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Vitamin D Receptor Gene Polymorphism and the Risk of Multiple Sclerosis in South Eastern of Iran

Mehrnaz Narooie-Nejad · Maryam Moossavi · Adam Torkamanzehi · Ali Moghtaderi · Saeedeh Salimi

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Abstract Multiple sclerosis is one of the most widespread demyelinating diseases of the central nervous system. Environmental and genetic factors are collaborating in triggering MS. The role of vitamin D receptor (VDR) gene and its polymorphisms are highlighted as susceptible components. The aim of the present study was to examine the association of single nucleotide polymorphism (SNP)—BsmI and FokI—in VDR gene and MS susceptibility in the South Eastern Iranian population. Therefore, 113 MS patients and 122 controls were recruited in the study. Restriction fragment length polymorphism was performed to detect the SNPs. There were no significant differences in the polymorphism of FokI (rs2228570) in VDR gene among patients and controls ($P>0.05$), while a significant difference was observed in BsmI (rs1544410) polymorphism in healthy subjects and homozygous genotype-b/b- with MS ($P=0.025$). Results showed a protective association of homozygous genotype-b/b- of BsmI with MS susceptibility in a population in South Eastern of Iran.

Keywords VDR polymorphism · Vitamin D · Multiple sclerosis · PCR-RFLP

M. Narooie-Nejad
Genetic of Non-Communicable Disease Research Center, Zahedan University of Medical Sciences, Zahedan, Iran

M. Narooie-Nejad
Department of Genetics, Zahedan University of Medical Sciences, Zahedan, Iran

M. Moossavi (✉) · A. Torkamanzehi
Department of Biology, Sistan and Baluchestan University, Zahedan, Iran
e-mail: maryam_moossavi@yahoo.com

A. Moghtaderi (✉)
Department of Neurology, Zahedan University of Medical Sciences, Zahedan, Iran
e-mail: amoghtaderi@gmail.com

S. Salimi
Department of Clinical Biochemistry, Zahedan University of Medical Sciences, Zahedan, Iran

S. Salimi
Cellular and Molecular Research Center, Zahedan University of Medical Sciences, Zahedan, Iran

Introduction

Multiple sclerosis (MS) is an inflammatory disease of the central nervous system (CNS) characterized by inflammation, demyelination, and finally gliosis in the brain and spinal cord. It is followed by motor, sensory, and cognitive disabilities (Calabresi 2004; Mount 1973; VanAmerongen et al. 2004). Recurrent inflammatory process and its influence on neurological functions of the human CNS make the nature of periodic neurological episodes, which are characteristics for relapsing-remitting (RR) MS. However, the chronic progressive phase characterizes the progressive forms of MS (Navikas and Link 1996; VanAmerongen et al. 2004). Thus far, the etiology of MS is unknown; however, it is believed that genetic and environmental factors play a significant role in predisposing individuals to the disease (Niedziela et al. 2013). In 2011, the Iranian Ministry of Health and Medical Education (MOHME) registry listed 34,605 patients affected with MS in Iran (Izadi et al. 2013). Seventy-seven percent were female and MS prevalence rate was estimated 45/100,000 in the Iranian population. The most reported prevalence rate was in Isfahan province and the least in Sistan and Baluchestan Province (Moghtaderi et al. 2013a). Vitamin D as a secosteroid was hydroxylated in the liver to 25-hydroxyvitamin D 25(OH)D (Rajakumar 2003). This metabolite can be further hydroxylated to the biologically active metabolite, $1,25(OH)_2D$ in kidney, which is the hormonally

active form of vitamin D. The major role of vitamin D is in calcium homeostasis. The active form of vitamin D, stabilize serum calcium level by parathyroid hormone, through actions on bone, intestinal calcium absorption, and renal calcium excretion (Rajakumar 2003). Besides, vitamin D has been considered to have important impact in the development of the nervous system, and the active form of vitamin D has extensive immunomodulatory properties (Haussler et al. 2011). Vitamin D has an immunoregulatory and anti-inflammatory functions in human body (Thomasset 1994; Adorini and Penna 2008). It has been reported that immune-suppressor T regulatory cells are upregulated in the presence of 1, 25$(OH)_2$D (Gorman et al. 2007; Barrat et al. 2002). In the presence of 1,25$(OH)_2$D, low production of interferon-gamma (IFN-ϒ) and high production of anti-inflammatory cytokines such as interleukin IL-4, IL-5, and IL-10 are reported (Boonstra et al. 2001). So, it is expected that malfunctions of vitamin D metabolism may have been linked to the inflammation and demyelinating conditions of MS (Sioka et al. 2011). After entering the cell, 1, 25-$(OH)_2$D binds to vitamin D receptor (VDR), and then the complex is entered into the cell nucleus and acts as a ligand-activated transcription factor (VanAmerongen et al. 2004). Thus, 1,25-$(OH)_2$D performs its biological activities through VDR-mediated gene regulation (Brown et al. 1999; Hewer et al. 2013). The VDR gene (VDRG) is mapped on chromosome 12q13.1, consists of 9 exons, and included a number of allelic variants (Uitterlinden et al. 2004a). It seems that VDR polymorphisms influence the response of target cells to vitamin D and may play a role to MS susceptibility. The SNP database of the NCBI lists over 30 polymorphisms within the VDR gene. Only a limited number of these polymorphisms have been investigated to understand their relationship to autoimmune diseases, and also to MS disease (Uitterlinden et al. 2004a; Sioka et al. 2011; Fukazawa et al. 1999). This study was designed to investigate the association of two polymorphism, BsmI (rs1544410) and FokI (rs2228570), to MS susceptibility in Sistan and Baluchistan Province in southeast of Iran.

Material and Method

This case-control study was approved by the Zahedan University of Medical Sciences Ethics Committee. The individuals recruited in this study were a total of 113 confirmed MS patients (88 women and 25 men) according to McDonald criteria (Polman et al. 2005). The criteria was based on clinical manifestations, CSF analyses, visual evoked potential (VEP), and brain and spinal cord MRI studies of patients who referred to the Neurology Clinic of Imam Ali University Hospital in Zahedan, Sistan and Baluchistan Province, Iran. There were 122 healthy controls (94 women and 28 men) without any neurologic and systemic diseases. They did not have any relative with MS disease in their family. Patients and controls are all from South East of Iran, matched for age and gender as the patient group. All participants provided informed consent according to the Declaration of Helsinki and accepted codes of the university ethics committee.

VDRG Genotyping

DNA was isolated from peripheral blood leukocytes by salting out procedure (Hashemi et al. 2010). VDRG genotypes of FokI (rs2228570) and BsmI (rs1544410) polymorphisms were detected by using PCR-RFLP technique. The primer sequences are shown in Table 1. PCR was performed in a 25-μl final volume that contained 25 pmol of each primers, 0.1 mmol of dNTP (Fermentas, Lithuania), 0.5 μg of genomic DNA, 1.5 mmol/L of MgCl2, 2.5 μl of 10× PCR buffer, and 0.5 unit of Taq DNA polymerase (Ferments, Lithuania), according to the following protocol: initial denaturation at 96 °C for 6 min, followed by 35 cycles of denaturation at 96 °C for 1 min, annealing at 61 °C for BsmI and 70 °C for FokI for 45 s and extension at 72 °C for 1 min, and final extension at 72 °C for 6 min. PCR products were digested by relevant restriction enzymes. The DNA fragments were analyzed by electrophoresis on 2 % agarose gel, then ethidium bromide staining and visualized by UV radiation.

Statistical Analysis

The distribution of genotypes in patients and healthy controls was evaluated for deviation from Hardy-Weinberg equilibrium. Data were analyzed by using the statistical software SPSS 18 (SPSS, Chicago, IL). Differences in genotypic and allelic distribution between patients and controls were examined by chi-squared (χ^2) and independent sample *t* tests. In addition, the odds ratio (OR) and 95 % confidence intervals (CI) were also calculated. *P* values of less than 0.05 were considered statistically significant. Quantitative data were shown as mean±standard deviation.

Table 1 Primer sequences of BsmI and FokI polymorphisms

SNP	Primer sequence
BsmI	forward 5′ACTTGCATGAGGAGGAGCATGTC3′
	reverse 5′GGAGAGGAGCCTGTGTCCCATTTG3′
FokI	forward 5′AGCTGGCCCTGGCACTGACTCTGGCT3′
	reverse 5′ATGGAAACACCTTGCTTCTTCTCCCTC3′

Result

In the current study, 113 MS patients with a mean age of 32.4±8.9 years (88 females and 25 males) were genotyped for two SNPs in VDR gene. Control experiments were also performed in the same way, with a mean age 30.8±10.2 years (94 females and 28 males). There were no significant differences in gender and the mean age between MS patients and controls, as these two groups were matched in gender and age at the time of sampling. The calculated results of genotype and allele frequencies are given in Tables 2 and 3, respectively. Allelic and genotype frequencies for BsmI and FokI polymorphisms hold the Hardy-Weinberg equilibrium ($P>0.05$). According to calculated odds ratio and P value for each genotype, the genotypic frequency of FokI polymorphism did not show any significant difference among MS patients and control group ($P>0.05$).

However, the allelic frequency had remarkable differences in these two groups ($P=0.006$, OR=2.0, 95 % CI in 1.2–3.3). Genotype and allelic frequencies of BsmI polymorphism revealed statistical differences for b/b ($P=0.025$, OR=3.9, 95 % CI in 1.2–13.0), groups (Tables 2 and 3).

Table 3 Allele frequency of BsmI and FokI in MS patients and healthy controls

SNP	Allele	MS patients Number (%)	Healthy Controls Number (%)	*P* value	Odds ratio (95 % CI)
BsmI	B	168 (74.3)	155 (63.5)	0.012	1.7 (1.1–2.5)
	b	58 (25.7)	89 (36.5)		
FokI	F	178 (78.8)	215 (88.1)	0.006	2.0 (1.2–3.3)
	f	48 (21.2)	29 (11.9)		

Discussion

Although several studies have been conducted in this field for many years, there is still the question of whether vitamin D is protective against multiple sclerosis disease or not (Simpson et al. 2011). Because of the extent of the disease, which is inversely related to the geographical conditions such as the levels of sunlight exposure, and the fact that vitamin D is produced in the skin in response to sunlight, the role of vitamin D in relation to MS has been introduced and is being considered as a treatment strategy (Wang et al. 2012). However, different studies revealed controversial findings about the serum and CSF level of vitamin D in MS patients (Moghtaderi et al. 2013b). When the hormonal metabolite of vitamin D is produced in the kidney, it is counted as a ligand for VDR. Following the ligand-receptor complex formed in the cytoplasm, VDR acts as a ligand-activated transcription factor and causes the transcription of many genes (Jurutka et al. 2001). Therefore, the functions of vitamin D and VDR are closely associated with each other, and VDR operates the impact of vitamin D metabolism in regulating mineral homeostasis and the immune system responses (Durrin et al. 1999; Valdivielso and Fernandez 2006). Genetic variation of the VDR gene may have no impact on the structure of the VDR protein, but it may be a marker of disease modulation by affecting on the stability of the mRNA or protein translation pathway. VDR gene (chromosome 12q13.11, OMIM 601769) contains many polymorphic regions; two of these single nucleotide polymorphisms (SNP) are located in intron 8 (BsmI) and in exon 2 (FokI). FokI is a known DNA sequence variant at the start codon (ATG) of VDRG and produces a longer 3-amino acid protein. Most studies revealed that the short form of VDR protein is more active than the long form as a transcription factor (Smolders et al. 2009b; Valdivielso and Fernandez 2006). BsmI—a genetic variation at the 3′-end of the VDRG—has no known effect on VDR function, nevertheless, it is in association with some diseases. The association of these two polymorphisms with many diseases has been the subject of some investigations, and according to the population type and environmental situations, there are contradictions in results (Uitterlinden et al. 2004b). In the current study which was conducted on 113 patients (Tizaoui et al. 2014) with MS were investigated. No difference in the incidence of FokI genotypes was observed in patients with healthy controls. As shown in Tables 2 and 3, P value and OR of FokI genotypes confirm no association with MS disease. These results are compatible with the results of other studies in Canada (Steckley et al. 2000), Australia (Dickinson et al.

Table 2 Genotypic frequencies for BsmI and FokI polymorphisms in MS patients and healthy controls

SNP	rs	Genotype	MS patients Number (%)	Healthy Controls Number (%)	Odds ratio (95 % CI)	*P* value
BsmI	1544410	BB	59 (52.2)	45 (36.9)	Ref=1.00	
		Bb	50 (44.3)	65 (53.3)	1.7 (1.0–2.9)	0.051
		bb	4 (3.5)	12 (9.8)	3.9 (1.2–13.0)	0.025
FokI	2228570	FF	73 (64.6)	93 (76.2)	Ref=1.00	
		Ff	32 (28.3)	29 (23.8)	0.7 (0.4-1.3)	0.257
		ff	8 (7.1)	0 (0)	–	0.999

2009), Netherland (Smolders et al. 2009a; Smolders et al. 2008), and Spain (Garcia-Martin et al. 2013). In another study conducted in the United States of America, FokI revealed no association with MS; however, ff genotype showed protective association with MS in patients who received a high intake of vitamin D (Simon et al. 2010). A novel meta-analysis by Tizaoui et.al, reported that FokI polymorphism in VDR gene was marginally related with asthma risk which is in contrast to our result (Tizaoui et al. 2014). Another discrepancy with our result was seen in protective association of ff genotype with MS (Partridge et al. 2004). Our results showed that FokI/F allele was protective for MS, while FokI/f allele showed the positive association with MS (P=0.006). Frequency analysis of BsmI genotypes showed positive association for b/b with susceptibility to MS (P=0.025). As P=0.051 was obtained for B/b genotype, perhaps there is also a significant difference for this genotype in two study group, but proving this claim requires more numbers of samples. According to the results showed in Tables 2 and 3, BsmI b/b genotype seems to correlate with protective effect to MS while BsmI B/B genotype associates with susceptibility to MS. Moreover, BsmI/b allele was protective for MS, while BsmI/B allele showed the positive association with MS (P=0.012). In agreement with our study, Shojapuor et.al, reported positive association between BsmI-VDR polymorphism and susceptibility to multiple sclerosis in Markazi Province, Iran (Shojapuor et al. 2012). Furthermore, BsmI was significantly associated with asthma risk in Tizaoui et.al, meta-analysis (Tizaoui et al. 2014). Although, in two studies in Japan, overexpression of BsmI b/b genotype and BsmI/b allele were reported, which is in contrast with our results (Fukazawa et al. 1999; Sioka et al. 2011). Although the role of some VDR gene polymorphisms such as BsmI has not been identified in gene expression, it is located in the 3′-end of the gene and shows strong linkage disequilibrium with 3′-UTR and probably has an impact on the regulation of gene expression. Although FokI polymorphism locates in the start codon and causes a 3 amino acid longer protein, it is not associated with MS in most studies. The results of a recent study in the same cohort, as our study, showed no significant difference in the serum/CNS level of 25-hydroxyvitamin D3 (25-OH-D3) concentration between the MS patients and the control group (Moghtaderi et al. 2013b). Therefore, with the data available in the recent research and also our results about the association of VDR polymorphisms with MS mentioned above, it seems that there is still a need for further the investigations in this field to obtain more definitive results.

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Conflict of Interests The authors declare that there is no conflict of interests.

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