Sphingosine-1-phosphate receptor 1 polymorphism as a cause of Fingolimod unresponsiveness and Multiple Sclerosis initiation

Alaa Hassan Khaliel¹, Ahmed Abdul-Hassan Abbas², Anmar Oday Hatem³, Ahmed Sahib Abdulamir⁴

¹ M.Sc., Ministry of Health, Baghdad, Iraq
² Prof./Dept. of Microbiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq
³ M.B.Ch.B – FIBMS-FACP/Baghdad Teaching Hospital – MS Clinic, Baghdad, Iraq
⁴ Prof./Dept. of Microbiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq

Corresponding author: Alaa Hassan Khaliel, Email: alaaaltamemy79@gmail.com

Abstract

Background: Alot of immune cells have sphingosine-1-phosphate receptor (SIPR) which regulates its migration from lymph node to blood circulation. The SIPR polymorphisms have been the focus of some studies as genetic risk factor for multiple sclerosis and drug responsiveness. Aim: This study was aimed to show if the genetic variations of the S1P1 have a role in Fingolimod/Gilenya (FTY720) unresponsiveness and if S1P1 polymorphisms were considered as genetic predisposition factor for MS. Materials and methods: This Case control study involved: sixty-six (66) with multiple sclerosis (MS), their ages were range from 14 to 69 years. They attended to seek treatment in the MS outpatient’s clinic at Medical City- Baghdad Teaching Hospital in the period, which extended from December 2018 to March 2020. Patient was divided into two group resistant group (34) and response group (32) to fingolimod (Gelyinia) treatment. Results: The SIPR 1 gene sequencing revealed two SNPs rs3737577 and rs3737578 without significant differences between control group and patients, responder patients and non-responder patients on the level of genotype and allele frequency. A comparison between responsive and resistant patients revealed that the haplotype block TT was more frequent among non-responder patients (30.88%) than responder patients (15.63%) with a significant difference (OR= 2.41, 95%CI=1.03-5.64, p= 0.042). Conclusion: There is no relationship between SIPR1 gene polymorphism and the responsiveness to the fingolimod in MS patients, but the TT haplotype block in this gene may have a negative effect on response to that medication also this gene polymorphisms cannot considered as genetic risk factor for MS initiation.

Keyword: Multiple Sclerosis, SIPR1 and Fingolimod (Gelyinia)


Introduction

Multiple sclerosis (MS) is a chronic, inflammatory immune-mediated, neurodegenerative disorder. It is the leading non-traumatic cause of disability in young adults and affect three times women than men (1-2). The deep heterogeneity of MS is not restricted to the symptoms, but to histologic appearances of lesions, neuroradiologic and response to therapy (3). Over the past two decades, different novel disease-modifying drugs for multiple sclerosis (MS) have been approved. However, there are high differences in the patient response to the available medications, which is hypothesized to be partly attributed to genetics (4). Sphingosine-1-phosphate (S1P) is a normal bioactive lipid molecule and a common...
or second messenger in the immune systems and cardiovascular. By binding with its receptors, S1P can work as mediator of signaling during cell differentiation, migration, proliferation and apoptosis. Until 2010, there was no oral drug for MS patients. In 2010, FDA approved Fingolimod (Gilenya) for the treatment of relapsing multiple sclerosis (MS) and marketed as the first effective oral alternative to injection therapy. It is a sphingosine 1-phosphate receptor modulator, impairing egress of peripheral T and B cells from secondary lymphoid tissue into blood, which lead to reducing access to the central nervous system (CNS). Some studies suggesting neuroprotective effect. Few studies suggest that individual genetic variations of sphingosine -1- phosphate receptor can influence receptor function and, therefore, infer differential disease susceptibility and interaction with sphingosine -1- phosphate receptor targeted Therapeutics. Human S1PR1 variants have been reported to have functional heterogeneity in vitro, suggesting that S1PR1 function may influence fingolimod efficacy. There is a limitation in the references that referred to the polymorphisms in the S1PR1, its relationship with multiple sclerosis and interaction with the fingolimod treatment. All of studies investigated the polymorphisms this gene with another diseases like Graft-versus-host disease, asthma, acute respiratory distress syndrome ARDS lung diseases. The S1PR modulators are currently under clinical trials for diverse pathophysiological conditions. There are significant Trials in targeting various components of S1P signaling for different diseases. So, this study was aimed to show if the genetic variations of the S1P1 have a role in Fingolimod/Gilenya (FTY720) unresponsiveness and if S1P1 polymorphisms were considered as genetic predisposition factor for MS.

Materials and methods
This Case control study involved; sixty six with multiple sclerosis (MS) their ages were range from 14 to 69 years. They were attended for seeking treatment of fingolimod in the MS out patient’s clinic at Medical City- Baghdad Teaching Hospital in the period, which extended from December 2018 to March 2020. The diagnosis of each case was established according to MC Donald criteria done by a neurologist and confirmed by MRI and certain cases by oligoclonal band test in the CSF. Patients were subjected to questionnaire about name, age, sex, smoking, family history, medication, type of medication, number of relapses in the last year and first clinical signs during diagnosis. Patients were divided into two groups of responder and non-responder, the responsiveness to treatment was detected according to Rio criteria. The ethical committee of College of Medicine/Al-Nahrain University approved this study, and all samples were obtained with informed consent in accordance with the Ministry of Health declaration. Sixty volunteers after explaining the objective of the current study and agreed to accession of the study, 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 fingolimod for more than 1 year

Exclusion criteria
Patients whom not adhere for treatment and have a period of treatment discontinuous

Deoxyribonucleic acid extraction and SIPR1 Gene Sequencing:
Two ml of venous blood were drawn from patients and controls in EDTA tube for DNA extraction which used in gene sequencing for 66 patients and 60 control, the DNA kept in Eppendorf tube at -20°C till used. We used DNA extraction
Kit (Geneaid – Tiwan) and PCR Kit (Bioneer /Korea). A specific pair of primers (table 1) was used to amplify S1PR1 gene (EDG1) with an expected amplicon of 1331 bp. Gel electrophoresis of PCR products was done.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Product length</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>forward</td>
<td>5’-TCTCCAGCCAAGGAAAGC-3’</td>
<td>1331 bp</td>
<td>Conventional PCR</td>
</tr>
<tr>
<td>Reverses</td>
<td>5’-AGTAAAGAGCGCTTCCGG-3’</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (1): SIPR1 primer sequence

The coding sequence of the human S1PR1 gene was amplified by PCR using the human genomic DNAs as templates. The master mix which used is ready master mix (AccuPowerTM PCR premix/ Korea). One micro of each primer (forward 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 25μl with free nuclease distal water. The mixture was then vortexed for 10 seconds and put in thermocycler (Bioneer / Korea) which was previously programmed as shown in table 2.

Table (2): SIPR1 PCR amplification programme

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Time</th>
<th>Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial denaturation</td>
<td>95°C</td>
<td>5 mints</td>
<td>1X</td>
</tr>
<tr>
<td>Denaturation</td>
<td>94°C</td>
<td>45 second</td>
<td></td>
</tr>
<tr>
<td>Annealing</td>
<td>62°C</td>
<td>30 second</td>
<td>30 X</td>
</tr>
<tr>
<td>Extension</td>
<td>72°C</td>
<td>30 second</td>
<td></td>
</tr>
<tr>
<td>Final extension</td>
<td>72°C</td>
<td>7 mints</td>
<td>1 X</td>
</tr>
</tbody>
</table>

PCR product was sent for Sanger sequencing using ABI3730XL, automated DNA sequence, by Macrogen Corporation – Korea. The results were received by email then analyzed using genius software.

**Statistical analysis**

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

**Results**

The results which presented in this study were built on the analysis of 66 patients with MS on fingolimod, in comparison with 60 apparently healthy individuals considered as controls. Overall, there were no significant differences between patients and control in the frequency of different age group. Younger age group (<30 years) was the most affected group with MS representing 37% of the patients; while the older age group (≥50 years) was the least frequent, the mean age of patients group was 35.6±10.7(13-58) and in control group was 34.9 ±10.3(18-53) . Similarly, the groups were comparable regarding Sex distribution (Table 3)
Polymorphisms in S1PR1 gene were investigated for their association with the development of MS as well as with the drug resistance. A specific pair of primers was used to amplify S1PR1 gene with an expected amplicon of 1331 bp. Gel electrophoresis of PCR products is shown in figure 1. The PCR products were directly sequenced, the results of which revealed two SNPs.

![Gel electrophoresis of S1PR1 gene amplified with specific pair of primers using conventional PCR.](image)

**Figure (1):** Gel electrophoresis of S1PR1 gene amplified with specific pair of primers using conventional PCR. The PCR products were stained with ethidium bromide. Lanes 1-7 successful amplification with 1331 bp PCR product. M: molecular marker

rs3737578 polymorphism appeared in only two genotypes (TT and CT) in patients (responsive or resistance) and controls (Figure 2).

![Sequence analysis of the, thers3737578 forward strand.](image)

**Figure (2):** Sequence analysis of the, thers3737578 forward strand. The bases in the frames represent the polymorphism sites. The T in the upper frame represents homozygous wild type genotype (TT), the T in the lower frame represents the heterozygous genotype (CT)

Although the TT genotype was more frequent in patients than controls (89.3% vs. 72%), the difference was not significant (OR= 0.63, 95%CI=0.27-1.5, p= 0.295), as shown in table (4).

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**Table 3:-Demographic Characteristics of the Study Population**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Controls (60)</th>
<th>MS patients treated with Gelyinia (66)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;30</td>
<td>24(40%)</td>
<td>25(37.88 %)</td>
<td>0.749</td>
</tr>
<tr>
<td>30-39</td>
<td>12(20%)</td>
<td>19(28.79%)</td>
<td></td>
</tr>
<tr>
<td>40-49</td>
<td>18(30%)</td>
<td>16(24.24%)</td>
<td></td>
</tr>
<tr>
<td>≥50</td>
<td>6(10%)</td>
<td>6(9.09%)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>24(40%)</td>
<td>28(42.42%)</td>
<td>0.783</td>
</tr>
<tr>
<td>Female</td>
<td>36(60%)</td>
<td>38(57.58%)</td>
<td></td>
</tr>
</tbody>
</table>
Table (4): The frequency of different genotypes and allele of rs3737578 and rs3737577 polymorphism in MS patients and controls

<table>
<thead>
<tr>
<th></th>
<th>MS patients (66)</th>
<th>Controls (50)</th>
<th>P-value</th>
<th>OR(95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>rs3737578</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotypes</td>
<td>TT 13(19.7%)</td>
<td>TC 53(80.3%)</td>
<td>0.295</td>
<td>1.0 Reference</td>
</tr>
<tr>
<td>HWE</td>
<td>0.375</td>
<td>0.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alleles</td>
<td>C 119(90.15%)</td>
<td>T 13(9.85%)</td>
<td>0.329</td>
<td>1.0 Reference</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>rs3737577</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotypes</td>
<td>GG 36(54.55%)</td>
<td>GT 28(42.42%)</td>
<td>0.445</td>
<td>1.0 Reference</td>
</tr>
<tr>
<td></td>
<td>GC 2(3.03%)</td>
<td>TT 2(3.03%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HWE</td>
<td>0.208</td>
<td>0.633</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alleles</td>
<td>G 100(75.76%)</td>
<td>A 32(24.24%)</td>
<td>0.689</td>
<td>1.0 Reference</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HWE: Hardy–Weinberg equation.

Also, there was a non-significant difference in the frequency of TC genotype and C allele of this polymorphism among resistance patients (21.87% and 10.94%, respectively) compared with responsive patients (17.65% and 8.28%, respectively) as shown in table (5).

Table (5): The frequency of different genotypes and allele of rs3737578 and rs3737577 polymorphism in resistance and responsive patients to treatment with Gelyinia

<table>
<thead>
<tr>
<th></th>
<th>Resistance (34)</th>
<th>Responsive (32)</th>
<th>P-value</th>
<th>OR(95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>rs3737578</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotypes</td>
<td>TT 6(17.65%)</td>
<td>TC 28(82.35%)</td>
<td>0.154</td>
<td>1.0 Reference</td>
</tr>
<tr>
<td></td>
<td>6(17.65%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HWE</td>
<td>0.573</td>
<td>0.487</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alleles</td>
<td>C 62(91.18%)</td>
<td>T 6(8.82%)</td>
<td>0.684</td>
<td>1.0 Reference</td>
</tr>
<tr>
<td></td>
<td>6(8.82%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>rs3737577</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotypes</td>
<td>GG 19(59.37%)</td>
<td>GT 13(40.63%)</td>
<td>0.445</td>
<td>1.0 Reference</td>
</tr>
<tr>
<td></td>
<td>GC 17(50%)</td>
<td>TT 17(44.12%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HWE</td>
<td>0.149</td>
<td>0.577</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alleles</td>
<td>G 51(79.69%)</td>
<td>A 13(20.31%)</td>
<td>0.307</td>
<td>1.0 Reference</td>
</tr>
<tr>
<td></td>
<td>13(20.31%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HWE: Hardy–Weinberg equation.
The three genotypes of rs3737577 polymorphism (GG, GT and TT) are shown in figure 3.

Figure (3): The frequency of different genotypes of Rs3737577 polymorphism in patients and controls.

The variation in the distribution of these genotypes, as well as in alleles, between patients and controls was too small to reach a significant level (table 4).

The comparison between the frequency of different genotype and allele of rs3737577 polymorphism revealed no significant differences between responsive and non-responsive patients as shown in table 5.

The frequency of the most common three haplotype blocks was comparable between patients and controls with no significant differences, table 6.

<table>
<thead>
<tr>
<th>Haplotype blocks</th>
<th>Controls (n=50)</th>
<th>Patients (n=66)</th>
<th>p-value</th>
<th>OR(95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>15(15%)</td>
<td>13(9.85%)</td>
<td>0.236</td>
<td>0.62(0.28-1.39)</td>
</tr>
<tr>
<td>TG</td>
<td>64(64%)</td>
<td>89(67.42%)</td>
<td>0.586</td>
<td>1.16(0.67-2.01)</td>
</tr>
<tr>
<td>TT</td>
<td>20(20%)</td>
<td>30(22.73%)</td>
<td>0.617</td>
<td>1.18(0.62-2.23)</td>
</tr>
</tbody>
</table>

A comparison between responsive and resistant patients revealed that the haplotype block TT was more frequent among resistant patients (30.88%) than responsive patients (15.63%) with a significant difference (OR= 2.41, 95%CI=1.03-5.64, p= 0.042), while there were no significant differences between the two groups in the frequency of haplotype TG or CG (Table 7).

<table>
<thead>
<tr>
<th>Haplotype blocks</th>
<th>Responsive (n=32)</th>
<th>Resistance (n=34)</th>
<th>p-value</th>
<th>OR(95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>7(10.94%)</td>
<td>6(8.82%)</td>
<td>0.684</td>
<td>0.79(0.25-2.48)</td>
</tr>
<tr>
<td>TG</td>
<td>47(73.44%)</td>
<td>42(61.76%)</td>
<td>0.154</td>
<td>0.58(0.28-1.22)</td>
</tr>
<tr>
<td>TT</td>
<td>10(15.63%)</td>
<td>21(30.88%)</td>
<td>0.042</td>
<td>2.41(1.03-5.64)</td>
</tr>
</tbody>
</table>

Discussion

The sequencing of S1PR1 gene lead to the distinction of two types of SNPs rs3737578 and rs3737577.

The TT genotype of rs 3737578 was more frequent in the MS patients than control group (89.3% vs. 72%) respectively. Nevertheless, the increase was not significant. Beside there are no significant differences between the patients and control in concern of the rs 3737577. Hideru Obinata et al 2014 suggested another two SNPs may change the S1PR1 function, and suggested that individual genetic variations of S1PR 1 can effect receptor function and, therefore, may lead to increase disease risks of MS and interact with S1P 1 -targeted therapeutics (12). There is a limitation in the references that referred to the polymorphisms in the S1PR1, its relationship with multiple sclerosis and interaction with...
the Fingolimod treatment. Zhao, J et al 2020 (10) they studied the S1PR1 and its relationship with the acute respiratory distress syndrome and they found association of 2 SNPs other than that in current study and allot of studies analyzed this gene polymorphism and its interaction with asthma disease. Since the approval of Fingolimod in 2010 as first oral medication for active relapsing remitting MS, it has made a revolution in MS treatment, 57% reducing the annual relapsing rate in the patients (13). The reason of unresponsiveness in some patients until now is unclear. In present study, the statistical analysis of SIPRI genotyping results showed no significant differences between detected SNPs in responsive and non-responsive patients on fingolimod, but the comparison between responsive and resistant patients revealed that the haplotype block TT was more frequent among resistant patients (30.88%) than responsive patients (15.63%) with a significant difference. The TT haplotype block may be interfering with the S1PR1 configuration and that leads to resistant to fingolimod and interfere with the receptor function responsiveness. Moheghi and Nasrin 2017(14) suggested that responding to fingolimod was not associated with S1PR1 polymorphysim. The detected SNPs in this study mentioned in little studies but they were associated with other diseases other than multiple sclerosis (9-12).

Conclusion
There is no relationship between SIPR1 gene polymorphism and the responsiveness to the Fingolimod in MS patients, but the TT haplotypes block in this gene may have a negative effect on response to that medication also this gene polymorphisms cannot considered as genetic risk factor for MS initiation.

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Acknowledgment: 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

Conflict of interest: Authors declares there is no conflict of interest.

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