THE ROLE OF IBD5 LOCUS ON CHROMOSOME 5q31 REGION IN THE DEVELOPMENT OF CROHN'S DISEASE AND ULCERATIVE COLITIS IN HUNGARIAN POPULATION SAMPLES

PhD thesis

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Introduction

The ulcerative colitis and the Crohn's disease are the idiopathic chronic inflammatory diseases of the gastrointestinal tract. While the ulcerative colitis affects exclusively the colon, the Crohn's disease can spread to any part of the digestive canal from the mouth to the anus. In case of the development of Crohn's disease most commonly both the small intestine as well as the colon are affected jointly (40-50%). In 30-40% of the cases only the small intestine, whereas in 15-25% of the cases only the colon is affected. Upper gastrointestinal localisation is relatively rare.

Both the ulcerative colitis and the Crohn's disease are multifactorial clinical pictures. In their development environmental, immunological as well as genetic factors are considered to play important roles.

Family observations show that the inflammatory bowel disease occurring in family anamnesis is the most explicit risk factor concerning the development of the disease. In close relatives of Crohn's patients the probability of developing the same disease is 2,2-16,2%, developing IBD is 5,2-22,5%. In case of ulcerative colitis the development of the same disease can be expected in 5,7-15,5% of the cases, while the probability of developing IBD is present in 6,6-15,8% of the cases.

Genetic factors play important roles in triggering inflammatory bowel diseases; however none of the clinical pictures can be traced back to one single genetic variant. The wide-spread use of genome wide screening and the linkage map technique have proved to be especially informative methods in the examination of genome sections associated with IBD.

The role of IBD5 region of chromosome 5q31 in the development of IBD has been studied by several authors. The organic cation transporter (OCTN) 1 and 2 genes (SLC22A4 and SLC22A5, encoding OCTN1 and OCTN2, respectively), which are responsible for the two-way transport of carnitine and other organic cations, are located in the IBD5 region. The SLC22A4 variant allele goes with the increase of organic cation transport funtion, whereas in the case of the SLC22A5 allele the activation of the promoter of the gene triggered by heat-shock proteins changes.

Peltekova and colleagues described the TC haplotype in the 5q31 region, which is determined by the polymorphism of the SLC22A4 gene C1672T (rs1050152) and the SLC22A5 gene G-207C (rs2631367). The team has proved that haplotype confers susceptibility for Crohn's disease. Silverberg and colleagues studied the role of other functional variants in 5q31 region in a relatively large population. They have discovered

genetic association between the IBD and the IGR2096a_1 (rs12521868), as well as the IGR2198a_1 (rs11739135) loci.

The aims of the research

The aim of our work was to examine the role f the IBD5 region in the case of ulcerative colitis and Crohn's disease in Hungarian population. We aimed at investigating the role of the IGR2096a_1 (rs12521868), IGR2198a_1 (rs11739135), IGR2230a_1 (rs17622208) genetic variants as well as the SLC22A4 (rs1050152), SLC22A5 (rs2631367) genetic variants in conferring susceptibility to IBD.

We were trying to find an answer to the question, whether in the Hungarian population the OCTN1, OCTN2 genetic variants as well as the TC haplotype described by Peltekova and colleagues had a determinative role in the development of idiopathic inflammatory bowel disease or other minor alleles scrutinized by Silverberg and colleagues. In order to find the answer we chose and examined the IGR2096a_1, the IGR2198a_1, and the IGR2230a_1 genetic variants.

Patients and methods

Our research was based on a Hungarian population of patients suffering from ulcerative colitis and Crohn's disease, which practically represented the population of the whole country. The samples were collected in Békéscsaba, Budapest, Miskolc, Pécs, Szombathely, and Zalaegerszeg. Our genetic database is part of the national genetic database.

In order to scrutinize IGR2096a_1, IGR2198a_1, SLCA22A4, SLCA22A5 the samples of 217 Crohn's patients (101 males, 116 females, mean age 39,5±1,0 years), and 252 patients with ulcerative colitis (110 males, 142 females, mean age 46,7±1,0 years), while for scrutinizing IGR2230a_1 the samples of 200 Crohn's disease patients (97 males, 103 females, mean age 39,4±1,1 years) and 246 patients with ulcerative colitis (108 males, 138 females, mean age 44,0±1,1 years) were processed. Both the patients with Crohn's disease and those with ulcerative colitis had typical clinical symptoms. The diagnosis in all cases was based on the clinical picture, endoscopic and histological findings.

A total of 290 clinically healthy control subjects (159 males, 131 females, mean age 40,0±0,8 years), in the case of scrutinizing IGR2230a_1 187 individuals (105 males, 81 females, mean age 37,7±0,8 years) were collected. The subjects of the research gave their written consent with no exception for use of their DNA for genetic study. The study was approved by the National Ethics Committee (ETT TUKEB) and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

At the location of the involvement of the patients blood sample was taken and placed into EDTA containing tubes in order to avoid blood clotting. Before taking the blood sample, ulcerative colitis had been diagnosed by using colonoscopy together with histological biopsy, whereas Crohn's disease was diagnosed by using ileo-colonoscopy and intestine X-ray examination.

DNA extraction was performed by using standard desalting method .The starting point of the analysis of the individual variants was the DNA amplification performed via polymerase chain reaction (PCR) which in the case of IGR2096a_1 rs12521868, IGR2198a_1 rs11739135 and SLC22A5 rs2631367 mutations was followed by RFLP method, while the determination of SLC22A4 rs1050152 variants was performed by direct sequencing using an ABI 3100 automatic sequencer.

During PCR reactions the following primer pairs were employed which had been designed by our team:

In the case of *IGR2096a_1 rs12521868 variant* the forward primer was:

5'-CAAGATTTCTGCCATAGCCTCCT-3',

the reverse prime was:

5'-GGAGGGTGGTGTAGCCAGAGTAG-3';

In the case of *IGR2198a_1 rs11739135 variant* the forward primer was:

5'-AGACACTGGGACATCATCTGTCTG-3',

the reverse primer was:

5'-GGGCAATTCTATGAGGACATTTAGA-3';

In the case of *SLC22A5 geners2631367 variant* the forward primer was:

5'-GCCGCTCTGCCTGCCAGC-3',

the reverse primer:

5'-GGTCGCTATCAGGAACACGGAGGA-3';

In the case of *SLC22A4 gene rs1050152 variant* the forward primer was:

5'-AGAGAGTCCTCCTATCTGATTG-3',

the reverse primer:

5'-TCCTAGCTATTCTTCCATGC-3';

In the case of *IGR2230a_1 rs1762208 the* forward primer was:

5'-CAGAAGAATGCCCTTGATGTG-3',

the reverse primer was:

5'-TCAGAAGCTGTCCATCCCAC-3'.

During the DNA amplification the following thermo parameters were employed: predenaturation at 95ºC for 2 minutes, 35 recurring cycles with the following steps: denaturation at 95ºC for 30 seconds, primer binding at 58ºC for 45 seconds (in the case of SLC22A4 rs1050152 at 54ºC for 30 seconds), polymerisation at 72ºC for 45 seconds, then after the cycles a final chain extension at 72ºC for 5 minutes.

In the case of the IGR2096a_1 rs12521868, IGR2198a_1 rs11739135 and SLC22A5 rs2631367, IGR2230a_1 rs1762208 mutations the amplicons were digested by the following allele-specific restriction endonucleases: *Tru1I* (IGR2096a_1), *Hin1II* (IGR2198a_1) and *HpaII* (SLC22A5), *Ddel* (IGR2230a_1).

Every amplicon contained an obligate cleavage site to control the efficacy of the enzyme digestion. For the IGR2096a_1, normal genotype (GG) resulted in two fragments of the 269 bp sized PCR product with the length of 81 bp and 188 bp.

For the homozygous genotype (TT) fragments with the length of 32, 81 and 156 bp were detected, while for the heterozygous genotype all fragments were seen. During the examination of IGR2198a_1 polymorphism in the case of normal genotype (GG) the restriction endonuclease cleaved the 280 bp sized PCR product into fragments with the length of 38, 60, and 1832 bp. In patients with homozygous genotype (CC) the cleavage resulted in DNA fragments sized 60 and 202 bp. For the heterozygous genotype all fragments were visible. At the presence of SLC22A5 normal genotype (GG) during the digestion fragments with the length of 31bp, 42 bp and 313 bp were detected. In heterozygous genotype DNA fragments sized 31 bp, 42 bp, 313 bp ad 355 bp were detected, while in homozygous genotypes cleavage products sized only 31 bp and 355 bp fragments were identified. During the examination of IGR 2230a_1 polymorphism in the case of G allele sections with the length of 122, 128, and 188 bp were detected, while in the case of A allele fragments of 128 bp and 310 bp fragments were measured.

The separation of the cleavage products took place with the use of gelelectroforesis, etidium-bromide staining and UV light.

For the exploration of associations between the disease and the examined genetic variants, χ^2 test and multivariate regression analysis were carried out with the use of SPSS 11.5 programme. For the examination of genetic linkage Haploview 4.1 programme was employed.

Results

Our team investigated the role of the OCTN1 and OCTN2 cation transporters located in the IBD5 region and the TC haplotype described as risk factor by Peltekova and colleagues in the development of Crohn's disease and ulcerative colitis. Besides that, the susceptibility nature of other variants located in IBD5 region was also suggested, among which the role of IGR2198a_1, IGR2096a_1, and the IgR2230a_1 were examined.

The frequency of the IGR2096a_1 T allele (47,2%) and the IGR2198a_1 C allele (45,9%) were significantly higher in the case of Crohn's disease compared to those in the control group (38,2%, 37,7%). These two alleles were also more common in the case of ulcerative colitis (41,8%, 42,0%), although significant difference could not be detected. The frequency of IGR2230a_1 A allele in the case of Crohn's disease (48,5%) as well as at ulcerative colitis (47,1%) proved to be higher compared to those in the control population (44,6%), but no significant difference was detected.

The frequency of the SLCA22A4 T allele (in Crohn's disease 45,4%, in ulcerative colitis 45,4%), and the SLCA22A5 C allele (in Crohn's disease 49,7%, in ulcerative colitis 47,4%) did not show difference compared to those in the control group (39,3%, 46,0%).

While examining the linkage disequilibrium values in pairs among the scrutinized variants the most significant association was noticed between the SLC22A5 (rs2631367) and the IGR2230a_1 (rs17622208) , which was (r^2>0,8).

During our research we could not detect any association concerning the OCTN1, and the OCTN2 genetic variants neither in the case of Crohn's disease nor ulcerative colitis. When examining the minor variants located in the IBD5 region, significant association was detected between Crohn's disease and the IGR2096a_1, the IGR2198a_1 genetic variants. Concerning ulcerative colitis no significant difference was detected. In the case of the IGR2230a_1 genetic variant no significant difference was detected neither in the case of Crohn's disease nor ulcerative colitis. Our results can be associated with the suggestions of Noble and colleagues who consider the role of these minor alleles more significant in the development of Crohn's disease than the role of the TC haplotype scrutinized by Peltekova and colleagues.

The detected linkage disequilibrium values among the examined variants are rather high, which certainly suggests a close linkage among the variants of the genes. Among the examined polymorphisms of IBD5 locus, the SLC22A5 rs2631367 and the IGR2230a_1 rs17622208 showed the closest linkage, which coincides with the fact that the distance is the shortest among these mutations on the 5th chromosome. This suggests that it might be more practical to choose only one of the mutations with high LD value for the examination.

Conclusions

1. The IGR2096a_1 T variant located in the IBD5 region, which was examined in Hungary for the first time can be a risk factor in the development of Crohn's disease, similarly to the results of Silverberg, Noble and Latiano.

2. We have not been able to find association between the IGR2096a_1 T variant and ulcerative colitis, our results show a match to the results found in a Scottish population examined by Noble and colleagues, and an Italian population examined by Latiano and colleagues.

3. We examined the IGR2198a_1 C variant for the first time in Hungary, which has proved to be a risk factor in the development of Crohn's disease, corresponding with the previous international findings.

4. The IGR2198a_1has not been found to confer susceptibility to ulcerative colitis in the Hungarian population similarly to the results in the Scottish population.

5. The IGR2230a_1 A variant, which was examined in Hungary for the first time by our team, has not proved to be a risk factor in the development of Crohn's disease, showing disassociation to findings published in international publications.

6. Similarly to Nobel and colleagues, we have not been able to find association between the IGR2230a_1 A variant and the development of ulcerative colitis.

7. We have found linkage between the SLC22A5 (rs2631367) and the IGR2230a_1 (rs17622208), which is (r^2>0,8).

Publications

Publications which have served as a base for the thesis

1. **Lakner L.**, Csöngei V., Sarlós P., Járomi L., Sáfrány E., Varga M., Orosz P., Magyari L., Bene J., Miheller P., Tulassay Zs., Melegh B.: IGR2096a_1 T and IGR2198a_1 C alleles on IBD5 locus of chromosome 5q31 region confer risk for Crohn's disease in Hungarian patients. Int. J. of Colorectal Dis., 2009; 24:503-507. ***Impact factor: 1,767***

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Total impact factor: 15,906

(From that the own publications which have served as a base for the thesis: 4,199)

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