Lack of Correlation between CCL5 -28C/G Functional Polymorphism and Multiple Sclerosis in Tunisian Patients

Nadia Ben-Fredj1, Walid Ben-Selma2,*, Saber Chebel1, Mahbouba Frih-Ayed3, Mahmoud Letaief1, Aouni Mahjoub1 and Jalel Boukadida2

1Laboratory of Transmissible Diseases and Biological Active substances, LR99-ES27, Faculty of Pharmacy, University of Monastir, Avicenne street 5000, Monastir, Tunisia
2Laboratory of Microbiology and Immunology, UR02SP13, Farhat Hached University Hospital, Sousse, Tunisia
3Department of Neurology, Fattouma Bourguiba University Hospital, Monastir, Tunisia

Abstract

Multiple sclerosis is a chronic demyelinating disease of the human central nervous system (CNS) of a still unknown etiology. CCL5 is localized in white matter tracts undergoing demyelination, suggesting that this chemokine participates in the pathogenesis of disease by attracting inflammatory cells into the CNS. The CCL5 -28C/G functional polymorphism have been reported to be associated with multiple sclerosis, however, evidence remains conflicting. In the current study, we investigated distribution of the CCL5-28C/G in 51 patients with multiple sclerosis in comparison to 162 healthy blood donors. The data revealed no significant differences in the distribution of the CCL5-28C/G polymorphism in multiple sclerosis patients compared with the control group. To conclude, our study showed no association between CCL5 -28C/G polymorphism and risk development of multiple sclerosis in Tunisian patients.

Keywords: Multiple sclerosis; Polymorphism; CCL5

Introduction

Multiple sclerosis is the most common chronic inflammatory demyelinating and neurodegenerative disease of the central nervous system (CNS) [1]. MS usually affects young adults and women more frequently than men and is clinically characterized by subsequently appearing neurologic deficits (relapses) and complete or incomplete recoveries (remissions) [2]. About 80% of multiple sclerosis patients start with a relapsing–remitting course that, over time, transforms into a secondary progressive course. In a smaller group of patients (20%), multiple sclerosis begins with a primary progressive [3].

The immunopathogenesis of multiple sclerosis is not completely understood, both polygenic and environmental factors contribute to disease onset and/or clinical exacerbation [2,4,5]. Viral pathogens have been implicated in the etiology and pathogenesis of multiple sclerosis [6]. Among those, strong data implicates Epstein-Barr Virus (EBV) a human DNA virus, as we [7] and others investigators have recently reported [8-12]. In addition to infection, genetic influence on multiple sclerosis is substantial, as evidenced by the 20-fold risk increase for siblings of multiple sclerosis patients [13]. Part of the genetic risk is explained by the MHC class II locus (HLA-DR15) [14]. In 2007 several novel risk alleles for multiple sclerosis were identified by a genome-wide association (GWA) study [4] and others confirmed the susceptibility loci by meta-analyses and replication [15]. These findings indicate that analyzing candidate genes could be an interesting approach in the search for multiple sclerosis susceptibility genes, but that other genetic risk factors such chemokines and their receptors [16] still need to be studied in different ethnic human groups.

CCL5 belongs to the family of CC chemokines, which are involved in immunoregulatory and inflammatory processes owing to their ability to recruit, activate and co-stimulate T cells and monocytes [17,18]. In addition to the trafficking effect, CCL5, like other CC chemokines, plays an important role in co-stimulation of T-cell proliferation [19,20] and activation of the T cells localized in the inflammatory lesion [21]. This chemokine have been detected within the CNS of multiple sclerosis patients as well as within active plaque lesions, suggesting that these molecules contribute to demyelination by attracting targeted populations of leukocytes into the CNS [22-24].

Several functional polymorphisms in the CCL5 gene have been described [25,26]. Of these SNP, the -28C/G (rs2280788) occurring in proximity to the promoter region was associated with increased transcriptional activity and subsequent CCL5 expression in human cell lines [26]. This polymorphism was associated with an increased risk to several inflammatory diseases such as asthma [25,27], sarcoidosis [28], rheumatoid arthritis [29], atopic dermatitis [30], diabetes type 1 [31] and infectious diseases, including HIV [32] and active tuberculosis as we have recently reported [33]. However, association between CCL5 polymorphism and multiple sclerosis remain controversial. Some studies in the literature revealed an increased risk to multiple sclerosis [34,35].

Tunisia is considered as a low zone of prevalence for multiple sclerosis according to the data obtained from the Atlas of multiple sclerosis resources in the world 2008 reported by WHO [36]. In the present study, we investigated association between CCL5 -28C/G functional polymorphism and multiple sclerosis.

Materials and Methods

Patients and controls

This study included 51 defined multiple sclerosis patients, enrolled at the department of Neurology, Fattouma Bourguiba Hospital, Monastir, Tunisia, and 162 healthy controls who had donated blood
at the Regional Center for Blood Transfusion, CHU Farhat Hached, Sousse, Tunisia (Table 1). In the patient group, there were 18 men (mean age, 43.31 years; age range, 24–64; SD 16.970) and 33 women (mean age, 35.81 years; age range, 19–54; SD 6.363). The mean age at disease onset was 32.81 years (SD 14.849, range 16–58), the mean disease duration was 5.46 years (SD 8.485, range 1–15 ), and mean Extended Disability Status Scale (EDSS) score was 2.921 (SD 1.414, range 1–8).

Diagnostic criteria incorporate magnetic resonance imaging (MRI), cerebrospinal fluid (CSF) and evoked potentials testing. 25 of multiple sclerosis patients were receiving interferon β treatment, but none of them were receiving steroid treatment prior to blood sampling.

In the control group of healthy blood donors, there were 140 men (mean age, 37 years; age range, 20–52, SD 9.36) and 22 women (mean age, 33 years; age range, 22–49; SD 7.47).

This study was approved by the local ethics committee and all of the participants gave informed consent before the experimental procedures.

**Genotyping**

Genomic DNA was extracted from peripheral blood samples collected on EDTA anticoagulant using spin column technique of QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany), according to the manufacturer’s instructions, eluted in 100 μL of water and subsequently quantified using Nanodrop spectrophotometer (UV-Visible NanoDrop 1000; Thermo Fisher Scientific Inc.) and standardized to 100 ng/mL.

The polymerase chain reaction (PCR)–restriction fragment length polymorphism (RFLP) method was used as described previously [37] for genotyping the CCL5-28C/G SNP.

Genomic DNA (100 ng) was amplified in a 25 mL PCR reaction under the following cycling conditions: denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 55 s, with a final extension at 72°C for 10 min. Amplifications were performed in a MyCycler thermal cycler (Bio-Rad).

The presence of wild type 'C' allele introduces a restriction enzyme site for HincII enzyme and yields 152 and 23 bp fragments. Five microdots of the PCR products were digested with 5U of HincII (Promega) at 37°C for 3 h. The digestion products were analyzed on 4% agarose gels (Sigma) containing ethidium bromide (0.5 mg/mL) (Sigma) and visualized under UV illumination using the Gel Doc XR (Bio-Rad).

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Controls (n=162) (%)</th>
<th>Multiple sclerosis (n=51) (%)</th>
<th>P</th>
<th>OR (CI 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alleles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>65 (20)</td>
<td>19 (19)</td>
<td>0.1</td>
<td>0.91 (0.5-1.66)</td>
</tr>
<tr>
<td>C</td>
<td>259 (80)</td>
<td>83 (81)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>10 (6)</td>
<td>3 (6)</td>
<td>3.6</td>
<td>0.92 (0.19-3.91)</td>
</tr>
<tr>
<td>CG</td>
<td>48 (30)</td>
<td>13 (25)</td>
<td>0.11</td>
<td>0.88 (0.4-1.93)</td>
</tr>
<tr>
<td>CC</td>
<td>107 (64)</td>
<td>35 (69)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The CC genotype served as the reference category, GG or CG vs. CC

*: Fisher exact test

Table 1: RANTES -28C/G allele and genotype frequencies (n, %) in multiple sclerosis cases and controls.

**Statistical analysis**

The genotype distributions of each polymorphism were tested for Hardy–Weinberg equilibrium using the χ²-test in multiple sclerosis patients and controls [38].

Statistical analyses were performed using statistical software (Epi Info software version 3.2.2). The distribution of CCL5 polymorphism between multiple sclerosis patients and healthy controls were compared by χ² or Fisher’s exact test. p<0.05 was considered significant. Odds ratios (OR) and 95% confidence intervals (CI) were also calculated whenever χ² or Fisher’s exact test was significant.

**Results**

**Hardy-Weinberg equilibrium**

In this study, evaluation of Hardy–Weinberg equilibrium showed that genotype frequencies of CCL5-28C/G polymorphism was in Hardy–Weinberg equilibrium in multiple sclerosis group and healthy blood donors (P<0.05).

**No association of the CCL5 -28C/G polymorphism with multiple sclerosis**

The 28G allele frequencies were approximately similar in multiple sclerosis patients and control groups (Table 1), with no significant statistical difference. No difference was found in the distribution of genotype frequencies between multiple sclerosis cases and controls (Table 1).

**Discussion**

Multiple sclerosis is a chronic demyelinating disease of the human central nervous system of a still unknown etiology; however, it has been suggested to be affected by an interaction between genetic, environmental and geographical factors [39,40]. Strong evidence from genetic epidemiologic studies over the past 3 decades suggests that environmental factors affect susceptibility to multiple sclerosis at a broad population level [14]. Even exposed to the same environment, individual susceptibility to multiple sclerosis may be different, which indicates that genetic factors are important in the pathogenesis of multiple sclerosis. Therefore, understanding the genetic basis of multiple sclerosis is essential for the development of therapeutic strategies. Linkage and association studies have found that chemokines and chemokines receptor are involved in the pathogenesis of multiple sclerosis [16]. To the best of our knowledge, this is the first study investigating the association between CCL5-28C/G genetic polymorphism and susceptibility to multiple sclerosis Tunisian populations. The present study showed that neither alleles nor genotypes of this SNP were associated with increased risk development of multiple sclerosis.

CCL5, a member of the beta (C-C) chemokine family, this chemokine plays an important role in the development of inflammation via its ability to chemoattract leukocytes and modulate their functions. CCL5 is a chemoattractant for many cell types, including unstimulated CD4⁺CD45RO⁻ T cells, stimulated CD4⁺ and CD8⁺ lymphocytes, natural killer (NK) cells, basophiles, dendritic cells, monocytes and microglia. It can also be an activator for T cells, monocytes and NK cells [41,42]. Several studies have implicated CCL5 in multiple sclerosis pathogenesis and the expression of CCL5 was observed in...
perivascular inflammatory foci in multiple sclerosis brain [43]. In actively demyelinating multiple sclerosis plagues CCL5 expression was restricted to the blood vessel endothelium, perivascular cells and astrocytes [44]. CCL5 level in the cerebrospinal fluid was increased during active multiple sclerosis [24]. Additionally, Glass et al. showed that antibody targeting of the CC chemokine ligand 5 results in diminished leukocyte infiltration into the central nervous system and reduced neurologic disease in a viral model of multiple sclerosis [45].

Genetic associations of CCL5 SNPs with asthma, atop dermatisis, sarcoidosis, multiple sclerosis and other inflammatory and autoimmune diseases have been reported [25, 27-31]. Therefore, CCL5 could be considered a gene generally predisposing to autoimmune disease. To test this hypothesis, we investigated CCL5-28 C/G functional SNP in 51 multiple sclerosis patients in comparison to control blood donors. We did not find any association between this SNP and multiple sclerosis. However, our study did not support the recently published data in which authors reported that the 28C/G SNP in the promoter region was associated with multiple sclerosis susceptibility in USA and Netherland [34,35]. These findings could be related to genetic heterogeneity or population stratification within each ethnicity. In addition, interactions with other polymorphisms may be of great importance to understand the genetic mechanisms controlling development of multiple sclerosis. In fact, analysis of polymorphisms in candidate genes has established that, at least in some populations, variations in for example, nucleotide variation—in the HLADR1, interleukin 7 receptor (IL7RA), the interleukin 2 receptor (IL2RA), the CD58 and the c-type lectin domain family 16 member A (CLEC16A) genes [46-48].

In summary, this is the first study demonstrating the no association of CCL5-28C/G functional SNP with multiple sclerosis in Tunisian patients. Our results suggest that the CCL5-28GG genotype is not a risk factor for development of this disease. As the genetic control of the autoimmune disorders seems to be polygenic [46], it is interesting to study other functional polymorphisms affecting other genes, including CCR5 receptor and CXCL10 chemokine that may ameliorate the comprehension of multiple sclerosis development in our country.

Acknowledgement

Financial support was provided by the Ministry of Higher Education, Scientific Research and Technology (UR02SP13) of Tunisia.

Disclosure and Conflict of Interests

The authors state they have no conflict of interest.

References

infection and disease progression in the Multicenter AIDS Cohort Study. AIDS 14: 2671-2678.


