Prevalence of SLC22A4 1672T and SLC22A5 -207C Combination Defined TC Haplotype in Hungarian Ulcerative Colitis Patients

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Ulcerative colitis (UC) is a chronic inflammatory disease of the gastrointestinal tract. The aim of this study was to verify the prevalence rate of the haplotype called TC, determined by combination of two functional alleles of OCTN cation transporter genes (SLC22A4 1672T and SLC22A5 -207C combination variants) in ulcerative colitis patients and unrelated healthy controls. The “TC haplotype” has recently been suggested to confer risk for UC. A total of 121 unrelated Hungarian subjects with UC and 110 matched controls were genotyped for the two single nucleotide polymorphisms. The genotypes were determined by using PCR/RFLP assay and direct sequencing. The SLC22A4 1672T allele frequency was 46.7% in the patients with UC and 46.4% in the controls, whereas the SLC22A5 -207C allele occurred in 48.8% of the patients and 51.4% of the controls. The prevalence of the TC haplotype was 19% in the patient group and 22.7% in controls. Since there was no accumulation of the TC haplotype in the patient group, our observation suggests that carrying the TC haplotype is not associated with a higher risk for UC in the Hungarian population.

Key words: ulcerative colitis, OCTN1, OCTN2, SLC22A4, SLC22A5, TC haplotype

Introduction

Ulcerative colitis (UC) is an inflammatory bowel disease (IBD) that leads to chronic inflammation of the digestive tract.1 UC is restricted to the colon, characteristically starting in the rectum and then spreading proximally in a continuous fashion with mucosal ulcerations.2,3 The risk of colorectal cancer increases with 2% after 10 years, 8% after 20 years, and 18% after 30 years.4

The pathogenesis of IBD is very complex and both environmental and genetic factors contribute to its etiology.5,6,11,22,30 Environmental factors may be smoking, infectious agents, diet, drugs, stress and social status.9,12 IBD may develop in a susceptible individual when the normal host-microbial interactions are dysregulated.13 Studies have demonstrated that relatives of persons with either Crohn’s disease (CD) or UC are at increased risk for developing either form of IBD.4,29 Genome-wide linkage analyses and candidate gene-based association studies have identified IBD loci on chromosomes 1, 5, 6, 12, 14, 16 and 19.11,24,25 The DLG5 gene, encoding a scaffolding protein that plays a role in epithelial cell integrity, is associated with UC.15 The variant G113A in the DLG5 gene causes R30Q substitution resulting in defect of the intestinal epithelial barrier function.16 Two variants of the TLR4 gene, Asp299Gly and Thr399Ile, are also associated with UC and participate in the innate immune response.24 HLA class II gene alleles HLA-DRB1*0103 and DRB1*1502 also show association with UC, predisposing to an altered regulation of immunologic mechanisms.24
Two variants of the OCTN cation transporter, C1672T in the SLC22A4 gene and G-207C in the SLC22A5 gene, were shown to alter the functions of these genes and have been suggested to be disease-causing mutations within the IBD5 locus. These polymorphisms together determine the so-called TC haplotype which has been suggested to be a susceptibility variant for CD. So far several reports have been published on the significance of the TC haplotype; the results are controversial. In several reports have been published on the significance of the TC haplotype; the results are controversial. In several studies in different populations such as German, Swedish and other Caucasian, Greek, Italian, Scottish, Spanish, Swedish and other Caucasian showed an association of this haplotype with Crohn’s disease. Peltekova et al reported on two novel functional Crohn’s disease-associated single nucleotide polymorphisms: the C1672T substitution in exon 9 of the SLC22A4 gene and the G-207C transversion in the promoter region of the SLC22A5 gene; these two SNPs together define the so-called TC haplotype. Several studies in different populations such as German, Greek, Canadian, Italian, Scottish, Spanish, Swedish and other Caucasian showed an association of this haplotype with Crohn’s disease. Peltekova et al. examined the Flemish population and found that OCTN variants were associated with perianal and penetrating CD. In a recent study we examined the association between these two functional variants of the OCTN cation transporter genes and Crohn’s disease in pediatric and adult patients. There were no significant differences in the allele frequencies either for SLC22A4 1672T or SLC22A5 -207C SNPs. The TC haplotype frequency was 19% in the patient group and 22.7% in the controls (Table 1).

**Materials and Methods**

We examined 121 patients with typical symptoms and diagnosis of ulcerative colitis (47 males, 74 females; mean age 47.8±1.37 years) and 110 clinically healthy controls (59 males, 51 females; mean age 46.7±1.87 years). The control subjects did not receive any drug treatment, while UC patients were treated with various drugs, such as sulfasalazine, 5-aminosalicylic acid, budesonide, methylprednisolone or azathioprine. During the entire study period the guidelines and regulations approved by the local Ethics Committee and the Helsinki Declaration of 1975 were followed. The diagnosis of ulcerative colitis was based on typical symptoms, endoscopic and histological findings in every case.

Genomic DNA was extracted from peripheral blood using standard desalting method. We examined two SNPs: one in the OCTN1 cation transporter SLC22A4 gene C1672T (rs1050152) in exon 9, and another one in the promoter region of the OCTN2 cation transporter SLC22A5 gene G-207C (rs2631367).

For genotyping we used a simple PCR/RFLP assay and direct sequencing. For the PCR amplification the following primers were used: for SLC22A4 C1672T, forward primer, 5’-TGA CAG GAA AGA ATG AAA AGC C-3’, reverse primer, 5’-TTT CAC TTT CTG CAT CTG CTC T-3’; for SLC22A5 G-207C, forward primer, 5’-GCC GCT CTG CCT GCC AGC-3’, reverse primer, 5’-GCT CGC TAT CAG GAA CAC GGA GGA-3’. PCR amplifications were performed on MJ Research PTC 200 thermal cyclers using the following conditions: predenaturation for 2 min at 95 °C, followed by 35 cycles of denaturation for 30 sec at 95 °C, annealing for 30 sec at 54 °C for SLC22A4 and 58 °C for SLC22A5, primer extension for 30 sec at 72 °C, and final extension at 72 °C for 5 min. The amplicons were digested by allele-specific restriction endonucleases, MnlI for SLC22A4 C1672T and HpaII for SLC22A5 G-207C. For the 1672C allele, MnlI cleaves the 358-bp PCR product into 62-bp, 101-bp and 195-bp fragments. If the mutation was present, a 163-bp and a 195-bp fragment could be detected. For the SLC22A5 SNP, the PCR product was 386 bp long, and was digested with HpaII. The GG genotype resulted in 31-bp, 42-bp and 313-bp bands. In GC genotype the digestion resulted in 31-bp, 42-bp, 313-bp and 355-bp digestion products. For patients with CC genotype, 31-bp and 355-bp fragments were seen. The restriction fragments were separated by electrophoresis on 3% agarose gels containing ethidium bromide, and visualized by UV illumination.

For direct sequencing we used the same primers and an ABI PRISM 3100 AVANT Genetic Analyzer.

**Results**

The results are shown in Table 1. Allele frequencies followed the Hardy-Weinberg equilibrium for both single nucleotide variants both in the patients and controls.

We found that for SLC22A4 C1672T the T allele frequency was not significantly different between UC patients and healthy controls (Table 1). For SLC22A5 G-207C the C allele frequency was also not significantly different in the UC population compared to the control group (Table 1). There were no significant differences in the allele frequencies either for SLC22A4 1672T or SLC22A5 -207C SNPs.

The TC haplotype frequency was 19% in the patient group and 22.7% in the controls (Table 1).

**Discussion**

Two main types of chronic inflammatory bowel disease are Crohn’s disease and ulcerative colitis. Peltekova et al. reported on two novel functional Crohn’s disease-associated single nucleotide polymorphisms: the C1672T substitution in exon 9 of the SLC22A4 gene and the G-207C transversion in the promoter region of the SLC22A5 gene; these two SNPs together define the so-called TC haplotype. Several studies in different populations such as German, Greek, Canadian, Italian, Scottish, Spanish, Swedish and other Caucasian showed an association of this haplotype with Crohn’s disease. Vermeire et al. examined the Flemish population and found that OCTN does not play a role in the susceptibility to IBD, either CD or UC, but plays a role in the phenotypic expression of the disease; OCTN variants were associated with perianal and penetrating CD. In a recent study we examined the association between these two functional variants of the OCTN cation transporter genes and Crohn’s disease in pediatric and adult patients. There were no significant differences...
Table 1. Comparison of the allele frequencies of SLC22A cation transporter genes in patients with ulcerative colitis and controls

<table>
<thead>
<tr>
<th>SLC22A4</th>
<th>Patients n=121</th>
<th>Controls n=110</th>
</tr>
</thead>
<tbody>
<tr>
<td>exon 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>38 (31.4%)</td>
<td>35 (31.8%)</td>
</tr>
<tr>
<td>C1672T</td>
<td>53 (43.8%)</td>
<td>48 (43.6%)</td>
</tr>
<tr>
<td>CT</td>
<td>30 (24.8%)</td>
<td>27 (24.5%)</td>
</tr>
<tr>
<td>GC</td>
<td>30 (24.8%)</td>
<td>27 (24.5%)</td>
</tr>
<tr>
<td>T allele frequency</td>
<td>46.7%</td>
<td>46.4%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SLC22A5</th>
<th>Patients n=121</th>
<th>Controls n=110</th>
</tr>
</thead>
<tbody>
<tr>
<td>promoter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>33 (27.3%)</td>
<td>25 (22.7%)</td>
</tr>
<tr>
<td>region</td>
<td>58 (47.9%)</td>
<td>57 (51.8%)</td>
</tr>
<tr>
<td>G-207C</td>
<td>30 (24.8%)</td>
<td>28 (25.5%)</td>
</tr>
<tr>
<td>C allele frequency</td>
<td>48.8%</td>
<td>51.4%</td>
</tr>
<tr>
<td>TC haplotype</td>
<td>23 (19.0%)</td>
<td>25 (22.7%)</td>
</tr>
</tbody>
</table>

in the allele frequencies of SLC22A4 C1672T and SLC22A5 G-207C mutations in Hungarian patients with CD, compared to unrelated healthy controls.3

In the limited number of publications on UC, Palmieri et al.18 found that the TC haplotype frequency was increased in both CD and UC, and the TC haplotype may influence some clinical features of IBD. Waller et al.11 reported that the OCTN variants were as strongly associated with UC as they were with CD. We found that the TC haplotype was not associated with UC in the Hungarian population, therefore, we conclude that these SNPs do not necessarily confer susceptibility to UC. Tosa et al.13 examined patients with UC and CD in a Japanese population, and they also found that the TC haplotype is not associated with IBD. Our findings show that it may depend on the population whether this haplotype causes susceptibility to UC. Other nearby SNPs in the SLC22A4 and SLC22A5 genes within the IBD5 locus region are statistically candidates in terms of their association to CD. Further studies are required on a larger population to understand the role of the IBD5 locus in the susceptibility to IBD.

References