CTLA4 CT60 gene polymorphism is not associated with differential susceptibility to pemphigus foliaceus

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Abstract

Pemphigus foliaceus is an organ-specific autoimmune disease characterized by autoantibodies against the extracellular region of desmoglein 1, a protein that mediates intercellular adhesion in desmosomes. Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) is a key negative regulator of the T cell immune response, playing an important role in T cell homeostasis and maintenance of peripheral tolerance. Polymorphisms in the CTLA4 gene have been associated with autoimmune diseases and the functional CT60 single nucleotide polymorphism (rs3087243, also named 6230G > A) has been proposed to be a causal variant in several of these diseases. The aim of this study was to ascertain whether this polymorphism is associated with inter-individual variation in susceptibility to pemphigus foliaceus. The population sample in this case-control association study comprised 248 patient and 367 controls. We did not found a significant association of pemphigus foliaceus with the CT60 variants. We conclude that the CTLA4 CT60 polymorphism is not an important factor for pemphigus foliaceus pathogenesis in the population analyzed.

Key words: CTLA4, CT60 polymorphism, pemphigus, fogo selvagem, autoimmunity.

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The 2q33 chromosome region harbors genes that encode costimulatory molecules, such as the cytotoxic T-lymphocyte-associated protein 4 (CTLA4), the cluster of differentiation molecule 28 (CD28) and the inducible costimulatory molecule (ICOS), all of which play crucial roles in T cell activation and regulation (Ling et al., 2001). CTLA-4 is a key molecule expressed by activated T cells that transduces an inhibitory signal after binding to CD80 or CD86 on antigen-presenting cells. CTLA-4 appears to inhibit immune responses by several mechanisms, besides being a critical mediator in peripheral tolerance. Based on its crucial role in immunological homeostasis, CTLA4 has become one of the main genes of research interest for association studies and has been considered as a target for immunotherapy (Scalapino and Daikh, 2008). Polymorphisms in CTLA4 are associated with a very wide range of inflammatory and autoimmune diseases (for a review, see Kristiansen et al., 2000; Gough et al., 2005). Several SNPs have been identified in the 2q33 region comprising CD28, CTLA4 and ICOS genes (Ueda et al., 2003). The authors found associations between polymorphisms in the segment that includes the 3’ region of the CTLA4 gene with Graves disease, hypothyroidism and autoimmune type I diabetes. Among these polymorphisms, the SNP CT60G>A (rs3087243, also denominated 6230G>A), was the most-associated marker. The CT60*G allele was correlated with higher disease susceptibility and lower mRNA levels of the soluble alternative splice form of CTLA-4 (sCTLA-4). Recent studies also suggested that polymorphisms in the CTLA4 gene may influence the development of autoimmune diseases. Palacios et al. (2008) concluded that regulatory SNPs in the CTLA4 gene exert a strong influence on expression levels of both known CTLA-4 isoforms. Moreover, association between CT60 polymorphism and variation in the frequency of regulatory T cells has been reported. Individuals homozygous for allele CT60*A showed an increase from 30-40% in the frequency of regulatory T cells. Although the basic mechanism connecting the CT60*A allele with an increase in regulatory T cells has not yet been established, these differences reveal a relationship between CT60 polymorphism and variation in adaptive immune responses (Atabani et al., 2005).

Endemic pemphigus foliaceus (PF), also known as fogo selvagem (meaning ‘wild fire’), is an organ-specific autoimmune disease characterized by autoantibodies against desmoglein 1 protein and by loss of adhesion between keratinocytes, leading to intraepithelial blisters of the skin (Warren et al., 2000). Several candidate genes have been analyzed for associations with PF. The HLA class II
genes (Pavoni et al., 2003) and the CD40L gene (Malheiros and Petzl-Erler, 2009) showed the strongest associations. In addition, interactions between some of the genes analyzed have been reported (Martel et al., 2002; Malheiros and Petzl-Erler, 2009). Two SNPs in the CTLA4 gene, -318C>T and 49A>G, had been previously analyzed, although no association with PF disease susceptibility was observed (Pavoni et al., 2006).

Based on the reported associations between susceptibility to autoimmune diseases and CTLA4 polymorphisms, especially the CT60 SNP, the aim of this study was to extend analysis of this candidate gene to evaluate whether the CT60 polymorphism is a factor contributing to differential genetic susceptibility to pemphigus foliaceus in the Brazilian population.

We analyzed 248 patients and 367 controls without history of the disease, all unrelated to each other. Diagnosis was according to clinical and histological criteria. Patients and controls were contacted at Hospital Adventista do Pêndigo, Campo Grande, Mato Grosso do Sul State, Brazil. Additional controls were contacted in Curitiba, Paraná State, Brazil. Patients and controls were matched for ancestry. Seventy percent of the individuals were of predominantly European and 30% of predominantly African ancestry. The male:female ratio was close to one, viz. 47% of the patients and 45% of the controls were males. According to age of disease onset, distribution was as follows: from 0 to 9 years, 4.2%; 10-14 years, 8.3%; 15-19, 13.1%; 20-24, 11.3%; 25-29, 9.5%; 30-34, 9.5%; 35-39, 12.9%; 40-44, 8.3%; 45-49, 10.1%; 50-59, 5.4%; 60-69, 4.2%; 70-84, 3.6%. Written informed consent was obtained from all the participants. The study received approval by the Human Research Ethics Committee, in accordance with Brazilian Federal Laws.

Genotyping was performed by polymerase chain reaction amplification followed by restriction fragment length polymorphism analysis (PCR-RFLP). The primers and PCR conditions were the same as those described by Teutsch et al. (2004). Allelic, genotypic and allele carrier frequencies (i.e., the frequency of individuals having the allele in either homozygosity or heterozygosity) were estimated by direct counting. Hardy-Weinberg equilibrium was assessed with the Guo and Thompson (1992) method implemented in the ARLEQUIN version 3.11 software package (Excoffier et al., 2005). Comparisons between frequencies in the patient and control population samples were performed by analysis of contingency tables by the chi-square test of independence. The strength of the association was estimated by the odds ratio (OR), using Woolf’s method. The p value of 0.05 was adopted as the significance limit for all statistical tests. The genotype taken as reference was CTLA4 CT60 G/G. Therefore, by definition, the OR for this genotype equals 1 and the OR for A/G and A/A approach the risk of these genotypes relative to the G/G genotype.

Allelic, genotypic and allele carrier frequencies are presented in Table 1. Genotype frequencies were in Hardy-Weinberg equilibrium (p = 0.115 and p = 0.829 in patient and control population samples, respectively). No significant association between PF disease status and CT60 variants was detected.

Associations previously described between susceptibility to some autoimmune diseases and CT60 polymorphism have been interpreted considering the possible effect of this SNP in the alteration of the ratio of CTLA-4 splicing isoforms, and that elevated levels of sCTLA-4 have already been detected in certain autoimmune disorders (Ueda et al., 2003; Saverino et al., 2007; Kawasaki et al., 2008). In addition, the hypothesis has been raised that sCTLA-4 is involved in control of T cell activation, its levels being regulated by genetic variation in chromosome region 2q33 (Kaartinen et al., 2007). Although several diseases have been associated with CT60 polymorphism (see above), further studies of the same and other autoimmune diseases in various populations have failed to detect associations, thus corroborating with our results (for example, Chang et al., 2007; Tsukahara et al., 2008). One reason for these conflicting results may be differences in allelic and haplotypic frequencies of this marker among populations. Another explanation is that the set of genes contributing to the establishment of different autoimmune diseases is not the same, and that the CTLA4 gene is important in some diseases but not in others.

In this study, we expanded our previous findings regarding variation in the CTLA4 gene by analyzing CT60 polymorphism. Our results lead to the conclusion that genetic variation in CTLA4 does not play an important role in PF susceptibility and that the effect of variations in this gene differs among autoimmune diseases. We do not exclude the involvement of the CTLA-4 molecule in PF

### Table 1 - Genotypic, allele carrier, and allelic frequencies for the CT60 SNP in patients and controls.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Frequency (%)</th>
<th>OR</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n = 248)</td>
<td>Controls (n = 367)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>37.9</td>
<td>33.2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>A/G</td>
<td>43.1</td>
<td>49.6</td>
<td>0.76</td>
<td>0.53-1.09</td>
</tr>
<tr>
<td>A/A</td>
<td>19.0</td>
<td>17.2</td>
<td>1.03</td>
<td>0.65-1.64</td>
</tr>
<tr>
<td>Allele carriers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G+</td>
<td>81.0</td>
<td>82.8</td>
<td>0.89</td>
<td>0.58-1.35</td>
</tr>
<tr>
<td>A+</td>
<td>62.1</td>
<td>66.8</td>
<td>0.82</td>
<td>0.58-1.14</td>
</tr>
<tr>
<td>Alleles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>59.5</td>
<td>58.0</td>
<td>1.06</td>
<td>0.84-1.34</td>
</tr>
<tr>
<td>A</td>
<td>40.5</td>
<td>42.0</td>
<td>0.94</td>
<td>0.75-1.19</td>
</tr>
</tbody>
</table>

1Odds ratio; 2Confidence interval; 3Probability values.
CT60 and pemphigus susceptibility

pathogenesis, but show that CT60 genotypes have no significant impact on pemphigus foliaceus disease susceptibility.

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References


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