Frequencies of the G-protein β3 subunit C825T polymorphism and the δ 32 mutation of the chemokine receptor-5 in patients with multiple sclerosis

Claus G. Haasea,*, Stephan Schmidtb, Pedro M. Faustmannac

aDepartment of Neurology, University Hospital Essen, Essen, Germany
bDepartment of Neurology, University Hospital Bonn, Bonn, Germany
cDepartment of Neuroanatomy and Molecular Brain Research, Ruhr-University Bochum, Bochum, Germany

Received 21 June 2002; received in revised form 15 July 2002; accepted 16 July 2002

Abstract

In the pathogenesis of multiple sclerosis (MS) genetic factors are known to influence autoreactive T-cell-actions like proliferation and chemotaxis across the blood–brain barrier via chemokine receptors (CCR) and G-protein coupled activating mechanisms. For the first time, we studied the frequencies of a recently described C825T polymorphism in the G-protein encoding gene for the β3 subunit (GNB3) together with frequencies of a 32-base-pair deletion in the CCR5 gene (Δ32 CCR5) in patients with MS (n = 253: relapsing-remitting (RR), n = 124 and chronic progressive course, n = 129). Apart from a trend to a reduced frequency of Δ32 CCR5 and increased GNB3 825T polymorphism in primary chronic progressive patients, numbers did not reach statistical significance in any group of MS. These results could not support differences in the genetic background of MS based on that CCR5 mutation or the described GNB3 polymorphism.

Keywords: Multiple sclerosis; Genetics; G-protein; Chemokine receptor; Δ32 chemokine receptors 5; Chemotaxis

Disturbed T lymphocyte reactions are known to contribute to lesion formation in multiple sclerosis and numerous attempts have been made to analyze genetic factors responsible for relapses as well as for chronic progression of the disease. Chemotactic factors are involved in the pathogenesis of multiple sclerosis (MS) as part of the innate immune system [3]. Leukocytes and CD4+ T cells in particular, play an important role in the pathogenesis of the acute and chronic inflammation of the central nervous system seen in MS. The mechanisms guiding auto-reactive lymphocytes to their targets include adhesion molecules, chemotactic factors and receptor mediated signaling. Chemokines induce cell migration and activation by binding to specific G-protein- coupled cell-surface receptors on target cells. Their action is then exerted via Gi-type G proteins and βγ-dimers released from activated α subunits initiated signal transduction pathways eventually resulting in chemotaxis [6]. Another mediator of lymphocyte migration in inflammatory diseases and infections the chemokine receptor (CCR) 5 has been found to play a potential role in leukocytes migration with its ligands the macrophage inflammatory proteins (MIP) 1α and 1β. In particular, individuals who are homogeneous for a 32-base-pair deletion could not produce a functioning receptor [8]. In MS protection from inflammation by mutation to Δ32 CCR5 could not be found [2]. However it was associated with a lower risk of recurrent clinical disease activity [9]. Disease activity in conjunction with enhanced chemotaxis of human neutrophils and enhanced T cell response as well as increased immunoglobulin formation has been associated with a specific G protein polymorphism at subunit β [4,7,11,12]. This ubiquitously expressed β3 subunit polymorphism at position C825T on the gene GNB3 has recently been found to be associated with enhanced G-protein signalling [11], and could be found to be involved in several epidemiological relevant diseases [10,11].

We conducted a study on the prevalence of Δ32 CCR5 and the G-protein β3 subunit 825T polymorphism in patients with MS. After informed consent was obtained, venous blood samples were drawn from 253 patients with...
definite multiple sclerosis. Half of the patients ranged from 18–42 years of age, the other half was 43–75 years old. Disability was scored by Kurtzkes Expanded disability score (EDSS) in 144 patients, with a score of 0–5 in 50% and 5.5–9.0 for the rest. In 145 patients data on disease duration was obtained, showing that 53% had a disease for up to 10 years, 83% had a disease duration for longer than 20 years (Table 1). DNA genotyping was performed after extracting genomic DNA using appropriate reagents according to the manufacturer’s instructions (Quiagen, Hilden, Germany). GNB3 C825T allele and δ32 CCR5 status were determined after PCR amplification and restriction analysis as previously described [9,10]. No additional control subjects were considered as database has been provided regarding frequencies and distribution of G-protein β3 subunit 825T polymorphism and δ32 CCR5 mutation [2,9,11]. Data was analyzed using SPSS 10.0 for Windows™ using Analysis of the Variance (ANOVA) and unpaired t-test for normal distributed variables. Non-parametric methods were used for variables without normal distribution. Correlation was estimated using the Pearson-test. Significant differences were employed with \( P < 0.05 \). In nine patients paired analysis was not done due to technical reasons.

Clinical findings revealed significant differences between chronic and relapsing remitting patients regarding age, gender, EDSS and time of disease duration. Male patients were relatively more frequent in the group with a primary progressive course and to a lesser extent in the group with a secondary chronic progressive course. Frequency of δ32 CCR5 mutation in different clinical course of multiple sclerosis showed a comparable carrier rate and homozygosity frequency between patients with RR, SCP and PCP course of MS (Table 1), and equaled the numbers previously reported [2] in healthy control subjects. No differences were found between patients carrying δ32 CCR5 and patients with normal CCR5 regarding age, sex, EDSS score or disease duration. Carrier rate and homozygosity frequency of the G-protein β3 subunit 825T polymorphism were comparable in patients with RR, SCP and PCP course of MS, and equaled the number reported on healthy control subjects from the literature [10,11]. No differences were found for patients carrying the G-protein β3 subunit 825T polymorphism with respect to age, gender, EDSS score or disease duration. No correlations were detected of neither mutation or polymorphism with any clinical parameter, including disease duration or EDSS score, or with course of disease. No correlation between δ32 CCR5 mutation and G-protein β3 subunit 825T polymorphism was found, either.

Genetic predisposition to enhanced and dysregulated immunologic functions has been described for multiple sclerosis with respect to several regions [3,5]. The Human Leukocyte Antigen (HLA) genes, in particular class II (DR2 (DRb1*1501)) regions on chromosome six have been identified to influence the susceptibility for MS but a disease modifying effect on age of onset, severity and type of clinical course remained controversial [5]. For non-HLA genes like TNF and CCR5 there was also some evidence provided with respect to their influence on disease course and severity which was not yet confirmatory [1,2,5,9]. Similarly, in our study we could not identify GNB3 825T polymorphism or δ32 CCR5 to act as susceptibility factors with regard to the clinical course of MS, in particular comparing chronic and relapsing-remitting courses. Though no separate healthy age/ gender-matched control group has been formed, our findings are in keeping with previous studies on δ32 CCR5 [2,9] and on GNB3 825T polymorphism [10,11]. The risk of recurrent clinical disease activity could not be addressed in our study as it was reported for the mutant δ32 CCR5 [9]. Moreover patients with a chronic course of MS are known to have an already reduced disease activity. In particular in our patients with a course of primary chronic progressive MS, the frequencies of GNB3 825T polymorphism and δ32 CCR5 seemed to dissociate to the potentially unfavorable state of low δ32 CCR5 [1,9] and increased GNB3 825T polymorphism [4,12,13] frequencies. However, due to the relative small number of patients in this group, frequencies of PCP did not differ significantly from the other patient groups or healthy controls, extracted from the literature [11].

Further studies are warranted examining patients with MS

---

**Table 1**

Clinical data and frequencies of the δ32 mutation in the CCR5 and frequencies of the G-protein β3 subunit C825T polymorphism in patients with different courses of MS

<table>
<thead>
<tr>
<th>MS course</th>
<th>CCR5 (^a)</th>
<th>CCR5-δ32 + δ32</th>
<th>GNB3-CC</th>
<th>GNB3-TC + TT</th>
<th>Sex (mean ± SD)</th>
<th>Age (years) (mean ± SD)</th>
<th>EDSS (mean ± SD)</th>
<th>Duration (years) (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR: ( n = 117 )</td>
<td>96</td>
<td>21</td>
<td>60</td>
<td>57</td>
<td>93</td>
<td>24</td>
<td>37.7 ± 8.8</td>
<td>1.8 ± 1.0</td>
</tr>
<tr>
<td>SCP: ( n = 84 )</td>
<td>69</td>
<td>15</td>
<td>44</td>
<td>40</td>
<td>52</td>
<td>32</td>
<td>47.1 ± 11.5</td>
<td>5.8 ± 1.8</td>
</tr>
<tr>
<td>PCP: ( n = 42 )</td>
<td>36</td>
<td>6</td>
<td>18</td>
<td>24</td>
<td>19</td>
<td>23</td>
<td>55.0 ± 10.7</td>
<td>6.2 ± 1.5</td>
</tr>
<tr>
<td>SUM: ( n = 243 )</td>
<td>165</td>
<td>36</td>
<td>104</td>
<td>97</td>
<td>164</td>
<td>79</td>
<td>43.5 ± 11.9</td>
<td>4.7 ± 2.5</td>
</tr>
</tbody>
</table>

\(^a\) MS, multiple sclerosis; RR, relapsing remitting course; SCP, secondary chronic progressive course; PCP, primary chronic progressive course; CCR5, chemokine receptor 5 allele; CR5δ32; δ32-32 base pair mutation; GNB3-C825T, polymorphism in the gene GNB3; CC, homozygous; CT, heterozygous for 825T polymorphism; TT, homozygous for 825T polymorphism; EDSS, expanded disability status scale.
with regard to their immunological and clinical response to the immunomodulatory therapies available, including T-and B-cell actions. With respect to their potential role for chemotaxis and leukocytes trafficking studies to clarify their association to the expression of IL-8 and MIP1α and β need to be performed in the future.

We wish to thank Prof. E. Kreuzfelder, Ph.D. and Prof. W. Siffert, M.D. for technical support.


