *IFNGR1**G results in three products, of 13 bp, 43 bp and 114 bp. The amplification-created restriction site method was used to type the *IFNGR2* Gln64Arg polymorphism, as described previously (Gao *et al.*, 1999). The primer sequences 5′-CAGCTGCCCGCTCCTCAG-3′ and 5′-GGCTTAC-TATTTAAACTGGACT-3′ amplify a 129-bp fragment. Endonuclease digestion with *Hin*fI results in invariant fragments of 27 bp and 107 bp when allele Gln64 is present and produces 27-, 20- and 80-bp products when allele Arg64 is present. PCR products were separated on a 3% agarose gel stained with ethidium bromide, and visualized by ultraviolet transillumination.

Statistical analysis

Primary data were the observed genotype and allele frequencies for the three loci. We analysed (by using the χ^2-test) whether the distributions of genotypes and alleles deviated from Hardy–Weinberg equilibrium. As the genes have related functions, we analysed the effects of all three loci simultaneously, on susceptibility to MS and disease characteristics, and tested for the presence of interactions between the loci (epistasis). As we are the first researchers to study *IFNGR1* and *IFNGR2* genotypes in MS, we have no a priori hypothesis of which alleles should be analysed. Therefore, we analysed carriership for both alleles. For the *IFNG* polymorphism, we dichotomized alleles into allele 12 and 'others'. Allele 12 was chosen because this allele correlates with high IFN-γ production. Regression analysis was used to investigate the associations of the combination of allele carriership at all three loci with onset type (relapsing vs. progressive onset) and age at onset of disease. To study the effect of all three polymorphisms simultaneously on the time to EDSS 6.0, we performed Cox's proportional hazard regression. MRI parameters, in relation to genotypes, were analysed using analysis of variance (ANOVA) with Bonferroni correction for multiple comparisons. In addition, linear regression analysis was performed with each of the MRI parameters as the dependent variable, and carriership of alleles at all loci as the three determinants. In the regression analyses we corrected for gender, disease duration and onset type of disease, and log transformations were carried out where necessary.

Results

The clinical characteristics are summarized in Table 1. Owing to technical difficulties, a number of genotypes could not be assessed with certainty and were therefore excluded from the analyses. The number of individuals who were typed successfully for each polymorphism are as follows: *IFNG*, 466 patients and 163 controls; *IFNGR1*, 476 patients and 160 controls; and *IFNGR2*, 509 patients and 180 controls. As the missing genotypes seemed to be randomly distributed in patients and controls, and the allele and genotype frequencies did not deviate from Hardy–Weinberg equilibrium, we consider that the validity of our results is not compromised.

Genotype and allele distributions

Allele and genotype distributions at the *IFNG*, *IFNGR1* and *IFNGR2* gene polymorphisms are shown in Tables 2

Table 1. Clinical characteristics of multiple sclerosis (MS) patients and controls

	Controls (n = 193)	MS patients (n = 509)	RR (n = 227)	SP (n = 180)	PP (n = 102)
Women/Men	104/89	322/187	157/70	106/74	59/43
Age (years)	37.4 ± 11.5	46.4 ± 11.2	39.9 ± 8.5	49.0 ± 9.0	56.1 ± 11.5
EDSS (mean)	—	4.6 ± 2.1	3.3 ± 1.7	5.9 ± 1.6	5.5 ± 1.7
EDSS ≥ 6.0	—	198	27	113	58
Disease duration (years)	—	12.5 ± 8.1	9.0 ± 5.7	16.7 ± 8.7	12.5 ± 7.7

Data are expressed as mean ± standard deviation of age, Expanded Disability Status Scale (EDSS) and disease duration.
PP, primary progressive; RR, relapsing-remitting; SP, secondary progressive.

Table 2. Allele frequencies of interferon-γ (*IFNG*), and interferon-γ receptor 1 (*IFNGR1*) and interferon-γ receptor 2 (*IFNGR2*) gene polymorphisms

	Controls	MS (all)	RR	SP	PP
IFNG					
Allele 12 (190 bp)	45	44	46	41	47
Allele 13 (192 bp)	45	45	42	50	43
Allele 14 (194 bp)	6	6	8	4	6
Allele 15 (196 bp)	3	4	4	5	4
Allele 16 (198 bp)	0	0	0	0	0
IFNGR1					
Allele Val14	88	83	82	83	83
Allele Met14	12	17	18	17	17
IFNGR2					
Allele Gln64	86	86	88	86	83
Allele Arg64	14	14	12	14	17

Results are expressed as percentage allele frequency.
bp, base pairs; PP, primary progressive; RR, relapsing-remitting; SP, secondary progressive.

and 3. For the *IFNG* gene polymorphism, five alleles were identified, ranging from 12 to 16 CA-repeats. Alleles 12 and 13 were the most common alleles; allele 16 was only observed in one healthy individual.

Susceptibility

There were no differences in allele frequencies or allele carriership in the polymorphisms in *IFNG*, *IFNGR1* and *IFNGR2* between patients and controls, and none of the polymorphisms were associated with gender.

Table 4. Interferon-γ receptor 2 (*IFNGR2*) allele Arg64 and disease onset

	Allele Arg64		
IFNGR2	Carriers	Non-carriers	Total *n* (%)
Progressive onset *n* (%)	34 (34.7)	64 (65.3)	98 (100)
Relapse onset *n* (%)	93 (23.8)	298 (76.2)	391 (100)
Total	127 (100)	362 (100)	489 (100)

$P = 0.028$ (χ^2-test); odds ratio (progressive onset and allele Arg64), 1.46; 95% confidence interval: 1.05–2.02.

Disease course

No differences in allele distribution or allele carriership were identified for age at onset and time to reach EDSS 6. However, Table 4 shows that patients with a progressive onset were more frequently carriers of allele *IFNGR2**Arg64, compared to patients with a relapse onset [34.7% vs. 23.8%, odds ratio (OR) 1.46, 95% CI: 1.05–2.02].

MRI

The clinical characteristics of the patients with MRI data and the whole patient group are comparable (Table 5). The main MRI features, in relation to the *IFNG* and *IFNGR* genotypes, are presented in Table 6. A somewhat lower BHR was seen in MS carriers of *IFNGR2* allele Arg64 compared to non-carriers ($P = 0.016$). No differences were observed for measures of atrophy or T1- and T2-lesion load in relation to the *IFNG* or *IFNGR* polymorphisms. To study the effects of genotype on MRI parameters, we performed ANOVA and found a

Table 3. Genotype frequencies of interferon-γ (*IFNG*), and interferon-γ receptor 1 (*IFNGR1*) and interferon-γ receptor 2 (*IFNGR2*) gene polymorphisms

	Controls (%)	MS patients (%)	RR (%)	SP (%)	PP (%)
IFNG genotype					
190/190	22.1	18.7	20.1	15.6	19.8
190/192	37.4	42.7	43.1	44.4	39.5
190/194	5.5	4.9	4.8	3.1	8.1
190/196	2.5	3.9	4.3	2.5	5.8
190/198	0.6	0	0	0	0
192/192	22.7	19.1	14.8	23.8	20.9
192/194	3.7	5.6	8.1	4.4	2.3
192/196	4.3	3.4	2.9	4.4	2.3
194/194	1.2	0.6	1.0	0	1.2
194/196	0	0.6	1.0	0.6	0
196/196	0	0.4	0	1.3	0
IFNGR1					
Val14/Val14	75.6	69.3	68.4	71.5	69.0
Val14/Met14	23.8	26.9	27.8	23.6	28.7
Met14/Met14	0.6	3.8	3.8	4.9	2.3
IFNGR2					
Gln64/Gln64	73.9	74.0	78.0	73.2	65.3
Gln64/Arg64	25.0	24.8	20.6	25.7	34.7
Arg64/Arg64	1.1	1.2	1.3	1.1	0

Table 5. Clinical characteristics in all patients with multiple sclerosis (MS) and magnetic resonance imaging (MRI) subgroups

	MS patients *n* = 509 (%)	Atrophy[a] *n* = 99 (%)	T1/T2 lesion load[b] *n* = 107 (%)
Women/Men	322/187 (63/37)	55/44 (56/44)	55/52 (51/49)
Age (years)	46.4 ± 11.2	48.9 ± 11.9	49.8 ± 10.6
EDSS (mean)	4.6 ± 2.1	4.9 ± 2.0	5.4 ± 1.9
EDSS ≥ 6.0	198 (38.9%)	43 (43.4%)	59 (55.1%)
Disease duration (years)	12.5 ± 8.1	12.9 ± 6.7	14.3 ± 6.7

[a]Atrophy: patients with serial ventricular fraction (VF) and parenchymal fraction (PF) data.
[b]T1/T2 lesion load: patients with serial T1 and T2 lesion load data.
EDSS, Expanded Disability Status Scale.

Table 6. Magnetic resonance imaging (MRI) features in relation to interferon-γ (*IFNG*), interferon-γ receptor 1 (*IFNGR1*) and interferon-γ receptor 2 (*IFNGR2*)

	ΔVF/year × 10^{-3} (*n* = 99)	ΔPF/year × 10^{-3} (*n* = 99)	ΔT1/year (cm^3) (*n* = 107)	ΔT2/year (cm^3) (*n* = 107)	BHR[a] (*n* = 107)
IFNG genotype					
190/190	0.77	−6.16	0.356	0.842	0.247
190/192	1.44	−5.74	0.617	0.701	0.254
190/194	1.09	−11.10	0.146	−0.203	0.198
190/196	1.15	−5.53	1.198	4.587	0.257
192/192	0.80	−5.42	0.301	0.870	0.244
192/194	0.34	−4.05	0.610	0.477	0.388
192/196	1.80	−15.89	0.604	−1.125	0.132
P-value	0.911	0.957	0.399	0.026[b]	0.451
IFNGR1					
Val14/Val14	1.18	−6.32	0.489	0.702	0.255
Val14/Met14	0.58	−4.38	0.412	0.743	0.244
Met14/Met14	1.73	−12.16	1.059	1.121	0.208
P-value	0.266	0.386	0.327	0.927	0.836
IFNGR2					
Gln64/Gln64	1.03	−7.13	0.552	0.783	0.266
Gln64/Arg64	1.01	−3.16	0.287	0.960	0.177
Arg64/Arg64	1.09	−10.07	0.019	−0.084	0.115
P-value	0.999	0.345	0.292	0.851	0.016[c]

n, number of patients; PF, parenchymal fraction; VF, ventricular fraction.
[a]BHR (black hole ratio) = T1 lesion volume ÷ T2 lesion volume (baseline descriptive).
[b]$P < 0.05$, 190/196 genotype compared with other genotypes. [Analysis of variance (ANOVA) with Bonferroni correction for multiple comparisons.]
[c]$P < 0.05$, carriers vs. non-carriers of allele Arg64 (ANOVA).

significantly higher yearly increase in T2 lesion volume for patients with a 190/196 genotype at IFNG, compared with other genotypes ($P = 0.026$, ANOVA with Bonferroni correction for multiple comparisons).

Combinatorial analyses of the effects of the three loci on clinical and MRI characteristics

Here, we investigated the combined effects of carriership of allele *IFNG**12 and both alleles of the *IFNGR1* and *IFNGR2* gene polymorphisms by entering all polymorphisms as independent variables in the regression model. The results for the different outcomes (dependent variables) are summarized in Table 7. The combined loci had no influence on the risk of developing MS. However, several trends for an effect of the single polymorphisms, after correction for the presence of the others, were observed. Carriership of *IFNGR2**Arg64 was associated with progressive onset; both *IFNG**12 and *IFNGR2**Arg64 were associated with a slower progression (time to EDSS 6.0); and allele *IFNGR1**Met14 and allele *IFNGR2**Gln64 were associated with, respectively, later and earlier disease onset. Weak associations of allele *IFNG**12 and allele *IFNGR1**Val14 with a lower ventricular fraction, and of allele IFNGR2*Arg64 with a lower T1-lesion load, were observed.

After correction for multiple testing, none of the observed associations was significant.

Analysis of interaction

We tested all two-way interactions, but obtained no evidence for a significant contribution to susceptibility to MS, onset type, age at onset, or time to reach EDSS 6.0.

Table 7. Results of combinatorial regression analyses: influence of carriership of alleles of the interferon-γ (*IFNG*), interferon-γ receptor 1 (*IFNGR1*) and interferon-γ receptor 2 (*IFNGR2*) genes on susceptibility to multiple sclerosis (MS); clinical and magnetic resonance imaging (MRI) parameters

Carriership	MS vs. controls	Progressive onset	Onset age	Time to EDSS 6	Ventricular fraction	T1-lesion load
*IFNG**12	$P = 0.53$	$P = 0.52$	$P = 0.77$	$P = 0.05$[c]	$P = 0.05$[d]	$P = 0.82$
*IFNGR1**Val14	$P = 0.12$	$P = 0.38$	$P = 0.93$	$P = 0.70$	$P = 0.06$[d]	$P = 0.56$
*IFNGR1**Met14	$P = 0.31$	$P = 0.38$	$P = 0.04$[b]	$P = 0.44$	$P = 0.35$	$P = 0.68$
*IFNGR2**Arg64	$P = 0.48$	$P = 0.03$[a]	$P = 0.38$	$P = 0.09$[c]	$P = 0.86$	$P = 0.03$[e]
*IFNGR2**Gln64	$P = 0.76$	$P = 0.60$	$P = 0.07$[b]	$P = 0.79$	$P = 0.31$	$P = 0.62$

Separate regression analyses were performed for each clinical and MRI parameter (dependent variable), with carriership of the alleles listed as independent variables. Therefore, every column represents the results of the regression model for that specific parameter.
[a]Higher frequency of progressive vs. relapsing onset for carriers of *IFNGR2**Arg64.
[b]Later onset age for *IFNGR1**Met14 carriers; earlier onset age for *IFNGR2**Gln64 carriers.
[c]Longer time to Expanded Disability Status Scale (EDSS) 6 (i.e. slower progression) for carriers of either allele.
[d]Lower ventricular fraction (i.e. less central atrophy) for carriers of either allele.
[e]Lower T1 lesion load (i.e. less destructive lesions) for carriers of *IFNGR2**Arg64.

Discussion

Genome screens suggest that several genes are involved in MS susceptibility. In addition, there is some evidence that genetic factors modify the disease course. Although most of the studies investigating candidate genes produced negative results, some suggested small effects that await confirmation (Kantarci *et al*., 2002). As genetic heterogeneity and the small contribution of each disease allele hamper the detection of effects of polymorphisms in complex diseases such as MS, several authors have suggested that simultaneous analysis of candidate genes is necessary (Schork *et al*., 1995; Comings, 1998). Very recently, multilocus statistics were proposed to circumvent the problem of multiple testing that troubles association studies when considering several loci (Bohringer *et al*., 2003).

Susceptibility

We observed no effect of polymorphisms in *IFNG*, *IFNGR1*, or *IFNGR2* on the susceptibility to MS, in concordance with a study in Nordic MS patients (Dai *et al*., 2001). However, disease-associated alleles may differ between populations, as demonstrated by Goris *et al*., who showed a north–south trend across Europe for the two most frequent *IFNG* alleles (Goris *et al*., 1999). In addition, although no overall association with MS susceptibility was shown, they observed that the *IFNG I2* allele (13 CA-repeats) was more often transmitted to a subgroup of Sardinian patients.

Disease course

A previous study addressing the *IFNG* polymorphism in MS detected no differences between benign, intermediately disabled, or severely disabled patient cohorts (Dai *et al*., 2001). We did not observe significant associations with disease course, but we do report a number of trends. Patients carrying the *IFNGR2**Arg64 allele more often had a progressive onset as opposed to a relapse onset. As both the IFN-γ R1 chain (binding IFN-γ) and the IFN-γ R2 chain are needed for signal transduction (Pestka, 1997), it is conceivable that polymorphisms in either of the *IFNGR* genes alter receptor function. This assumption is supported by the observation that B cells of both healthy individuals and SLE patients bearing the *IFNGR1* Met14/Val14 genotype show a decreased response to IFN-γ (Tanaka *et al*., 1999). With no such data available for the *IFNGR2* polymorphism, we can only speculate that this polymorphism also changes receptor function, making patients more prone to a progressive disease onset. Interestingly, in patients carrying the *IFNGR2* allele, Arg64, we found a decreased BHR, suggesting less destructive lesion formation. In addition, this allele was associated with a trend towards slower progression to EDSS 6.0. MRI data provide important additional disease parameters. In our patients there was no significant association of gene polymorphisms in *IFNG*, *IFNGR1* and *IFNGR2* with MRI parameters. However, we observed weak evidence for a reduction of atrophy (lower VF) by *IFNGR1**Val14 and *IFNG**12. In a recent study in MS, no correlation was found between serum cytokine levels (including IFN-γ) and MRI parameters (Kraus *et al*., 2002). Although the role of cytokines in MS pathogenesis is beyond question, correlations with MRI parameters appear to be hard to detect. This lack of correlation between MRI-detectable inflammation and clinical phenotype has been attributed to the independence of the neurodegenerative process in MS (Martino *et al*., 2002). The same authors questioned the notion that inflammation is always detrimental, and concluded that, despite the proinflammatory genotype in MS, the net effect of the inflammatory process depends on the timing of its protective and destructive components. We conclude that in the investigated population, the *INFG* intron 1 gene polymorphism is unlikely to play a major role in MS susceptibility or disease course. The *IFNGR1* and *IFNGR2* gene polymorphisms do not exert an important influence on MS susceptibility, but the *IFNGR2* polymorphism may be associated with a progressive disease onset.

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