Interleukin-4 receptor alpha polymorphisms in autoimmune myasthenia gravis in a Caucasian population

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A B S T R A C T

Autoimmune myasthenia gravis is a T-cell–dependent, antibody-mediated, rare neuromuscular disorder. Interleukin-4, acting via interleukin-4 receptor alpha, plays a pivotal role in B-cell differentiation and antibody production and has been implicated to influence disease progression in experimental autoimmune myasthenia gravis. Polymorphisms of the interleukin-4 receptor alpha gene have been shown to be associated with various autoimmune diseases. We compared the distribution of three polymorphisms of the interleukin-4 receptor alpha gene (S503P, rs1805015, Q576R, rs1801275, 17SV, rs1805010), all affecting interleukin-4 signaling, in two cohorts of myasthenia gravis patients with ethnically matched controls. Although the distribution of the S503P and Q576R polymorphisms did not differ significantly between the groups, the frequency of the GG rare homozygote genotype of the 17SV polymorphism was significantly higher in patients with myasthenia gravis. Our data suggest that the reduced responsiveness to interleukin-4 because the 17SV polymorphism may contribute to the pathogenesis of myasthenia gravis.

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1. Introduction

Pathologic muscle fatigue, the characteristic symptom of autoimmune myasthenia gravis (MG), is caused by autoantibodies directed against different proteins of the neuromuscular junction, most commonly antinicotinic acetylcholine receptor (AChR) antibodies in 70% to 90% of the cases, less frequently anti–muscle-specific kinase (MuSK) antibodies. By modulating the synthesis of high affinity antibodies, CD4+ T cells and their secreted cytokines play a vital role in the pathomechanism of MG. T helper 1 (Th1) cytokines may induce the synthesis of IgGs capable of fixing complement, and thus, facilitating the destruction of the neuromuscular junction. Th2 cytokines have anti-inflammatory properties, and have diverse effects on MG (for review, see Conti-Fine et al. [1]). Among these cytokines, interleukin-4 (IL-4) is a potent growth and differentiation factor for B cells and stimulates class-switching and autoantibody production [2] but has also been suggested to take part in the modulation of regulatory T-cell functions [3,4]. The signal of IL-4 is mediated by the interleukin-4 receptor alpha (IL4Ra) chain which is part of both the IL4R and the IL13R. Of the seven nonsynonymous polymorphisms found in the coding sequence of IL4Ra gene in the Caucasian population, three (17SV, S503P, Q576R) have been shown to modulate the signal transduction pathway of IL-4 via STAT6 protein or by synergizing with STAT6 actions [5–7]. The role of these polymorphisms in different immune disorders has been well established, and associations have been demonstrated in exacerbation of allergic asthma [8], multiple sclerosis [9] and erosive rheumatoid arthritis [10]. Here we report a case-control study, where we investigated the association of these polymorphisms with MG.

2. Subjects and methods

2.1. Patients and controls

For the initial genotype analysis clinical data and blood samples were collected from 164 Hungarian MG patients treated in 2 major Hungarian centers, the Neurology Department of Debrecen University and the Centre for Molecular Neurology at Semmelweis University, Budapest. The clinical data and samples of these patients were used to build the NEPSYBANK (Hungarian Neurological and Psychiatric Biobank) [11]. To confirm data, a second cohort consisting of further 96 Hungarian MG patients was analyzed; these samples and clinical data were obtained from 2 other major MG centers, the Neurology Departments of Pécs University and the Jahn Ferenc Teaching Hospital, Bu-
Table 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients, cohorts 1 + 2</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, n (% of all)</td>
<td>164 / 96</td>
<td>299</td>
</tr>
<tr>
<td>Male</td>
<td>33 / 38 (27)</td>
<td>97 (32)</td>
</tr>
<tr>
<td>Female</td>
<td>131 / 58 (73)</td>
<td>202 (68)</td>
</tr>
<tr>
<td>Age (mean ± SD, years)</td>
<td>49 ± 32.2 and 56.9 ± 15.8 (Range: 18–78 and 18–84)</td>
<td>37.9 ± 13.31 (Range: 18–86)</td>
</tr>
<tr>
<td>Age at onset (mean ± SD, years)</td>
<td>36.02 ± 13.04 and 46.3 ± 13.6 (Range: 7–80 and 17–80)</td>
<td>–</td>
</tr>
<tr>
<td>AChR antibody positivity n (%)</td>
<td>118 / 96 (82% of all cases)</td>
<td>–</td>
</tr>
<tr>
<td>Distribution of weakness n (%) [1]</td>
<td>31 / 0 (12% of all cases)</td>
<td>–</td>
</tr>
<tr>
<td>Ocular</td>
<td>133 / 96 (88% of all cases)</td>
<td>–</td>
</tr>
</tbody>
</table>

2.4. Statistical analysis

Genotype association and Cochran-Armitage tests were performed using an online Hardy–Weinberg equilibrium (HWE) calculator (https://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl) based on Wigginton et al. [12]. As age and gender differed significantly in the control and patients groups; correction was made with logistic regression using SPSS 15.1 (SPSS, Inc., Chicago, IL). The strength of association was represented by exponentiation of the B value (ExB) in logistic regression models with 95% confidence interval (CI). p Values <0.05 were considered significant. Since three loci were analyzed, multiple comparisons have also been done. The Bonferroni significance level was p < 0.017.

3. Results

All polymorphisms in this study were in Hardy–Weinberg equilibrium. Distributions of the 3 examined polymorphisms were evaluated in groups of patients with MG and healthy controls. Frequencies of the IL4RA genotypes were also compared among controls and patients grouped according to anti-AChR positivity, gender (women only), age at onset (>40 years, and distribution of muscle weakness (generalized form as first symptom). In logistic regression models containing age and gender, none of the examined SNPs were significantly associated with MG. Different combinations of heterozygous and homozygous groups within one genotype were also analyzed. In case of the S503P and Q576R polymorphisms the number of individuals harboring the rare homozygote form was very low in both the patient and control groups. Thus, for more accurate analysis the combination of the rare homozygote and heterozygote groups was needed, but it did not reveal any association in case of these polymorphisms in the combined analysis either (data not shown).

However, concerning the 175V polymorphism, the rare GG genotype, which results in 2 copies of the V75 allele, was significantly associated with MG (p = 0.023, ExB = 1.78, CI: 1.08–3.08). This association could also be seen in the “women only” group (p = 0.019, ExB = 1.88, CI: 1.36–3.4) and in the subgroup of patients with anti-AChR antibody positivity (p = 0.027, ExB: 1.84, CI: 1.07–3.16). No association could be found in the other investigated groups. The significant associations were also analyzed in a second cohort of MG patients. Here, a significant association could be confirmed with MG (p = 0.042, ExB = 1.91, CI: 1.02–3.55) and the anti-AChR antibody positive subgroup (as in this cohort all MG patients were also anti-AChR antibody positive, p = 0.042, ExB = 1.91, CI: 1.02–3.55). However, no association could be found in the “women only” subgroup (p = 0.297), probably because of the small sample size. Comparison of the distribution of the GG genotype between the combined (merged) MG cohorts and control group also gave a significant difference in the case of the whole MG group (p = 0.017, ExB = 1.74, CI: 1.1–2.72). Similarly, significant differences have been detected in the cases of the anti-AChR-positive (p = 0.018, ExB = 1.77, CI: 1.1–2.84) and the “women only” subgroups (p = 0.024, ExB = 1.81, CI: 1.09–3.3) (association of these subgroups are shown in Table 2). After Bonferroni correction, however, a significant difference in the 175V GG genotype distribution could only be demonstrated when comparing the control group with the merged MG cohorts, but could not be found when either the individual cohorts or subgroups were analyzed separately.

In the anti-AChR negative patients, only three patients were anti-MuSK positive with radioimmunoprecipitation, thus this subgroup was not analyzed further. Where appropriate, analysis was also done with excluding this small population, however the results did not change significantly.

4. Discussion

IL-4, acting through IL4RA, has multiple roles in the pathomechanism of MG [3,4,13,14]. While stimulating isotype switching...
and antibody production, IL-4 probably also has a protective effect in experimental MG, possibly by the induction of peripheral tolerance via regulatory T-cells [3,4,13,14].

The first IL-4 gene polymorphism association study in MG investigated a Swedish cohort. Analysis of a dinucleotide polymorphism of the second, and a variable number of tandem repeat of the third intronic regions of the IL-4 gene, with a putative role in influencing IL-4 levels, showed no association with MG [15].

Here we investigated the association of 3 functional polymorphisms of the IL-4RA gene with MG. Although the S503P and Q576R polymorphisms showed no association, and although no allelic association could be found with the I75V SNP either, we found that the homozygous G-allele carriage of the I75V polymorphism, resulting in the V75 variation, was significantly higher in the MG population, the women-only subgroup and in patients with anti-AChR antibodies.

In a study performed in rheumatoid arthritis patients, individuals with such homozygous GG genotype had more severe disease with bone erosions compared with patients carrying zero or one G allele [10]. The 175V amino acid substitution in the extracellular domain of the protein, enhances IL4R signaling [7], with the I75 variant being a “gain of function” mutation and thus having a greater signal transduction capacity via STAT6 compared with the V75 variant [10,16].

In this study performed in myasthenia gravis patients, we report that the GG genotype is associated with the disease. The reduced IL-4 signal transduction on CD4+ T cells from V75F/V75 could either cause a shift in the Th balance towards Th1 responses, or reduce the function of regulatory T-cells, which could influence the pathomechanism of the disease. However, the exact role of this polymorphism in the pathogenesis of human autoimmune MG will need to be further investigated. Also, due to the small population size of our anti-MuSK positive MG patients, we could not perform correct statistical analysis. It would be interesting to see however, where there is also association in this subgroup, as this antibody is mostly of the IgG4 subclass, which is an IL-4 driven Th2 isotype [17].

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