Pharmacogenomic study in patients with multiple sclerosis

Responders and nonresponders to IFN-β

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ABSTRACT

Objectives: We aimed to investigate the association between polymorphisms located in type I interferon (IFN)-induced genes, genes belonging to the toll-like receptor (TLR) pathway, and genes encoding neurotransmitter receptors and the response to IFN-β treatment in patients with multiple sclerosis (MS).

Methods: In a first or screening phase of the study, 384 polymorphisms were genotyped in 830 patients with MS classified into IFN-β responders (n = 416) and nonresponders (n = 414) according to clinical criteria. In a second or validation phase, the most significant polymorphisms associated with IFN-β response were genotyped in an independent validation cohort of 555 patients with MS (281 IFN-β responders and 274 nonresponders).

Results: Seven single nucleotide polymorphisms (SNPs) were selected from the screening phase for further validation: rs832032 (*GABRR3*; $p = 0.0006$), rs6597 (*STUB1*; $p = 0.019$), rs3747517 (*IFIH1*; $p = 0.010$), rs2277302 (*PELI3*; $p = 0.017$), rs10958713 (*IKBKB*; $p = 0.003$), rs2834202 (*IFNAR1*; $p = 0.030$), and rs4422395 (*CXCL1*; $p = 0.017$). None of these SNPs were significantly associated with IFN-β response when genotyped in an independent cohort of patients. Combined analysis of these SNPs in all patients with MS (N = 1,385) revealed 2 polymorphisms associated with IFN-β response: rs2277302 (*PELI3*; $p = 0.008$) and rs832032 (*GABRR3*; $p = 0.006$).

Conclusions: These findings do not support an association between polymorphisms located in genes related to the type I IFN or TLR pathways or genes encoding neurotransmitter receptors and the clinical response to IFN-β. Nevertheless, additional genetic and functional studies of *PELI3* and *GABRR3* are warranted. *Neurol Neuroimmunol Neuroinflamm* 2015;2:e154; doi: 10.1212/NXI.0000000000000154

GLOSSARY

EDSS = Expanded Disability Status Scale; **GABA** = γ-aminobutyric acid; **IFN** = interferon; **MS** = multiple sclerosis; **SNP** = single nucleotide polymorphism; **TLR** = toll-like receptor.

Interferon β (IFN-β) is one of the most widely prescribed disease-modifying therapies for patients with relapsing-remitting multiple sclerosis (MS) and has demonstrated positive effects on reducing disease activity.[1] However, IFN-β is only partially effective, and a significant proportion of patients with MS do not respond to this treatment.[2] Despite

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many efforts to identify allelic variants influencing the response to IFN-β,[3] to date there is a lack of genetic biomarkers reliably associated with therapeutic response.

A previous IFN-β transcriptomic study conducted by our group (Cemcat) implicated genes belonging to the type I IFN pathway and genes related to the toll-like receptor (TLR) pathway in the clinical response to IFN-β.[4] The 2 genome-wide pharmacogenomic studies published thus far in relation to the response to IFN-β[5,6] reported the involvement of type I IFN– responsive genes[5] and the overrepresentation of genes coding for glutamate and γ-aminobutyric acid (GABA) receptors among the polymorphisms that best discriminated between IFN-β responders and nonresponders, suggesting a relationship between neuronal excitation and response to IFN-β.[5,6]

Building on these observations, in the present study we aimed to genotype an extensive panel of polymorphisms located in genes related to the type I IFN and TLR pathways and genes coding for GABA and glutamate receptors in a multicenter cohort of IFN-β responders and nonresponders in order to investigate their potential association with the response to this treatment.

METHODS

Definition of response to IFN-β therapy. Patients with relapse-onset MS treated with IFN-β and with a follow-up of at least 2 years were classified as responders and nonresponders. Responders were patients with no relapses and no increase in the Expanded Disability Status Scale (EDSS) score over the follow-up period. Nonresponders were patients having 1 or more relapses during the follow-up period and an increase in the EDSS score of at least 1 point confirmed at 6 months.[7] We further classified the response of a subgroup of patients according to the number of relapses during the first 2 years of treatment without considering EDSS progression. Responders had no relapses during the follow-up period. Two different criteria were considered for nonresponders: (1) 1 or more relapses during the 2-year follow-up (criteria 1), and (2) 2 or more relapses over the follow-up period (for this criterion, patients with only 1 relapse were not considered in the analysis; criteria 2).

Information on the IFN-β neutralizing antibody status was not available for patients included in the study.

Single nucleotide polymorphism selection. Genes related to the type I IFN and TLR pathways and genes coding for GABA and glutamate receptors were selected through bibliographical searches, and a total of 384 biologically informative single nucleotide polymorphisms (SNPs) were selected for genotyping using Select Your SNPs, an online tool for selecting tagSNPs from a roster of candidate genes.[8]

Study design. In a first or screening phase of the study (phase I), 384 SNPs were genotyped in 830 patients with MS classified according to their response to IFN-β. There were 416 responders and 414 nonresponders. Genomic DNA samples were obtained using standard methods and genotyping was performed using an Illumina GoldenGate assay at Progenika Biopharma SA (Bizkaia, Spain).

Overall genotyping success rate was 95%. The full list of SNPs and probes is provided as table e-1 at Neurology.org/nn.

In a second or validation phase of the study (phase II), the most significant SNPs obtained in phase I were genotyped in an independent cohort of 555 patients with MS (281 responders and 274 nonresponders to IFN-β). Genotyping was performed using TaqMan assays at the Cemcat (Barcelona, Spain). Genotyping success rate was 97%.

A summary of demographic and baseline clinical characteristics of all patient cohorts is shown in table 1.

Statistical analysis. Data processing, missingness, Hardy-Weinberg analysis, and allele frequency comparisons between responders and nonresponders to IFN-β were performed with SNPator (www.snpator.org).[9] Possible stratification owing to different population origin was assessed using the Cochran-Mantel-Haenszel test implemented in PLINK (http://pngu.mgh.harvard.edu/~purcell/plink/).

Standard protocol approvals, registrations, and patient consents. The study was approved by the corresponding local ethics committee, and all patients gave their informed consent.

RESULTS

In the first phase of the study, a total of 384 SNPs were genotyped in 830 patients with MS classified according to their clinical response to IFN-β treatment. The results of the allelic association analysis for all the study cohorts (combined and stratified by MS center) are shown in table e-2. The most significant SNPs associated with the response to IFNβ were selected for further validation (table 2): (1) rs832032 (*GABRR3*; $p = 0.0006$), (2) rs6597 (*STUB1*; $p = 0.019$), (3) rs3747517 (*IFIH1*; $p = 0.010$), (4) rs2277302 (*PELI3*; $p = 0.017$), (5) rs10958713 (*IKBKB*; $p = 0.003$), (6) rs2834202 (*IFNAR1*; $p = 0.030$), and (7) rs4422395 (*CXCL1*; $p = 0.017$).

In a second phase of the study, these 7 SNPs were genotyped in an independent cohort of 555 patients with MS classified according to the same clinical criteria as in phase I. As depicted in table 2, allelic analysis in this validation cohort showed a lack of statistically significant associations between selected polymorphisms from phase I and the response to IFN-β (figure e-1). Combined analysis in the whole cohort of patients with MS (N = 1,385; table 2) revealed 2 SNPs associated with the response to IFN-β with p values below 0.01: rs2277302, a synonymous polymorphism located in the *PELI3* gene; and rs832032, a polymorphism located in the *GABRR3* gene that results in a premature stop codon and protein truncation.

Table 1 Summary of demographic and baseline clinical characteristics for all the cohorts of patients with MS (responders and nonresponders to IFN-β treatment)

		N		Female/male (% female)		Age, y		Disease duration, y		EDSS		IFN-β (1/2/3)[a]	
Phase	Sets	R	NR	R	NR	R	NR	R	NR	R	NR	R	NR
I	Toulouse	67	68	53/14 (80.1)	47/21 (69.1)	28.9 (8.0)	27.9 (8.7)	4.5 (5.4)	4.8 (5.8)	2.0 (1.2)	1.7 (1.3)	15/39/13	16/36/16
	Serbia	24	24	17/7 (70.8)	15/9 (62.5)	31.9 (6.4)	33.3 (7.8)	3.6 (3.5)	6.1 (4.9)	0.9 (0.8)	2.3 (0.9)	12/0/12	14/0/10
	Bochum	4	4	2/2 (50.0)	2/2 (50.0)	37.8 (4.6)	36.0 (9.8)	3.8 (3.8)	4.3 (3.6)	2.1 (1.1)	3.9 (2.5)	0/1/3	1/1/2
	Rostock	43	43	36/7 (83.7)	36/7 (83.7)	40.9 (9.9)	38.3 (11.4)	5.0 (4.8)	4.6 (5.8)	1.8 (1.6)	3.0 (1.7)	12/9/22	6/6/31
	Newcastle	32	32	25/7 (78.1)	25/7 (78.1)	42.0 (11.1)	35.2 (12.2)	6.3 (9.1)	6.0 (5.6)	—	—	16/2/14	16/2/14
	San Francisco	2	2	2/0 (100)	2/0 (100)	40.5 (6.4)	41.5 (6.4)	1.9 (0.9)	3.7 (4.1)	1.0 (1.4)	1.5 (0.7)	1/1/0	1/1/0
	Milan	6	6	4/2 (66.7)	3/3 (50.0)	39.6 (8.8)	40.8 (15.8)	3.7 (5.1)	6.3 (7.2)	1.5 (0.8)	2.5 (1.8)	4/2/0	4/2/0
	Madrid PH	20	20	12/8 (60.0)	14/6 (70.0)	37.7 (8.3)	32.7 (9.8)	4.6 (2.7)	7.2 (2.8)	—	—	10/5/5	10/8/2
	Madrid SC	29	30	21/8 (72.4)	15/15 (50.0)	33.4 (7.4)	34.1 (7.7)	5.3 (4.8)	5.1 (6.5)	2.0 (1.6)	2.1 (1.3)	12/7/10	12/5/13
	Malaga	32	32	23/9 (71.9)	23/9 (71.9)	40.0 (9.5)	44.0 (10.8)	16.0 (4.0)	15.0 (7.6)	1.0 (1.3)	2.0 (1.4)	10/17/5	9/16/7
	Bilbao	28	25	21/7 (75.0)	18/7 (72.0)	36.5 (9.8)	34.0 (8.4)	8.0 (7.4)	8.1 (8.2)	2.2 (2.0)	2.5 (1.5)	13/3/12	11/5/9
	Barcelona	129	128	103/26 (79.8)	99/29 (77.3)	40.8 (8.8)	40.9 (10.3)	13.7 (7.2)	13.3 (7.3)	2.4 (1.8)	5.3 (2.1)	38/37/54	34/36/58
	All phase I	416	414	319/97 (76.7)	299/115 (72.2)	37.5 (4.1)	36.5 (4.6)	6.4 (7.0)	7.0 (3.5)	1.7 (0.5)	2.7 (1.1)	143/123/150	134/118/162
II	Madrid RC	33	41	27/6 (81.8)	29/12 (70.7)	38.0 (9.4)	35.9 (9.2)	6.2 (6.1)	3.8 (5.2)	1.6 (0.8)	1.8 (1.3)	21/4/8	22/13/6
	Malaga	129	114	82/47 (63.6)	75/39 (65.8)	45.5 (11.1)	46.8 (11.1)	16.3 (7.9)	17.3 (7.6)	1.4 (1.5)	1.7 (1.4)	48/27/48	41/38/3
	Rostock	43	43	36/7 (83.7)	36/7 (83.7)	37.7 (10.2)	35.5 (11.1)	3.0 (0.9)	3.1 (0.9)	1.8 (1.3)	2.2 (1.1)	9/17/17	6/7/30
	Amsterdam	76	76	55/21 (72.3)	50/24 (67.6)	35.5 (8.3)	35.0 (9.4)	4.0 (4.2)	4.2 (4.0)	2.5 (1.3)	3.1 (1.4)	24/27/25	18/25/31
	All phase II	281	274	200/81 (71.2)	190/84 (6.9)	40.6 (11.0)	40.14 (11.8)	9.8 (8.7)	9.9 (8.8)	1.8 (1.4)	2.2 (1.5)	102/75/98	87/83/70
Both phases	All cohorts	698	688	519/178 (74.5)	489/199 (71.1)	38.6 (10.6)	37.7 (11.6)	8.9 (8.0)	8.7 (7.9)	1.8 (1.5)	2.7 (2.1)	245/198/248	221/201/232

Abbreviations: EDSS = Expanded Disability Status Scale; IFN = interferon; Madrid PH = Hospital Universitario Puerta de Hierro, Madrid; Madrid RC = Hospital Ramón y Cajal, Madrid; Madrid SC = Hospital Clínico San Carlos; NR = nonresponder; R = responder.
Unless otherwise indicated, data are expressed as mean (SD).
[a] IFN-β (1a intramuscular/1b subcutaneous/1a subcutaneous).

Table 2 Summary of results of allele association analysis

SNP	Chr	Gene	MA	MAF	Screening cohort[a]			Validation cohort[b]			Combined cohorts[c]		
					p Value	OR (95% CI)	A[d]	p Value	OR (95% CI)	A[d]	p Value	OR (95% CI)	A[d]
rs832032	3	*GABRR3*	*T*	0.21	0.0006	1.55 (1.21–1.99)	*A*	0.745	1.06 (0.76–1.46)	*T*	0.006	1.31 (1.08–1.59)	*A*
rs10958713	8	*IKBKB*	G	0.36	0.0030	1.38 (1.11–1.70)	G	0.601	1.07 (0.83–1.39)	G	0.016	1.22 (1.04–1.43)	G
rs3747517	2	*IFIH1*	G	0.26	0.0104	1.35 (1.07–1.69)	*A*	0.602	1.08 (0.81–1.43)	G	0.065	1.18 (0.99–1.40)	*A*
rs2277302	11	*PELI3*	*A*	0.20	0.0172	1.36 (1.05–1.75)	*A*	0.310	1.17 (0.87–1.58)	*A*	0.008	1.29 (1.07–1.56)	*A*
rs4422395	4	*CXCL1*	G	0.15	0.0173	1.42 (1.06–1.89)	*A*	0.338	1.17 (0.85–1.62)	G	0.289	1.12 (0.91–1.38)	*A*
rs6597	16	*STUB1*	*A*	0.12	0.0193	1.47 (1.06–2.03)	*A*	0.746	1.06 (0.74–1.52)	C	0.040	1.27 (1.01–1.60)	*A*
rs2834202	21	*IFNAR1*	*A*	0.27	0.0303	1.29 (1.03–1.62)	G	0.509	1.10 (0.83–1.44)	G	0.048	1.19 (1.00–1.41)	G

Abbreviations: Chr = Chromosome; CI = confidence interval; IFN = interferon; MA = minor allele; MAF = minor allele frequency; OR = odds ratio; SNP = single nucleotide polymorphism. *GABRR3* (gamma-aminobutyric acid [GABA] A receptor, rho 3) rs832032 results in a premature stop codon and protein truncation. *STUB1* (STIP1 homology and U-box containing protein 1, E3 ubiquitin protein ligase) rs6597 is an intronic polymorphism. *IFIH1* (interferon induced with helicase C domain 1) rs3747517 is a nonsynonymous coding polymorphism. *PELI3* (pellino E3 ubiquitin protein ligase family member 3) rs2277302 is a synonymous polymorphism. *IKBKB* (inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta) rs10958713 is an intronic polymorphism. *IFNAR1* (interferon [alpha, beta and omega] receptor 1) rs2834202 is located in the 3′ untranslated region. *CXCL1* (chemokine [C-X-C motif] ligand 1) rs4422395 is an intergenic polymorphism.

[a] Corresponds to phase I of the study (n = 830).

[b] Corresponds to phase II of the study (n = 555).

[c] Corresponds to combined analysis in both cohorts (N = 1,385).

[d] A corresponds to the allele associated with the response to IFN-β.

A classification of the response to IFN-β based only on the number of relapses during the first 2 years of treatment was performed in a subgroup of 526 patients with MS from the screening cohort. It did not result in stronger associations with the response to IFN-β relative to the combined classification including relapses and EDSS progression (table e-3 for criteria 1; table e-4 for criteria 2).

DISCUSSION Previous pharmacogenomic studies have suggested a role for genes responsive to type I IFNs, genes related to the TLR pathway, and genes coding for neurotransmitter receptors in the response to IFN-β treatment in patients with MS.[4–6] Prompted by these observations, we genotyped an extensive panel of polymorphisms positioned in genes within these pathways in a large multicenter cohort—the largest thus far published in the field—of responders and nonresponders to IFN-β. Classification of patients with MS into IFN-β responders and nonresponders was conducted after 2 years of follow-up according to common stringent criteria based on the presence of relapses and progression of the EDSS score. These clinical criteria were similar to those used in previous studies.[4–6]

Despite the statistically significant associations with the response to IFN-β observed for some of the SNPs genotyped in the screening phase of the study, none of these associations remained significant following genotyping in an independent cohort of responders and nonresponders to IFN-β. While the reasons for this lack of association observed between selected polymorphisms from the screening phase and the response to IFN-β are manifold, a lack of statistical power to detect statistically significant associations in the validation cohort cannot totally be ruled out. In fact, combined analysis in the whole cohort of 1,385 patients with MS revealed significant associations for 2 polymorphisms, rs832032 and rs2277302.

Rs832032 is located in the *GABRR3* gene and encodes a subunit of the GABA(C) receptor. It is interesting that this polymorphism results in a premature stop codon and subsequent protein truncation. At present, there is no evidence in the literature of a functional relationship between GABA(C) receptors and IFN-β mechanism of action.

Rs2277302 is a synonymous SNP located in the *PELI3* gene, which codes for an E3 ubiquitin protein ligase involved in TLR signaling. In a recent publication,[10] Peli3-deficient mice expressed increased levels of endogenous IFN-β in response to TLR3 activation, and an autoregulation mechanism was proposed in which PELI3 inhibited type I IFNs by targeting IFN regulatory factor 7. These findings are in agreement with previous data from our group revealing

increased baseline levels of endogenous IFN-β in peripheral blood cells from IFN-β nonresponders.[11]

Overall, results from this study do not support an association between polymorphisms positioned in genes belonging to the type I IFN or TLR pathways or genes coding for GABA or glutamate receptors and the clinical response to IFN-β. Findings with rs832032 and rs2277302 polymorphisms warrant further studies exploring the role of *GABRR3* and *PELI3* in the response to IFN-β in larger cohorts of patients with MS.

AUTHOR CONTRIBUTIONS

M.F. Bustamante: study concept and design, manuscript drafting and revision, genotyping, analysis and interpretation of the data. C. Morcillo-Suárez: manuscript drafting and revision, statistical analysis, analysis and interpretation of the data. S. Malhotra: manuscript revision, genotyping. J. Rio: manuscript revision, contribution of patients and clinical data. L. Leyva: manuscript revision, contribution of patients and clinical data. O. Fernández: manuscript revision, contribution of patients and clinical data. U.K. Zettl: manuscript revision, contribution of patients and clinical data. J. Killestein: manuscript revision, contribution of patients and clinical data. D. Brassat: manuscript revision, contribution of patients and clinical data. J.A. García-Merino: manuscript revision, contribution of patients and clinical data. A.J. Sánchez: manuscript revision, contribution of patients and clinical data. E. Urcelay: manuscript revision, contribution of patients and clinical data. R. Alvarez-Lafuente: manuscript revision, contribution of patients and clinical data. L.M. Villar: manuscript revision, contribution of patients and clinical data. J.C. Álvarez-Cermeño: manuscript revision, contribution of patients and clinical data. X. Farré manuscript revision, statistical analysis supervision, analysis and interpretation of the data. J. Lechner-Scott: manuscript revision, contribution of patients and clinical data. K. Vandenbroeck: manuscript revision, contribution of patients and clinical data. A. Rodríguez-Antigüedad: manuscript revision, contribution of patients and clinical data. J.S. Drulovic: manuscript revision, contribution of patients and clinical data. F. Martinelli Boneschi: manuscript revision, contribution of patients and clinical data. A. Chan: manuscript revision, contribution of patients and clinical data. J. Oksenberg: manuscript revision, contribution of patients and clinical data. A. Navarro: manuscript revision, statistical analysis supervision, analysis and interpretation of the data. X. Montalban: manuscript revision, contribution of patients and clinical data, study concept and design, analysis and interpretation of the data. M. Comabella: manuscript drafting and revision, study concept and design, analysis and interpretation of the data, study supervision.

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