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No Association between Single-Nucleotide Polymorphisms of The *S1PR1* Gene or Interleukin-17 Levels with Fingolimod Response in A Small Group of Iranian Relapsing-Remitting Multiple Sclerosis Patients: A Case-Control Study

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Abstract

Objective: Multiple sclerosis (MS) has a multi-factorial etiology involving genetic factors. Fingolimod (Gilenya ®, FTY720) modulates the G-protein-coupled sphingosine 1-phosphate (S1P) receptors, *S1PR1*, 2, 3, 4 and 5. Variation in the human *S1PR1* coding sequence results in heterogeneity in the function of the receptor. Interleukin-17, producing CD4$^+$ T cells, tends to be increased after treatment with Fingolimod. The aim of the study was to investigate single-nucleotide polymorphisms (SNPs) in the *S1PR1* gene or interleukin-17 (IL-17) levels in a small group of Iranian relapsing-remitting MS patients treated with Fingolimod.

Materials and Methods: In this case-control study, the genomic DNA of 94 MS patients treated with Fingolimod was extracted and Sanger sequencing was performed on polymerase chain reaction (PCR) products to detect variants in the *S1PR1* gene. Quantification of IL-17 from the serum of the patients was performed using a commercially available enzyme-linked immunosorbent assay (ELISA).

Results: Among 94 relapsing-remitting MS patients treated with Fingolimod, 69 (73.4%) were responders and 25 (26.6%) were non-responders. There were four novel and five common SNPs in the *S1PR1* gene and no significant association between SNP genotype and drug response was detected. In a subset of 34 patients, there was no significant difference in IL-17 serum concentrations before or after treatment and no association with *S1PR1* polymorphisms was determined.

Conclusion: This study is the first in Iran to investigate association between SNPs of the *S1PR1* gene or IL-17 levels with fingolimod response in a small group of Iranian relapsing remitting MS patients. There was no association with *S1PR1* gene SNPs or IL-17 levels before or after treatment.

Keywords: Fingolimod, Interleukin-17, Multiple Sclerosis, Polymorphism, Sphingosine 1-Phosphate Receptor

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Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating and neurodegenerative disease of the central nervous system (CNS) with a multi-factorial aetiology involving an interaction of environmental (1) and genetic factors (2).

Sphingosine-1-phosphate (S1P) is a pleiotropic bioactive lipid. It is a crucial regulator of many physiological processes. S1P is produced by sphingosine kinase 1 or 2 (sK1, sK2) initially in red blood cells, platelets and endothelial cells (3). S1P up-regulates neurotrophic gene expression, which protects hippocampal neurons against excitotoxic cell death (4). The function of S1P is mediated by five specific G-protein coupled receptors (GPCRs) named S1PR1 to S1PR5 (S1P receptors 1 to 5) that bind to S1P and dihydro-S1P with high affinity (5).

The *S1PR1* gene is on chromosome 1p21 and it is expressed in a wide variety of cells (6). It regulates the trafficking of T and B lymphocytes (7) and the other haematopoietic cells including natural killer (NK) T-cells, dendritic cells, macrophages, neutrophils, haematopoietic progenitors, mast cells and osteoclasts (8). It is also involved in vascular development and maintenance

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(9). S1PR subtypes have different G-protein coupling, and their associated downstream signalling pathways are implicated in cell proliferation, survival, migration, and neural cell communication (10). Inhibition of S1PR signalling prevents dendritic destabilization and denervation-induced dendrite loss (11).

Variation in the human *S1PR1* coding sequence has been demonstrated to result in heterogeneity in the function of the receptor and impact on the interaction of S1PR1 with Fingolimod. Non-synonymous single nucleotide polymorphism (SNP), Arg^{120} to Pro, failed to transmit S1P-induced intercellular signals, such as calcium increase and activation of P44/42 MAKPK and Akt. Another mutation (Arg^{13} to Gly) was demonstrated to be protective against coronary artery disease in high cardiovascular risk populations, while the patients carrying this mutation had a significantly lower percentage of multi-vessel coronary obstruction (12). Reactive astrocytes in MS lesions, when cultured under pro-inflammatory conditions, strongly express S1PR1 and 3 (13).

Nagahashi et al. (14) found a mechanism involving the SphK1/S1P/S1PR1 axis Associated with obesity, inflammation, breast cancer progression and metastasis. Overexpression of S1PR1, S1PR2 and S1PR3 in bladder tumour, suggested that the S1PR profile in tumour biopsies was a promising marker in the diagnosis of bladder carcinoma (15).

Fingolimod is an orally administered drug applied for relapsing-remitting MS (RRMS). Fingolimod is a successful therapy for RRMS which decrease relapses, disability progression, new brain lesions and loss of brain volume (16).

Fingolimod has been reported to modulate the B cell cytokine profile, increasing the ratio of transforming growth factor-beta (TGF-β) and IL-4 to pro-inflammatory cytokines (17). The increased ratio of regulatory B cell subsets may be involved in influencing the cytokine environment and disease progression. Inhibition of S1PR signalling may prevent dendritic destabilization and denervation-induced dendrite loss (11). It also protects from neuro-inflammation by blocking the effect of S1PR1 expression in astrocytes which helps the blood brain barrier (18). Kim et al. (19) demonstrated that prolonged pFTY720 binding to S1PR1 triggered receptor internalization and degradation *in vivo*. Moreover, FTY720 had protective effects on oligodendrocytes.

Among 381 MS patients who were classified according to the no evidence of disease activity-3 (NEDA-3) criterion at 2 years, Ferrè et al. (20) found a genetic model containing 123 SNPs which could predict Fingolimod response with an area under the ROC curve (AUROC=0.65 in the independent test set. By adding clinical data, the model accuracy was improved to AUROC=0.71, meaning that combining clinical and genetic data, by applying machine learning methods, was beneficial to predict response to Fingolimod.

Interleukin-17 (IL-17) belongs to a family of cytokines, including IL-17A, IL-17B, IL-17C, IL-17D, IL-17E and IL-17F. Studies in MS patients demonstrated that IL-17 levels were particularly increased during relapse time, both in the blood and the CNS (21). IL-17 was involved in ectopic lymphoid follicle formation in the CNS (22).

In a model with phosphorylation-deficient receptors of the *S1PR1* gene, mice developed severe experimental autoimmune encephalomyelitis, as a result of the production of autoreactive T helper 17 cells (Th17) in peripheral blood and nervous system with the ability to produce IL-17 (23), while this mechanism may be pathogenic in MS. In an Iranian population, mRNA levels of *IL-17* have been reported to be significantly increased in MS patients. mRNA levels of *S1PR1* and *IL-10* were also significantly lower compared to the healthy controls (HCs) (24). Li et al. (25) found that MSCs-Exos reversed the increased Th17/Treg in Aplastic Anaemia $CD4^+$ T cells, as previously reported. A recent study showed that expression of SMAD7 and S1PR1 in $CD4^+$ T cells in peripheral blood were biomarkers of MS and Crohn's disease (26).

In this study, we investigated the polymorphism of the *S1PR1* gene. Moreover, we studied the association of *S1PR1* gene polymorphisms and levels of IL-17 before and after treatment with Fingolimod, in a small group of Iranian relapsing-remitting MS patients.

Materials and Methods

Subjects

In this case-control study, a total of 94 MS patients (69 female, 76.7%, and 21 male, 23.3%), treated with Fingolimod (0.5 mg orally once daily), were recruited from the Research Institute of Neurological Diseases, Imam Khomeini Hospital, Tehran University of Medical Science (Tehran, Iran). All of the MS patients were diagnosed according to McDonald criteria (27). The responder patients in this study referred to evidence of delayed disability progression, decreased deterioration from baseline in the Extended Disability Status Scale score and/or reduced MRI markers of disease progression (16). The Ethical approval for this study was granted from the Institute of Research Review Board at Tehran University of Medical Sciences, Tehran, Iran (91-02-24-1823-66824).

DNA extraction and *S1PR1* polymerase chain reaction

The genomic DNA of patients was extracted from peripheral blood cells by QIAamp DNA Blood Mini Kit (Qiagen, USA) according to the manufacturer's instructions.

The primers listed in Table 1 were designed to amplify *S1PR1* introns, exons and flanking sequence in the 5′ and 3′ UTR regions of the gene, in addition to overlapping along the sequence of the *S1PR1* gene containing untranslated region, intronic, and exonic regions (Fig.1). *S1PR1* gene (NM-001400) was genotyped in MS patients (Ensemble genome browser: Transcript: *S1PR1*-001 ENST00000305352, Chromosome 1: 101702444-101707074 forward strand). This gene has two exons, one coding exon, and one intron; the length of exon one is 163 bps, intron 1153 bps, and exon two is 1377 bps. The transcript length is 2909 bps and the translation length is 382 residues).

Polymerase chain reaction (PCR) was performed as follows: 200 ng of genomic DNA were added to the final volume of 10 µl mastermix with final concentrations as follows: 0.25 µM of each dNTP, *Taq* DNA polymerase 0.05 U/µl, PCR buffer 10x; concentration of each primer was 1 µM for *S1PR1-1, -2, -3, -4, -5, -6* and *-7*, but in *S1PR1-5* primers, concentration for each primer was 0.8 µM.

Thermocycling conditions for the primer pairs *S1PR1-1* were as follows: initial denaturation for 5 minutes at 95°C, followed by two cycles at 95°C for 30 seconds, annealing at 69°C for 30 seconds, extension at 72°C for 45 seconds, followed by two cycles at 95°C for 30 seconds, annealing at 67°C for 30 seconds, extension at 72°C for 45 seconds, followed by 45 cycles at 95°C for 30 seconds, annealing at 65°C for 30 seconds, extension at 72°C for 45 seconds and a final extension at 72°C for 5 minutes. Variations in the touchdown PCR conditions were as shown in Table S1 (See Supplementary Online Information at www.celljournal.org) for the other primer pairs.

The amplified products were analysed by electrophoresis using a 1.8% (w/v) agarose gel containing (5 µg/µl) SafeView dye (NBS Biological, UK) and visualized under ultraviolet light (G:Box Gel Image Analysis Systems, UK).

Table 1: The primer sequences used for *S1PR1* genotyping

Primer name	Primer sequence (5´-3´)	Amplicon size (bp)
S1PR1	F1: TGGCTCTTTCCCTGACTCT	577
	R1: AAACATACTCCCTTCCCGCA	
	F2: CCAAGAAATTCCACCGACCC	480
	R2: GCTCCGAGTCCTGACCAAG	
	F3: CTGTCCAGCTGCTCCACC	497
	R3: CTGCGGCTGAATTCCATGC	
	F4: GGCAAATTCAAGCGACCCAT	466
	R4: TTAGTCTTTGAGGAGGGGCC	
	F5: GGTCTAGCATTGTCAAGCTCC	470
	R5: GAGAGGAAGGATCCTGGCTA	
	F6: GCTGAGGCCAAAGTTTCCAT	498
	R6: GCTTTTAAGGGCACAAGAGGG	
	F7: TCATTTCAAGCAACAACATGGT	493
	R7: CCAAAGAGCTACACAATCCAGT	

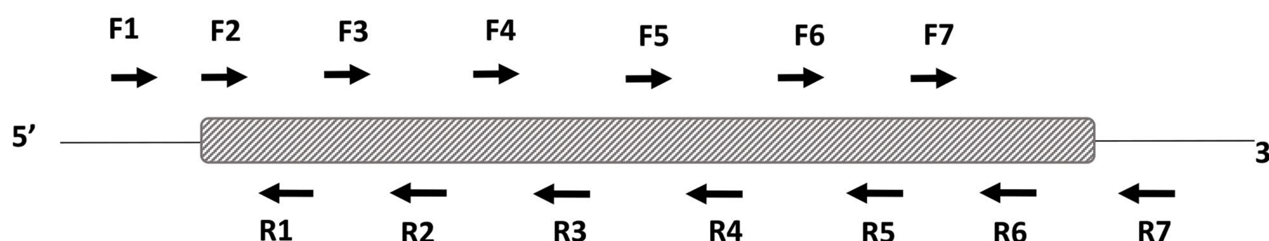

Fig.1: Overlapping primers along the sequence of *S1PR1* gene containing untranslated region, intronic, and exonic regions.

Direct Sanger sequencing of polymerase chain reaction products

Briefly, PCR products were purified using ExoSAP, containing Exonuclease I, (New England BioLabs, USA) and Shrimp Alkaline Phosphatase (USB Products Affymetrix, USA). Two µl of ExoSAP stock (containing 7.5 units Exonuclease I and 12.5 units Shrimp Alkaline Phosphatase) was added to 5 µl of PCR product. The reaction was incubated at 37ºC for 40 minutes and then at 95°C for 5 minutes to deactivate the enzymes. 1 ng/µl (per 100 bp) of the PCR product from the clean-up step and 3.2 pmol/µl of sequencing primer (forward or reverse) were sent to Source Bioscience for Sanger sequencing (Cambridge, UK).

IL-17 detection in patient sera

Detection of IL-17 in patient sera was performed using the IL-17 Cytoscreen kit (Invitrogen, USA), according to the manufacturer's instructions. Absorbance was measured at 450 nm, 405 nm, and 630 nm using an ELISA reader (Reader type ELx808; Bio Tek, USA) for both standard curve and experimental samples. IL-17 concentration in each experimental sample was calculated using the standard curve.

Statistical analysis

Statistical Package for the Social Sciences (SPSS, version 22, IBM Corp., USA) was used for statistical analysis. The Chi-Squared test and Fisher's Exact test were used for qualitative data and the paired t test for quantitative data. Allele frequencies of *S1PR1* were tested for Hardy-Weinberg equilibrium (HWE) using the chi-squared test. The Hardy-Weinberg testing was carried out using an online calculator (Michael C., 2008). $P<0.05$ were considered statistically significant. Odds ratios (OR) were calculated and given with 95% confidence intervals (95% CI).

Results

Age, sex, and disease duration

Among the 94 relapsing-remitting MS patients treated with Fingolimod, 69 (73.4%) were responders and 25 (26.6%) were non-responders (Table S2, See Supplementary Online Information at www.celljournal.org).

There was no significant association between the gender of MS patients treated with Fingolimod and their response (whether they were responder or non-responder; data not shown).

S1PR1 genotyping data

Sanger sequencing

Among the 94 MS patients, three common variants (frequency ≥ 5%), two uncommon variants (frequency >1% and <5%) and four novel SNPs were detected by Sanger sequencing. These results are shown in Table 2. The agarose electrophoresis images are shown in Figure 2. The NOVEL1 and NOVEL2 SNPs were found in responders and NOVEL3 and NOVEL4 in non-responders. All novel variants were located in the 3′ UTR of the *S1PR1* gene. Sequencing electrophorograms of rs114115083 and novel variant 1, in MS patients are shown in Figures 3 and 4.

Table 2: Chromosome position, SNP name, alleles, type of variant and minor allele frequency of the five known and four novel *S1PR1* SNPs ordered by location in the gene

Position in chromosome	SNP	Alleles	Type of variant	Minor allele frequency*
101238940	rs3737578	T/C	5-prime UTR	11%
101238976	rs3737577	G/T	5-prime UTR	15%
101239016	rs61734752	C/A	Missense	2%
101240681	NOVEL3	G/T	3-prime UTR	-
101240690	rs114115083	T/C	3-prime UTR	1%
101240850	rs55872049	C/A	3-prime UTR	5%
101241054	NOVEL4	G/T	3-prime UTR	-
101241183	NOVEL1	A/G	3-prime UTR	-
101241436	NOVEL2	T/A	3-prime UTR	-

SNP; Single-nucleotide polymorphisms, UTR; Untranslated region, and *; Frequency of the second most frequent allele in 1000 Genome project phase 3 combined population.

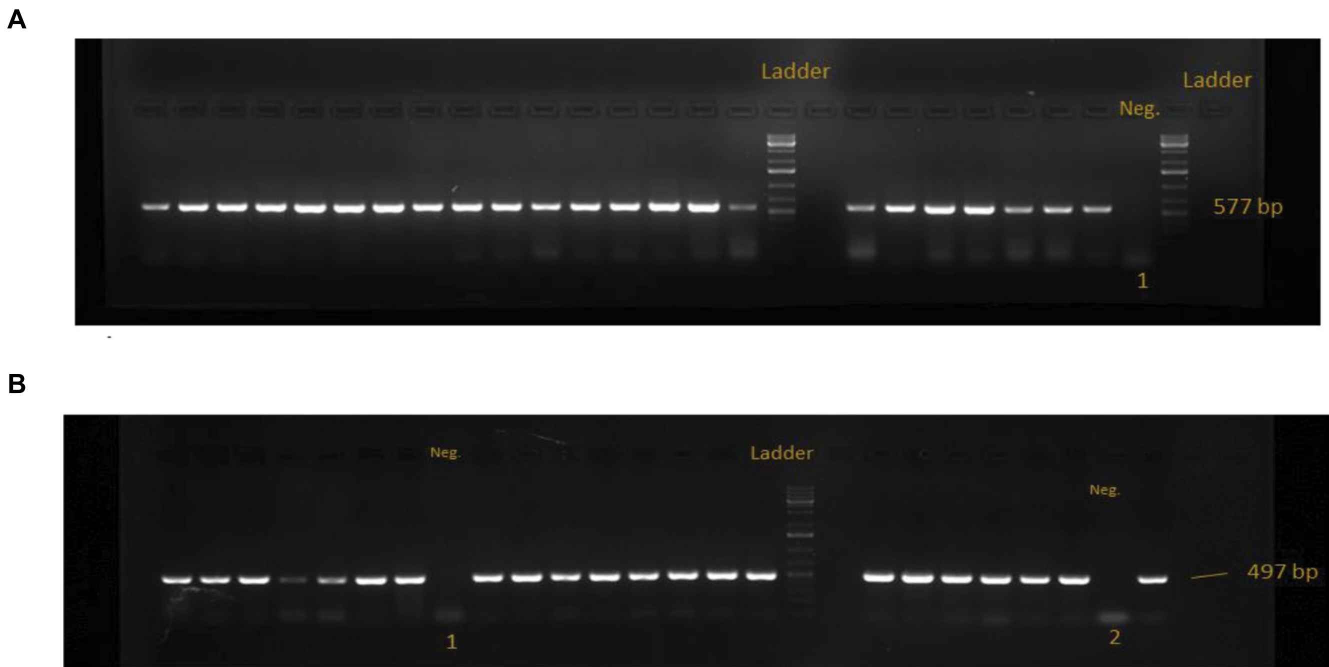

Fig.2: Agarose gel electrophoresis image of *S1PR1* gene amplicons. Amplicon sizes are as following: **A.** Primer 1: 577 bp and **B.** Primer 3: 497 bp. Neg.; Negative control marked as lane 1 or 2.

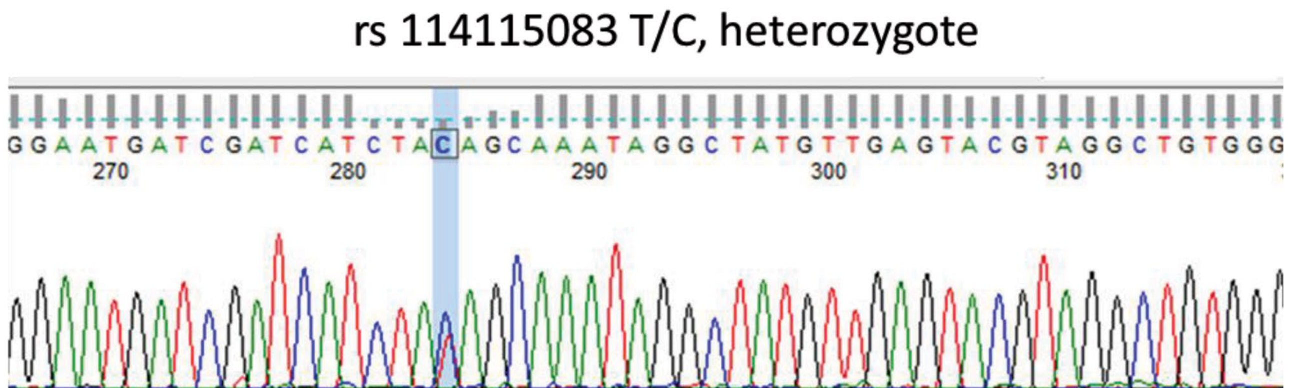

Fig.3: Sequencing electropherograms of rs114115083 in multiple sclerosis (MS) patients. The nucleotide "T" was substituted by "C" in this variant.

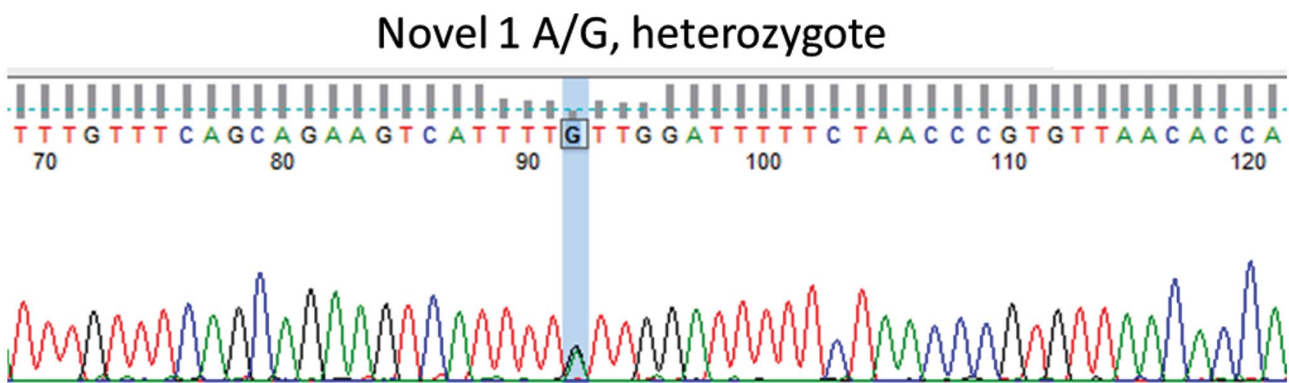

Fig.4: Sequencing chromatogram of novel variant 1 in multiple sclerosis (MS) patients. The nucleotide "A" was substituted by "G" in this variant.

Statistical analysis of *S1PR1* genotyping data

Quality control of rs3737577 and rs3737578 variant genotyping

HWE calculation gave P values of 0.126 and 0.851 for rs3737577 and rs3737578 respectively. This calculation was not possible for the rarer variants.

Statistical analysis of known and novel variants

The results of testing for the association of variants with Fingolimod response are shown in Table S3 (See Supplementary Online Information at www.celljournal.org). No significant association was observed for any variants (P>0.05 in all tests).

Concentration of serum IL-17 in multiple sclerosis patients treated with Fingolimod

Sera, collected from the patients, were obtained before and after treatment with Fingolimod. The mean and median of IL-17 levels in the serum of both responder and non-responder groups before and after treatment are shown in Table S4 (See Supplementary Online Information at www.celljournal.org).

There was no statistically significant difference (P<0.05) in IL-17 concentration between the patients before and after treatment with Fingolimod using a paired t test.

Discussion

The frequency of response to Fingolimod treatment in the MS patients was about three times more than non-responders (frequency of response was 73.4 vs. 26.6% for non-response). Although the frequency of females in the non-responder group (80%) was slightly more than in the responder group (75.4%), there was no significant association between gender and response to Fingolimod (P: 0.643, odds ratio: 1.306, 95% CI: 0.422-4.047).

Sanger sequencing identified four novel SNPs and five common SNPs. Two novel SNPs were found in the responder group and two in the non-responder group. Detected SNPs were in the 5′ UTR, and the 3′ UTR. Additionally, one SNP (rs61734752) was a coding region missense variant (C/A) while it was considered to be a benign substitution (28).

The common SNPs are not significantly associated with response to Fingolimod. However, there is likely to be insufficient statistical power to test for association in this study, so this cannot be ruled out for any of them. While the study is fundamentally underpowered, any smaller effects due to confounding would be undetectable, as they would be non-significant.

Moreno-Torres et al. (29) found that MS patients treated with Fingolimod displayed lymphopaenia together with a reduction in naïve T cell (TN), central memory T cells (TCM), memory B and natural killer (NK) bright cells and relative growth in effector memory T cell (TEM), terminally differentiated effector T cell (TEMRA), native B, transitional B, plasma blast, NK, and NK T cells (NKT) in the peripheral blood. They did not reveal difference in the expression levels of the genes coding CCR7, CD45RA, CD31, and PTK7 surface markers (CCR7, PTPRC, PECAM1 and PTK7) between the responder and non-responder patients before treatment.

Fingolimod applies a potent anti-inflammatory effect by reducing the proportion of cytokine producer T and B cells. This effect happened at both cellular and molecular levels, as Fingolimod improved a downregulation of genes, such as CD40L, CD 40, IRF4, CR2, IL-23A, CD2, IL-17A, and IL-17D. Likewise, the cytokine activity pathways were downregulated after treatment. In MS patients, a high proportion of NK bright cells has recently been associated with stable MRI. A higher percentage of these cells before treatment was associated with a good response, while they were reduced to a lesser degree after treatment in the responder compared to the non-responder patients. Fingolimod raised the proportion of NK cells. It also upregulated NCAM1 (CD56) and FCGR3A (CD16a) at the transcriptional level (29).

Fingolimod downregulated genes were involved in oxidative stress and suppression of stress-fiber formation, by the rhoA-ROCK1-NFKB pathway, showing that the anti-oxidant effect of Fingolimod was not related on innate immune activation (29). In the MS patients, lower serum levels of reactive oxygen species (ROS) were noticed in the patients treated with Fingolimod compared to the first-line-treated patient, and a relation between ROS levels and disease duration was shown (30). The decline in lymphocyte activation in response to Fingolimod may come up with a decrease in FOXP3 expression levels. Moreover, growth in the expression of IL-10, IL-10RA, IL-10RB, IL-15, and TNFRSF1A displayed a strong inductive effect of regulatory mechanisms in response to Fingolimod.

Upregulation of the apoptotic pathway implied that Fingolimod induced programmed cell death and it may contribute to lymphopenia. Overexpression of ADA2, SGK1, and BCL2L13 after six months of treatment in only responder patients, may suggest a differential effect of Fingolimod on the mechanisms of cell proliferation and differentiation (29). Tumour necrosis factor receptor super family member 13B (TNFSF13) and its ligand B cell activating factor have also been shown to contribute in the potential role of proliferation and differentiation of B cells in MS (31). Moreno-Torres et al. (29) found an important downregulation of IRF4 (a highly expressed molecule in activated B cells), CD27 (a memory B cell marker) and CXCR5, and a decline in the proportion of memory B cells after treatment. Moreover, a higher proportion of plasma blasts and overexpression of CXCL13 before treatment in the patients with NEDA response, showed that manipulation of B cell activation played an important role in the clinical response to Fingolimod. Immunoglobulin receptor *FCRL1* and *FCRL2* genes in the responder patients, despite the growth in the production of immunoglobulins, had a lower

activity of their receptors which is related to radiological activity in MS (32). This is in line with the fact that Fingolimod made a better effect in patients with a more inflammatory component. Moreover, Moreno-Torres et al. (29) determined a reduction in IL-2 producing cells and a downregulation of the IL-2RA gene in PBMCs of treated MS patients. Moreover, a decrease in IL-2 producing cells between NEDA and evidence of disease activity (EDA) patients was found, but no difference was noticed at the transcription level.

The study of Malhotra et al. (33) suggested that Fingolimod treatment may have benefit effects via inhibiting NLRP3 inflammasome activation in peripheral blood mononuclear cells in MS patients.

Hoffmann et al. (18) found that FTY-p induced neuroprotective factors, including leukaemia inhibitory factor (LIF), IL-11, and heparin-binding EGF-like growth factor (HBEGF). It can also suppress tumor necrosis factor (TNF) induced inflammatory cytokines BAFF and CXCL10. Moreover antiviral proteins like 2´-5´-oligoadenylate synthetase 2 (OAS2) and myxovirus resistance 1 (MX1).

Proschmann et al. (34) found people with MS (PwMS) treated with Fingolimod and HCs respond to acute exercise; they had a comparable temporal pattern of increased immune cell counts, immediately after exercise and a return to baseline levels within 1 hour.

Long intergenic non-coding RNA (Linc) 00513 has been recently reported as a novel regulator of the type 1 signalling pathway. Polymorphisms in the promoter region of Linc00513 (“G” for rs205764 and “A” for rs547311) have been associated with an overexpression of Linc00513 and growth in the downstream signalling activity of the type 1 IFN pathway (35).

In the Amin et al. (36) study, the MS patients carrying polymorphisms at rs547311 displayed a significantly higher disability score compared to the patients who carried the major allele. No significant difference was noticed in the other models of inheritance, suggesting that a single or double "A" allele(s) are equally harmful for a patient’s EDSS. Moreover, polymorphism at rs205764 showed to have no association with the EDSS. These findings are compatible with patients disability and the study which detected rs205764 and rs547311 as novel regulators of IFN signalling. Several other variants have been associated with differences in EDSS for MS patients, including rs17445836 in the interferon regulatory factor-8 gene, rs3087456 and rs4774 in class-II trans-activator gene, rs1049269 in transferrin gene, and rs1494555 in interleukin-7 receptor gene.

The 3´ UTR is the regulatory noncoding part of an mRNA containing a potential role in gene expression (37). The 3´ UTRs carry a special class of noncoding variants which may influence post-transcriptional and translational processes. Causal peripheral blood cis-expression Quantitative Trait Local (eQTL) presence is 4-fold enhanced in 3´ UTRs, a level comparing with that of promoter elements. Among the all tissues on the genotype-tissue expression project (GTEx), eQTLs in 3´ UTRs were shown to be 2-fold enhanced, which was the largest enhancement across all non-coding regions. Untranslated regions harbour the greatest enhancement of GWAS heritability (5-fold) of all non-coding classes, whereas transcription start sites, confirm an important role for post-transcriptional activities in human regulatory variation. Griesemer et al. (38) developed the Massively Parallel Reporter Assay for 3´ UTRs (MPRAu) to evaluate allelic expression differences for thousands of 3´ UTR variants simultaneously, in a high-throughput, accurate, and reproducible method. MPRAu tool functionally identifies 3´ UTR variants. It is applied to characterize 2368 3´ UTR variants that modulate transcript abundance among six cell lines. They developed MPRAu which is a strong predictive model of 3´ UTR function and characterized a novel way of 3´ UTRs regulation. They anticipate this model will be a common experimental paradigm to validate variants of unknown significance and rare variants in future. They found additional evidence applying 3´ UTR tiling and endogenous allelic replacement for three variants (rs1059273, rs705866, and rs34448361) with very important results to realize human disease and evolution.

The results in our study suggested that response to treatment with Fingolimod was not associated with *S1PR1* gene polymorphism in our study population. Further investigations with a greater sample size are required to thoroughly determine the effect of polymorphism in *S1PR1* on MS.

Serum levels of IL-17A and IL-17F in Iranian MS patients by comparing with HCs have a significant rise, moreover, a significant positive correlation of IL-17F serum levels with the number of relapses has been reported (39). However, our study did not find any significant differences in total IL-17 levels before and after treatment.

In addition, a significant decrease in IL-10, IL-27 and TGF-beta, has been reported but with no significant difference in IL-17 and IL-23 levels between the MS patients and healthy controls. This is in line with the results of our study in IL-17 serum levels. Higher IL-17, IL-19, and IL-4 producing CD^+ T cell percentages were reported in pre-treatment MS patients compared to the healthy controls, while the IL-17 producing CD^+ T cell percentages contribute to show a transient rise at 2 weeks of Fingolimod therapy (40). This suggested that the detection of variation in IL-17 levels may depend on the time serum sample collection from the patient after treatment.

Additional studies with larger sample sizes and multiple sampling time points should be the next stage to definitely confirm whether Fingolimod treatment affects the level of IL-17 in the control and MS patient sera.

Conclusion

This study is the first in Iran to investigate association between SNPs of the *S1PR1* gene or IL-17 levels with fingolimod response in a small group of Iranian relapsing remitting MS patients. The response to treatment was not associated with S1PR1 gene SNPs or IL-17 levels before or after treatment.

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Authors' Contributions

N.M.; Investigation, Writing the original draft, Review, Editing, and Visualization. P.S.; Validation, Editing, and Review. A.J.W.; Supervision, Review, Editing, and Validation. All authors read and approved the final manuscript.

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