Iraqi JMS

Published by Al-Nahrain College of Medicine
P-ISSN 1681-6579
E-ISSN 2224-4719
Email: iraqijms@colmed.nahrainuniv.edu.iq
http://www.colmed-alnahrain.edu.iq
http://www.iraqijms.net
Iraqi JMS 2022; Vol. 20(1)

The Impact of Very Late Antigen 4 Polymorphism on Drug Responsiveness in Patients with Multiple Sclerosis Initiation

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Abstract

Background Very late antigen 4 (VLA4) integrin facilitates the immune cells migration to central nervous system (CNS) through blood brain barrier (BBB), so the polymorphism in this gene may be considered as genetic risk factor for multiple sclerosis (MS) occurrence. It may interact with the responsiveness level of Natalizumab.

Objective To show if VLA4 single nucleotide gene polymorphism (SNP) (C-269-A) considered as genetic predisposition factor for MS and if have a role in Natalizumab (Tysabri) drug non-responsiveness.

Methods Sixty-six (66) person with MS and 60 healthy persons involved in this study, their ages were range from 14 to 67 years. They attended to seek treatment in the MS outpatient's clinic, at Baghdad Teaching Hospital, Medical City Complex from December 2018 to March 2020. Patient were divided into two group; resistant group (34) and response group (32). The VLA-4 SNP polymorphism investigated by sequence specific primer polymerase chain reaction (SSP-PCR) technique.

Results The VLA4 gene SSP-PCR genotyping revealed no significant differences between patients and control group also between responder patients and non-responder to Natalizumab.

Conclusion VLA-4 polymorphisms at the level of SNP at positions 269 (C/A) have no role in MS susceptibility or Natalizumab responsiveness.

Keywords MS, Natalizumab, VLA-4

Citation Khaliel AH, Abbas AA, Hatem AO, Abdulamir AS. The impact of Very Late Antigen 4 polymorphism on drug responsiveness in patients with multiple sclerosis initiation. Iraqi JMS. 2022; 20(1): 83-89. doi: 10.22578/IJMS.20.1.11

List of abbreviations: SSP-PCR = sequence specific primer polymerase chain reaction, VLA = Very late antigen, VCAM-1 = Vascular cell adhesion molecule

Introduction

Multiple sclerosis (MS) is the most common neurological immune-mediated disorder of the central nervous system (CNS) that affects patients in the most active and productive time of their lives by causing physical disability and mental retardation [(1)]. The disease is characterized by inter-individual differences in its course and response to immunomodulatory therapy [(2,3)]. It believed that MS is initiated by immune dysregulation triggered by genetic and environmental factors [(4)].

The very late antigen 4 (VLA-4) (alpha 4: beta 1; CD49d / CD29) - approved symbol (ITGA4) integrin is a gene with protein product involved in both cellular adhesion to extracellular matrix and cell-cell interactions [(5)].

Integrin α4β1 (VLA 4) is an integrin dimer. It is consisting of CD49d (alpha 4) and CD29 (beta 1), it is expressed on the cell surfaces of

progenitor cells, stem cells, T cells, B cells, natural killer cells, monocytes, eosinophils and neutrophils. It functions to consolidate an inflammatory response by the immune system through assisting in the movement of leukocytes to tissue that requires inflammation, and it is the main player in cell adhesion [6,7]. After a chemotactic agent or other stimuli activate the leukocytes, the VLA-4 will adhere to its appropriate ligand. The primary ligands of VLA-4 include vascular cell adhesion molecule 1 (VCAM-1) and fibronectin [8].

In MS, the VLA-4 integrin is essential for T cell passing to the brain. It allows the cells to penetrate the blood brain barrier (BBB) that normally limits immune cell access. In many studies, the researchers found the severity of MS is positively correlated with the expression of alpha four. One approach to prevent an autoimmune reaction has been to block the action of VLA-4 so that self-reactive T-cells are unable to enter the brain and thus unable to attack myelin protein [9].

The polymorphic α4-subunit of VLA-4 gene represents a good target for association with MS. Previous study investigated the association between VLA-4 gene polymorphisms and MS on 275 patients and 255 controls, focused on two genetic polymorphisms of the α4-subunit; the first, a single point mutation at position 3061 producing an arginine (CGG) to glutamine (CAG) trans version, the second, a C to A transversion at position 269 in the promoter region of exon [10].

Natalizumab (NTZ) is a humanized monoclonal IgG4 antibody blocking the a4-integrin subunit, its acts as a α4 integrin antagonist to prohibit leukocyte trafficking into the central nervous system [11,12]. It is approved by United States Food and Drug Administration (FDA) for the treatment of relapsing–remitting multiple sclerosis (RRMS). Recently, a study established to identify pharmacogenetic factor(s) associated with MS patients' response to NTZ, this study found that the variant (rs2304166) in GP6 gene is associated with poor response to NTZ in homozygous CC genotype MS patients [13].

So, we designed this study to identify the genetic impact of VLA-4 gene on MS initiation and NTZ responsiveness.

Methods

Sixty-six patients (66) with MS were involved in this case control study. Their ages were range from 14 to 67 years. They were attended for seeking treatment in the MS outpatient's clinic, at Baghdad Teaching Hospital, Medical City Complex in the period extended from December 2018 to March 2020.

They diagnosed according to McDonald criteria [14] by a neurologist and the diagnosis confirmed by magnetic resonance imaging and some cases by oligoclonal band test in the cerebrospinal fluid. Patients were subjected to questionnaire about name, age, sex, smoking, family history, medication, number of relapses in the last year, type of medication, and first clinical signs during diagnosis.

According to Rio criteria [15], the patients were divided into two groups, group I (32) responder to NTZ (Tysabri) and group II (34) non-responder to NTZ (Tysabri). The Institutional Board Review (IRB) committee of College of Medicine, Al-Nahrain University approved this study, and all samples were obtained with permission of Ministry of Health declaration.

After explaining the objective of the current study and agreed to accession of the study, sixty volunteers were involved as controls, their sex and ages were matched with patients' group were included in this work as control. All of them received no treatment with no complaint of other chronic or systemic diseases; not suffering from any neurological signs in the last 2 years their age range was (16-68) years.

Inclusion criteria

Multiple sclerosis patients on NTZ for more than 1 year.

Exclusion criteria

We excluded the patients whom not stick to treatment and have a period of treatment discontinuous.

The detection of ITGA4 single nucleotide gene polymorphism (SNP) rs113276800 (−269C/A) in the present study was done by the amplification-refractory mutation system (ARMS). Two ml of venous blood were drawn from patients and controls in EDTA tube for DNA extraction, which used in SSP-PCR for 66 patients and 60 control, the DNA kept in Eppendorf tube -20℃ till used.

Kits

1. DNA extraction Kit (Geneaid, Taiwan)
2. PCR Kit (Bioneer, Korea)

Procedure

Molecular detection of ITG4 SNP- rs113276800 (−269C/A) in blood sample was done by polymerase chain reaction ARMS. The master mix which used is ready master mix (Accupower PCR premix/ Korea). One microliters of each primer (foreword and reverse as in table 1) and three microliter of template DNA were added to the master mix tube. The final volume was adjusted to 20 ul with free nuclease distal water. The mixture was then vortexed for 10 seconds and put in thermocycler (Bioneer, Korea), which was previously programmed with the following (Table 2).

Table 1. VLA4 primer sequence

Polymorphism	Primer	Sequence	Product length	Method
C 269-A EX 1	269 C	5′-ACGCTCCGCCCGCGGTGGGC-3′	251 bp	PCR-SSP (ARMS)
	269 A	5′-ACGCTCCGCCCGCGGTGGGA-3′		
	269 R	5′-CAGCAACAGCATCACCGTCT-3′		

Table 2. VLA-4 gene amplification PCR program

Temperature	Time	Cycle
95°C	5 minutes	1X
94°C	45 seconds	
61°C	30 seconds	30 X
72°C	30 seconds	
72°C	7 minutes	1 X

Statistical analysis

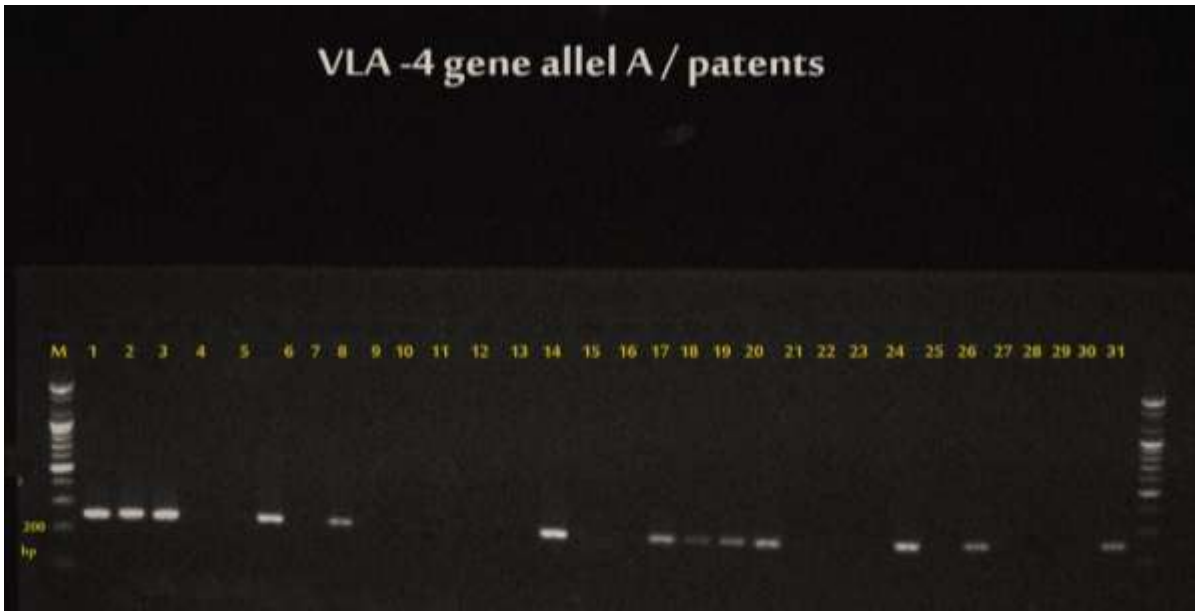
The statistical package for the social sciences V26 (SPSS Inc., Chicago, USA) was used. By comparing the observed and expected frequencies (Chi-square test), the polymorphisms were tested for deviation from Hardy Weinberg Equilibrium (HWE). The association between genotype and risk of MS and drug responsiveness was valued by calculation of odds ratio (OR) with 95% confidence interval (95% CI). Statistical significance was set at a p value <0.05.

Results

There were no significant differences between patients and control in the frequency of different age group.

The ARMS was used to amplify the VAL4 gene using specific set of primers. The PCR products are shown in figure 1 (A & B).

A



B

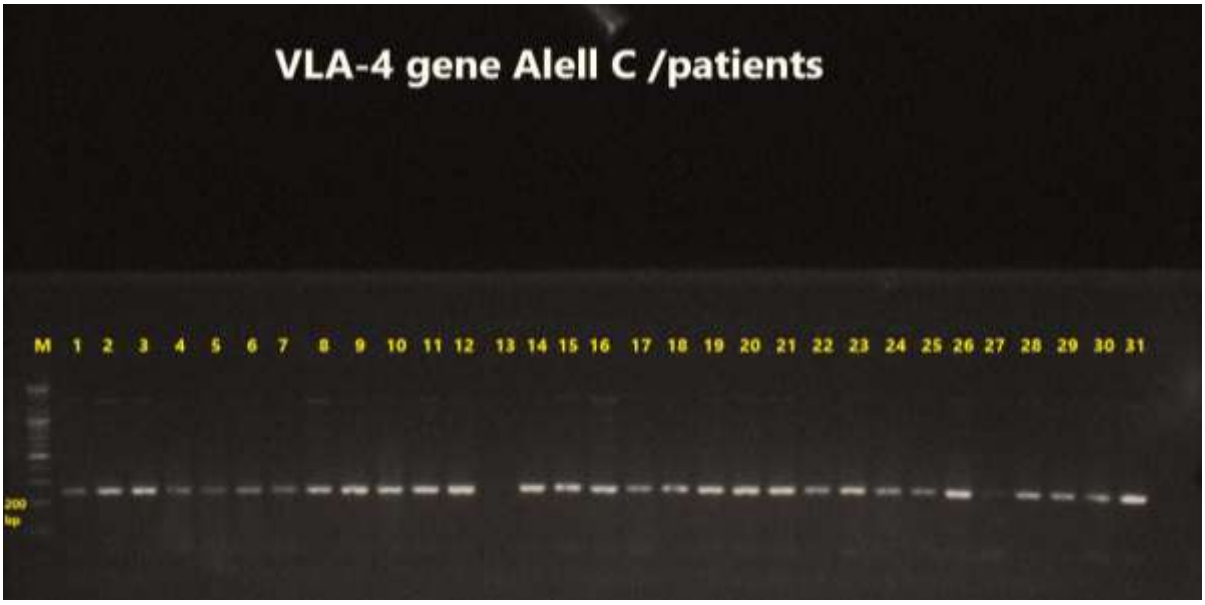


Figure 1. PCR products of VLA-4 Gen; A: A allele in patients, B: C allele in patients

The genotype and allele frequency of VAL4 SNP in patients and controls are shown in table 3. The frequency of different genotypes and alleles of this SNP was almost similar between patients and controls with no significant differences.

Likewise, the distribution of genotypes and allele of VAL4 SNP in responsive and non-responsive patients was almost identical with no significant differences (Table 4).

Table 3. The genotype and allele frequency of VAL4 SNP in patients and controls

VAL4		Controls (50)	Patients (66)	*P*-value	OR (95% CI)
Genotypes	CC	26 (52.0%)	34 (51.52%)	0.587	1.0 Reference
	CA	23 (46.0%)	28 (42.42%)	0.330	0.33 (0.03-3.1)
	AA	1 (2.0%)	4 (6.06%)	0.302	0.3 (0.03-2.92)
	HWE	0.109	0.573		
Alleles	C	75 (75.0%)	٩٦ (72.73%)	0.697	1.0 Reference
	A	٢٥(٢٥.0%)	٣٦ (27.27%)		1.13 (0.62-2.04)

Table 4. The frequency of different genotypes and allele of VAL4 polymorphism in responsive and non-responsive patients

VAL4		Responsive (32)	Non-responsive (34)	*P*-value	OR (95% CI)
Genotypes	CC	16 (50.0%)	18 (52.94%)	0.972	1.0 Reference
	CA	14 (43.75%)	14 (41.18%)	0.911	0.89 (0.11-7.06)
	AA	2 (6.25%)	2 (5.88%)	1.000	1.0 (0.12-8.13)
	HWE	0.346	0.736		
Alleles	C	46 (71.88%)	50 (73.53%)	0.831	1.0 Reference
	A	18 (28.12%)	18 (26.47%)		0.92 (0.43-1.98)

Discussion

As MS initiation needs to cross of immune cells into the CNS, the VLA-4 gene may be considered as a probable candidate genetic risk factor for susceptibility to MS. Therefore, the current work studied the association between SNP at positions 269 (C/A) in the VLA4 gene and the risk of MS. Only a few studies have analyzed the genetic predisposition of VLA-4 (α4β1 integrin) to chronic inflammatory diseases of CNS, including MS.

Ďurmanová et al. (2018) studied the ITGA4 gene polymorphism encoding the VLA-4 α4 subunit with increased risk of Alzheimer's disease, they observed no statistically significant differences in concern ITGA4 −269C/A gene polymorphism (rs113276800) between patients and control [16]. Taher et al (2018) have investigated the rs1143676 (+3061A/G) of VLA-4 gene polymorphism and its association with MS risk in Iranian population, their result showed significant differences in genotype and allele frequencies between the MS patients and healthy subject [17]. Correia et al. (2009) found an association between rs155100 SNP located in the intron 9 of the integrin α4 gene and autism [18].

The current study showed no significant differences between MS patients and healthy control group in concern to C/A transversion at position 269 in the promoter region of exon 1 , this result also obtained by Andreoli et al. (2007), Ďurmanová et al. (2015) [10,19].

On the other hand, the present study revealed the homozygous AA genotype was detected in 2% and 6.06% in control and patients respectively. This outcome disagrees with Hilger-Eversheim et al. (2000), who suggested that no homozygous 269 AA genotype could be observed as the 269 (C/A) polymorphism is

located in the α4 promoter region near the AP-2 binding sites, the AA variant may be responsible for the negative gene expression causing the functional impairment of the α4 subunit [(20)].

In the present study, the 269 (C/A) polymorphism genotyping showed no significant differences between the responder and non-responder patient. This polymorphism never studied as a cause of NTZ (Tysabri) unresponsiveness.

Until 2019, there was only two studies analyzed the pharmacogenetic reasons of NTZ (Tysabri) unresponsiveness [(21,22)]. Recently, a study has established to identify pharmacogenetic factor(s) associated with MS patients' response to NTZ (Tysabri), which found that the variant (rs2304166) in GP6 gene is associated with poor response to NTZ (Tysabri) in homozygous CC genotype MS patients. Al-Mojel et al. (2019) investigated the possible inference of genes encoding detoxification enzyme GSTP1 and NQO1 polymorphisms on NTZ (Tysabri) response in MS, this study concluded a significantly increased frequency of double NQO1 an2d GSTP1 mutant polymorphisms in non-responders compared to the responders [(23)].

In conclusion, VLA-4 polymorphisms at the level of SNP at positions 269 (C/A) have no role in MS susceptibility or NTZ responsiveness.

Acknowledgement

A greeting for medical staff and sub-staff in the MS clinic in Baghdad Teaching Hospital for their help in samples collection and providing data concerning patients and eliminating all difficulties and many thanks for Al-Hayat Society for MS patient.

Author contribution

Khaliel and Dr. Abbas designed the study and did the laboratory works. Dr. Abdulamir designed the primers. Dr. Hatem determined the responsiveness to Natalizumab criteria and detected the responsiveness group and the non-responsiveness group.

Conflict of interest

Authors declares there is no conflict of interest.

Funding

Self-funded.

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Received Nov. 8th 2020
Accepted Dec. 16th 2020