

Supplementary Information for

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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Table S1: Touchdown PCR conditions showing the temperature and the number of cycles at that particular temperature used for the *S1PR1* primers

Steps of PCR	Temperature (°C)	Time	Number of cycles
Initialization	95	5 minutes	1
Denaturation	95	30 seconds	2
Annealing	X+4	30 seconds	
Extension	72	Z	
Denaturation	95	30 seconds	2
Annealing	X+2	30 seconds	
Extension	72	Z	
Denaturation	95	30 seconds	Y
Annealing	X	30 seconds	
Extension	72	Z	
Final hold	72	5 minutes	1

X; 65°C for primers *S1PR1-1,2,3,5*, 63°C for primer *S1PR1-4*, 68°C for primer *S1PR1-6*, 59°C for primer *S1PR1-7*, Y; 45 for primer *S1PR-1,4,7*, 35 for primers *S1PR1-2,3,5,6*, Z; 45 seconds for primers *S1PR1-1,4,7*, 30 seconds for primers *S1PR1-2,3,5*, 25 seconds for primers *S1PR1-6*, and PCR; Polymerase chain reaction.

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Table S2: Frequency of demographic and clinical data of MS patients treated with fingolimod

Demographic and clinical data	Number (%) or mean \pm SD
Gender	
Female	69 (76.7)
Male	21 (23.3)
Age (Y)	
At presentation (Y)	30.9 \pm 5.9
At onset of disease (Y)	23.6 \pm 6.0
Disease characteristics	
Disease duration (Y)	7.3 \pm 3.9
EDSS value	2.54 \pm 1.14
Fingolimod response	
Responder	69 (73.4)
Non-responder	25 (26.6)
Relapses during treatment	
One relapse	20 (23.8)
Two relapses	5 (6.0)
No relapse	58 (69.0)
Serious adverse effect	1(1.2)

MS; Multiple sclerosis and EDSS; Expanded disability status scale.

Table S3: Statistical analysis of association of SNPs with responding to fingolimod in MS patients

Variant	Non-responder genotype numbers (n=25)			Responder genotype numbers (n=69)			P value	Odds ratio (95% CI)
rs3737577	15 (G/G)	10 (G/T+T/T)	25 (total)	45 (G/G)	24 (G/T+T/T)	69 (total)	0.64	0.80 (0.31-2.05)
rs3737578	17 (T/T)	8 (T/C+C/C)	25 (total)	50 (T/T)	19 (T/C+C/C)	69 (total)	0.67	0.81 (0.30-2.18)
rs114115083	0 (T/C)	25 (T/T)	25 (total)	1 (T/C)	68 (T/T)	69 (total)	1.00*	1.02 (0.99-1.04)
rs55872049	1 (C/A)	24 (C/C)	25 (total)	0 (C/A)	69 (C/C)	69 (total)	0.27*	0.96 (0.89-1.04)
rs61734752	1 (C/A)	24 (C/C)	25 (total)	0 (C/A)	69 (C/C)	69 (total)	0.27*	0.96 (0.89-1.04)
NOVEL1	25 (A/A)	0 (A/G)	25 (total)	68 (A/A)	1 (A/G)	69 (total)	1.00*	1.02 (0.99-1.04)
NOVEL2	0 (T/A)	25 (T/T)	25 (total)	1 (T/A)	68 (T/T)	69 (total)	1.00*	1.02 (0.99-1.04)
NOVEL3	24 (G/G)	1 (G/T)	25 (total)	69 (G/G)	0 (G/T)	69 (total)	0.27*	0.96 (0.89-1.04)
NOVEL4	24 (G/G)	1 (G/T)	25 (total)	69 (G/G)	0 (G/T)	69 (total)	0.27*	0.96 (0.89-1.04)

MAF for rs3737577 was 0.22 and for rs3737578 was 0.154. For P value, test is either Chi-squared test or Fisher's Exact Test indicated with *. SNP; Single-nucleotide polymorphism and MS; Multiple sclerosis.

Table S4: The mean and median of IL-17 levels in serum of responder and non-responder group of RRMS patients before and after treatment with fingolimod

Group	Before treatment (pg/ml)	After treatment (pg/ml)
Responder (n=23)		
Mean	147.6 ± SE: 33.6	103 ± SE: 10.4
Median	86.5	96
	Minimum	52.24
	Maximum	763.19
Non-responder (n=11)		
Mean	116.6 ± SE: 21.2	113.4 ± SE: 13.7
Median	105	91.7
	Minimum	45.57
	Maximum	286.05