Association of Toll-like receptor 4 (*TLR4*) gene polymorphism with multiple sclerosis (MS) in Iranian patients

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Abstract

Objective: Multiple sclerosis (MS) is a chronic debilitating disease with unknown pathogenesis. Recent studies indicated that pathogen recognition receptors such as Toll-like receptor 4 (TLR4) may have a role in pathogenesis of MS. The aim of the study was to evaluate the association of rs1927911 polymorphism in TLR4 gene with MS.

Methods: Four hundred subjects including 200 MS patients and 200 healthy individuals were recruited in the study. Patients were included secondary-progressive (SP), primary-progressive (PP) and relapsing-remitting (RR) subtypes. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was performed to identify rs1927911 genotypes in TLR4 gene.

Results: The mean age of healthy and MS group was 34.22 ± 1.3 and 33.2 ± 0.98, respectively. The frequency of TT, TC and CC was 29/52, 132/128 and 39/20 respectively in MS compared to healthy subjects. Genotype and allele distribution were significantly different between the both groups ($P<0.05$). In addition, TC (OR= 1.849, 95 % CI= 1.105-3.095, P=0.019) and CC (OR= 3.497,95 % CI=1.728-7.076, P=0.001) genotypes had increased the risk of MS.

Conclusion: Our findings showed a significant relationship between rs1927911 polymorphism in TLR4 gene and MS. We concluded that rs1927911 genotype variations may increase the risk of MS. Further studies in other populations are recommended to support our findings.

Keywords: **Gene variation, Multiple sclerosis, Toll-like receptor**

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INTRODUCTION

Multiple sclerosis (MS) is an autoimmune disease of central nervous system and spinal cord. According to global statistics about 2.2 million people in 2016 (1) and a total of 2.8 million people in 2020 are estimated that suffered from MS (35.9 per 100000 population) and the mean age of affected peoples is 32 years (2). The number of patients has increased from 2013 and estimations show that females are twice as likely as men (2).

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The major manifestation of the disease is demyelination of white and grey matter of the central nervous system[3]. The exact etiology of the disease is unknown. However, genetic, environmental and infectious agents have been suggested in previous studies as influential factors contributing to the development of MS[4]. In addition, it appears that the main mechanism of injury is inflammation[3]. Previous studies demonstrated the role of Toll-like receptors (TLRs) in neurodegenerative disorders such as MS[5-7]. TLRs transcription change due to up-regulation of pro-inflammatory genes in brain and contribute in neurodegeneration related to aging[8]. Recently pathogen recognition receptors such as TLR4 (TLR4) have been indicated in the pathogenesis of MS[9].

TLR4 gene (Gene ID: 7099), also known as TOLL; CD284; TLR-4; ARMD10, is locate on the long arm of chromosome 9 (9q33.1). The protein encoded by this gene plays a role in pathogen recognition, obesity and activation of innate immunity[10]. TLR4 is expressed on the cell surface of innate immune cells or in the cytoplasmic vesicles of these cells[11]. Hence, it responds to pathogen-associated molecular patterns (PAMPs) that are expressed on infectious agents and damage- associated molecular patterns (DAMPs)[12]. TLR4 activates immune cells trough MYD88 and TRIF intracellular signaling pathways[13].

Recent studies indicated that TLR4 has a critical role in the pathogenesis of MS[14]. TLR4 and CD40 triggers a signaling pathway in regulatory B cells leading to production of IL-10 that consequently reduce the severity of the disease. It has been shown that TLRs are expressed in the glial cells of CNS of MS patients[15]. Moreover, studies with knockout models indicated TLR2, TLR9 and MyD88 deficiency had protective roles in neuroinflammatory models, whilst models with TLR4, TLR2 and TRIF deficiency showed an aggravating disease. These findings indicated the complexity of TLRs interplay in developing MS[8].

As of 17 April 2022, 3575 polymorphisms have been reported in TLR4 gene region in dbSNP (https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=7099). rs1927911 polymorphism with minor allele frequency > 0.4 (MAF=0.4002) is located on noncoding region of TLR4 gene. This polymorphism has been investigated in Alzheimer's disease[16], Parkinson's disease[17], Autoimmune Thyroid Disease[18], rheumatoid arthritis[19], Vitiligo[20], T2DM risk[21], cervical cancer[22], Guillain-Barré syndrome (GBS)[23], Autoimmune pancreatitis[24] and other diseases. However, there was no study that investigated this polymorphism in MS patients. We investigated rs1927911 polymorphism in intronic region of TLR4 to know that whether there is association between this polymorphism and MS.

METHODS

Subjects

Four hundred age- and sex-matched subjects including 200 MS patients and 200 healthy subjects who referred to Peymanaieh hospital (Jahrom city, Iran) were enrolled in this case-control study. Patients whose disease was confirmed by a neurologist were included in the study. Patients were included secondary-progressive (SP), primary-progressive (PP) and relapsing-remitting (RR) subtypes. Subjects with a history of inflammatory underlying disease, autoimmune disease, cancer and familial history of MS were excluded from the study. The study protocol was confirmed by the ethics committee of Jahrom University of Medical Sciences. A written informed consent form was filled out by all participants.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

Five milliliter whole blood was drowning from all the subjects in EDTA-containing tubes and immediately transferred to the central lab of Jahrom University of Medical Sciences and stored in -20. Extraction of DNA was performed by salting-out method[25]. Detection of rs1927911 genotypes in intron region of TLR4 was performed by PCR-RFLP.

Reactions were performed in a final volume 20 μl containing 10 μl Taq DNA Polymerase Master Mix Red (AMPLIQON, Cat no: A180301), 3 μl genomic DNA (0.2 μg), 1 μl forward primer (TCACTTTGCTCAAGGGTCAA; 1μM), 1μl reverse primer (AAACCTGCATGCTCTGCAC; 1μM) and 5 μl PCR grade water. Temperature cycles were set for 35, after incubation at 94°C for 5 minutes (94°C for 45_{sec}, 58°C for 45_{sec}, and 72°C for 30_{sec}). A final extension was applied for 10 minutes. PCR products were applied to digestion by Sty I (Thermo Scientific™ , 10 U,16 h overnight). Digested

products were run on 3% agarose gel and visualized by green viewer on an UV transluminator.

Statistical analysis

Statistical analysis was performed by SPSS v.18 (Chicago). Kolmogorov-Smirnov test was used to investigate normality of the data. Hardy-Weinberg equilibrium was performed to survey allele distribution. Multinomial logistic regression was performed to analyze the probability of categorical membership. The numeric data were reported as mean± Standard Error (SE). Student t and Chi square tests were applied to investigate the differences between groups, genotype and allele variations. *P* value less than 0.05 was considered to be significant.

RESULTS

Study population

400 subjects including 200 MS and 200 healthy subjects were investigated in the study. The subjects were in the range of 15 to 60 years. The mean age of healthy and MS groups was 34.22 ± 1.3 and 33.2 ± 0.98, respectively. The differences between the groups were not statistically significant (P>0.05). 39.1 % and 60.9 % of subjects in control group were respectively men and women. In addition, 29.2 % and 70.8 % of MS patients were respectively men and women. The distribution of sex between the groups was not statistically different (P>0.05). 23.6 %, 1.4 % and 75 % of the patients were SP, PP and RR subtypes, respectively. Moreover, 23.6 %, 26.4 %, 34.7 %, 15.3 % of patients had a history of < 1 year, 1-5 year, 6-10 year, and >10 year, respectively. 23.6 % of patients had a familial history of MS.

Genotype/Allele distribution

rs1927911 genotypes and allele distributions are summarized in Table 1. TT, TC and CC genotype distributions were statistically different between MS and healthy groups (P=0.002). Moreover, T and C allele distributions were different between the groups (P=004). Genotype distribution was not statistically different between MS (SP, PP and RR) subtypes(P>0.05). In addition, TC (OR= 1.849, 95 % CI= 1.105-3.095, P=0.019) and CC (OR= 3.497, 95 % CI= 1.728-7.076, P=0.001) genotypes had increased the risk of MS.

DISCUSSION

The main finding of our study was that genotype and allele distribution of rs1927911 polymorphism in the non-coding region of TLR4 had an association with MS. The number of people suffered from MS is increasing worldwide[26]. Recent studies indicated that current therapeutic options for MS are disappointing because the exact mechanism of MS is unknown[27]. It is believed that the interactions between gene and environment may be one of the most important factors in etiology of MS[26].

Toll like receptors have a critical role in innate and acquired immune response. Previous studies suggested that polymorphism within TLR gene could affect signaling pathways related to TLRs and increase the risk of autoimmune diseases[28]. Evidences regarding the role of TLR4 in pathogenesis of MS have been collected in some studies. Identification of gene polymorphism within TLR4 could increase our knowledge of MS pathogenesis.

Korner group[29] investigated the association of Asp299Gly polymorphism in TLR4 with MS. Their findings showed that there is no association between

Table 1. Genotype and allele distribution of rs1927911 polymorphism in the study population.

Allele / Genotype		Healthy subjects (n = 200)[b]	MS[a] Subjects (n = 200)[b]	P-value
Allele	T	232 (58%)	190 (44.4%)	P = 0.004[*]
	C	168 (42%)	210 (55.6%)	
Genotype	TT	52 (26 %)	29 (14.5 %)	P = 0.002[*]
	TC	128 (64%)	132 (66 %)	
	CC	20 (10 %)	39 (19.5 %)	

[a]Multiple Sclerosis [b]n=number of subjects that studied in healthy and patient groups

[*]Statistically significant value. Chi-square test was performed to compare alleles and genotypes between healthy and patient groups.

this polymorphism and MS subtypes and severity of the disease which was in agreement with Reindl findings[30]. However, Korner et.al showed that peripheral blood mononuclear cells (PBMCs) from heterozygote Asp299Gly have a lower proliferation in response to Lipopolysaccharides (LPS) stimulation[29].

We searched in PubMed, Google, and dbSNP databases, and found that there are only a few studies regarding the TLR4 polymorphisms and MS. To the best of our knowledge, this is the first study that investigated rs1927911 polymorphism in Iranian MS patients. This polymorphism has been investigated in many other diseases. Zhao group investigated rs1927911 polymorphism in sporadic Parkinson diseases in Han Chinese population[17]. They showed that there were no differences in genotype and allele distributions between the groups. However, our findings indicated that rs1927911 heterozygote TC and homozygote CC had a higher distribution between MS patients. Hence, we found that there is an association between rs1927911 genotype and allele distribution with MS in Iranian patients. Frequency of C allele was significantly higher in MS group compared to control group. Cho group[18] surveyed rs1927911 in Koran pediatric patients to dedicate an association with autoimmune thyroid disease. They showed that the frequency of C allele in grave disease is significantly higher than control group which is in agreement with our finding.

Davis group[19] investigated rs1927911 in patients suffered from rheumatoid arthritis to study the relation of polymorphism with disease progression. They found an association between rs1927911 genotypes and disease activity. They concluded that this relation is independent of other covariates. We found that TC and CC allele respectively had an odds ratio 1.84 and 3.49. These findings indicated that rs1927911 heterozygote and mutant homozygote genotypes may be risk factors for MS. Some studies indicated that TLR4 knockout models had an aggravating disease which indicated the critical roles of TLR4 in the developing of MS[8].

In a systematic review, Moura group[22] investigated the TLR4 and TLR9 polymorphisms including rs1927911 in association with cervical cancer. Their Analysis by bioinformatics tools showed that studied polymorphisms in TLR4 could change intracellular signaling and patterns of proteins in immune cells. They concluded that these polymorphisms could increase the risk of cervical cancers. Gene variations of TLR2, TLR3 and TLR4 have been investigated by Dutta group[23] in Guillain-Barré syndrome (GBS). They showed no increased risk of GBS in relation to TLR4 polymorphism which is in contrast to our findings.

CONCLUSION

Our findings indicate that the distribution of alleles and genotypes of rs1927911 in TLR4 gene are different between MS patients and healthy subjects. Also, the result showed that this polymorphism may increase the risk of MS. Conclusion with limited number of studies regarding the role of special polymorphism in developing of MS is difficult. However, we didn't find another study that investigated this polymorphism in MS patients, so further studies in other populations are needed to support our findings. Since this is the first study that investigated rs1927911 in MS, the results should be interpreted with cautions.

Statements and Declarations

Authors' contribution:

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Abdolreza Sotooteh Jahromi, Saiedeh Erfanian and Abazar Roustazadeh. The first draft of the manuscript was written by Abdolreza Sotooteh Jahromi, Saiedeh Erfanian, Abazar Roustazadeh and Sobhan Safavi and all authors commented on previous versions of the manuscript. Saiedeh Erfanian and Sobhan Safavi assisted in laboratory measurements. All authors read and approved the final manuscript.

Consent to participate: All participants filled out a written consent form. In addition, a written informed consent was given by the parents/legally authorized representatives of the minor subjects (under 16 years of age), both patients and controls, involved in the study.

Consent for publication: Not applicable

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Ethical approval: Ethics committee of Jahrom University of Medical sciences approved the study. All methods were performed in accordance with the relevant guidelines and regulations. The research ethics number was IR.JUMS.REC.1398.024.

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