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ORIGINAL ARTICLE

Association of SHMT1, MAZ, ERG, and L3MBTL3 Gene Polymorphisms with Susceptibility to Multiple Sclerosis

Seyede Zahra Nazari Mehrabani, et al. ***[full author details at the end of the article]***

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Abstract

Multiple sclerosis (MS) is the most common inflammatory and chronic disease of the central nervous system (CNS). A complex interaction between genetic, environmental, and epigenetic factors is involved in the pathogenesis of MS. With the advancement of GWAS, various variants associated with MS have been identified. This study aimed to evaluate the association of single-nucleotide polymorphisms (SNPs) rs4925166 and rs1979277 in the *SHMT1*, *MAZ* rs34286592, *ERG* rs2836425, and *L3MBTL3* rs4364506 with MS. In this case–control study, the association of five SNPs in *SHMT1*, *MAZ*, *ERG*, and *L3MBTL3* genes with relapsing–remitting MS (RR-MS) was investigated in 190 patients and 200 healthy individuals. Four SNPs including *SHMT1* rs4925166, *SHMT1* rs1979277, *MAZ* rs34286592, and *L3MBTL3* rs4364506 were genotyped using PCR–RFLP and genotyping of ERG rs2836425 was performed by tetra-primer ARMS PCR. Our findings showed a significant difference in the allelic frequencies for the four SNPs of *SHMT1* rs4925166, *SHMT1* rs1979277, *MAZ* rs34286592, and *ERG* rs2836425, while there were no differences in the allele and genotype frequencies for *L3MBTL3* rs4364506. These significant associations were observed for the following genotypes: TT and GG genotypes of *SHMT1* rs4925166 (OR 0.47 and 1.90, respectively) genotype GG of *SHMT1* rs1979277 (OR 0.63), genotype GG of *MAZ* rs34286592 (OR 0.61), TC and CC genotypes of *ERG* rs2836425 (OR 1.89 and 0.50, respectively). Our study highlighted that people who are carrying genotypes including GG (SHMT1 rs4925166) and TC (ERG rs2836425) have the highest susceptibility chance for MS, respectively. However, genotypes TT (*SHMT1* rs4925166), CC (*ERG* rs2836425), GG (*MAZ* rs34286592), and GG (*SHMT1* rs1979277) had the highest negative association (protective effect) with MS, respectively. *L3MBTL3* rs4364506 was found neither as a predisposing nor a protective variant.

Seyede Zahra Nazari Mehrabani and Mohammad Hossein Shushizadeh contributed to the work equally.

Keywords Multiple sclerosis · Association study · Single-nucleotide polymorphisms · *SHMT1* · *MAZ* · *ERG* · *L3MBTL3*

Introduction

Multiple Sclerosis (MS) (Online Mendelian Inheritance in Man (OMIM) 126,200) is a neurological disease characterized by inflammation and CNS demyelination (Abdollahzadeh et al. 2018; Hollenbach and Oksenberg 2015). MS etiology is unclear; however, complex interactions between genetic and environmental factors are thought to be involved in the pathogenesis of the disease (Abdollahzadeh et al. 2016; Dyment et al. 2004). Epidemiological studies have shown that genetic factors are responsible for the increased incidence observed in relatives of affected individuals (Dyment et al. 2006; Hemminki et al. 2009) and the most known single risk is *HLA-DRB1* gene, in particular, the DRB1* 15: 01 allele (Sawcer et al. 2011; Schmidt et al. 2007). Technological development has evolved in recent decades in the field of genetic research. Genome-wide association studies (GWAS) and the emergence of next-generation sequencing (NGS) have led to significant advances in our understanding of molecular and genetics principles of disease development (Gibson 2010; Meldrum et al. 2011).

Although GWAS studies are a common and robust approach to MS genetic study, identified associated alleles have only described a proportion of MS heritability (Bashinskaya et al. 2015; Sawcer et al. 2014). This "missing heritability" indicates that the underlying causes of MS cannotonly be genetic, and recentstudies have highlighted the very significant role of epigenetic which bridges the environment andgenetics (Huynh and Casaccia 2013; Huynh et al. 2014; Küçükali et al. 2015). One of the efforts in the field of MS-epigenetic studies is identification of SNPs which have an influence on the activity of genes that directly or indirectly contribute to epigenetic changes. Andlauer Group has conducted the most recent research in this area in 2016 which led to the identification of new SNPs located in loci that are involved in epigenetic regulations (Andlauer et al. 2016).

SNP rs4925166, which had the greatest association with MS in the recent report by Andlauer Group, was the first SNP and its association with MS was aimed to be studied in the current investigation. SNP rs4925166 is located within an intron of the *TOP3A* gene but it acts as an eQTL for the adjacent *SHMT1* gene and the genotype of this SNP can strongly affect the expression of *SHMT1* (Andlauer et al. 2016). *SHMT1* (Serine Hydroxymethyltransferase 1) or *CSHMT* is located at 17p11.2 with 14 exons (Schirch and Peterson 1980). Given the involvement of serine hydroxymethyltransferase in providing one-carbon units for the synthesis of thymidylate, methionine, and purines in the cytoplasm, it plays an essential role in DNA methylation, DNA synthesis, and repair (Girgis et al. 1997; Wang et al. 2006).

The second target SNP in the present work is located in the *SHMT1* gene which is known as SNP rs1979277. It is thought to affect protein by changing the leucine amino acid at position 474 to phenylalanine (Leu474Phe) (Heil et al. 2001). Several studies have shown that this polymorphism does not affect the activity of *SHMT1*,

but it interferes with nuclear transduction and subsequently makes defects in thymidylate synthesis (Anderson and Stover 2009; Woeller et al. 2007).

The third SNP selected for the current research is SNP rs34286592 located in the intron of the *MAZ* (MYC-related zinc finger protein) gene (Andlauer et al. 2016). The MAZ transcription factor is an inflammation-responsive protein and is believed to be involved in the pathogenesis of MS by regulation of a wide range of genes including amyloid A (Ray and Ray 1996), *VEGF* (Ray et al. 2007), p21 (Ray et al. 2004), several matrix metalloproteases (*MMPs*) (Ray et al. 2005), *c-myc* (Bossone et al. 1992), and serotonin 1A receptor (Parks and Shenk 1996). A large number of inflammatory stimuli which have also been studied in MS can also activate the MAZ protein (Ray et al. 2000; Ray and Ray 1997).

The fourth SNP selected in this study was rs2836425, which was firstly reported in the study of Andlauer as the second strongly related SNP with MS in the German population. This SNP is an intronic variant located in *ERG* gene [ETS (erythroblast transformation-specific)-related gene] (Andlauer et al. 2016). *ERG* contains 18 exons and is located at 21q22.2 which encodes erythroblast transformation-specific transcription factors (Loughran et al. 2008). The ERG transcription factor targets a variety of genes that play crucial roles in both the immune and neurological systems, two systems which are principally involved in the pathogenesis of MS. Involvement in transcriptional regulation via ESET-mediated histone methylation may highlight the possible epigenetic effects of *ERG* in the pathogenesis of MS (Liu et al. 2002). The Fifth and last SNP studied in the current population, was SNP rs4364506, which is located in the intronic region of *L3MBTL3* [Lethal(3)malignant brain tumor-like protein 3] gene (Andlauer et al. 2016). *L3MBTL3* or *MBT*-1 has 30 exons and is located in a cytogenetic region of 6q23.1. The *L3MBTL3* gene is a member of the putative Polycomb Group (PcG) and includes the methyl-lysine reader Malignant Brain Tumor (MBT) domain which is responsible for detecting mono and di-methyl methylcellulose from H3 and H4 histone tails. MBT-domain proteins are associated with the suppression of gene expression and their dysregulation is associated with various diseases (Bonasio et al. 2010; Meier et al. 2012; Tang et al. 2013; Zhang et al. 2013).

According to previously reported investigations, the *SHMT1, MAZ, ERG*, and *L3MBTL3* genes are thought to be involved in the pathogenesis of MS. Therefore, the selected SNPs located in the mentioned gene could be the genetic carriers of the susceptibility to MS in the individuals. Hence, we were encouraged to design the present case–control study aimed to investigate the association of rs4925166 and rs1979277 in the *SHMT1, MAZ* rs34286592, *ERG* rs2836425, and *L3MBTL3* rs4364506 with MS in an Iranian population.

Materials and Methods

Patients and Controls

In this study, 190 MS patients were selected according to the last revised McDonald's criteria (Table 1) from Imam Khomeini and Sina hospitals (Tehran, Iran) which

Table 1 The McDonald criteria for diagnosis of multiple sclerosis

Clinical presentation	Additional data needed
2 or more attacks (relapses) 2 or more objective clinical lesions	None; clinical evidence will suffice (additional evidence desirable but must be consistent with MS)
2 or more attacks 1 objective clinical lesion	Dissemination in space, demonstrated by MRI Or a positive (cerebrospinal fluid) CSF and 2 or more MRI lesions consistent with MS Or further clinical attack involving different sites
1 attack 2 or more objective clinical lesions	Dissemination in time, demonstrated by MRI Or second clinical attack
1 attack 1 objective clinical lesion (monosymptomatic presentation)	Dissemination in space demonstrated by MRI Or positive CSF and 2 or more MRI lesions consistent with MS **and** Dissemination in time demonstrated by MRI Or second clinical attack
Insidious neurological progression suggestive of MS (primary progressive MS)	One year of disease progression (retrospectively or prospectively determined) and Two of the following Positive brain MRI (nine T2 lesions or four or more T2 lesions with positive VEP) Positive spinal cord MRI (two focal T2 lesions) Positive CSF

all were relapsing-remitting (RR-MS). Two hundred healthy people were selected as controls which were consistent with the patient group regarding gender and age as it is shown in Table 2. The written consent form was obtained from all participants in the study before participation. The study was approved by the National Ethics Committee and Principles of Helsinki Declaration were followed. Precise information related to the frequency, sex ratio, and age of affected people and controls, as well as the inclusion and exclusion criteria, are presented in Table 2.

DNA Extraction and SNPs Genotyping

Five ml peripheral blood was taken from each participant and DNA extraction was performed using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany), and the purity and quality of the extracted DNA were determined by nanodrop and electrophoresis. SNPs of *SHMT1* rs4925166, *SHMT1* rs1979277, *MAZ* rs34286592, and *L3MBTL3* rs4364506 were genotyped using PCR–RFLP and genotypes of *ERG* rs2836425 were determined using tetra-primer ARMS PCR method. The sequences of primers required for the amplification and genotyping of the above SNPs are shown in Table 3.

Table 2 Demographic information of participants and inclusion/exclusion criteria

p value	Control	Case	Properties
0.664	144/56 (72:28)	133/57 (70:30)	F/M (%F:%M)
0.285	33.9 ± 10	34.8 ± 6	Mean age ±SD
Including criteria			
Selection of patients as defined by 2005 revised McDonald criteria			
All the patients were relapsing-remitting			
Lack of consanguinity between patients			
Have the same sex and age distribution between patients and controls			
Have the same race			
Absence of individuals with other autoimmune diseases in the family			
Sign written informed consent prior to participating in the study			
Excluding criteria			
Other forms of MS			
Affected related patients			
Subjects with different races			
Individuals with other chronic autoimmune diseases or immunodeficiency syndrome in the family			
Diagnosis of AIDS, Hepatitis B and C, and active systemic infection of bacterial, viral, and fungal.			
Any of the following neurologic/psychiatric disorders			

Table 3 The sequences of primers required for the amplification and genotyping of the selected SNPs

SNPs	Primers' sequence (5′→3′)
rs4925166	
Forward primer	AGGTAAATGCAATCCAATGGCT
Reverse primer	GCCTCCCAAAGTGCTAGGAT
rs1979277	
Forward primer	AGCAGCTCATCCATCTCTCA
Reverse primer	TGTGTAGTGTGGGGTGACTC
rs34286592	
Forward primer	ACGGTATCCTTGGTAGCCTG
Reverse primer	GGTCACAAGGCAAGGTTTCC
rs4364506	
Forward primer	TCGCCTTGGTAAATAGAGTACTC
Reverse primer	GATACAAGCCATGCACGTCC
rs2836425	
Forward inner primer (C allele)	GTTACACTTTTCCCCAAAATGAAATCCCAC
Reverse inner primer (T allele)	GGGCTTCTTACGCCAGGTGAAAGCTA
Forward outer primer (5′-3′)	GACAAACCAAACAGGCACAGTCTCATGT
Reverse outer primer (5′-3′)	GGATTCTGATTCTCAGCACATCCAAACA

The PCR reactions with a final volume of 25µl were performed in Veriti® Thermal Cycler (Applied Biosystems, USA) as follows: initial denaturation at 95 °C for 6 min, 35 cycles of denaturation at 95 °C for 30 s, annealing for 30 s at 62 °C (*SHMT1* rs4925166), 59 °C (*SHMT1* rs1979277), 60 °C (*MAZ* rs34286592 and *L3MBTL3* rs4364506), and 68 °C (*ERG* rs2836425), extension at 72 °C for 30 s, and a final extension at 72 °C for 6 min. Finally, the PCR products of four SNPs *SHMT1* rs4925166, *SHMT1* rs1979277, *MAZ* rs34286592, and *L3MBTL3* rs4364506 were digested with specific restriction enzymes and were electrophoresed on a 2.5% agar gel. PCR Products of SNP *ERG* rs2836425 were also directly electrophoresed on 2.5% agarose gel. Table 4 shows the genotypes patterns of each SNP.

Statistical Analysis

We used the χ^2 test to evaluate differences in the frequency distributions of the genotypes or alleles, between the cases and controls. The Student's *t* tests were performed to distinguish differences in the mean values of age and gender between the cases and controls. Odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated for assessing the risk of MS. All tests were two-sided and a p value of < 0.05 was defined as the significance level. The statistical analyzes were performed by using the Statistical Package for the Social Sciences (SPSS) version 16.0.

Results

In our study, 190 RR-MS patients (133 females and 57 males) with a mean age of 34.8 ± 6 and 200 healthy individuals (144 females and 56 males) with a mean age of 33.9 ± 10 were selected from the Iranian population which were not significantly different from each other and were comparable in terms of their gender ($p = 0.664$) and age ($p = 0.285$) distribution (Table 2). This population was genotyped for five SNPs of *SHMT1* rs4925166, *SHMT1* rs1979277, *MAZ* rs34286592, *L3MBTL3* rs4364506, and *ERG* rs2836425, wherein the results of the genotypes

Table 4 Band patterns generated by SNP genotyping in gel electrophoresis analysis

SNP (enzyme-#Cat.)	Common heterozygotes	Heterozygotes	Rare homozygotes
Rs4925166 (Hpy188I-**R0617S**)	GG	TG	TT
	399-100	399-216-183-100	216-183-100
Rs1979277(EarI-**R0528S**)	GG	AG	AA
	187-109	296-187-109	296
Rs34286592 (HpyAV-**R0621S**)	GG	AG	AA
	224-131-36	224-167-131-36	224-167
Rs2836425 (no enzyme)	CC	CT	TT
	211-414	211-259-414	259-414
Rs4364506 (PciI-**R0655S**)	GG	AG	AA
	270-153-127	397-270-153-127	397-153

Table 5 Allele and genotype frequency in patient and control groups

Alleles and genotypes	Case (%)	Control (%)	*p* value	OR (95% CI)
SNP rs4925166	MAF = 0.3243 Minor (reference) allele = T			
T	91 (23.95)	141(35.25)	**0.0006**	0.58(0.42–0.79)
G	289 (76.05)	259(64.75)	**0.0006**	1.73 (1.27–2.36)
TT	13 (6.84)	27 (13.50)	**0.0333**	0.47 (0.24–0.94)
TG	65(34.21)	87(43.50)	0.0606	0.66 (0.45–1.02)
GG	112(58.95)	86(43.00)	**0.0017**	1.90 (1.27–2.85)
SNP rs1979277	MAF = 0.2278 Minor (reference) allele = A			
A	108 (28.42)	83(20.75)	**0.0131**	1.52 (1.09–2.11)
G	272(71.58)	317(79.25)	**0.0131**	0.66 (0.47–0.92)
AA	18(9.47)	11(5.50)	0.1394	1.80 (0.83–3.91)
AG	72(37.89)	61(30.50)	0.1242	1.40(0.91–2.12)
GG	100(52.64)	128(64.00)	**0.0231**	0.63 (0.42–0.94)
SNP rs34286592	MAF = 0.0889 Minor (reference) allele = A			
A	61(16.05)	43(10.75)	**0.0304**	1.59 (1.04–2.41)
G	319(83.95)	357(89.25)	**0.0304**	0.63 (0.41–0.96)
AA	6(3.16)	3(1.50)	0.2866	2.14 (0.53–8.69)
AG	49(25.79)	37(18.5)	0.0838	1.53 (0.94–2.48)
GG	135(71.05)	160(80.00)	**0.0405**	0.61 (0.38–0.98)
SNP rs2836425	MAF = 0.1326 Minor (reference) allele = T			
T	73 (19.21)	45 (11.25)	**0.0021**	1.88 (1.26–2.80)
C	307 (80.79)	355 (88.75)	**0.0021**	0.53 (0.36–0.80)
TT	8 (4.21)	4 (2.00)	0.2166	2.15 (0.64–7.27)
TC	57 (30.00)	37 (18.5)	**0.0084**	1.89 (1.18–3.03)
CC	125 (65.79)	159 (79.5)	**0.0026**	0.50 (0.31–0.78)
SNP rs4364506	MAF = 0.1887 Minor (reference) allele = A			
A	80 (21.05)	105 (26.25)	0.0886	0.75 (0.54–1.04)
G	300 (78.95)	295 (73.75)	0.0886	1.33 (0.96–1.86)
AA	10 (5.26)	16 (8.00)	0.2821	0.64 (0.28–1.45)
AG	60 (31.58)	73 (36.50)	0.3058	0.80 (0.53–1.22)
GG	120 (63.16)	111 (55.50)	0.1244	1.37 (0.92–2.06)

MAF minor allele frequency

are shown in Table 5. Additionally, both the control and affected populations were analyzed for each SNP in terms of Hardy–Weinberg equilibrium and all were in equilibrium ($p > 0.05$) as it is shown in Table 6.

As it is indicated in Table 5, there is a significant difference in the allele frequencies of SNPs of *SHMT1* rs4925166, *SHMT1* rs1979277, *MAZ* rs34286592, and *ERG* rs2836425 between patient and healthy subjects ($p < 0.05$). However, significant differences were observed neither in allele nor genotype frequencies for *L3MBTL3* rs4364506 ($p > 0.05$).

Table 6 Results of Hardy–Weinberg equilibrium test

Group	Rare homozygotes	Heterozygotes	Common heterozygotes	χ^2	*p* value
SNP rs4925166	TT	TG	GG		
Case					
Obs.	13	65	112	0.304	0.859
Exp.	11	69	110		
Control					
Obs.	27	87	86	0.190	0.909
Exp.	25	91	84		
SNP rs1979277	GG	AG	AG		
Case					
Obs.	18	72	100	0.331	0.847
Exp.	16	77	97		
Control					
Obs.	11	61	128	1.186	0.552
Exp.	7	67	126		
SNP rs34286592	AA	AG	GG		
Case					
Obs.	6	49	135	0.135	0.9349
Exp.	5	51	134		
Control					
Obs.	3	37	160	0.256	0.8800
Exp.	2	39	159		
SNP rs2836425	TT	TC	CC		
Case					
Obs.	8	57	125	0.105	0.9488
Exp.	7	59	124		
Control					
Obs.	4	37	159	0.272	0.8727
Exp.	3	40	157		
SNP rs4364506	AA	AG	GG		
Case					
Obs.	10	60	120	0.143	0.931
Exp.	9	63	118		
Control					
Obs.	16	73	111	0.258	0.8789
Exp.	14	77	109		

In *SHMT1* rs4925166, the frequency of T allele was higher in the case group than control group ($p = 0.0006$) and T allele (OR 0.58, 95% CI 0.42–0.79) and TT genotype (p value = 0.0333, OR 0.47, 95% CI 0.24–0.94) were identified as the protective genetic elements in the development of MS disease; however, the G allele ($p = 0.0006$; OR 1.73, 95% CI 1.27–2.36) and GG genotype (p value = 0.0017,

OR 1.90, 95% CI 1.27–2.85) played a predisposing role. However, there was no significant difference in the frequency of heterozygotes between affected and healthy subjects ($p = 0.0606$).

G allele of *SHMT1* rs1979277 played a protective role ($p = 0.0131$, OR 0.66, 95% CI 0.47–0.92), nonetheless, allele A had a predisposing effect on the disease (p value = 0.0131, OR 1.52, 95% CI 1.09–2.11). For this SNP, there was no significant difference in the frequency of homozygote AA and heterozygotes ($p = 0.1394$ and $p = 0.1242$, respectively), however, the frequency of homozygotes GG was significantly different between control (64%) and patient (52.5%) groups, respectively, wherein it was negatively associated with the risk of MS ($p = 0.0231$, OR 0.63, 95% CI 0.42–0.94).

Regarding *MAZ* rs34286592, the frequency of predisposing A allele in healthy and MS subjects was 10.75% and 16%, respectively ($p = 0.0304$, OR 1.59, 95% CI 1.04–2.41), while the G allele showed protective effect (OR 0.63, 95% CI 0.41–0.96). In this SNP, the GG genotype only showed a negative correlation with MS ($p = 0.0405$, OR 0.61, 95% CI 0.38–0.98).

For *ERG* rs2836425, there was a highly significant difference in the allele frequencies between the patients and controls (p value =0.0021) and the ORs for the T and C alleles were 1.88 (95% CI 1.26–2.80) and 0.53 (95% CI 0.36–0.80), respectively. The CC genotype had a very strong protective role against MS ($p = 0.0026$; OR 0.50, 95% CI 0.31–0.78) and the TC genotype showed a strong susceptibility to MS ($p = 0.0084$; OR 1.89, 95% CI 1.89–3.03). However, there was no significant difference for GG genotype between case and healthy subjects ($p = 0.2166$). Bold values in Table 5 indicate the significant p values (p values less than 0.05).

Related to the last studied SNP (*L3MBTL3* rs4364506), no significant differences were found in the allele and genotype distribution between patient and healthy subjects ($p > 0.05$).

Discussion

MS is a chronic neurological disease with inflammation and autoimmune characteristics that affect parts of the CNS, leading to the degeneration of axons (Compston and Coles 2008; Simons et al. 2014). Despite major research in the past few decades, the etiology of MS remains elusive. The multifactorial nature of the disorder and limited availability of appropriate brain tissue samples, where the active demyelination and neurodegeneration can be detected, are the major issues in the disease pathology (Lassmann 2014). The best kind of studies to identify SNPs associated with multifactorial diseases, such as MS, are GWAS. Regarding MS, at least 16 GWAS have been reported globally, which have been mentioned in the previous literature (Abdollah Zadeh et al. 2017; Sawcer et al. 2014; Andlauer et al. 2016; Giacalone et al. 2015). These studies have identified more than 100 common SNPs that have a remarkable association with MS and a significant number of these SNPs are found in genes that play vital roles in immunological processes (Abdollah Zadeh et al. 2017; Sawcer et al. 2014). In addition to a wide variety of genetic and environmental factors that can importantly complicate the understanding of the MS

pathogenesis, epigenetic factors should also be considered, wherein the environment can interact with the genome through changes in the epigenetic patterns and affect the phenotype (Guintivano and Kaminsky 2016). In the case of MS, low concordance rates in MZ twins, principally in mother-to-child transmission model of the disease (parent-of-origin effect), and the twice occurrence of MS in women highlight the role of epigenetic causes (Andlauer et al. 2016; Küçükali et al. 2015). In the present study, the association of rs4925166 and rs1979277 in the *SHMT1*, *MAZ* rs34286592, *ERG* rs2836425, and *L3MBTL3* rs4364506 with MS was assessed in an Iranian population, wherein, genotypes GG (*SHMT1* rs4925166) and TC (*ERG* rs2836425) conveyed the strongest (positive) susceptibility to MS, respectively. However, genotypes TT (*SHMT1* rs4925166), CC (*ERG* rs2836425), GG (*MAZ* rs34286592), and GG (*SHMT1* rs1979277) had the highest negative association (protective effect) with MS, respectively. *L3MBTL3* rs4364506 was found neither as a predisposing nor protective variant.

The first studied SNP in the current work was rs4925166 (G>T) in the *SHMT1* gene which showed the strongest association with MS disease. The allele frequencies in this SNP showed a significant difference between the affected and healthy subjects ($p = 0.0006$), the T allele was a protective variant (OR 0.58), and G allele was a predisposing genetic element (OR 1.73). Besides, individuals with TT genotype versus combined TG+GG genotypes were protected against MS (OR 0.47). However, people with GG genotype versus TT+TG were highly susceptible to MS (OR 1.90). In fact, GG and TT genotypes had the most positive and negative correlations with MS, respectively. The second SNP, rs1979277 (G>A) in the *SHMT1* gene, found to be significantly different in the allele frequencies between patients and controls ($p = 0.0131$), and the allele A and allele G were predisposing and protective variants, respectively (OR 1.52 and OR 0.66, respectively). Among the genotypes, the GG genotype only showed significant differences and played a protective role in the development of MS ($p = 0.0231$, OR 0.63). *SHMT1* rs4925166, as an eQTL factor, can affect the expression of the SHMT1 gene and SNP rs1979277 can also affect the SHMT1 protein by changing the leucine amino acid at position 474 to phenylalanine (Leu474Phe) (Heil et al. 2001). The *SHMT1* gene encodes serine hydroxymethyltransferase which plays an essential role in the DNA methylation, DNA synthesis, and repair (Girgis et al. 1997). In addition, the enzyme is also involved in the futile folate cycle and indirectly synthesis of s-adenosylmethionine (SAM), therefore, any perturbation in this metabolic pathway can lead to a defect in the synthesis of SAM, which is a typical messenger of the methyl group and may cause epigenetic changes, in particular, abnormal DNA methylation (Naushad et al. 2011). Furthermore, the conversion of homocysteine to methionine is one of the most important roles of the folate cycle, wherein the abnormal function of the cycle results in homocysteine accumulation as a neurotoxic amino acid in many of the neurodegenerative abnormalities such as MS (Zhu et al. 2011). Therefore, based on our assumption, the SNPs of rs4925166 and rs1979277 in *SHMT1* can be involved in the pathogenesis of MS by the changes in gene expression and function of the SHMT1 protein. Since SNPs rs4925166 and rs1979277 in *SHMT1* showed a protective and predisposing role in the development of MS, respectively, it can be concluded that they might balance the effect of each other on susceptibility to MS in an unknown way. However,

further studies, especially functional approaches, are needed to confirm this hypothesis. *SHMT1* rs4925166 was studied for the first time in the Iranian population and the association of *SHMT1* rs1979277 with MS was investigated in a case–control approach for the first time in the world. Besides, our findings confirmed the results of the GWAS conducted by Andlauer et al. (2016).

The third variant studied in the present research was SNP rs34286592 (G>A) in the *MAZ* gene. According to the genotyping results, there was a significant difference in the allele frequencies between the affected and healthy subjects ($p=0.0304$). Alleles A and G played a predisposing (OR 1.59) and protective (OR 0.63) role, respectively. Similar to *SHMT1* rs1979277, only the GG genotype showed a significant difference and had a protective function ($p=0.0405$, OR 0.61). Consistent with the study conducted by the Andlauer group, our findings confirmed the role of this variant. Throughout the reports on *c-MYC*, *MAZ* is thought to be over-expressed in lesions and T lymphocytes of patients with MS (Mycko et al. 2003; Satoh et al. 2005). In addition, this transcription factor is believed to be involved in the pathogenesis of neurological diseases especially MS by regulating the expression of many other genes (Okamoto et al. 2002). The association of SNPs located in *NR1* and the expression of *RDIy*, two important target genes of *MAZ*, in B and T lymphocytes, precursor cells, and some neuroblastoma have been shown to be related to the severity and development of MS disease (Rossi et al. 2013; Ramsay and Gonda 2008; Sawcer et al. 2011; Zhou and Ness 2011). According to these evidence, we can conclude the genetic variants located in *MAZ* gene might be associated with the susceptibility to MS by influence on the protein expression and function.

The fourth target SNP in the present study was SNP rs2836425 (C>T) in the *ERG* gene. The allele frequencies were significantly different between MS and healthy subjects ($p=0.0021$) and the C allele was protective (OR 0.53), while the T allele played a predisposing role to MS (OR 1.88). In relation, TC and CC genotypes showed positive associations (OR 1.89) and negative (OR 0.50) with MS disease, respectively. According to our results, TC and CC genotypes had the highest positive and negative correlation with MS after GG and TT genotypes of *SHMT1* rs4925166. The *ERG* transcription factor targets a variety of genes, particularly nuclear factor- κB (NF-κB) gene, that play important roles in both the immune and neurological systems, the two major systems involved in the pathogenesis of MS (Hoesel and Schmid 2013; Miterski et al. 2002; Beinke 2004; Berenson et al. 2001). Furthermore, NF-κB has also several roles in the CNS, specifically in synaptic transmission (Guerrini et al. 1995; Kaltschmidt et al. 1993; Meberg et al. 1996), neuronal plasticity (Frade et al. 1996; Tong and Perez-Polo 1996), and neuronal development (Kurata et al. 1993; Sheppard et al. 1995). However, the study of Baarsen et al. on peripheral blood lymphocytes (PBLs) using microarray analysis, did not show a significantly altered expression of NF-κB-specific genes between MS patients and controls (Van Baarsen et al. 2006). Liu Yang's study showed that the ERG transcription factor is probably involved in transcriptional regulation via ESET-mediated histone methylation (Liu et al. 2002). The *ESET* or *SETDB1* gene encodes a specific histone methyl transferase called H3K9 methyl transferase, which epigenetically regulates gene silencing and transcriptional repression (Ryu et al. 2006). Therefore, due to the roles of *ERG* in the regulation of immune and neurological system, it can be

concluded that this gene and its genetic variants are associated with MS through genetic/epigenetic effects.

The last examined variant was SNP rs4364506 in the *L3MBTL3* gene which found no significantly different in allelic and genotypic frequencies ($p > 0.05$). To confirm the association between this SNP and MS, this study should be conducted in other populations and it might be concluded that SNP rs4364506 in the *L3MBTL3* gene is not the accurate SNP associated with the disease and the other SNPs in the LD are the real correlated SNPs. In a population, the frequency of different haplotypes of adjacent SNPs is varied; therefore, an SNP in a population may be associated with the disease and it may not be related in the other population. The lack of haplotype assessment in the present report could be considered as one of the limitations of the study. The *L3MBTL3* gene is a member of the putative Polycomb group (PcG) which is responsible for detecting mono and di-methyl monocyclic lysine in H3 and H4 histone tails. The homologous MBT-domain proteins are associated with the suppression of gene expression and their maladaptation is associated with various diseases (Bonasio et al. 2010). Interestingly, a recent GWAS has shown a strong association between SNPs rs6569648 and rs6899976 in the *L3MBTL3* gene with height (Lango Allen et al. 2010). Therefore, the *L3MBTL3* gene, as a mediator gene, is thought to play an important role in epigenetic changes and due to its extremely diverse roles could be involved in MS pathogenesis, and assessment of the relation of the *L3MBTL3* variants is required to be performed in different populations and functional phase.

Regarding contradictory results of the present study with the others, we are aware of several limitations including ethnicity differences, vast geographical diversity, interaction with other genetic or environmental factors, and clinical heterogeneity. Therefore, lack of significant association in some of the studied SNPs might be due to the population stratification, inter-population genetic variation, false-positive studies, false-negative studies, or true variability in association with different populations, small sample size, and need to the greater statistical power. Combined effects of the different genotypes of each SNP have been evaluated, nonetheless, haplotype or combine genotype analyzes can also be considered as the further limitations of the present work. However, the distance between the studied SNPs and LD of SNPs was assessed in haploreg (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php), wherein, no LD was observed for any of SNPs. *MAZ* rs34286592, *ERG* rs2836425, and *L3MBTL3* rs4364506 are located in different chromosomes, therefore haplotype analysis cannot be performed for these SNPs. Given the distance of SNPs, rs4925166 and rs1979277 in the *SHMT1* are not located in a haplotype block.

Conclusion

This study examined the association between MS and SNPs located in genes that somehow involved with epigenetic changes. Having/not having an association with MS is not a clear sign of having/not having a role in the disease. Therefore, morestudies are needed to confirm the findings, especially repeating the study in other populations, examining the presence of LD in the desired SNP with other SNP(s),

and functional studies are suggested to assess the role of SNPs in the target gene expression. Another point to be noted is that epigenetic changes are tissue-specific and cannot be investigated by association and linkage studies, however, studies similar to our work can be helpful in the starting point for mapping genes and chromosomal regions that might be involved in the pathogenesis of MS and might open up a new window to the epigenetic world of multifactorial autoimmune diseases such as MS. Increasing evidence including the epigenetic regulation of genes related to inflammation and neurodegeneration, epigenetic changes in MS-associated inflammation and myelination promoters, and key studies in the EAE model have confirmed the importance of epigenetic factors in the MS pathogenesis. Another point of the current research is that although the SNPs located in exon and regulatory areas are often investigated, it should be noted that most of the SNPs found in the genome are located in intron and intergenic regions that can play a crucial role in susceptibility to MS through vital pathways, such as eQTL, miRNAs, and noncoding RNAs, and epigenetic pathways. Finally, it is important to mention that the discrepancies between our results and the Andlauer Group might be due to the different sample sizes and different ethnicities.

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Affiliations

Seyede Zahra Nazari Mehrabani[1] · Mohammad Hossein Shushizadeh[2] · Mohammad Foad Abazari[3] · Maryam Nouri Aleagha[3] · Abbas Ardalan[4] · Rasoul Abdollahzadeh[5] · Asaad Azarnezhad[5,6]

✉ Rasoul Abdollahzadeh
RASOUL142857@gmail.com

✉ Asaad Azarnezhad
Azarnezhad@gmail.com

1 Department of Molecular Genetics, Islamic Azad University, East Tehran branch, Tehran, Iran

2 Doctor of Clinical Lab Science, Pasteur Medical Lab Shush Danial Iran, Dezful Medical University, Dezful, Iran

3 Department of Genetics, Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran

4 Department of Biology, Faculty of Sciences, Arak University, Arak, Iran

5 Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

6 Cellular and Molecular Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran