**ASSOCIATION STUDY OF CASITAS B-LINEAGE LYMPHOMA PROTO-ONCOGENE B (CBLB) GENE VARIANT AND MULTIPLE SCLEROSIS**

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**ABSTRACT**

**Introduction**: Multiple sclerosis is a complex inflammatory demyelinating disorder of central nervous system (CNS). Several genetic loci have been shown to be linked with this disorder. Among them is the Casitas B-lineage lymphoma proto-oncogene b (CBLB) gene whose protein product participates as a negative regulator of adaptive immune responses. In the current study we aimed at evaluation of the association between the rs12487066 single nucleotide polymorphism (SNP) within CBLB gene and MS in a population of Iranian patients.

**Materials and methods**: We designed a case-control association study registering 410 unrelated patients with sporadic MS and 428 healthy matched controls. Genotyping of rs12487066 was performed using tetra-primer amplification refractory mutation system PCR (T-ARMS-PCR).

**Results**: No statistically significant difference was observed in allele and genotype frequencies between the MS patients and healthy subjects.

**Conclusion**: Although the rs12487066 SNP has been shown to be associated with MS in other populations, our study suggest that this SNP is not linked with MS in Iranian population.

**Keywords**: Multiple sclerosis, polymorphism, CBLB.

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**Introduction**

Multiple sclerosis is a complex inflammatory demyelinating disorder of central nervous system (CNS) caused by T cell mediated destruction of myelin sheath1. A T helper-1 (Th1)-type skewing of the immune response towards proinflammatory cytokines (e.g. IL-2, interferon (IFN)-γ, IL-12 and TNF-α) has been implicated in the pathogenesis of MS2. Most of MS patients experience a numerous-year period of relapses and remissions of neurological deficit (relapsing–remitting MS [RRMS]), which is distinguished by a confirmed immune response and specific magnetic resonance imaging (MRI) features3.

As a complex multifactorial disorder, several genetic loci have been shown to be implicated in the pathogenesis of MS. A substantial portion of the MS risk variants participate in T cell proliferation or regulation of their activation which highlights the significance of dysregulation of T cell activation and function in MS pathogenesis4. The Casitas B-lineage lymphoma proto-oncogene b (CBLB) gene has been shown by genome wide association studies as a risk locus for MS5, 6. The protein coded by this gene has been shown to be a negative regulator of adaptive immune responses7. Its down-regulation has been demonstrated in CD4+ T cells from RRMS patients during relapse. The rs12487066 within this gene has been noted as a MS risk-related
single nucleotide polymorphism (SNP). The risk allele of this SNP has been shown to decrease CBLB expression levels and change the effects of type I IFNs on human CD4+ T cell proliferation (4). Consequently, in the current study we aimed at evaluation of the rs12487066 association with MS in a population of Iranian patients.

**Materials and methods**

**Patients**

We designed a case-control association study registering 410 unrelated patients with sporadic RRMS and 428 healthy matched controls. RRMS patients were selected from patients referred to Tehran Hospitals or registered by MS society of Iran. The diagnosis of RRMS was confirmed by specialized neurologists based on the revised McDonald criteria (8). Control group comprised healthy volunteers without MS or other inflammatory demyelinating diseases. Control subjects were matched with patients in the terms of sex, age-distribution and ethnic background. All participants signed written informed consent. Patients’ clinical and demographic data including age, sex, disease duration, age at onset and disease severity based on the Expanded Disability Status Scale (EDSS) (9) have been gathered as well. The current study was approved by the local ethical committee.

**Genotyping**

Genotyping of rs12487066 was performed using tetra-primer amplification refractory mutation system PCR (T-ARMS-PCR). FlexCycler (Analytik Jena, Germany) system was used for PCR. PCR was carried out using Taq 2x red master mix (Ampliqon, Denmark) with specific primers which were designed using the primer 1 software (10). The primer sequences are listed in Table 1. The PCR program comprised a primary denaturation at 95 °C for 5 minutes, followed by 35 cycles of 95 °C for 45 seconds, 58 °C for 35 seconds, and 72 °C for 45 seconds, with the final extension of 72 °C for 5 minutes. In order to confirm T-ARMS-PCR results we sequenced 10% of samples by using ABI 3730xl DNA analyzer (Macrogen, Korea).

<table>
<thead>
<tr>
<th>Primer sequence</th>
<th>Tm</th>
<th>Annealing temperature</th>
<th>PCR product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward inner primer</td>
<td></td>
<td>64 °C</td>
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<tr>
<td>(C allele): TAGATAAAGCTTAAGGACTAAGTAAAAGTGCTC</td>
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<tr>
<td>Reverse inner primer</td>
<td></td>
<td>64 °C</td>
<td>245 bp (T allele)</td>
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<td></td>
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<tr>
<td>Forward outer primer</td>
<td></td>
<td>64 °C</td>
<td>384 bp (two outer primers)</td>
</tr>
<tr>
<td>(GAATTCTCTGGAATTCTGGAATTCTGGGG)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reverse outer primer</td>
<td></td>
<td>64°C</td>
<td></td>
</tr>
<tr>
<td>TATTGCCTGAGTGGTTGTTTCGAGGAAA</td>
<td></td>
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</tr>
</tbody>
</table>

Table 1: Primer sequences and PCR conditions used for the rs12487066 genotyping.

**Results**

Demographic and clinical characteristics of study participants are shown in Table 2. The genotype distribution rs12487066 polymorphism in the controls and patients were in agreement with Hardy-Weinberg equilibrium (P>0.05). Sanger sequencing completely confirmed the genotyping results obtained by T-ARMS-PCR (data not shown). The genotype frequencies of the rs12487066 polymorphism for the MS patients and healthy subjects are demonstrated in Table 3. No statistically significant difference was observed in allele and genotype frequencies between the MS patients and healthy subjects.

**Discussion**

The CBLB rs12487066 has been shown to be a functional SNP within this gene whose risk allele alters binding of the transcription factor C/EBPb...
and leads to decreased CBLB expression in human CD4+ T cells\(^4\). CBLB is a RING-family E3 ubiquitin ligase which negatively control T cell receptor (TCR) and B cell receptor (BCR) function\(^7,12\). More importantly, Cblb knocked out mice have been shown to be vulnerable to experimental autoimmune encephalomyelitis\(^13\), which is an animal model for MS. However, in the current study we could not find any association between this SNP and risk of MS in an Iranian population. Different studies including large GWAS studies have described three CBLB MS risk SNPs comprising rs12487066\(^4\), rs9657904 from the CBLB promoter\(^5\) and rs2028597\(^6\).

However, a more recent ImmunoChip study did not confirm the association between either of these three SNPs and risk of MS\(^6\). It has been suggested that the ethnic origin of the population affects the results of GWAS studies leading to different results based on the assessed population\(^4\). In addition, a single variant such as the assessed SNP in the current study is expected to act just as a minor participant in the complex phenotype of MS, while its prominence and function for disease expression increases in definite genetic and environmental backgrounds\(^4\). This fact provides another explanation for the observed disagreement between the result of our study and the previous results regarding the role of rs12487066 in the pathogenesis of MS.

This discrepancy between the results of association studies of MS related SNPs in different population has been noted previously as well. This fact might point out to both different allele and genotype frequencies in diverse populations and genetic heterogeneity of MS\(^17\).

In addition, although Stürner et al. detected an association between rs12487066 and MS, they did not rule out a functional interaction between reported independent CBLB SNPs associated with MS. So they highlighted the necessity for a more comprehensive representation of ultimately tagged additional SNPs which could originate from high resolution sequencing data\(^4\). In brief, future studies are necessary in other ethnic groups to assess the association between the risk of MS and the rs12487066 SNP as well as other sequence variants within this gene.

### References


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