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Short Report

NLRP3 polymorphisms and response to interferon-beta in multiple sclerosis patients

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Abstract: We aimed to investigate whether NLR family, pyrin domain containing 3 (NLRP3) polymorphisms are associated with the response to interferon-beta (IFNβ) in multiple sclerosis (MS) patients. A total of 14 NLRP3 polymorphisms were genotyped in a cohort of 665 relapsing-remitting MS patients recruited across 5 centers and classified into responders and non-responders according to clinical-radiological criteria after 1 year of IFNβ treatment. A meta-analysis failed to demonstrate significant associations between the response to IFNβ and NLRP3 polymorphisms. These findings do not support a role of polymorphisms located in the NLRP3 gene and the response to IFNβ in MS patients.

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Introduction

Inflammasomes are multi-protein complexes involved in the maturation of proinflammatory cytokines.[1] One of the best characterized inflammasomes, the NLR family, pyrin domain containing 3 (NLRP3), was recently reported to be associated with the response to interferon-beta (IFNβ) in multiple sclerosis (MS) patients, based on the findings of higher NLRP3 messenger RNA expression levels in peripheral blood mononuclear cells from IFNβ non-responders compared to responders.[2] The same study showed a trend for association between the NLRP3 polymorphism rs35829419 and the response to IFNβ according to clinical criteria.[2] In this study, we genotyped additional NLRP3 polymorphisms in order to further investigate a potential association between the NLRP3 gene and the response to treatment using more stringent clinical-radiological criteria.

Patients and methods

A total of 665 MS patients were recruited from 5 centers (Milan, $n = 389$; Barcelona, $n = 107$; Madrid, $n = 83$; Rostock, $n = 68$; Newcastle, $n = 18$). Inclusion criteria for the study were as follows: (1) age > 18 years and MS diagnosis according to McDonald criteria;[3] (2) treatment naive patients receiving IFNβ for at least 6 months and with a follow-up longer than 1 year; (3) relapsing-remitting disease course at the time of treatment onset. Patients who switched between IFNβ formulations were retained in the study. The study was approved by the corresponding local ethics committees, and written informed consent was obtained from all participants.

Criteria of response to IFNβ were applied after 1 year of treatment based on clinical-radiological criteria.[4] Non-responders were patients fulfilling any of the three of the following criteria: (1) presence of relapses, (2) increase of one or more points in the Expanded Disability Status Scale (EDSS) score, and (3) presence of ≥3 active lesions (new or enlarging T2 lesions or contrast-enhancing lesions) on the 1-year brain magnetic resonance imaging (MRI). Patients with no evidence of disease activity according to these variables were considered IFNβ responders.

Selection of NLRP3 single-nucleotide polymorphisms and genotyping

Taking advantage of 1000Genomes Project data, an initial list of variants segregating in CEU (Europeans) population was obtained considering the region

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Table 1. Summary of demographic and baseline clinical characteristics for all the cohorts of MS patients responders and non-responders to IFNβ treatment.

Sets	Milan		Barcelona		Madrid		Rostock		Newcastle	
	R	NR	R	NR	R	NR	R	NR	R	NR
N (%)	279 (72.8)	104 (27.2)	39 (49.4)	40 (50.6)	41 (55.4)	33 (44.6)	49 (74.2)	17 (25.8)	13 (76.5)	4 (23.5)
Age at onset (years)	31.2 (9.4)	29.0 (9.5)	30.8 (8.1)	27.7 (9.1)	32.0 (9.8)	34.2 (9.8)	36.1 (8.9)	34.3 (7.9)	35 (13.0)	32.5 (17.9)
F/M (%F)	175/104 (62.7)	73/31 (70.2)	32/7 (82.0)	26/14 (65.0)	36/5 (87.8)	23/10 (69.7)	42/7 (84)	14/3 (82.4)	11/2 (84.6)	3/1 (75%)
Duration (years)	11.8 (7.5)	12.9 (7.5)	4.6 (5.4)	4.0 (4.3)	5.2 (5.3)	4.0 (5.0)	1.9 (3.4)	3.0 (5.3)	5.8 (7.7)	7.3 (7.2)
EDSS[a]	1.7 (0–7)	1.6 (0–4)	1.9 (0–4)	2.1 (1–6)	1.6 (0–6.5)	1.7 (0–4.5)	1.7 (0–4)	1.8 (1–3)	1.6 (0–2)	2.5 (0–7.5)
Relapses[a,b]	1.4 (1–4)	1.6 (0–5)	2.1 (1–5)	2.0 (0–4)	1.2 (0–4)	1.3 (0–3)	1.2 (0–5)	1.3 (0–5)	1.6 (1–3)	1.3 (1–2)
IFNβ[c]	69/40/170	28/12/64	12/6/21	16/8/16	27/4/10	15/7/11	7/27/15	5/7/5	2/3/8	1/2/1

MS: multiple sclerosis; IFNβ: interferon-beta; R: responders; NR: non-responders; EDSS: Expanded Disability Status Scale; F: female; M: male.
Data are expressed as mean (standard deviation) unless otherwise stated.
[a]Data are expressed as mean (min–max).
[b]Refers to the number of relapses in the previous 2 years before treatment onset.
[c]Type of IFNβ (1a intramuscular/1b subcutaneous).

spanning 5 Kb NLRP3 gene start and gene end. Among all the obtained variants, only 26 were found to be annotated in at least one of the several used functional and conservation databases. Finally, only 14 variants had a valid TaqMan code and were considered for further analysis.

Genotyping was conducted using a TaqMan Open Array Genotyping System (Thermo Fisher Scientific, Waltham, USA). DNA samples were loaded at 50 ng/ mL and amplified according to the manufacturer's instructions. The autocalling method as implemented in the TaqMan Genotyper software version 1.3 was used to assign genotypes. As quality controls, a genotype call rate of 0.90 and an individual call rate of 0.80 were used as threshold.

Statistical analyses

Gender, age at onset, disease duration, number of relapses in the 2 years before treatment onset, and baseline EDSS were tested as possible confounding variables within each cohort using the *t*-test or Mann–Whitney test when appropriate for continuous data and the chi-square test for ordinal data and comparing responders and non-responders. All single-nucleotide polymorphisms (SNPs) were tested for association with response using PLINK-v1.07.[5] Statistical significance of individual genotype data was calculated using a logistic regression model within each cohort adjusted for any clinical variable identified as differentially distributed across responders and non-responders. Odds ratios (ORs) and 95% confidence intervals (CIs) are reported. PLINK-v1.07 was also used for evidence of deviation from Hardy–Weinberg equilibrium within each cohort. A meta-analysis across the cohorts was performed assuming a per-allelic model and using both fixed- and random-effects meta-analysis approaches.[6]

Results

A total of 619 patients were included in the analysis while 46 patients were excluded due to low call rate. A total of 421 patients (68.0%) were classified as responders and 198 patients (32.0%) as non-responders. Table 1 summarizes the main demographic and clinical characteristics of patients. No differences were observed between responders and non-responders except for the number of relapses in the 2 years before treatment onset in the Milan cohort ($p = 0.03$); thus, this variable was included as covariate in the analysis.

Among the 14 selected NLRP3 polymorphisms, the following were excluded: (1) rs4925547 and

Table 2. Summary of individual and combined analyses of the study cohorts.

SNP	MA	Milan OR (CI)	p	Barcelona OR (CI)	p	Madrid OR (CI)	p	Rostock OR (CI)	p	Newcastle OR (CI)	p	Meta-analysis OR (fixed)	p (fixed)	OR (random)	p (random)	I^2
rs121908147	A	0.77 (0.21–2.84)	0.70	a	a	1.25 (0.08–20.77)	0.88	6.40 (0.54–75.62)	0.14	b	b	1.23	0.707	1.23	0.707	0.0
rs7525979	T	1.17 (0.66–2.09)	0.59	0.61 (0.16–2.36)	0.47	0.93 (0.19–4.46)	0.92	1.02 (0.19–5.66)	0.98	2.66 (0.41–17.24)	0.30	1.11	0.669	1.11	0.669	0.0
rs180177471	A	2.12 (0.29–15.63)	0.46	b	b	a	a	b	b	a	a	2.12	0.462	2.12	0.462	0.0
rs148478875	T	2.67 (0.90–7.93)	0.08	a	a	b	b	b	b	a	a	2.67	0.077	2.67	0.077	0.0
rs35829419	A	0.83 (0.36–1.92)	0.67	1.54 (0.40–5.96)	0.53	0.39 (0.04–3.89)	0.42	b	b	a	a	0.91	0.789	0.91	0.789	0.0
rs12070953	C	0.57 (0.35–0.95)	0.03	1.60 (0.53–4.84)	0.41	2.24 (0.92–5.45)	0.08	0.49 (0.10–2.52)	0.40	3.50 (0.28–43.16)	0.33	0.88	0.507	1.12	0.770	59.3
rs12065526	A	0.56 (0.34–0.91)	0.02	1.15 (0.51–2.56)	0.74	2.18 (0.86–5.50)	0.10	0.93 (0.22–3.93)	0.92	3.50 (0.28–43.16)	0.33	0.86	0.420	1.08	0.822	53.0
rs12086048	T	0.56 (0.34–0.91)	0.02	1.06 (0.46–2.46)	0.89	2.46 (0.95–6.39)	0.06	0.84 (0.20–3.48)	0.81	3.50 (0.28–43.16)	0.33	0.84	0.361	1.08	0.831	55.9
rs4925663	T	1.22 (0.87–1.72)	0.25	0.43 (0.22–0.84)	0.014	0.69 (0.31–1.54)	0.37	0.91 (0.37–2.22)	0.84	1.58 (0.22–11.24)	0.65	0.95	0.705	0.83	0.438	51.8
rs11583410	C	1.11 (0.8–1.53)	0.54	1.90 (1.01–3.57)	0.048	0.71 (0.35–1.47)	0.36	0.82 (0.34–1.97)	0.66	0.77 (0.12–4.87)	0.79	1.11	0.435	1.10	0.556	16.6

SNP: single-nucleotide polymorphism; OR (CI): odds ratio (95% confidence interval); I^2: I^2 heterogeneity index; MA: minor allele; R: responders; NR: non-responders; IFNβ: interferon-beta.
OR is calculated referring to the minor allele.
Meta-analysis was performed using both fixed- and random-effects approaches.
Less stringent clinical-radiological IFNβ response criteria considering as responders patients with Rio score 0 or 1 and non-responders patients with Rio score 2 or 3 did not result in significant associations with the response to treatment (data not shown).
[a]Monomorphic.
[b]Minor allele present only in one group (R or NR).

rs34298354 had a call rate < 0.90, (2) rs201229629 was monomorphic in the entire cohort, and (3) rs139814109-T allele was not present in the non-responder cohort, thus it was not possible to calculate an association. A final list of 10 SNPs was included in the analyses. None of them showed deviation from Hardy–Weinberg equilibrium ($p > 0.05$).

As shown in Table 2, individual cohort analysis only revealed marginal associations with the response to IFNβ for the NLRP3 polymorphisms rs12070953, rs12065526, and rs12086048 in the Italian cohort and for rs4925663 and rs11583410 in the Barcelona cohort. However, in the meta-analysis, none of the SNPs genotyped were significantly associated with the response to IFNβ. A trend for association was observed in the meta-analysis for SNP rs148478875; however, this effect was exclusively driven by the results in the Italian cohort, since the polymorphism was found to be monomorphic in the other cohorts most likely due to the low frequency of the minor allele.

Discussion

In an earlier study,[2] the NLRP3 polymorphism rs35829419 showed a trend for association with the response to IFNβ in MS patients classified into responders and non-responder according to clinical criteria based on the presence of relapses and progression on the EDSS score after 2 years of treatment. Motivated by these findings, in this study, we selected additional SNPs across the NLRP3 gene in order to investigate a relationship between inflammasome polymorphisms and response to treatment in MS patients classified into responders and non-responders according to more appropriate stringent clinical-radiological criteria.[4] Combined analysis of the five MS cohorts did not show a significant association between rs35829419 and IFNβ response, and genotyping of additional NLRP3 polymorphisms also failed to demonstrate significant associations with treatment response. Altogether, these results do not support a role for NLRP3 polymorphisms and IFNβ response in MS patients.

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