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ARTICLE

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. (Pathology Oncology Research Vol 13, No 1, 53–56)

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.[7] UC is restricted to the colon, characteristically starting in the rectum and then spreading proximally in a continuous fashion with mucosal ulcerations.[2,8] The risk of colorectal cancer increases with 2% after 10 years, 8% after 20 years, and 18% after 30 years.[6]

The pathogenesis of IBD is very complex and both environmental and genetic factors contribute to its etiology.[5,11,20,22,29,32] 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.[15] 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]

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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.[23] These polymorphisms together determine the so-called TC haplotype which has been suggested to be a susceptibility variant for CD.[19] So far several reports have been published on the significance of this haplotype; the results are controversial.[7,16,17,21,26,30] In a recent study we examined these two SNPs in Hungarian patients with CD, and found no significant differences from controls in the allele frequencies.[3] Besides CD, the TC haplotype has also been suggested to confer risk for UC.[18,31]

Our aim was to examine the prevalence of these two described SNPs in Hungarian patients with ulcerative colitis, and test the possible significance of TC haplotype in the development of the disease.

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 administration, while UC patients were treated with various drugs, such as sulfosalazine, 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'-GGT 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[19] 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,[27] Greek,[7] Canadian,[16] Italian,[18] Scottish,[17,21] Spanish,[14] Swedish[26] and other Caucasian[1,10] showed an association of this haplotype with Crohn's disease. Vermeire et al[30] 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.[3] There were no significant differences

Table 1. **Comparison of the allele frequencies of SLC22A cation transporter genes in patients with ulcerative colitis and controls**

			Patients n=121	*Controls n=110*
SLC22A4	*exon 9 C1672T*	*CC*	38 (31.4%)	35 (31.8%)
		CT	53 (43.8%)	48 (43.6%)
		TT	30 (24.8%)	27 (24.5%)
	T allele frequency		46.7%	46.4%
SLC22A5	*promoter region G-207C*	*GG*	33 (27.3%)	25 (22.7%)
		GC	58 (47.9%)	57 (51.8%)
		CC	30 (24.8%)	28 (25.5%)
	C allele frequency		48.8%	51.4%
	TC haplotype		23 (19.0%)	25 (22.7%)

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[31] 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[28] 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

1. *Babusukumar U, Wang T, McGuire E, et al:* Contribution of OCTN variants within the IBD5 locus to pediatric onset Crohn's disease. Am J Gastroenterol 101:1354-1361, 2006
2. *Bene J, Komlosi K, Havasi V, et al:* Changes of plasma fasting carnitine ester profile in patients with ulcerative colitis. World J Gastroenterol 12:110-113, 2006
3. *Bene J, Magyari L, Talian G, et al:* Prevalence of SLC22A4, SLC22A5 and CARD15 gene mutations in Hungarian pediatric patients with Crohn's disease. World J Gastroenterol 12:5550-5553, 2006
4. *Bonen DK, Cho JH:* The genetics of inflammatory bowel disease. Gastroenterology 124:521-536, 2003
5. *Danese S, Fiocchi C:* Etiopathogenesis of inflammatory bowel diseases. World J Gastroenterol 12:4807-4812, 2006
6. *Eaden JA, Abrams KR, Mayberry JF:* The risk of colorectal cancer in ulcerative colitis: a meta-analysis. Gut 48:526-535, 2001
7. *Gazouli M, Mantzaris G, Archimandritis AJ, et al:* Single nucleotide polymorphisms of OCTN1, OCTN2, and DLG5 genes in Greek patients with Crohn's disease. World J Gastroenterol 11:7525-7530, 2005
8. *Giallourakis C, Stoll M, Miller K, et al:* IBD5 is a general risk factor for inflammatory bowel disease: replication of association with Crohn disease and identification of a novel association with ulcerative colitis. Am J Hum Genet 73:205-211, 2003
9. *Jantchou P, Monnet E, Carbonnel F:* [Environmental risk factors in Crohn's disease and ulcerative colitis (excluding tobacco and appendicectomy)] (in French). Gastroenterol Clin Biol 30:859-867, 2006
10. *Leung E, Hong J, Fraser AG, et al:* Polymorphisms in the organic cation transporter genes SLC22A4 and SLC22A5 and Crohn's disease in a New Zealand Caucasian cohort. Immunol Cell Biol 84:233-236, 2006
11. *Machida H, Tsukamoto K, Wen CY, et al:* Association of polymorphic alleles of CTLA4 with inflammatory bowel disease in the Japanese. World J Gastroenterol 11:4188-4193, 2005
12. *Mahid SS, Minor KS, Soto RE, et al:* Smoking and inflammatory bowel disease: a meta-analysis. Mayo Clin Proc 81:1462-1471, 2006
13. *Mahida YR, Rolfe VE:* Host-bacterial interactions in inflammatory bowel disease. Clin Sci (Lond) 107:331-341, 2004
14. *Martinez A, Del Carmen MM, Mendoza JL, et al:* Association of the organic cation transporter OCTN genes with Crohn's disease in the Spanish population. Eur J Hum Genet 14:222-226, 2006
15. *Medici V, Mascheretti S, Croucher PJ, et al:* Extreme heterogeneity in CARD15 and DLG5 Crohn disease-associated polymorphisms between German and Norwegian populations. Eur J Hum Genet 14:459-468, 2006
16. *Newman B, Gu X, Wintle R, et al:* A risk haplotype in the Solute Carrier Family 22A4/22A5 gene cluster influences phenotypic expression of Crohn's disease. Gastroenterology 128:260-269, 2005
17. *Noble CL, Nimmo ER, Drummond H, et al:* The contribution of OCTN1/2 variants within the IBD5 locus to disease susceptibility and severity in Crohn's disease. Gastroenterology 129:1854-1864, 2005
18. *Palmieri O, Latiano A, Valvano R, et al:* Variants of OCTN1-2 cation transporter genes are associated with both Crohn's disease and ulcerative colitis. Aliment Pharmacol Ther 23:497-506, 2006
19. *Peltekova VD, Wintle RF, Rubin LA, et al:* Functional variants of OCTN cation transporter genes are associated with Crohn disease. Nat Genet 36:471-475, 2004
20. *Pena AS:* Contribution of genetics to a new vision in the understanding of inflammatory bowel disease. World J Gastroenterol 12:4784-4787, 2006
21. *Russell RK, Drummond HE, Nimmo ER, et al:* Analysis of the influence of OCTN1/2 variants within the IBD5 locus on disease susceptibility and growth indices in early onset inflammatory bowel disease. Gut 55:1114-1123, 2006
22. *Satsangi J, Jewell D, Parkes M, et al:* Genetics of inflammatory bowel disease. A personal view on progress and prospects. Dig Dis 16:370-374, 1998
23. *Silverberg MS:* OCTNs: will the real IBD5 gene please stand up? World J Gastroenterol 12:3678-3681, 2006
24. *Siminovitch KA:* Advances in the molecular dissection of inflammatory bowel disease. Semin Immunol 18:244-253, 2006

25. *Taylor KD, Yang H, Rotter JI:* Inflammatory bowel disease. II. Gene mapping. Mol Genet Metab 74:22-44, 2001
26. *Torkvist L, Noble CL, Lordal M, et al:* The contribution of OCTN1/2 variants within the IBD5 locus to Crohn's disease in the Swedish population. Gastroenterology 128:A449, 2005
27. *Torok HP, Glas J, Tonenchi L, et al:* Polymorphisms in the DLG5 and OCTN cation transporter genes in Crohn's disease. Gut 54:1421-1427, 2005
28. *Tosa M, Negoro K, Kinouchi Y, et al:* Lack of association between IBD5 and Crohn's disease in Japanese patients demonstrates population-specific differences in inflammatory bowel disease. Scand J Gastroenterol 41:48-53, 2006
29. *Urcelay E, Mendoza JL, Martinez A, et al:* IBD5 polymorphisms in inflammatory bowel disease: association with response to infliximab. World J Gastroenterol 11:1187-1192, 2005
30. *Vermeire S, Pierik M, Hlavaty T, et al:* Association of organic cation transporter risk haplotype with perianal penetrating Crohn's disease but not with susceptibility to IBD. Gastroenterology 129:1845-1853, 2005
31. *Waller S, Tremelling M, Bredin F, et al:* Evidence for association of OCTN genes and IBD5 with ulcerative colitis. Gut 55:809-814, 2006
32. *Yang H, Taylor KD, Rotter JI:* Inflammatory bowel disease. I. Genetic epidemiology. Mol Genet Metab 74:1-21, 2001