MULTIPLE SCLEROSIS JOURNAL | MSJ

Original Research Paper

Pharmacogenomics of response to interferon-beta and glatiramer acetate in Multiple Sclerosis: A multi-centric study

Multiple Sclerosis Journal
2026, Vol. 32(7) 747–758
DOI: 10.1177/13524585261417130

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Abstract

Background: Multiple sclerosis (MS) treatment response varies significantly, hindering effective management and necessitating better predictive biomarkers. Pharmacogenomics offers a promising avenue to identify genetic markers that, combined with clinico-demographic predictors, could enhance personalized therapeutic decisions.

Objectives: This multi-center study investigated genetic determinants of response to interferon-beta (IFN-β) and glatiramer acetate (GA) in European ancestry patients with relapsing–remitting MS (RRMS).

Methods: After harmonization and quality control, we tested over 6 million genetic variants. We used negative binomial regression, meta-analysis, and gene-set enrichment to link variants, genes, and biological pathways to treatment response.

Results: In 679 GA and 1614 IFN-β patients, the top GA variant was rs2053696A in MAP3K1 ($p = 3.97*10^{-9}$), involved in key signaling pathways. Another significant GA signal was in WWOX. For IFN-β, no genome-wide significant variants were found, but suggestive signals emerged near ZMIZ1, ZCCHC7, and other genes linked to immune function and interferon signaling. Gene-set analysis revealed IL-17 regulation for GA and ion channel pathways for IFN-β.

Conclusion: This study identified novel genetic variants for GA response, implicating genes in crucial pathways. For IFN-β, suggestive signals point to immune and interferon-related genes. These findings enhance our understanding of genetic influences on RRMS treatment response, while highlighting pharmacogenomic research challenges.

Keywords: Multiple sclerosis, pharmacogenomics, interferons, glatiramer acetate

Date received: 16 July 2025; revised: 2 December 2025; accepted: 23 December 2025

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Introduction

Multiple sclerosis (MS; MIM 126200) is a disease of the central nervous system (CNS) characterized by chronic inflammation, demyelination, and axonal loss.[1] It is a complex multifactorial disorder, with both genetic and environmental factors playing a role in disease susceptibility, and a polygenic architecture underlying its risk.[2]

The disease is highly heterogeneous in its clinical expression and marked inter-individual differences have been observed in response to treatments.

Since the advent of disease-modifying therapies (DMTs), the treatment landscape for MS has significantly evolved, offering to patients a range of options to manage their condition. However, the clinical

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response to these therapies varies considerably among individuals and the response of each patient remains largely unpredictable.[3] The paucity of pre-treatment biomarkers that can help in the early identification of non-responders is a critical issue in the management of MS patients. The timely identification of patients with partial response to therapies is of utmost importance, especially considering the current portfolio of approved DMT in MS, with the final aim of counteracting the trial-and-error approach in assigning patients to treatment and supporting therapeutic decisions.

Pharmacogenomics can be a valuable tool in fulfilling this unmet need, and genetic markers, together with the biological processes they pinpoint and coupled with clinico-demographic and magnetic resonance imaging (MRI) predictors, could provide insight into patients' responsiveness to treatments.[4]

However, an issue that has plagued pharmacogenomic studies is the small sample size,[5] since the difficulty in collecting large cohorts of well-phenotyped patients have hampered constitution of sufficiently powered cohorts.

In this study, we report the results of a genome-wide meta-analytic screen of genetic markers of response to two well-established first-line drugs (interferon-beta (IFN-β) and glatiramer acetate (GA)), taking advantage of cohorts available within the multi-centric effort of the MultipleMS consortium.

IFN-β, available as IFN-β1b and IFN-β1a formulations, has been the first licensed drug for the treatment of relapsing–remitting MS (RRMS). Clinical trials demonstrated its efficacy in reducing annualized relapse rate by approximately 30.[6] IFN-β, an endogenous cytokine, exerts its therapeutic effect through anti-inflammatory mechanisms, modulating various biological responses. It mimics the action of natural interferons, known for their antiviral and anti-inflammatory properties.

GA has been the second DMT approved for the prevention of MS relapses and has been found to reduce relapse rate by 30% and time to first relapse.[7]

For both IFN-β and GA, non-responder rates hover near 50%, highlighting the significant individual variability in patient response to these therapies.[8] Analyses were conducted variant-wise, gene-wise, and gene-set-wise, with the aim of eliciting shared and specific genetic determinants of response across the two drugs. Finally, we sought to exploit findings from previous pharmacogenomic studies of response to IFN-β and GA, to validate putatively associated markers.

Patients and methods

Study population

This is a multi-centric study, combining patients of European ancestry exposed to the two DMTs of interest. More precisely:

- Patients treated with IFN-β were recruited from Belgium, Denmark, Germany, Italy, Norway, and Sweden;
- Patients treated with GA were recruited from Germany, Italy, Norway, and Sweden.

Given the heterogeneity in clinical data collection, a harmonization effort was put in place using data dictionaries: details regarding this procedure have already been described in another study of our group.[9]

Inclusion criteria required the availability of baseline variables like age and disease duration at DMT start, sex, and the number of relapses 2 years before DMT start. We excluded patients with a progressive clinical course (primary or secondary) at treatment initiation.

If a patient had previously received multiple different drugs, we excluded those who had had three or more agents, or those who had had a second-line treatment (e.g. fingolimod, natalizumab, ofatumumab, cladribine, anti-CD19, oral/intravenous immunosuppressant) within the preceding 12 months. The study was approved by the local ethical committees and all participants provided their written informed consent to participate in this study.

Response to treatment

There is inherent uncertainty in defining response to treatment in MS and a standardized definition is lacking. To model response to treatment, we used the number of relapses experienced by patients during first-line DMT exposure. This definition allowed to include a larger number of patients as part of the international effort of the MultipleMS consortium, given the limited or lacking availability of MRI and Expanded Disability Status Scale (EDSS) data among the majority of centers. The observation period lasted from treatment initiation either to treatment stop or to the 2-year follow-up visit.

A relapse was defined on a clinical basis by the treating neurologists as the occurrence of new or worsened neurological symptoms or signs persisting for at least 24 hours in the absence of fever or infections and preceded by a 4-week stable period, using appropriate EDSS and MRI assessment if deemed.

Quality control

The study cohort was derived from a subset of a larger dataset from the MultipleMS project. Initial pre-imputation steps are described elsewhere.[10] Details on post-imputation quality control (QC) steps on a per-sample and per-marker base are described in the Supplementary Methods.

Association analysis

Negative binomial regression models were fitted to test for association between genotypes and the number of relapses during treatment, assuming allelic additive effects. Models included the logarithm of treatment duration as an offset variable and were adjusted for gender, age and disease duration at treatment start, pre-treatment relapse rate, and the first five eigenvectors from principal component analysis (PCA).

Summary statistics were aggregated across centers using fixed-effect meta-analysis with inverse-variance weighting. Details can be found on Supplementary Methods.

Given the relatively low replicability rate in the field of MS pharmacogenomics, we conducted a replication effort, leveraging findings from the field that were gathered from a review paper[8] both for GA and IFN-β. We thus investigated results from one pharmacogenomic genome-wide association study (GWAS) for GA ($n=11$ tested single-nucleotide polymorphisms (SNPs)) and five studies for IFN-β ($n=42$ tested SNPs).

Gene-set analysis

Gene-wise statistics were computed using MAGMA and meta-analyzed with weighted Stouffer's procedure. A critical choice in gene-based analysis is the assignment of SNPs to genes, since inclusion of noisy variants can be detrimental to association analyses. We thus assigned SNPs to the target gene applying a proximity rule and a functional rule that integrated regulatory information from blood and CNS tissues and cells from four sources. These results were used for gene-set analysis against Gene Ontology Biological Processes (GO-BP). Further details on gene-based and gene-set analysis are provided in the Supplementary Methods.

Results

Association analysis

Clinico-demographic variables of the study cohorts are reported in Table 1: it can be observed that there is high variability in the sample size of the cohorts for each study. Specifically, for GA, Italy (ITA) was the most numerous cohort ($N=375$), followed by GER ($N=179$), and Sweden/Norway (SWE/NOR) ($N=50$). For IFN-β, ITA had the highest sample size ($N=805$), followed by GER ($N=540$), Denmark (DEN) ($N=202$), SWE/NOR ($N=164$) and Belgium (BEL) ($N=67$). Furthermore, we observed heterogeneity for the three drugs across centers in baseline variables: male/female ratio, age, disease duration, EDSS at drug start, and relapses 2 years before drug start.

For each study, fixed-effect inverse-variance weighted meta-analysis of effect sizes (rate ratios, RR) was performed on variants present in at least one participant cohort: we tested 6,717,276 for IFN-β and 6,008,208 for GA. Overall association patterns are reported in Manhattan plots (Figure 1). For each DMT, top independent variants ($p<5*10^{-6}$ after clumping) are showed in Tables 2 and 3.

For GA, the most significant association was rs2053696A located in the intronic region of the MAP3K1 gene, coding for mitogen-activated protein kinase 1 ($p=3.96510^{-9}$, RR = 3.87). Figure 2 shows this variant's genomic context. A significant eQTL effect was observed for this variant in whole blood from eQTLGen. Other top associated variants beyond genome-wide significance were rs13287324C located in the intergenic region between genes OSTF1 and PCSK5 on chromosome 9 ($p=1.01*10^{-8}$, RR 2.12, Supplementary Figure S1) and rs2667609G, located in the intronic region of WWOX gene on chromosome 16 ($p=1.05*10^{-8}$, RR = 2.83, Supplementary Figure S2).

No variants in IFN-β meta-analysis exceeded the genome-wide significance threshold and we focused on the five most highly ranked signals. More precisely, the top associated variant was rs34766672 T ($p=1.90*10^{-7}$, RR = 3.02, Supplementary Figure S3), an intronic SNP in gene ZMIZ1, which shows a distal promoter interaction with gene POLR3A as of Open Target Genetics database. The second

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Table 1. Clinical and demographic characteristics of the study cohorts for the two DMTs.

	Glatiramer acetate				Interferons					
	GER	ITA	SWE/NOR	P-value	BEL	DEN	GER	ITA	SWE/NOR	*p*-value
	(*n*=254)	(*n*=375)	(*n*=50)		(*n*=67)	(*n*=202)	(*n*=540)	(*n*=805)	(*n*=164)	
Gender				0,035						0,015
Female	179 (70.5%)	268 (71.5%)	44 (88%)		50 (74.6%)	137 (67.8%)	357 (66.1%)	562 (69.8%)	131 (79.9%)	
Male	75 (29.5%)	107 (28.5%)	6 (12%)		17 (25.4%)	65 (32.2%)	183 (33.9%)	243 (30.2%)	33 (20.1%)	
Age at drug start, years	34.8 (8.8)	35.8 (8.9)	38.7 (8.4)	0,014	34.3 (9.9)	35.1 (8.5)	34.2 (9.1)	33.7 (8.5)	40.1 (9.0)	<0.001
Disease duration at drug start, years	3.0 (3.8)	7.6 (6.5)	7.9 (7.0)	<0.001	5.6 (6.3)	4.9 (5.7)	3.0 (4.8)	5.5 (5.8)	8.6 (7.1)	<0.001
EDSS at drug start[a]	1.5 (2.5)	1.5 (1.0)	2 (1.5)	0,001	2 (5.0)	2.5 (1.5)	1.5 (1.5)	1.5 (1.0)	2 (1.5)	0,002
DMT before drug start										
No[b]	155 (61.1%)	207 (55.2%)	29 (58%)	ns	57(85.1%)	202(100%)	473 (87.6%)	632 (78.5%)	139 (84.7%)	<0.001
Yes[b]	99 (39.9)	168 (44.8%)	21 (42%)		10 (14.9%)	0 (0%)	67 (12.4%)	173 (21.5%)	25 (15.3%)	
Relapses 2 years before drug start	1.2 (0.9)	1.2 (1.1)	1.0 (1.1)	ns	1.9 (1.2)	2.3 (1.1)	1.1 (0.7)	1.5 (1.1)	0.6 (0.9)	<0.001
Relapses 2 years	0.5 (0.8)	0.5 (0.8)	0.7 (0.9)	ns	0.9 (1.1)	1.0 (1.5)	0.5 (0.8)	0.7 (1.0)	0.4 (0.7)	<0.001

DMT: disease-modifying treatment; GER: Germany; ITA=Italy (combined OSR and UPO cohorts); SWE/NOR: Sweden/Norway; BEL: Belgium; DEN: Denmark. Values are presented as mean (standard deviation), unless otherwise stated.
[a]Median (inter-quartile range).
[b]No: naive patients; Yes: patients with preceding first-line or second-line DMT (see inclusion criteria in Patients and Methods section).

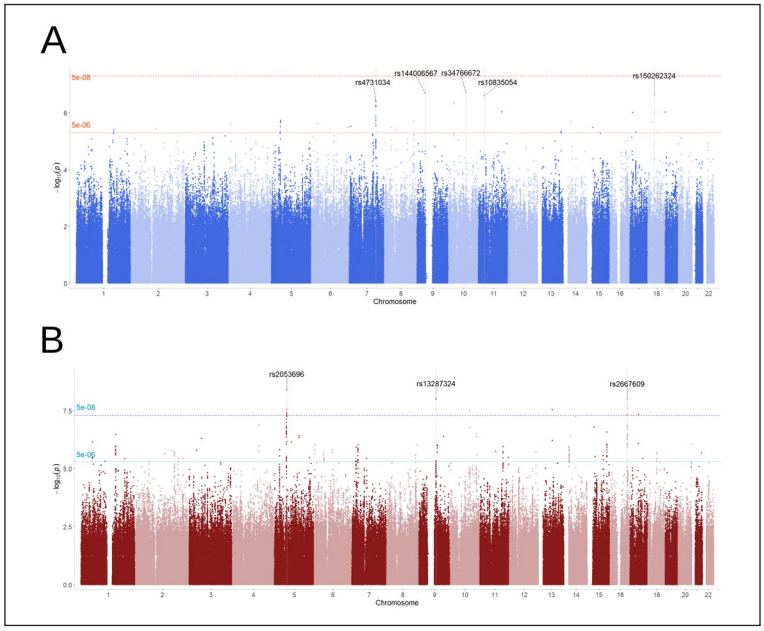

Figure 1. The Manhattan plot of -log10(p) of associations from fixed-effect meta-analysis for the two DMTs is shown ((a): GA, (b): IFN-β).
The genome-wide and suggestive level of significance are set at $\alpha = 5*10^{-8}$ and $\alpha = 5*10^{-5}$, respectively, and are illustrated by dashed lines. For GA analysis, rsIDs of the three associated SNPs exceeding genome-wide significance are reported, whereas for IFN-β, variants significant at suggestive level and discussed in the manuscript are marked.

variant was rs144006567G ($p = 2.0*10^{-7}$, RR = 4.71, Supplementary Figure S4), an intronic SNP located in gene ZCCHC7 which shows strong eQTL effect on whole blood for POLR1E gene and a distal promoter interaction with gene PAX5. The third top associated variant was rs150262324 C ($p = 2.391*10^{-7}$, RR = 3.51, Supplementary Figure S5), in intronic region of gene TRAPPC8, which shows strong eQTL effect on whole blood for this gene and for RNF138. The fourth variant was rs10835054 T ($p = 2.50*10^{-7}$, RR = 0.7, Supplementary Figure S6) for which an eQTL effect was detected for its proxy (rs11 029623A) for genes ANO3 and SLC5A12. Finally, the SNP rs4731034A ($p = 3.79*10^{-7}$, RR = 1.58, Supplementary Figure S7) was located in the intergenic region between FAM3C and PTPRZ1 genes.

Of note, in both meta-analyses we observed a specificity in the association signals, since top signals detected in association with response to IFN-β were not associated with GA, and vice versa (Tables 2 and 3).

Finally, we tested for replication loci that had been previously investigated in MS pharmacogenomics (Table 4). For IFN-β, out of 42 selected SNPs, 3 were successfully replicated: the intergenic variant rs16898029T ($p = 0.00593$, RR = 0.71), rs2237562C ($p = 0.047$, RR = 0.89) an intronic variant in gene GRM3, and rs1475919A ($p = 0.029$, RR = 1.14) an intronic variant of gene GRIK2.[11] For GA, none of the 11 SNPs was replicated at nominal significant threshold; however, for rs16886004G mapping in intronic region of MAGI2, we could observe a trend of association with a consistent direction of effect ($p = 0.071$, RR = 1.23): nevertheless, we could not observe association for MAGI2 at gene-wise level (Supplementary Table 2). Supplementary Table 3 overall summarizes the replication pattern observed for the 53 tested variants for the two DMTs.

Gene-set analysis

Gene-set analysis under the competitive hypothesis did not yield significant results after multiple testing correction. However, data analysis showed top associated gene sets primarily related to ion channel regulation. Specifically, top three GO terms for IFN-β response (Supplementary Table 4) included: "Sodium ion export across plasma membrane" ($p = 1.11*10^{-4}$), "IMP metabolic process" ($p =$ "Establishment or maintenance of transmembrane electrochemical gradient"($p = 3.36*10^{-4}$), For GA, the top enriched GO term was "Negative regulation of interleukin 17 production" ($p = 4.76*10^{-4}$) (Supplementary Table 5).

Table 2. List of suggestive SNPs ($p < 5*10^{-7}$) from meta-analysis of GA cohorts.

SNP	CHR	Position	A1	A2	MAF	*p*-value	RR	Genomic context	Gene(s)	*N*	*p*-value IFN-β	RR IFN-β	I^2
rs2053696	5	56168571	A	G	0.02665	3.97E-09	3.8741	intronic	*MAP3K1*	3	0.7707	1.0516	0
rs13287324	9	78013530	C	T	0.07691	1.01E-08	2.1233	intergenic	*OSTF1;PCSK5*	2	0.9657	0.995	0
rs2667609	16	78548574	G	T	0.04581	1.05E-08	2.8288	intronic	*WWOX*	3	0.311	1.1317	54.27
rs9563944	13	62403600	A	G	0.11656	2.86E-08	2.4231	intergenic	*LINC02339;LINC00358*	3	0.5251	1.0887	7.52
rs148477335	10	92224796	A	G	0.03466	3.26E-08	10.623	ncRNA_intronic	*LINC02653*	2	0.2593	1.3726	6
rs144133346	17	37162261	G	T	0.06002	4.62E-08	4.669	intergenic	*FBXO47;LINC02079*	2	0.5095	0.8643	0
rs113481211	14	52648526	G	A	0.04205	5.62E-08	4.0492	intergenic	*NID2;*	2	0.1815	1.3314	0
rs72682611	4	122870537	T	C	0.0344	1.30E-07	6.7256	intronic	*TRPC3*	2	0.1799	1.4783	28.2
rs144500865	15	30297714	A	G	0.02457	1.61E-07	4.3744	intergenic	*TJP1;GOLGA8J*	2	0.9299	0.9777	81.18
rs16886024	5	55980571	A	G	0.08729	2.52E-07	1.9227	intergenic	*C5orf67;LOC105378979*	3	0.9709	1.0032	0
rs8030899	15	88331209	T	C	0.1408	2.60E-07	0.4175	intergenic	*LINC00052;NTRK3*	3	0.2064	1.1013	0
rs116954556	10	122744547	T	G	0.01161	3.04E-07	6.706	intergenic	*WDR11;FGFR2*	2	0.9122	0.9689	0
rs569932	1	160725854	T	C	0.29481	3.41E-07	1.6257	intergenic	*SLAMF7;LY9*	3	0.3661	1.052	43.84
rs115305913	5	113528825	T	G	0.0256	3.73E-07	4.9711	intronic	*KCNN2*	2	0.1561	1.6016	0
rs117110990	9	113090647	G	A	0.02247	4.07E-07	5.3522	intronic	*TXNDC8*	2	0.137	1.3305	0
rs4983171	14	26887002	T	C	0.02262	4.12E-07	3.1321	intergenic	*STXBP6;NOVA1*	3	0.723	1.0639	0
rs147401623	3	56758381	T	C	0.05212	4.93E-07	7.4417	intergenic	*TASOR;ARHGEF3*	2	0.836	1.076	0

SNP: Single Nucleotide Polymorphism; CHR: chromosome; A1: effect allele (same as minor allele); A2: major allele; MAF: minor allele frequency; RR: rate ratio meta-analyzed from single-stratum negative binomial regression models, using minor allele as effect allele; Genomic context, neighboring genes, and distance for each SNP have been derived using ANNOVAR; N: Number of cohorts contributing to the meta-analysis for each SNP; p-value IFN-β and RR IFN-β report association statistics of GA in IFN-β cohorts; I2: percentage of total variation across cohorts due to heterogeneity.

Table 3. List of suggestive SNPs ($p < 5*10^{-6}$) from meta-analysis of IFN-β cohorts.

SNP	CHR	Position	A1	A2	MAF	*p*-value	RR	Genomic context	Gene(s)	*N*	*p*-value GA	RR GA	I^2
rs34766672	10	80797216	T	C	0.04868	1.90E-07	3.0234	ncRNA_ intronic	*ZMIZ1-AS1*	3	0.6749	1.2449	58.4
rs144006567	9	37225201	G	A	0.0198	2.00E-07	4.714	intronic	*ZCCHC7*	2			0
rs150262324	18	29511164	C	T	0.02137	2.39E-07	3.5134	intronic	*TRAPPC8*	3			66.3
rs10835054	11	26691664	T	C	0.16033	2.50E-07	0.7036	UTR3	*SLC5A12*	5	0.3039	1.1146	23.3
rs4731034	7	121298574	A	G	0.03779	3.80E-07	1.587	intergenic	*FAM3C; PTPRZ1*	5	0.8682	1.0289	41.6
rs180773215	10	25229948	C	T	0.01062	4.19E-07	5.195	intronic	*PRTFDC1*	2			0
rs1939894	11	107494204	C	T	0.49758	9.30E-07	0.7571	intronic	*ELMOD1*	3	0.2114	0.8888	0
rs72986440	19	342205	A	C	0.01967	9.60E-07	4.0898	intronic	*MIER2*	2			0
rs77260345	17	12985554	G	A	0.02337	9.73E-07	2.2316	intergenic	*ELAC2; LINC02093*	4	0.5991	1.2305	21.6
rs12187833	5	40704827	T	C	0.02668	1.87E-06	1.7455	intergenic	*PTGER4; TTC33*	5	0.2244	1.2809	0
rs6980814	8	134387355	C	A	0.32334	1.88E-06	0.7594	intergenic	*NDRG1; ST3GAL1*	5	0.6189	0.9546	0
rs113968648	14	33673779	G	C	0.02707	2.06E-06	1.8499	intronic	*NPAS3*	4	0.005734	1.763	3.3
rs150817880	18	14254137	A	G	0.01610	2.09E-06	4.4962	intergenic	*ANKRD20A5P; CYP4F35P*	2			48.5
rs149969836	6	27105958	T	C	0.02447	2.41E-06	3.6931	downstream	*H2BC12*	2			0
rs59128242	4	7064529	G	A	0.066	2.56E-06	1.4605	intronic	*GRPEL1*	5	0.1757	0.7808	57.8
rs17163287	7	7136077	C	A	0.29961	2.96E-06	1.5629	ncRNA_ exonic	*LOC100131257*	2	0.3549	0.8751	0
rs4708628	6	168433319	C	T	0.33974	3.18E-06	1.5617	intronic	*KIF25*	2			0
rs11631807	15	26187167	A	G	0.2444	3.21E-06	1.3321	ncRNA_ intronic	*LINC02346*	4	0.91	1.0126	0
rs78415264	8	28152818	A	G	0.05345	3.35E-06	0.5108	intergenic	*ELP3;*	5	0.8778	0.9703	0
rs116213854	2	115120041	A	C	0.01072	3.67E-06	4.2141	intergenic	*LINC01191; DPP10*	2			63.9
rs6424955	1	172469471	T	C	0.10569	3.87E-06	0.7053	intergenic	*C1orf105;*	5	0.0899	0.8153	0
rs13249358	8	54512490	C	A	0.2833	4.07E-06	1.6669	intergenic	*LOC100507516; ATP6V1H*	3			68.7
rs146554808	13	106566719	C	T	0.05002	4.51E-06	2.8315	intergenic	*LINC00343; LINC00460*	2			52.2
rs2945394	17	25883972	G	A	0.14432	4.72E-06	1.3777	intronic	*KSR1*	4	0.3027	0.8788	16.3

SNP: Single Nucleotide Polymorphism; CHR: chromosome; A1: effect allele (same as minor allele); A2: major allele; MAF: minor allele frequency; RR: rate ratio meta-analyzed from single-stratum negative binomial regression models, using minor allele as effect allele; Genomic context, neighboring genes, and distance for each SNP have been derived using ANNOVAR; N: Number of cohorts contributing to the meta-analysis for each SNP; p-value GA and RR GA report association statistics of IFN-β in GA cohorts; I2: percentage of total variation across cohorts due to heterogeneity.

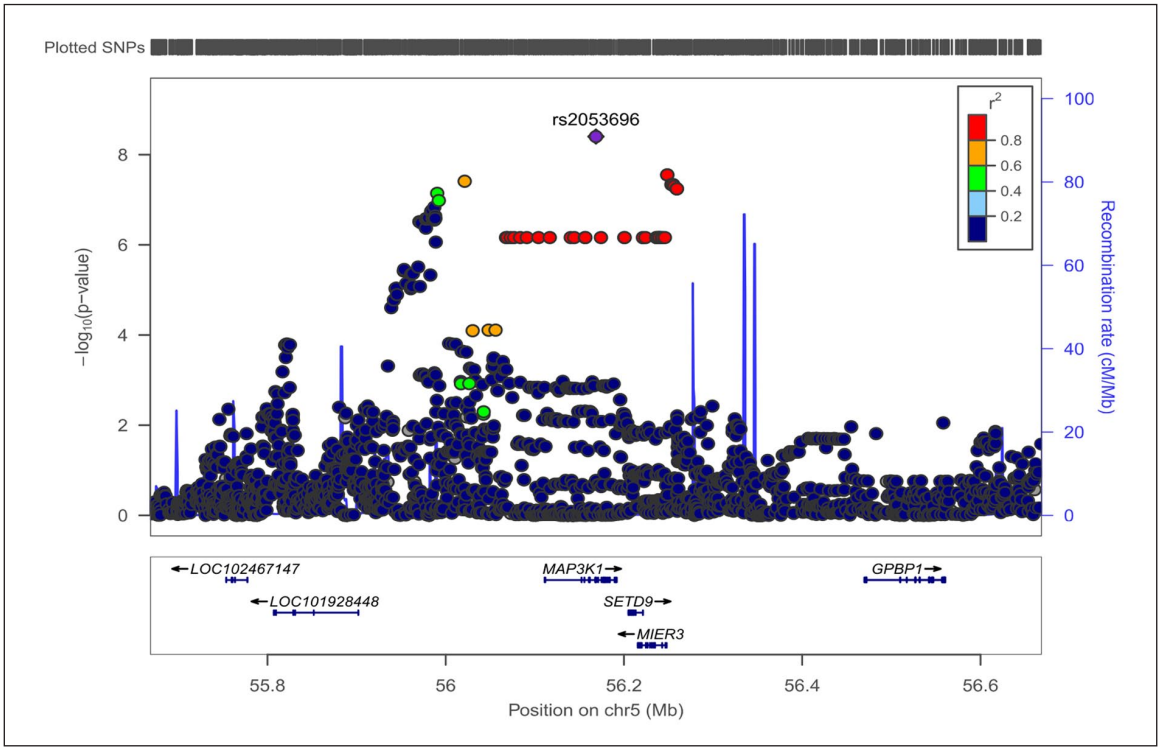

Figure 2. Regional plot of top associated locus from fixed-effects meta-analysis in GA cohorts.
The plot shows the genomic context of top associated signal (lead variant: rs2053696A, $p = 3.97*10^{-9}$, rate ratio = 3.87), located in the intronic region of MAP3K1 gene. The plot was generated with LocusZoom tool (http://locuszoom.sph.umich.edu), using hg19 genome assembly. The -log10(p) of associations from fixed-effect meta-analysis is reported on left y-axis and the recombination rate on right y-axis, over the genomic position. Each symbol represents one SNP, with the most associated SNP marked in purple and shading of the other points based on the linkage disequilibrium metrics with the top SNP are reported in the legend. Genes' positions are shown below the plot.

Table 4. Nominally associated variants from previous MS pharmacogenomic studies of interferon and glatiramer acetate.

SNP	Effect allele	Study PMID	DMT	Gene	*p*-value	OR	Replication *p*-value	Replication RR
rs16898029	T	26644206	Interferon	intergenic	6.00E-06	0.21	5.931E-03	0.78
rs2237562	C	26644207	Interferon	*GRM3*	2.21E-04	0.47	4.787E-02	0.89
rs1475919	A	26644207	Interferon	*GRIK2*	1.89E-04	2.37	2.892E-02	1.14
rs16886004	G	28569182	Glatiramer Acetate	*MAGI2*	3.3E-05	5.56	0.07171	1.23

OR: odds ratio from previous study on dichotomous endpoint; RR: rate ratio of number of relapses in this study.

Discussion

This multi-centric study aimed to uncover the genetic factors influencing response to IFN-β and GA, two foundational first-line DMTs for RRMS. Despite their proven efficacy in reducing relapse frequency, a considerable portion of patients show suboptimal responses, leading to treatment changes. This variability highlights the need to better understand DMT mechanisms and the molecular basis of suboptimal response.

GA's mechanism, though not fully clear, is believed to involve immunomodulation, leading to anti-inflammatory and neuroprotective effects.[12] GA may shift T-cell balance toward an anti-inflammatory phenotype, increasing anti-inflammatory like IL-10 and TGF-β, while decreasing pro-inflammatory IL-17.[13] Studies have linked specific Th2/Th1 cytokine patterns to GA response.[14] Our gene-set analysis supports this, although none of the top-ranked pathways achieved statistical significance after multiple testing

correction. The observed genetic enrichment in processes such as "Negative Regulation of Interleukin 17 production," "Response to metal ion," and "Response to zinc ion" suggests that pre-existing genetic variations in these pathways may be key determinants of therapeutic response to GA, correlating with its known mechanism of action and with significant pathological features of the disease.[15,16]

At the genome-wide level for GA, the most significant signal, rs2053696A, is an intronic variant of MAP3K1, a gene with known eQTL effects. MAP3K1 encodes a serine/threonine kinase and ubiquitin ligase crucial for MS-relevant pathways like mitogen-activated protein kinase (MAPK) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). Both MAPK and NF-κB pathways are vital for cellular signaling and inflammatory responses, and their phosphoproteomic signatures in MS patients are modulated by DMTs.[17] While the second top signal was not significant, the third genome-wide significant signal, rs2667609G, was found in WWOX. Notably, other variants in WWOX have been associated with MS and its expression in prior studies,[18] and WWOX was also linked to treatment response in our gene-based analysis. Still at the gene level, it should be noted that the third top associated gene, ATXN3, was also recently identified as associated with MS in a proteome-wide association study.[19]

For IFN-β, its therapeutic action is thought to involve reducing inflammation by modifying autoimmune cell balance and related cytokines, similar to GA. IFN-β binds type I interferon receptors (IFNAR1 and IFNAR2c), triggering phosphorylation via Jak1 and Tyk2, activating immunomodulatory and antiviral proteins. Although no genome-wide significant variants were identified, our data points to SNPs with eQTL effects on genes involved in inflammation and interferon-mediated signaling pathways.

Specifically, the top-ranked signal for IFN-β was rs34766672T, an intronic variant of *ZMIZ1*, a gene encoding a vitamin D-responsive transcription factor crucial for the TGF-beta inflammatory pathway. *ZMIZ1* is an MS risk factor and is downregulated in MS patients.[20,21] This variant also showed a distal promoter interaction with *POLR3A*, a gene known for neurological functions and its role in inflammasome regulatory networks and IFN-β response. The second top-ranked variant, rs144006567G, showed a strong eQTL effect with *POLR1E* (another POL1-related gene) and a distal promoter interaction with *PAX5*,[22] a critical regulator of B cells involved in adaptive immunity and autoimmune diseases, as well as JAK/STAT signaling.[23] B cells, a major target for MS immunotherapy, are reduced by IFN-β-induced FAS-mediated apoptosis.[24] The third top-ranked variant, rs150262324C, showed eQTL effects for *TRAPPC8* (linked to neurodevelopmental disorders) and *B4GALT6* (regulates astrocyte activation, a potential therapeutic target in neuroinflammatory disorders including MS[25]). The fourth top-ranked signal, rs10835054T, a 3′ UTR variant of *SLC5A12* (a sodium-coupled monocarboxylate transporter), acts as a mediator of selective control of T-cell motility.[26] Another Na+ transporter (SLC9A9) influencing T-cell differentiation to a pro-inflammatory fate was linked to interferon response in another pharmacogenomics study.[27]

Among the top associated GO terms in the gene-set analysis, two main themes emerged for IFN-β: sodium ion homeostasis and transmembrane electrochemical gradients maintenance. Several genes detected are related to ion transport across membranes, a theme also seen in previous IFN-β pharmacogenomics studies.[28] Sodium channels have long had a recognized role in the symptomatology of the disease, with suspected roles in causing permanent axonal destruction, and in modulating the intensity of immune activity.[29]

Regarding the replication of signals from previous studies, 4 out of 42 tested variants replicated. It is noteworthy that rs1475919A and the other three replicated variants stemmed from a study linking interferon response to extracellular ligand-gated ion channel regulation and immuno-regulatory processes (e.g. T-cell activation and specific cytokines).[11] Overall, our multi-centric study also observed a low level of replication. It is well known that MS pharmacogenomics has faced replication challenges due to a multiplicity of factors, ranging from limited power to lack of consensus on response definitions, and other sources of heterogeneity. First, many of the initial reports were from small candidate gene studies or small genome-wide screen (often with $N < 300$) that were thus severely underpowered. Small sample sizes increase the likelihood of type I errors (i.e. false positives), meaning that the initial findings may have been spurious and cannot be reliably reproduced in our larger cohort. Second, reproducibility might be impacted by lack of uniformity in response definition, with different studies using highly variable criteria to define "responder" and "non-responder." Furthermore, criteria often differ on the minimum required follow-up (e.g. 1 year, 2 years, or 4 years), which impacts the phenotype being captured. Subtle population stratification

effects in European populations may as well introduce variations in linkage disequilibrium that can impact the ability to successfully tag and replicate the true causal SNP. Finally, differences in genotyping platforms and protocols can be a major technical factor that can significantly impact the replication rate of pharmacogenomic findings. Older or less dense genotyping arrays used in the original studies covered different sets of SNPs. Differences in the accuracy of imputation that can depend on the specific array used and the reference panel and application of various QC thresholds can also impact the reproducibility of results. Overall, the synergic contribution of these factors might have hampered the replicability of results in our study.

Still, our study itself, despite the multi-centric design, carries the limitation of modest power for a genome-wide scan. This was partially mitigated by complementing single-variant analysis with gene- and gene-set-level screening. Furthermore, motivated by the relevance of regulatory information (demonstrated by GWAS signal enrichment in eQTL loci[30]), robust eQTL data in blood and CNS from large-scale resources were incorporated to enhance signal detection and refine SNP-gene assignments. Finally, we privileged the number of relapses to model treatment response using count regression, to maximize sample size across centers, offering higher power than classical dichotomization into responders and non-responders. On the other hand, we acknowledge that this choice came at a price of a decreased sensitivity of our endpoint in capturing all treatment effects of IFN-β and GA on disease activity. While relapses are in fact an indicator of acute inflammation, our outcome may indeed have been insensitive to critical subclinical disease activity (e.g. brain T2 lesions) or to disability worsening.

Furthermore, it should also be emphasized that differences in baseline clinico-demographic characteristics and disease activity prior to treatment initiation may have potential for confounding our pharmacogenomic associations, since these differences may modify the effects. However, at least for the two top associated variants for GA rs2053696 (GA) and rs34766672 (IFN-β), we did not observe a heterogeneity in effects, as measured by the I2 index.

In conclusion, even with limited success in replicating previously reported pharmacogenomic markers, our multi-centric study highlighted biologically meaningful genes and processes involved in treatment response for both GA and IFN-β. This offers potential avenues for future research aimed at personalized MS treatment. The study also underscores the inherent challenges of pharmacogenomic research in MS, including sample size limitations and phenotypic heterogeneity.

Acknowledgements

The authors are deeply grateful to the individuals and institutions that provided crucial support, both financial and technical, making this research possible. Particular thanks are extended to the MultipleMS consortium, funded by the European Union under the Horizon 2020 program.

Declaration of Conflicting Interests

The authors declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: A. Corona, F. Clarelli, K. Pääkkönen, A. Harroud, K. Shchetynsky, A. Zollo, A. Mingione, M. Sorosina, E. Mascia, A. Giordano, M. A. Rocca, F. Piehl, P. Stridh, B. Hemmer, A. Goris, B. Dubois, H. B. Søndergaard, F. Sellebjerg, S. D'alfonso, H. F. Harbo, T. Olsson, J. Saarela, A. Priori, M. Filippi, I. Kockum, F. Esposito, F. Martinelli Boneschi have no relevant or material financial interests to declare relating to the research described in this paper. A. Harroud has received consultancy fees from Biogen, Amgen, Novartis, and Pfizer; compensation for serving on a scientific advisory board for Amgen, and research funding from Amgen. A. Giordano received travel grant and consulting fees from Novartis. F. Piehl has received research grants from Denka, Janssen, Merck KGaA, Novartis, Pfizer and UCB, fees for serving on data monitoring committees in clinical trials with Lundbeck and Roche, and preparation of expert witness statement for Novartis. B. Hemmer served on advisory boards for Novartis, Polpharma und Hoffmann LaRoche, as well as DMSC boards for AllergyCare, Polpharma, Sandoz, Biocom, and TG Therapeutics. He received honoraries for counseling clients of the Gerson Lehrman Group and educational activities by neuro.today and patients.today. F. Sellebjerg has served on scientific advisory boards for, served as consultant for, received support for congress participation or received speaker honoraria from Biogen, Bristol Myers Squibb, Lundbeck, Merck, Novartis, Roche and Sanofi Genzyme. His laboratory has received research support from Biogen, Merck, Novartis, Roche and Sanofi Genzyme. M. Filippi received compensation for consulting services from Alexion, Almirall, Biogen, Merck, Novartis, Roche, Sanofi, Speaking activities from Bayer, Biogen, Celgene, Chiesi Italia SpA, Eli Lilly, Genzyme, Janssen, Merck-Serono, Neopharmed Gentili, Novartis, Novo Nordisk, Roche, Sanofi, Takeda, and TEVA; Participation in Advisory Boards for Alexion, Biogen, Bristol Myers Squibb, Merck, Novartis, Roche, Sanofi, Sanofi-Aventis, Sanofi Genzyme, Takeda; Scientific direction of educational

events for Biogen, Merck, Roche, Celgene, Bristol Myers Squibb, Lilly, Novartis, Sanofi Genzyme. He receives research support from Biogen Idec, Merck-Serono, Novartis, Roche. F. Esposito received consulting and speaking fees from Novartis, Sanofi Genzyme. F. Martinelli Boneschi in the last 5 years received honoraria for consulting and travel expenses from Roche and Novartis.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study has been funded by the European Union's Horizon 2020 Research and Innovation Program (grant MultipleMS, EURIA 733161). A. Harroud is supported by a Clinical Research Scholarship (349722) and a Clinician-Researcher Establishment Grant (358114) from the Fonds de Recherche du Québec Santé (FRQS), as well as a Future Leaders in Canadian Brain Research Grant from the Brain Canada Foundation. The views expressed herein do not necessarily represent the views of the Minister of Health or the Government of Canada. P. Stridh was supported by Horizon 2020 MultipleMS grant number 733161 and the Neuro Foundation. B. Hemmer received funding for research projects by Regeneron, Polpharma and Hoffmann LaRoche. He also received research funding from the EC as part of the Multiple MS and WISDOM Consortia, the Clinspect-M Consortium funded by the Bundesministerium für Bildung und Forschung and by the Deutsche Forschungsgemeinschaft as a member of the Munich Cluster for Systems Neurology (EXC 2145 SyNergy—ID 390857198). This study was supported by the Danish Multiple Sclerosis Society. F. Sellebjerg holds a professorship at the Faculty of Health Sciences and Medicine, University of Copenhagen sponsored by the Danish Multiple Sclerosis Society.

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Data availability statement

The datasets generated and analyzed during the current study are not directly shareable in a public repository due to their integration within multiple secure institutional clinical databases. Access to the summary statistics may be granted upon reasonable request to the corresponding author.

Supplemental material

Supplemental material for this article is available online.

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