

Interferon Regulatory Factor 5 Gene Variants and Pharmacological and Clinical Outcome of Interferon β Therapy in Multiple Sclerosis

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Genes Immun. 2011 Sep;12(6):466-72.

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ABSTRACT

Interferon- β (IFN β) therapy is effective in approximately half of the patients with in relapsing remitting multiple sclerosis (RRMS). Clinical non-responders were characterized by an increased expression of IFN response genes before the start of therapy and a lack of a pharmacologically induced increase in IFN-response gene activity. Because Interferon Regulatory Factor 5 (IRF5) is a master regulator of IFN-activity we performed a candidate gene study of IRF5 gene variants in relation to the pharmacological and clinical response upon IFN β treatment. We found that patients with the IRF5 rs2004640-TT and rs47281420-AA genotype exerted a poor pharmacological response to IFN β compared to patients carrying the respective G-alleles ($p= 0.0006$ and $p=0.0023$, respectively). Moreover, patients with the rs2004640-TT genotype developed more MRI-based T2 lesions during IFN β treatment ($p = 0.003$). Accordingly, an association between MRI-based non-responder status and rs2004640-TT genotype was observed ($p = 0.010$). For the rs4728142 AA-genotype there tends to be an association with more T2 lesions during IFN β treatment and MRI-based non-responder status however, this was not significant ($p = 0.103$ and $p = 0.154$, respectively). The clinical relevance of the rs2004640-TT genotype was validated in an independent cohort wherein a shorter time to first relapse was found ($p = 0.037$). These findings suggest a role for IRF5 gene variation in the pharmacological and clinical outcome of IFN β therapy that might have relevance as biomarker to predict the response to IFN β in MS.

INTRODUCTION

Interferon- β (IFN β) products were the first agents to show clinical efficacy in relapsing-remitting multiple sclerosis (RRMS).¹⁻³ However, clinical experience showed that IFN β therapy is effective for approximately half of the RRMS patients.⁴ Given the destructive nature of multiple sclerosis (MS), risk of adverse effects and considerable costs for therapy and alternative treatment options that have become available, there is a strong need to make predictions on success before start of therapy. Gene-based decisions hold great promise for individual tailoring of drug regimes in chronic diseases such as MS. Gene expression profiling in MS revealed considerable differences between patients with RRMS. We observed that a subgroup of patients with RRMS was characterized by an increased expression of an immune defense-response gene set, including a set of IFN type I response genes.⁵ Non-responders to IFN β treatment were characterized by an increased expression of IFN response genes before the start of therapy and a lack of a pharmacologically induced increase in IFN response gene activity.⁶ Accordingly, the expression of IFN response genes before the start of IFN β therapy was shown to be related to the clinical response.⁷ In search for genetic polymorphisms as predictors of drug response, we focused on regulators of the IFN pathway that could influence IFN-activity and downstream signaling and gene activation events. We hypothesized that genetic variation in such regulators might influence both the pharmacological and clinical response. Therefore, we studied Interferon Regulatory Factor 5 (IRF5), a master driver of the IFN pathway. IRF5 is a transcription factor important for the production of type I IFN, apoptosis, cell-cycle regulation, cell adhesion and pro-inflammatory reactions.⁸ IRF5 functions as a central mediator of Toll-like receptor signaling.⁹ Moreover, expression of IRF5 is induced after activation of the IFN type I receptor, indicative that IRF5 is not only important in the production of type I IFN, but also in the regulation IFN type I induced gene activity.¹⁰ Genetic variation in the IRF5 gene has been found to be strongly associated with systemic lupus erythematosus, a disease wherein type I IFNs are clearly associated with disease activity and severity, and IFN response gene activity.¹¹⁻¹³ The aim of this study was to investigate whether polymorphisms in the IRF5 gene are associated with the pharmacological response and, if such an association was found, if it is related to clinical response to IFN β in MS.

PATIENTS AND METHODS

PATIENTS

Patients with RRMS were included who met the diagnosis of MS by McDonald or Poser criteria;^{18,19} each subject was consented using documents approved by the Institutional Review Board of the respective institute (Partners Healthcare, VU University medical center (VUmc), Amsterdam, The Netherlands; Hospital Universitari Vall d'Hebron, Barcelona, Spain and the Institutional Review Board of Partners Healthcare, Boston). For our initial studies, patients with clinically definite RRMS (test group) were included from prospectively and systematically followed cohorts of patients from the VUmc (n = 30) and the Centre d'Esclerosi Multiple de Catalunya (CEM-Cat), (n = 45). A total of 30 patients of the VUmc from whom peripheral blood RNA before and after the start of therapy was available, were studied for their pharmacological response to IFN β . From a total of 73 patients (VUmc n = 28, CEM-Cat n = 45) DNA and two MRI scans during IFN β treatment at least 12 months apart were available (test group). For all patients the disability status was assessed annually using the Extended Disability Status Scale (EDSS). For genetic validation studies an independent group of 261 RRMS patients (validation group) who were treated with IFN β for at least 12 months was selected. A total of 176 patients were from Brigham and Women's Hospital (BWH) (Boston, MA, USA) and 85 came from the VUmc (Amsterdam, the Netherlands), were selected based on the availability of DNA and accurate clinical follow-up data. In this cohort, time to first relapse within the first 24 months of treatment was used as main clinical outcome measure.

BLOOD SAMPLING

From each VUmc patient of the test group (n = 30), blood was drawn before and during treatment (median 4 months; range from 1–13 months) into a PAXgene tube (PreAnalytix, Hilden, GmbH, Germany). RNA was isolated from PAXgene tubes as described elsewhere.⁶ From all VUmc patients in both cohorts, genomic DNA was extracted from anticoagulated peripheral blood using DNAzol according to the manufacturer's recommended protocol (Molecular Research Center, Inc., Cincinnati, OH, USA). For the CEM-Cat samples, DNA extraction was carried out by a standard phenol/chloroform purification method followed by an ethanol precipitation. For the BWH samples, the Qiagen 'Gentra Puregene Blood Kit Plus' (Valencia, CA, USA, cat#158489) was used to extract DNA samples from whole blood.

GENE EXPRESSION

RNA (0.5 mg) was reverse transcribed into complementary DNA using a Revertaid H-minus cDNA synthesis kit (MBI Fermentas, St Leon-Rot, Germany) according to the manufacturers' instructions. Gene expression levels of 10 IFN-induced genes (RSAD2, IFIT1, MxA, ISG15,

EPSTI1, IRF7, LY6E, OAS1, OAS3 and SERPING1), based on the type I IFN gene set as described previously,⁶ were determined simultaneously using Taqman Low Density Arrays (TLDA, Applied Biosystems, Foster City, CA, USA). TLDA are pre-loaded customizable 384-well micro fluidic cards for target class and pathway studies based on Taqman real-time PCR. 18S ribosomal RNA was used as a house-keeping gene. The average gene expression level of the 10 IFN-induced genes was used to determine the pharmacological response to IFN β therapy. Pharmacological response is defined as the average gene expression level of the IFN-induced genes during therapy divided by the average gene expression levels measured before start of the therapy.

GENOTYPING

The following IRF5 gene variants were determined in samples of the test cohort: rs2004640, a SNP that alters a consensus splice donor site; rs10954213 that leads to alternative poly-adenylation; rs4728142, located 5 kb upstream of the alternative exon 1a; and a 30bp insertion/deletion (indel) polymorphism in exon 6. SNPs at rs2004640, rs4728142 and rs10954213 were found to be associated with IRF5 mRNA expression.^{13,20} SNPs in the DNA samples obtained at the VUmc and CEM-Cat were genotyped using Taqman Genotyping Assay (rs2004640 by assay C 9491614_10; rs4728142 by assay C 2691222_10 (both from Applied Biosystems), and rs10954213 by a customized assay). The 30-bp indel was amplified as a 115/145 bp fragment using conventional PCR and separated on a 2.5% agarose gel and visualized using ethidium bromide staining. CEM-Cat patients in the test group and all patients in the validation group were genotyped only for SNPs rs2004640 and rs4728142. For the BWH subjects from Boston, genotypes were obtained using Sequenom MassArray platform and its iPLEX format (Sequenom Inc., San Diego, CA, USA).

MRI ACQUISITION AND ANALYSIS

MRI examinations were carried out at start of therapy and after at least 12 months of IFN β treatment, including dual-echo proton density (PD) and T2-weighted spin-echo images performed with a 1.5 Tesla scanner (Philips Medical Systems, Best, the Netherlands). All images were acquired in the axial orientation with 3-mm thick contiguous slices and a 3-mm gap. The number and change of T2 lesions on MRI were determined by designated readers at the Image Analysis Center, VUmc in Amsterdam or at CEM-Cat in Barcelona, following standard operating protocols based on described guidelines.²¹ Readers were blinded for clinical data and genotyping results. Patients were considered MRI-based non-responders, if one or more new T2 lesions had developed during 12 months of treatment. If the interval between MRI scans was greater than 12 months, the annualized number of new T2 lesions was used.

STATISTICAL ANALYSIS

Data were analyzed using the standard statistical software (SPSS, version 15.0, SPSS Inc., Chicago, IL, USA). Differences in pharmacological response between IRF5 genotypes were analyzed using Student's independent samples t-test with Welch's correction, if appropriate. IRF5 genotypes were correlated to MRI parameters (the occurrence of new T2 lesions during treatment and the (annualized) number of new lesions) using Kruskal–Wallis (between the three genotypes per SNP) and Mann–Whitney (between two groups per SNP: homozygous for risk allele versus others) where appropriate. The pre-specified main clinical outcome measure for analysis in the validation cohort was time to first relapse during the first 2 years of follow-up. Kaplan–Meier curves were constructed for the patients homozygous for the risk alleles versus others, and group differences were analyzed by the log-rank test.

RESULTS

ASSOCIATION OF IRF5 POLYMORPHISMS WITH THE PHARMACOLOGICAL RESPONSE TO IFN β

We investigated the relationship between four IRF5 gene-associated polymorphisms and the pharmacological response to IFN β therapy in 30 RRMS patients. The pharmacological response for each patient was calculated as the ratio of mean expression of 10 IFN β -response genes before and after the start of IFN β therapy.

Table 1. Patient characteristics.

Patient characteristics	Test group (n=75)		Validation group (n=261)	
	VUmc patients (n = 30)	CEM-Cat patients (n=45)	Boston patients (n=176)	VUmc patients (n=85)
Female n (%)	18 (60%)	28 (62%)	133 (76%)	59 (69%)
Male n (%)	12 (40%)	17 (38%)	43 (24%)	26 (31%)
Age at disease onset in years: mean (SD)	31.7 (9.1)	28.1 (8.3)	31.9 (9.0)	30.5 (8.2)
EDSS at start IFN β : median (IQR)	2.0 (2.0-3.1)	1.5 (1.0-2.25)	n.a.	2.5 (2.0-3.5)
Disease duration at start IFN β in years: mean (SD)	2.1 (2.0)	5.0 (4.3)	6.0 (7.5)	n.a.
Disease subtype n (%)	3 (10%)	0 (0%)	1 (0.006%)	2 (2%)
Clinically Isolated Syndrome	27 (90%)	45 (100%)	175 (99.994%)	83 (98%)
Relapsing-remitting				
<i>IFNβ product used</i>				
IFN β -1a 30 μ g IM n (%)	7 (23%)	15 (33%)	130 (74%)	25 (29%)
IFN β -1a 22 SC n (%)	4 (13%)	17 (38%)	15 (8.5%)	14 (16%)
IFN β -1a 44 μ g SC n (%)	11 (37%)			
IFN β -1b 250 μ g SC n (%)	8 (27%)	13 (29%)	31 (17.5%)	30 (35%)

EDSS = Extended disability Status Scale; IFN- β = Interferon beta. SD = standard deviation; IQR= interquartile range; IM = intramuscular; SC = subcutaneous.

Patients who are homozygous for the IRF5 rs2004640 T-allele revealed a low or absent induction of the 10 IFN β - response genes upon IFN β therapy compared with patients with the GT/GG genotype, who exerted a strong induction ($P = 0.0006$) (Figure 1).

A similar observation was made for the IRF5 rs4728142-AA genotype compared with the AG/GG genotype ($P = 0.0023$). For both, rs2004640 and rs4728142, no significant differences were observed between patients carrying one or two G alleles (data not shown). No significant differences were observed in pharmacological response between the rs10954213 and 30-bp indel gene variants. Observed associations remain significant after correction for multiple testing. Although IRF5 rs2400640 and rs4728142 are in strong linkage disequilibrium, haplotype analysis did not increase the significance of our findings (data not shown).

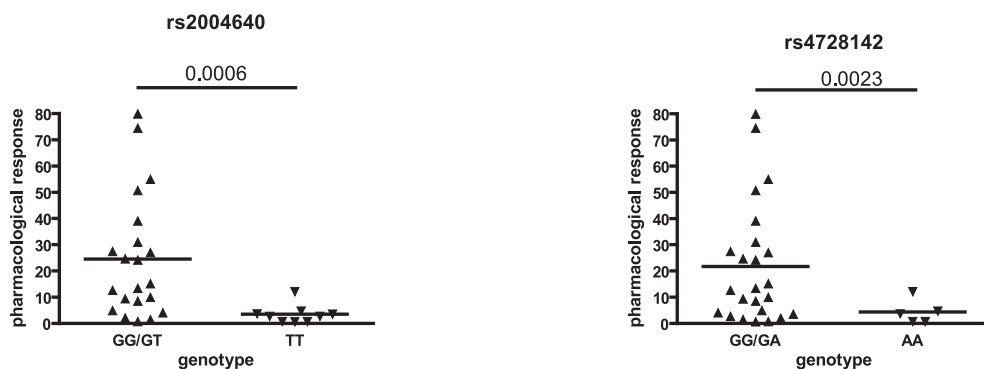


Figure 1. Relation between genotypes and pharmacological response to IFN β treatment. Pharmacological response is determined in the test cohort ($n=30$) and compared with genotypes rs2004640 (a) and rs4728142 (b). Pharmacological response is lower in patients homozygous for the rs2004640 T-allele (a) or rs4728142 A-allele (b) compared with patients with other genotypes.

ASSOCIATION OF IRF5 POLYMORPHISMS WITH MAGNETIC RESONANCE IMAGING (MRI)-BASED LESION LOAD AND CLINICAL RESPONDER STATUS

On the basis of the associations of the IRF5 rs2004640 and rs4728142 genotypes with the extent of the pharmacological response, we studied whether this was also reflected in the (sub)clinical treatment response as measured with magnetic resonance imaging (MRI). Therefore, we investigated the association between the IRF5 rs2004640 and rs4728142 genotypes and MRI-based lesion load and clinical responder status in a group of 75 patients with RRMS treated with IFN β (test group, see Table 1). Initial analysis showed a higher number of new T2-weighted lesions in the patients with the rs2004640-TT genotype compared to those with the other rs2004640 genotypes, that is, compared between the three genotypes (Kruskal–Wallis $P = 0.003$) and compared to the two other genotypes

Table 2. Association between *IRF5* genotypes and pharmacological and clinical response parameters.

	Genotype vs pharmacological response		Genotype vs MRI based lesion load		Genotype vs MRI based non- responders (≥ 1 new T2 lesions)		Genotype vs time to first relapse	
	rs2004640 TT	rs4728142 AA	rs2004640 TT	rs4728142 AA	rs2004640 TT	rs4728142 AA	rs2004640 TT	rs4728142 AA
VUmc patients (n=30)	p = 0.0006¹	p = 0.0023¹	p = 0.003²	p = 0.103 ²	p = 0.010²	p = 0.154 ²	N.A..	N.A.
VUmc + CEM-Cat patients (n=45)	N.A.	N.A.	p = 0.013³	p = 0.201 ³	p = 0.073 ³	p = 0.440 ³		
BWH+VUmc validation cohort (24 months follow up)	N.A.	N.A.	N.A.	N.A.	N.A..	N.A.	p = 0.037	N.S.
BWH validation cohort (long follow-up)	N.A.	N.A.	N.A.	N.A.	N.A.	N.A..	p = 0.030	p = 0.067

¹ Student t test, ²Kruskal-Wallis, ³ Mann-Whitney, N.A. = Not Applicable, N.S. = not significant
p < 0.05 are shown in bold

together (Mann–Whitney P = 0.013). No significant differences in MRI lesion load were found for rs4728142-AA compared to the other rs4728142 genotypes (Kruskal–Wallis P = 0.103 and Mann–Whitney P = 0.201) (Table 2).

Subsequently, we investigated whether the observed association between *IRF5*-genotypes and lesion load also applied to MRI-based clinical responder and non-responder status. This analysis revealed that 42 out of 73 patients were classified as non-responders, that is, they had developed one or more new T2 lesions in a one year interval or an annualized number of new T2 lesion of one or more during IFN β treatment. The other patients (n = 31) were classified as responders. A genetic comparison between the MRI-based responders and non-responders revealed an association between the rs2004640-TT genotype and the non-responder status (Kruskal–Wallis P = 0.010 and Mann–Whitney P = 0.073). No significant association was found between the rs4718142-AA genotype and the non-responder status (Kruskal–Wallis P = 0.154 and Mann–Whitney P = 0.440) (Table 2). Altogether, these findings suggest a role of the rs2004640 single nucleotide polymorphism (SNP) in determining the (sub) clinical treatment response to IFN β in RRMS.

ASSOCIATION OF *IRF5* POLYMORPHISM WITH TIME TO FIRST RELAPSE

To provide additional support for the association of *IRF5* genotypes with IFN β response status, we analyzed the rs2004640 and rs4728142 genotypes in an independent group of 261 RRMS patients (176 patients from Harvard University and 85 patients from the VUmc) on IFN β therapy (validation group). As limited MRI data were available for these patients, we used the time to first relapse as clinical outcome for response.

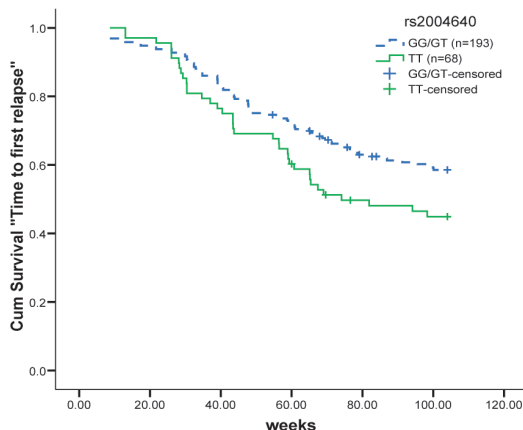


Figure 2. Cumulative relapse free survival in time in relation to IRF5 genotypes. Patients with TT genotype for IRF5 rs2004640 show a significant (log rank P value=0.037) shorter time to first relapse compared to patients with GT or GG genotype.

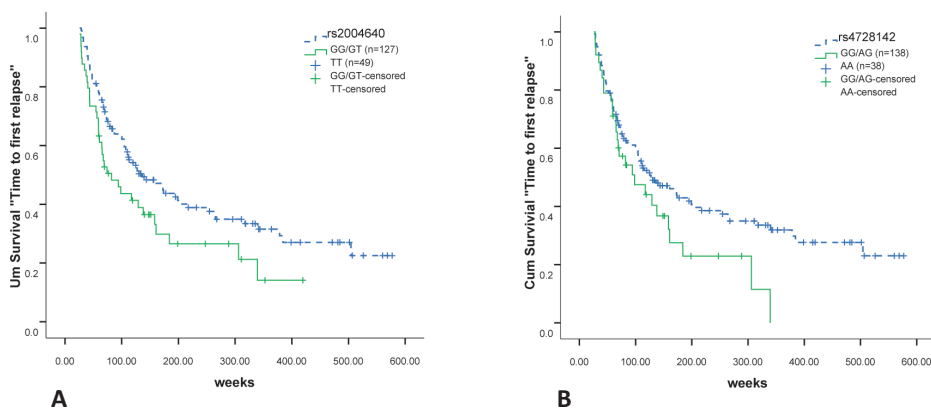


Figure 3. Cumulative relapse free survival in time for IRF5 genotypes in patients of the validation group with long follow-up from Brigham and Women's Hospital in Boston. (A) Patients with TT-genotype for rs2004640 show shorter time to first relapse (log rank P value=0.030) compared with other genotypes. (B) Patients with AA-genotype for rs4728142 show a trend towards a shorter time to first relapse (log rank P value=0.067) compared with other genotypes.

The analysis revealed that the IRF5 rs2004640 TT genotype was associated with a shorter time to first relapse (log rank P value = 0.037) (Figure 2). For rs4728142 no association was observed (data not shown).

Time to first relapse monitoring data was also available for longer observation periods than

24 months for the Harvard patient group. Analysis of these data showed again a shorter time to first relapse in the patients that were homozygous for the risk allele of rs2004640 (log rank P value = 0.030) (Figure 3a). In addition, a trend towards a shorter time to first relapse was observed in the patients homozygous for the rs4728142 A-allele (log rank P value = 0.067) (Figure 3b).

DISCUSSION

In this study, we present data that suggest a role for IRF5 gene variants on the clinical outcome of IFN β treatment in RRMS patients. Patients who are homozygous for the IRF5 rs2004640 T-allele or the rs4728142 A-allele exerted an absent or low pharmacological response, whereas those carrying the other genotypes had a significant increased response. The former also developed more new T2-weighted lesions than the ones with the other genotypes. There was also a trend that IRF5 rs2004640 TT positivity was associated with the responder status based on developed new T2 lesions. Evaluation of the relationship between IRF5 polymorphisms and clinical response, as defined by the time to first relapse in an independent validation group of RRMS patients, further confirmed these results. T2-weighted MRI images are very sensitive for the detection of lesional disease activity in MS and are routinely used in MRI-monitored studies and trials. For the clinical response measurement, MRI is considered to be an objective and preferable measure because of its sensitivity to disease change and reproducibility.¹⁴ In the test group, we observed that new MRI lesion activity during IFN β treatment correlated with the IRF5 rs2004640-TT genotype. However, as data on MRI lesion activity from the RRMS patients in the validation cohort was not available, we determined the clinical response by measurement of the time to first relapse. Because increased numbers of new T2-weighted lesions in patients treated with IFN β were found to correlate with a less favorable disease course,^{14,15} we reasoned that time to first relapse is a valid, although less-superior clinical response measure to evaluate the clinical responder status of IFN β . Despite the suboptimal response measure, we could validate the role of IRF5 rs2004640 in determining the clinical responder status on IFN β . The absence of validation for each specific clinical response measurement, that is, MRI-based response measurement and clinically based endpoint measurement (time to first relapse), might seem as a limitation of the study. However, we believe that the consistent pattern that is observed using pharmacodynamic analyses in combination with different response outcome measurements, using independent cohorts, strengthens our conclusion. We anticipate that future studies, making use of the preferable MRI-response measure would improve evidence for a role of IRF5 rs2004640 in predicting the response to IFN β in RRMS.

The activity of the type I IFN pathway is highly heterogeneous between patients with RRMS.⁶ Previously, we and others showed that levels of IFN activity in untreated patients negatively correlated with the pharmacological and clinical response towards IFN β .^{6,7} In systemic lupus erythematosus, an autoimmune disease in which type I IFN is associated with development and severity of the disease, a clear link between IRF5 polymorphisms, among these rs2004640, and disease has been shown.^{11,16} Besides a role for rs2004640 in conferring risk to systemic lupus erythematosus, we here provide evidence for a role of this SNP in determining the responsiveness towards IFN β in RRMS. Graham et al.¹¹ showed that the rs2004640 T-allele creates a consensus splice donor site leading to expression of a protein product bearing an alternative exon 1B protein sequence, with concomitant higher levels of IRF5 mRNA. As IRF5 has a complex and dual role in IFN production and signaling, functional genetic variation in IRF5 may affect signaling transduction, resulting in varying IFN- response gene activity, which might be linked to differential responsiveness to IFN β treatment in RRMS. Therefore, the increased expression of the exon 1B bearing IRF5 gene product, as a consequence of the rs2004640 T allele variant, might lead to a more sensitive IFN system. This could result in increased endogenous IFN response gene activity, which reaches saturation, and is therefore not further enhanced by administration of exogenous IFN β . Thus the genetic contribution of the IRF5 gene may explain the increased baseline status of IFN activity in RRMS that negatively correlates with the pharmacological and clinical effects of IFN β treatment. IRF5 polymorphism rs4728142, which is located 5 kb upstream of IRF5, was shown to be associated with MS.¹⁷ The exact function of this polymorphism has not been revealed. Rs4728142 is in high linkage disequilibrium with a 50 -CGGGG-30 indel that is located 64 kb upstream of IRF5. This insertion of 5 bp creates an additional binding site for the transcription factor SP1, which might implicate functional effects of rs4728142 alleles on IRF5 mRNA expression.

It is generally believed that essentially two phases of unresponsiveness might be identified: a mechanistic phase directly after the start of treatment, and a secondary phase that develops in initial responders during the course of therapy because of the occurrence of neutralizing antibodies (NAbs) that can reduce treatment efficacy of IFN β treatment. Because of the temporal aspects related to monitoring the clinical response, our findings might be linked to processes that are related to both the mechanistic phase and NAbs development. The finding of a significant association of rs2004640 and rs4728142 with the pharmacological response to IFN β is supportive for a mechanistic effect. Additionally, the observation that the association between rs2004640 and IFN β response status becomes more pronounced when the observation period exceeds 24 months hints to a role of IRF5 genetics in NAbs development. Therefore, information on the development and persistence of NAbs needs to be related to IRF5 genetics and IFN signaling in future studies. Unfortunately, NAbs titers were only inconsistently available for the current patient groups.

In conclusion, in this study we report on the identification of IRF5 polymorphisms that are associated with the pharmacological response to IFN β , as well as the development of new T2 lesions on MRI and the time to first relapse during IFN β treatment. The identification of genetic factors that predispose to the clinical response to IFN β treatment in RRMS is an important clinical finding, especially with the current availability of alternative drugs. If confirmed in future prospective studies, the determination of IRF5 genotypes could provide additional information for treatment decisions in RRMS patients.

ACKNOWLEDGEMENTS

We gratefully acknowledge the staff and patients of the departments of Neurology at the VUmc hospital, the Centre d'Esclerosi Multiple de Catalunya and Brigham and Women's Hospital who participated in this study. The study was supported by the Dutch MS Research Foundation (Grant 04-549 MS).

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