CTLA4/CT60 Polymorphism Is Not Relevant in Susceptibility to Autoimmune Inflammatory Intestinal Disorders

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ABSTRACT: The aim of this work was to investigate the possible influence of the recently described CT60 A/G dimorphism of the CTLA4 (cytotoxic T-lymphocyte antigen 4) gene in the susceptibility to two different autoimmune inflammatory intestinal disorders, inflammatory bowel disease (IBD) and celiac disease. We analyzed a case-control cohort composed of 528 Spanish patients with IBD (284 with Crohn disease and 244 with ulcerative colitis) and 454 unrelated healthy individuals, and additionally a group of 90 celiac disease families. CT60 genotyping was performed with a TaqMan 5' allelic discrimination assay. After comparing patients with IBD with the control population, we found no significant deviation in the distribution of the alleles or genotypes of CTLA4/CT60 dimorphism. In addition, by means of familial and case-control analysis, no evidence for a statistically significant association was observed between CTLA4/CT60 and celiac disease susceptibility. Therefore, our results suggest that the CTLA4/CT60 polymorphism does not play a major role in inflammatory intestinal disorders.


KEYWORDS: CTLA4; IBD; celiac disease; autoimmunity; polymorphism

ABBREVIATIONS

CTLA4 cytotoxic T-lymphocyte antigen 4
HLA human leukocyte antigen
IBD inflammatory bowel disease
OR odds ratio
SNP single nucleotide polymorphism
TDT transmission disequilibrium test

INTRODUCTION

Inflammatory bowel disease (IBD) and celiac disease are chronic autoimmune inflammatory intestinal disorders. The two main forms of IBD, Crohn disease and ulcerative colitis, share some clinical and pathologic features, although disease type can usually be distinguished by anatomic and histologic features [1]. The patients with IBD experienced a chronic relapsing inflammation of the gastrointestinal tract as a result of an inappropriate and exaggerated mucosal immune response to normal constituents of intestinal microflora [1]. In celiac disease, intolerance to dietary gluten leads to inflammation and atrophy of the mucosa [2].

Genetic factors are known to play an important role in determining susceptibility to both IBD and celiac disease [2, 3]. Inflammatory bowel disease epidemiologic and linkage studies have demonstrated that there are many contributing genes in disease susceptibility. Although the nucleotide-binding oligomerization domain 2 has been characterized as the IBD1 locus [4], the identification of other target genes is necessary to understand the mechanisms underlying IBD susceptibility [1, 3]. Regarding celiac disease, the human leukocyte antigen (HLA) genes have been demonstrated to have a relevant role in the genetic predisposition, with 90% of celiac...
patients bearing the DQ2 molecule (DQA1*05/DQB1*02). However, the results of haplotype sharing studies have revealed that the contribution of HLA genes for celiac disease genetic predisposition is no more than 40%, suggesting a role for non-HLA genes in celiac disease susceptibility [2, 5].

T cells play a major role in the mucosal pathogenesis of IBD and celiac disease as well as in the maintenance of oral tolerance [1, 2]. Therefore, molecules that mediate regulation of T-cell activity could influence disease susceptibility. The CTLA4 (cytotoxic T-lymphocyte antigen 4) molecule is a homologue for CD28, and both molecules and their common ligands (B7-1 and B7-2) constitute the B7/CD28-CTLA4 costimulatory pathway for T-cell activation. Whereas the CD28/ligand interaction plays a critical role in increasing and maintaining the T-cell response initiated through T-cell antigen receptor engagement, the CTLA4/ligand interaction has an inhibitory effect on T-cell activation and might contribute to peripheral tolerance [6]. Thus, CTLA4 is a good candidate gene for susceptibility to both IBD and celiac disease. Furthermore this gene maps within the 2q35 region, which has been demonstrated to be a chromosomal region that confers risk to both IBD and celiac disease [7, 8]. Several polymorphisms have been described in the CTLA4 gene [9–12]. Among them, a CT60 A/G dimorphism (simple nucleotide polymorphism [SNP] 3087245) has been recently associated with functional relevance and with susceptibility to a variety of autoimmune diseases [13].

The aim of this work was to investigate the possible influence of the recently described CT60 A/G dimorphism of the CTLA4 gene in the susceptibility to two different autoimmune inflammatory intestinal disorders, IBD and celiac disease.

SUBJECTS AND METHODS

Patients and Controls

We analyzed a case-control cohort composed of 528 Spanish patients with IBD (284 with Crohn disease and 244 with ulcerative colitis) recruited in two hospitals from South Spain (Hospital Virgen de las Nieves, Granada, and Hospital Puerta del Mar, Cádiz). The patients were recruited and diagnosed following standard criteria based on reviews of clinical, endoscopic, radiologic, and histopathologic reports [14]. A total of 454 unrelated healthy individuals were included as controls.

In parallel, a group of 90 celiac families characterized by the presence of a celiac disease–affected sibling was considered for the study. Samples were collected in two hospitals, “Hospital Materno-Infantil” and “Hospital Clínico Universitario” in Granada, Spain. Patients with celiac disease were diagnosed following the European Society for Paediatric Gastroenterology and Nutrition criteria for celiac disease [15]. The same 90 patients with celiac disease were also used for a case-control analysis by comparing them with 202 healthy controls. All study participants—patients with IBD, patients with celiac disease, and controls—were of white Spanish origin.

RESULTS

Table 1 lists the allelic distribution of CTLA4/CT60 polymorphism in patients with IBD and controls. The allelic distribution in controls was similar to that obtained in other Caucasian populations [20, 21]. Allele frequencies were in Hardy-Weinberg equilibrium in both the patient and control groups. After comparing patients with Crohn disease and patients with ulcerative colitis as two independent groups, with the control population, we found no significant deviation in the distribution of the alleles or genotypes of CT60 dimorphism. Similarly, when both groups were considered as a whole, no statistically significant difference was found between patients with IBD and controls (Table 1).

In addition, in both familial and case-control analyses, no evidence for a statistically significant association was observed between CTLA4/CT60 and celiac disease susceptibility. The TDT analysis revealed no significant
deviation of the transmission–no transmission pattern for the CT60 alleles (Table 2). Accordingly, results from case-control analysis demonstrated very similar allelic frequencies among celiac patients and controls, with no statistically significant differences (Table 3).

**DISCUSSION**

There are two main arguments to choose a good candidate gene in disease-association analyses, based on the positional or the functional implication of the gene in disease susceptibility. Here we have applied these two different strategies to investigate the possible implication of CTLA4/CT60 polymorphism in IBD and celiac disease susceptibility. Genotyping data in multiple SNPs of CTLA4 obtained in a study concerning other autoimmune diseases (Graves disease, autoimmune hypothyroidism, and type 1 diabetes) suggest that disease susceptibility maps to 6.1-kb 3' region of the CTLA4 gene [13], in particular the marker CT60 (SNP3087243), which demonstrated the strongest association. Interestingly, the CT60 G allelic variation was also reported to be correlated with lower mRNA levels of the soluble alternative splice form of CTLA4.

The CTLA4/CT60 polymorphism is a good functional candidate marker in IBD. It is known that the mucosal inflammation observed in patients with IBD results from a dysregulation in T-cell balance from an abnormally robust Th1 (Crohn disease) or Th2 (ulcerative colitis) response [1]. In this respect, the role of the CTLA4 gene might be very important as a regulator of T-cell activation and proliferation. Of note, a recent genome scan meta-analysis has shown that the 2q33 region confers susceptibility to IBD [8], demonstrating that the CTLA4/CT60 is a good positional candidate gene to IBD.

In this work, we investigated for the first time the role of CTLA4/CT60 polymorphism in IBD susceptibility, and no evidence of association was found. It is unlikely that our results could have arisen because of a type II error (false-negative results) because, with the sample size used, the IBD combined study had 95% power to detect the effect of a polymorphism, conferring an OR of 1.8 at the 5% significance level (assuming an allele frequency of 50% in the control population). Nevertheless, our study was underpowered to detect smaller effects such as an OR of 1.14 associated with type 1 diabetes [13]. Interestingly, our data are in accordance with those obtained in a study of IBD in a Dutch Caucasian population in which no association between HLA11001 49A/G and C-318T CTLA4 polymorphisms and IBD was observed [22]. These findings and our results suggest that CTLA4 polymorphism does not play a major role in IBD susceptibility. However, the contribution of other candidate genes located in close proximity to CTLA4 in susceptibility to IBD, like the CD28 and ICOS genes, could not be discarded. In fact, very recently, it has been demonstrated that patients with IBD hyperexpress ICOS in the gastrointestinal tract [23].

The role of the CTLA4 gene in celiac disease have been broadly analyzed because it is a very good positional and functional candidate gene to disease susceptibility [7, 24, 25]. Most of the studies have analyzed polymorphisms −318C/T, +49A/G in exon 1 and the ATn microsatellite, without consistent findings in all populations [20]. The present study found no association between CTLA4/CT60 polymorphism and celiac disease susceptibility. Our analyses in celiac disease case-control and familial cohorts have a 50% power to detect the effect of a polymorphism, conferring an OR of 2.0 (assuming an allele frequency of 50%). In accordance with our findings, recent studies have observed no association

**TABLE 1** Genotype and allelic frequencies of CTLA4/CT60 in patients with IBD

<table>
<thead>
<tr>
<th>Genotype or allele</th>
<th>Patients with IBD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crohn disease</td>
</tr>
<tr>
<td></td>
<td>(n = 284), n (%)</td>
</tr>
<tr>
<td>CT60 genotype</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>67 (23.6)</td>
</tr>
<tr>
<td>AG</td>
<td>143 (50.4)</td>
</tr>
<tr>
<td>GG</td>
<td>74 (26)</td>
</tr>
<tr>
<td>CT60 allele</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>277 (48.7)</td>
</tr>
<tr>
<td>G</td>
<td>291 (51.2)</td>
</tr>
</tbody>
</table>

**TABLE 2** Transmission pattern of CT60 alleles in patients with celiac disease

<table>
<thead>
<tr>
<th>Allele</th>
<th>Transmitted, n (%)</th>
<th>Not transmitted, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>71 (45.8)</td>
<td>72 (46.4)</td>
</tr>
<tr>
<td>A</td>
<td>84 (54.2)</td>
<td>83 (53.6)</td>
</tr>
</tbody>
</table>
TABLE 3 Genotypic and allelic frequencies of CTLA/CT60 in patients with celiac disease and in controls

<table>
<thead>
<tr>
<th>Genotype or allele</th>
<th>Patients with celiac disease (n = 90), n (%)</th>
<th>Controls (n = 202), n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT60 genotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>16 (17.9)</td>
<td>54 (26.7)</td>
</tr>
<tr>
<td>GA</td>
<td>52 (57.3)</td>
<td>101 (50)</td>
</tr>
<tr>
<td>AA</td>
<td>22 (24.7)</td>
<td>47 (23.3)</td>
</tr>
<tr>
<td>CT60 allele</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>84 (46.7)</td>
<td>209 (51.7)</td>
</tr>
<tr>
<td>A</td>
<td>96 (53.3)</td>
<td>195 (48.3)</td>
</tr>
</tbody>
</table>

[20, 26] or borderline significance [27] between CTLA4/CT60 polymorphisms and celiac disease predisposition.

All these observations suggest that the chromosome 2q33 linked susceptibility in celiac disease might be attributed to another genetic marker. Interestingly, recently, an implication of the ICOS gene in celiac disease predisposition has been described [26].

Although the CTLA4/CT60 polymorphism has been proposed as a common genetic marker for autoimmunity [6, 13], studies in autoimmune diseases like rheumatoid arthritis have failed to detect an implication of CTLA4/CT60 polymorphism in disease susceptibility [28, 29].

Our results in IBD and celiac disease are in accordance with these studies, suggesting that the role of CTLA4/CT60 in disease susceptibility might be different depending on the genetic background or environmental factors that influence complex genetic diseases.

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