Abstract  

TNFα and TNFβ, or linfotoxin (LTα), are two molecules playing an important role in inflammation. Their genes map on Chromosome 6, between the HLA class II and class I loci. Polymorphisms in, or near, TNF genes have been associated with susceptibility to several autoimmune diseases. Studies of TNF genes in celiac disease (CD) have presented contradictory results. We have assessed the role of TNFα and linfotoxin α (TNFβ) in CD and their relative value as CD markers in addition to the presence of DQ2. The TNFA –308 polymorphism and the polymorphism at the first intron of the LTA gene were typed in CD patients and healthy controls and the results were correlated with the presence of DQ2. Significant differences were found in genotype and allele frequencies for the TNFA and LTA genes between CD patients and healthy controls and the results were correlated with the presence of DQ2. Significant differences were found in genotype and allele frequencies for the TNFA and LTA genes between CD patients and healthy controls, with an increase in the presence of DQ2-positive CD patients and DQ2-positive controls are compared. In DQ2-positive individuals, allele 2 (A) in position –308 of the promoter of TNFA and allele 1 (G) of the NcoI RFLP in the first intron of LTA are additional risk markers for CD.

Keywords  

TNFα · LTA · DQ2-positive · Celiac disease

Introduction  

HLA is an important factor in celiac disease (CD) susceptibility. However, haplotypes that carry risk alleles for CD are frequent within the Caucasian population, and most of the individuals with DQ2 heterodimer do not show a gluten intolerance. This fact prevents DQ2 positivity from being used as a marker of gluten intolerance in the general population. The contribution of HLA to the familial risk of CD has been estimated at no more than 40% and, therefore, other unknown genetic factors should have a greater role. Genome-wide linkage studies have been performed with inconclusive results (Greco et al. 1998; Zhong et al. 1996), although they have highlighted interesting areas for future studies. It is thought that additional candidate genes might vary in different populations.

TNFα and LTα, or linfotoxin (TNFβ), are two molecules which play an important role in inflammation. Their genes are also located on Chromosome 6, between the HLA class II and class I loci. Polymorphisms in, or near, TNF genes have been associated with susceptibility to several autoimmune diseases. These polymorphisms are linked to high TNF secretion by blood mononuclear cells (Peña et al. 1998).

Studies on the influence of TNF genes in CD have yielded contradictory results (de la Concha et al. 2000; McManus et al. 1996a, b; Polvi et al. 1998b). We tried to assess the role of the TNFα and linfotoxin α (TNFβ) genes in CD within a Spanish population, and their relative value as CD markers in addition to the presence of DQ2.

Materials and methods  

Patients and controls

The study includes a total of 129 DNA samples from 66 non-related previously diagnosed CD patients, and 63 healthy controls from randomly enrolled healthy blood donors from the Hospital Universitario de Valladolid (HUV) blood bank. CD patients were diagnosed according to revised ESPGAN criteria (Walker-Smith et al. 1990), and previously typed for HLA-DQ2 alleles and divided into DQ2-positive CD patients (carriers of DQA1*0501 and DQB1*02 in cis or trans, n=56) and DQ2-negative CD patients (n=10). Healthy controls were also typed for HLA-DQ2. The TNFA–308 polymorphism and the polymorphism at the first intron of LTA were typed in CD patients and healthy controls and the results were correlated with the presence of DQ2.
tron of the LTA gene were typed in the CD patients and the control group. The control group was ethnically and geographically matched with the CD patient group. The study was approved by the Ethics Committee of the University Hospital and Faculty of Medicine.

**HLA-DQ typing**

**HLA-DQA1*0501 and –DQB1*02 genotyping** was performed using sequence-specific primers (SSP-PCR) as previously described (Olerup et al. 1993). Primers (Pharmacia Amershams, Sweden) were used at 0.25 µM, control primers for amplification (C3 and C5, complementary to codons 193–200, Pharmacia) at one fifth of the concentration of DQ primers and Taq DNA polymerase at 0.25 units (Pharmacia). The PCR mixture was run for 25 cycles (at 94, 55 and 72 °C, during 20, 50 and 20 s, respectively).

**TNFA –308 polymorphism genotyping**

The SSP-PCR method, with specific primers for each allele of the G to A polymorphism at -308 position, was used for TNFA –308 polymorphism genotyping. Conditions were previously described (Verjans et al. 1994). The TNFA*I allele defines G in position –308 and TNFA*I2 defines A. The control primers were the same as used in HLA-DQ typing. (Olerup et al. 1993).

**LTA NcoI polymorphism genotyping**

Genotyping for the polymorphism in the first intron of the LTA gene was carried out using RFLP-PCR and previously published primers and protocols (Moffatt and Cookson 1997). After amplification, 5 µl of PCR product was digested with 5 units of NcoI restriction enzyme (New England BioLabs) for 1 h, at 37 °C, in a total volume of 20 µl. The LTA*I allele is identified by two bands of 250 and 500 bp. LTA*I2 produces a single band of 750 bp (absence of restriction site).

All PCR and digestion products were separated on 2% agarose gels and visualized under UV light after ethidium bromide staining.

**Statistical analysis**

The χ² test with Yates’ correction was used to compare frequencies amongst groups. Fisher’s exact test was used in 2×2 tables.

**Table 1** TNFA promoter gene –308 polymorphisms: genotype frequencies, TNFA*I2 allele frequency and frequency of the presence of at least one TNFA*I2 allele per individual. Comparisons between CD patients and healthy controls, stratified by the presence of DQ2: χ² with Yates’ correction and Fisher’s exact test for 2×2 tables. Odd ratio (OR) and ethicologic fraction (EF) for the presence of TNFA*I2 are also listed. NS not significant

<table>
<thead>
<tr>
<th></th>
<th>TNFA –308 genotype</th>
<th>Allele frequency</th>
<th>TNFA*I2-positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1/1 1/2 2/2</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td>Healthy controls (HC)</td>
<td>51 14 0</td>
<td>P=0.0007 14</td>
<td>P&lt;0.0001 14</td>
</tr>
<tr>
<td>Celiac disease (CD)</td>
<td>78.5% 21.5% 0%</td>
<td>10.77% 27</td>
<td>30% 55.6%</td>
</tr>
<tr>
<td>HC DQ2-positive</td>
<td>14 2 0</td>
<td>P=0.0028 2</td>
<td>P=0.0045 2</td>
</tr>
<tr>
<td>CD DQ2-positive</td>
<td>13 21 2</td>
<td>6.25% 25</td>
<td>34.7% 23</td>
</tr>
<tr>
<td>HC DQ2-negative</td>
<td>35 12 0</td>
<td>N.S. 12</td>
<td>N.S. 12</td>
</tr>
<tr>
<td>CD DQ2-negative</td>
<td>7 2 0</td>
<td>11.1% 2</td>
<td>22.2%</td>
</tr>
</tbody>
</table>

**Results**

**HLA-DQA1*0501 and –DQB1*02**

Data on the presence of DQ2 alleles (DQA1*0501 and DQB1*02) in our CD population have been previously reported (Arranz et al. 1997; Polvi et al. 1998a): 93% of patients had both alleles (DQ2-positive) and the remainder (7%) showed only one or none of these alleles.

We identified the presence of DQ2 alleles in the healthy population in which TNFA and LTA gene polymorphisms were typed. DQA1*0501 was present in 34/63 of healthy controls (54%) and DQB1*02 in 30/63 (47.6%). 16/63 cases (25%) presented both alleles (DQ2-positive).

**TNFA –308 genotyping**

The frequency of the TNFA*I2 allele is increased in CD compared to healthy controls (30% vs 10.77%, χ²=17.6, P<0.0001) (Table 1). Genotype frequencies are also different between the groups, with an increase in the frequency of heterozygotes in CD compared with healthy controls (χ²=14.57, P<0.0007). We define “positivity” for allele TNFA*I2 as the presence of at least one allele of TNFA*I2 in one individual; the frequency of “positivity” for this allele is higher in CD than in healthy controls (55.6% vs 21.5%, χ²=13.45, P<0.0005; OR=4.46 –95%; CI: 1.94–10.29; EF=0.43).

Since TNF genes are in linkage disequilibrium with HLA, we divided the groups by the presence of DQ2. DQ2-positive is defined as the presence of both alleles HLA-DQA1*0501 and DQB1*02 in the same individual,