Susceptibility genetic variants in Hungarian morbus Crohn and ulcerative colitis patients

PhD thesis

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1. INTRODUCTION

1.1. Description of inflammatory bowel disease

There are two main types of inflammatory bowel disease (IBD), Crohn's disease (CD) and ulcerative colitis (UC). 20-25.000 people in Hungary have IBD. The disease may occur in persons adults between ages 20 and 40 in both sex, although women are more frequently affected. The pathogenesis of IBD is very complex and both environmental and genetic factors contribute to its etiology. Environmental factors may be infectious agents, drugs, poisons, smoking, alcohol, and bowel bacteria as well. The genetic background has a particular importance, people may have genetic susceptibility and show reduced resistance of the mucosa or abnormal activation of the immune system in the intestines. These together with environmental factors cause inflammation. IBD may develop in a susceptible individual when the normal host-microbial interactions are dysregulated. The normal host-microbial flora consists of 300-400 different strains of bacteria. This balance can overturn, when people have chronic inflammatory bowel disease. This time the concentration of certain bacteria, such as *Bacteroides* increases causing inflammation. External bacteria can be also responsible for IBD *(Listeria monocytogenes, Pseudomonas fluorescens, Escherichia coli, Clostridium difficile, Yersinia enterocolitica, cytomegalovirus, rotavirus).* There are many extraintestinal manifestations associated with inflammatory bowel disease, for instance eye and mouth inflammation, skin rash, gallstones, kidney stones, pancreatitis and joint pain.

1.1.1. Crohn’s disease

The disease is named after Burrill Crohn. CD is a chronic inflammation of uncertain etiology that can affect any portion of the digestive tract from mouth to anus. Inflammation occurs primarily in the ileum and colon, although any portion of the intestinal tract can be affected (oesophagus, gaster). Ileocolitis is the most common form affecting the ileum and the colon. Ileitis only affects the ileum, while granulomatous colitis only affects the colon. The intestinal inflammation can appear in the terminal ileum (terminal ileitis) or might occur in some parts of the ileum (regional enteritis) or the colon (Crohn-colitis). Symptoms include abdominal pain, diarrhea, bloody stools, fistulas, fever, loss of appetite, vomiting, weight loss, growth retardation, indisposition and late of puberty.

1.1.2. Ulcerative colitis

Ulcerative colitis is a disease that causes inflammation and ulcers in the lining of the rectum and colon. When the inflammation occurs in the rectum it is called proctitis. If the entire colon is affected it is called pancolitis. Proctosigmoiditis involves inflammation of the rectum and the sigmoid colon, while left–sided colitis involves inflammation that starts at the rectum and extends up the left colon (sigmoid colon and the descending colon). Symptoms range from abdominal pain, cramps, bloody diarrhea, tenesmus, fever, anemia, loss of body fluids and nutrients, to decreased level of protein and iron.

1.1.3. Comparison of the two diseases

The main difference between CD and UC is the area of the digestive tract they affect. CD can occur along the entire digestive tract and spread deep into the bowel wall (mucosa, submucosa, serosa). In contrast, UC usually only affects the top layer of the colon and rectum (mucosa, submucosa). Crohn's disease is often segmental and the rectum is frequently spared, while ulcerative colitis results in connected inflammation. In Crohn's disease the inflammation penetrates into deeper layers to such an extent that it can reach the external layer of the intestines and might stick to another part of the ileum and the colon or other organs. Fistulas are common in Crohn's disease and either occur in the perianal region or are internal forming between the intestinal structures or between the intestine and other organs. In rare instances the intestinal wall may rupture, allowing bacteria from the intestine to infect other organs causing illness. When the intestine has inflammation, the system can not function normally, this ultimately leads to loss of appetite and weight loss. It is characteristic of Crohn's disease, as the inflammation spreads to the ileum that has an imporant role in absorbing the nutrients.

2. AIMS

Searching for genetic variants that have an association to IBD.

2.1. CARD15 R702W, G908R, 1007finsC

The first identified susceptibility gene for CD was the CARD15 (NOD2) gene on chromosome 16. Studies conducted on Caucasian populations showed that three independent mutations were associated with CD within the NOD2/CARD15 gene: two missense mutations, the Arg702Trp in exon 4 and the Gly908Arg in exon 8; and one insertion, 1007finsC in exon 11. Interestingly, these mutations have not been found at all in Asian patients. Our aim was to analyse the possible connection between these three variants of the CARD15 gene and Crohn's disease to characterize our Hungarian adult samples. Several studies on pediatric CD population have reported an association between CARD15 mutations and CD in North America, in Germany, in Israeli Jewish patients and in an Italian cohort. Our objective was to investigate the prevalence of the three CARD15 mutations in Hungarian pediatric population with CD.

2.2. SLC22A4 C1672T, SLC22A5 G-207C

Peltekova reported on two novel functional Crohn's disease (CD) associated single nucleotide polymorphisms: the C1672T substitution in exon 9 in the SLC22A4 gene and the G-207C transversion in the promoter region in the SLC22A5 gene, these two SNPs together define the so called TC haplotype. Several studies on different populations such as German, Greek, Canadian, Italian, Scottish, Spanish, Swedish and other Caucasians showed that this haplotype is associated with Crohn's disease. Vermeire et al. examined Flemish population and found that OCTN does not play a role in the susceptibility to IBD. In the limited number of publications on UC, Palmieri et al. 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. reported that the OCTN variants were as strongly associated with UC as they were with CD. Tosa et al. examined patients with UC and CD in a Japanese population, and they found that the TC haplotype is not associated with IBD. Our aim was to examine the association

between these two functional variants of the OCTN cation transporter genes and IBD in adult and pediatric patients.

2.3. CTLA4 A+49G

The cytotoxic T-lymphocyte antigen 4 gene is a T cell receptor. Nistico identified a novel polymorphism +49A/G in exon 1, which associates with a Thr to Ala substitution at position 17 of the amino acid sequence. Several studies have reported controversial results on the association of the +49A/G SNP in the CTLA4 gene with type I diabetes, Grave's disease, rheumatoid arthritis, multiple sclerosis and celiac disease. CTLA4 is also a susceptibility gene for two main types of inflammatory bowel disease: Crohn's disease and ulcerative colitis as well. Machida and colleagues found that in Japanese population CTLA4 is one of the determinants of UC, and confers risk for the development of CD associated with fistula formation. Xia and colleagues found no association of the CTLA4 +49G SNP either with IBD in Dutch Caucasian patients or with UC in Chinese patients.

2.4. IL23R rs10889677 C/A, rs2201841 T/C, rs1884444 G/T

Oppmann discovered the IL-23 molecule in 2000 as a novel member of the IL-12 heterodimeric cytokine family. IL-23 plays a key role in the innate and T cell-mediated intestinal inflammation. Genetic studies in humans and mice uncovered a strong association of signaling pathways of IL-23 with IBD and other autoimmune and chronic inflammatory diseases. As an extension, Duerr and colleagues very recently have identified IL23 receptor gene as an inflammatory bowel disease-associated gene in a genome-wide association study. They examined series of SNPs in this gene region and found sets of variants (rs1004819, rs7517847, rs10489629, rs2201841, rs11465804, rs11209026, rs1343151, rs10889677, rs11209032, rs1495965, rs7530511, rs 1884444) that had association with the development of the disease in Jewish and non-Jewish populations. Our aim was to investigate the distribution of the IL23R gene rs10889677 C/A, rs2201841 T/C, and rs1884444 G/T in Hungarian IBD population.

3. MATERIALS AND METHODS

3.1. Patients

We have collected 442 adult IBD blood samples (201 CD, 241 UC) and 19 pediatric CD blood samples for our biobank (www.biobank.hu) from several regions of the country (Békéscsaba, Budapest, Pécs, Szombathely, Zalaegerszeg) since 2003. As controls 235 adult and 49 pediatric blood samples were collected. All control subjects were age- and sex-matched clinically healthy subjects.

3.2. Genetic analysis

Genomic DNA was extracted from peripheral blood leukocytes with a routine salting out method. We used polymerase chain reaction (PCR) with primers, Taq polimerase, dNTP, buffer and DNA to be amplified. We analysed the PCR product by electrophoresis visualized by UV transillumination. To identify the different genetic variants (CARD15, SLC22A4, SLC22A5, CTLA4, IL23R) we used RFLP method or direct sequencing. During the entire study period the guidelines and regulations

approved by the local Research-Ethical Committee of the Medical and Healthyscience College of Pecs in 10.07.2000., 04.02.2003., and 09.03.2004. were followed.

3.2.1. CARD15 R702W, G908R, 1007finsC

For the PCR amplification as well as for the sequencing the following primers were used: for R702W the forward primer was: 5'-GAG CCG CAC AAC CTT CAG ATC-3', and the reverse primer was: 5'-ACT TGA GGT GCC CAA CAT TCA G-3'; for G908R the forward primer was: 5'-GTT CAT GTC TAG AAC ACA TAT CAG G-3', and the reverse primer was: 5'-GTT CAA AGA CCT TCA GAA CTG G-3'; for 1007finsC the forward primer was: 5'-CCT TGA AGC TCA CCA TTG TAT C-3', the reverse primer was: 5'-GAT CCT CAA AAT TCT GCC ATT C-3'. Sequencing was performed in an ABI 3100 automatic sequencer.

3.2.2. SLC22A4 C1672T, SLC22A5 G-207C

For genotyping we used a simple PCR/RFLP assay and direct sequencing. For the PCR amplification as well as for the sequencing the following primers were used: for SLC22A4 C1672T the forward primer was: 5'-AGA GAG TCC TCC TAT CTG ATT G-3', and the reverse primer was: 5'-TCC TAG CTA TTC TTC CAT GC-3'; for SLC22A5 G-207C the forward primer was: 5'-AGT CCC GCT GCC TTC CTA AG-3', and the reverse primer was: 5'-GTC ACC TCG TCG TAG TCC CG-3'. For PCR/RFLP methods the following primers were designed and used: for SLC22A4 C1672T the forward primer was: 5'-TGA CAG GAA AGA ATG AAA AGC C-3', and the reverse primer was: 5'-TTT CAC TTT CTG CAT CTG CTC T-3'. For SLC22A5 G-207C the forward primer was: 5'-GCC GCT CTG CCT GCC AGC-3', and the reverse primer was: 5'-GGT CGC TAT CAG GAA CAC GGA GGA-3'. The amplicons were digested by allele-specific restriction endonucleases, *MnlI* for SLC22A4 C1672T and *HpaII* for SLC22A5 G-207C. The restriction fragments were separated by electrophoresis on 3% agarose gels containing ethidium bromide and visualized by UV transillumination.

3.2.3. CTLA4 A+49G

For genotyping we used PCR/RFLP methods. For the amplification of the target sequence the following primers were designed and used: 5'-CTT GAG GTT GTC TTT TCG AG-3' as the sense and 5'-TAC TAA ATA CCT GGC GCT CT-3' as the antisense primer. The amplicons were digested by allele-specific restriction endonuclease, *Bse XI*.

3.2.4. IL23R rs10889677 C/A, rs2201841 T/C, rs1884444 G/T

PCR-RFLP methods were applied to test the alleles of the IL-23 receptor gene using the following primers: for rs10889677 the forward was 5'-ATC GTG AAT GAG GAG TTG CC-3' and the reverse primer was 5'-TGT GCC TGT ATG TGT GAC CA-3', for rs2201841 the forward primer was 5'-GGC AAA AGG GAA TTG AGA GG-3' and the reverse primer was 5'-GGC CTA TGA TTA TGC TTT TTC CTG-3', for rs1884444 the forward primer was 5'-CAG TCT TTT CCT GCT TCC AGA CAT GAA TC-3' and the reverse primer was 5'-AAT AAA ATC ATA CTC TTG CCA ATG GCC C-3'. The amplicons were digested by allele-specific restriction endonucleases, *MnlI* for rs10889677, *HpyF3I* for rs2201841 and *PscI* for rs1884444.

3.3. Statistical analysis

Statistical analysis was carried out using SPSS 11.5. for Windows. For statistics the χ^2 method (cross-table analyses) and regression analysis were used to investigate the possible associations between the diseases and the polymorphisms studied.

4. RESULTS

4.1. CARD15 R702W, G908R, 1007finsC

We examined 100 patients with CD (47 males, 53 females, mean age: 37.3 years), and 94 clinically healthy control subjects (47 males, 47 females, mean age: 45.6 years). For 1007finsC we found significantly increased mutant allele frequency in the adult Crohn group compared to the adult controls (Table 1). 17% of the Crohn's disease patients were either heterozygous or homozygous for the mutation compared to 4.3% of controls (Table 1). There were no differences in the allele frequencies of R702W and G908R variants between the adult CD populations and the controls (Table 1).

We examined 19 pediatric patients with CD (14 males, 5 females, mean age: 13.4 years). This cohort was compared with 49 pediatric healthy control subjects (28 males, 21 females, mean age: 14.4 years). We found significantly increased homozygous genotype and mutant allele frequency for G908R and 1007finsC in pediatric patients compared to the pediatric controls (Table 2) . For R702W no significant difference was found between the genotype- and allele-distribution of the the pediatric CD group compared to the healthy controls (Table 2).

4.2. SLC22A4 C1672T, SLC22A5 G-207C

We examined 100 patients with CD (47 males, 53 females, mean age: 37.3 years), and 94 healthy control subjects (47 males, 47 females, mean age: 45.6 years). There were no differences in the allele frequencies of SLC22A4 C1672T and SLC22A5 G-207C mutations as compared the results of the adult CD populations to the controls (Table 3). There was no statistically significant difference in the prevalence of TC haplotype between the CD patients and controls (Table 3).

We examined 121 UC patients with typical symptoms and diagnosis of ulcerative colitis (47 males, 74 females; mean age 47.8 years) and 110 clinically healthy controls (59 males, 51 females; mean age 46.7 years). We found that in SLC22A4 C1672T the T allele frequency was not significantly different from healthy controls (Table 4). The SLC22A5 G-207C the C allele frequency was also not significantly different in the UC population compared to the control group (Table 4). We could not detect accumulation of SLC22A4 and SLC22A5 susceptibility variants nor the TC haplotype (Table 4).

We examined 19 pediatric patients with CD (14 males, 5 females, mean age: 13.4 years). This cohort was compared with 49 pediatric healthy control subjects (28 males, 21 females, mean age: 14.4 years). There were no significant differences in the allele frequencies either for SLC22A4 1672T or SLC22A5 -207C SNPs (Table 5).

4.3. CTLA4 A+49G

We examined 130 patients with CD (55 males, 75 females, mean age: 43.0) and 150 patients with UC (63 males, 87 females, mean age: 46.1). A total of 170 selected controls (49 males, 121 females, mean age: 57.7) were analyzed as well. We found no increased prevalence rate of the homozygous GG genotype, and no accumulation of the G allele alone, neither expressed as AG heterozygous genotype, nor as the G allele frequency, in any IBD type compared to the healthy, IBD free controls (Table 6).

4.4. IL23R rs10889677 C/A, rs2201841 T/C, rs1884444 G/T

The Crohn's disease group consisted of 190 subjects (88 males, 102 females, mean age: 39.6 years). A total of 220 subjects (115 males, 105 females, mean age: 41.7 years) served as controls. The rs10889677 AA homozygous genotype was significantly increased in patients with Crohn's disease, compared to the healthy controls (Table 7). Logistic regression analysis revealed that the AA genotype represented a 2.19 times higher risk for the development of CD. For rs2201840 the CC homozygous genotype and the C mutant allele frequency were significantly increased in the CD group compared to the controls (Table 7). Logistic regression analysis revealed that the CC genotype represented a 2.41 higher times risk for the development of CD. The prevalence of any allelic variants of the rs1884444 did not significantly differ in the CD group as compared with controls (Table 7).

Table 1: Comparison of the alleles of CARD15 genes in adult Crohn's disease patients and adult controls.

		Adult CD patients n=100	Adult controls n=94
CARD15 genotype			
R702W	CC	87 (87.0%)	87 (92.6%)
	CT	12 (12.0%)	5 (5.3%)
	TT	1 (1.0%)	2 (2.1%)
	T allele frequency	7.00%	4.79%
G908R	GG	94 (94.0%)	93 (98.9%)
	GC	6 (6.0%)	1 (1.16%)
	CC	-	-
	C allele frequency	3.00%	0.53%
1007finsC	– –	83 (83.0%)	90 (95.7%)
	– insC	15 (15.0%)	4 (4.3%)
	insC insC	2 (2.0%)	-
	Cins allele frequency	9.50%*	2.13%

*P<0.05

Table 2: Comparison of the alleles of CARD15 genes in pediatric Crohn's disease patients with pediatric controls.

		Pediatric CD patients n=19	Pediatric controls n=49
CARD15 genotype			
R702W	CC	18 (94.7 %)	46 (93.9 %)
	CT	1 (5.3 %)	2 (4.1 %)
	TT	-	1 (2.0 %)
	T allele frequency	2.63 %	4.08 %
G908R	GG	14 (73.7 %)	48 (98.0 %)
	GC	3 (15.8 %)	1 (2.0 %)
	CC	2 (10.5 %)*	-
	C allele frequency	18.4 %*	1.02 %
1007finsC	– –	13 (68.5 %)	46 (93.9 %)
	– insC	4 (21.0 %)	3 (6.1 %)
	insC insC	2 (10.5 %)*	-
	Cins allele frequency	21.1 %*	3.06 %

*P<0.05

Table 3: Comparison of the alleles of SLC22A4 and SLC22A5 cation transporter genes and the TC haplotype distribution in adult CD patients and adult controls.

		Adult CD patients n=100	Adult controls n=94
SLC22A4 genotype			
C1672T	CC	37 (37.0%)	28 (29.8%)
	CT	54 (54.0%)	45 (47.9%)
	TT	9 (9.0%)	21 (22.3%)
	T allele frequency	36.0%	46.3%
SLC22A5 genotype			
G-207C	GG	31 (31.0%)	19 (20.2%)
	GC	54 (54.0%)	51 (54.3%)
	CC	15 (15.0%)	24 (25.5%)
	C allele frequency	42.0%	52.7%
	TC haplotype	9 (9.0%)	19 (20.2%)

*P<0.05

Table 4: Comparison of the alleles of SLC22A4 and SLC22A5 cation transporter genes and the TC haplotype distribution in adult UC patients and adult controls.

		Adult UC patients n=121	Aqdult controls n=110
SLC22A4 genotype			
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 genotype			
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%)

*P<0.05

Table 5: Comparison of the alleles of SLC22A4 and SLC22A5 cation transporter genes and the TC haplotype distribution in pediatric CD patients and pediatric controls.

		Pediatric CD patients n=19	Pediatric controls n=49
SLC22A4 genotype			
C1672T	CC	4 (21.0 %)	12 (24.5 %)
	CT	11 (58.0 %)	25 (51.0 %)
	TT	4 (21.0 %)	12 (24.5 %)
	T allele frequency	50.0 %	50.0 %
SLC22A5 genotype			
G-207C	GG	3 (15.8 %)	10 (20.4 %)
	GC	7 (36.8 %)	26 (53.1 %)
	CC	9 (47.4%)	13 (26.5 %)
	C allele frequency	65.8 %	53.1 %

*P<0.05

Table 6: Prevalence of the alleles of CTLA4 gene in patients with Crohn's disease, ulcerative colitis and controls.

		CD patients n=130	UC patients n=150	Controls n=170
CTLA4 genotype				
+49A/G	AA	47 (36.2 %)	56 (37.3 %)	70 (41.2 %)
	AG	67 (51.5 %)	66 (44.0 %)	73 (42.9 %)
	GG	16 (12.3 %)	28 (18.6 %)	27 (15.9 %)
	G allele frequency	38.1 %	40.6 %	37.4 %

*P<0.05

Table 7: Interleukin-23 receptor rs10889677, rs2201841 and rs1884444 genotype and allele frequencies in CD patients and controls.

		CD patients n=190	Controls n=220
IL23R genotype			
rs10889677	CC	75 (39.5%)	96 (43.6%)
	CA	92 (48.4%)	111 (50.5%)
	AA	23 (12.1%)*	13 (5.91%)
	A allele frequency	36.3%	31.1%
rs2201841	TT	75 (39.5%)	101 (45.9%)
	CT	90 (47.4%)	106 (48.2%)
	CC	25 (13.2%)*	13 (5.91%)
	C allele frequency	36.8%*	30.0%
rs1884444	GG	57 (30.0%)	55 (25.0%)
	GT	132 (69.5%)	162 (73.6%)
	TT	1 (0.53%)	3 (1.36%)
	T allele frequency	35.3%	38.2%

*P<0.05

5. DISCUSSION

Inflammatory bowel disease is a multifactorial disorder characterized by non-specific inflammation of the digestive tract with several intestinal and extraintestinal manifestations. The development of these diseases are known to be influenced by both environmental factors and complex genetic predisposition. Our goal was to analyse the possible influence of 9 variants of 5 genes on Hungarian IBD population. PCR/RFLP method or direct sequencing were used for detecting the different genotypes.

We examined previously described CARD15 mutations in Hungarian adult and, for the first time in the literature, in Hungarian pediatric CD population. In the adult CD group the 1007finsC showed significantly increased heterozygous and homozygous genotype distribution and mutant allele frequency compared to the adult controls. The R702W and the G908R showed no significant difference in distribution between the adult CD population and the healthy controls. We confirmed that the 1007finsC is a risk factor for CD in the Hungarian population. Examining the pediatric CD group, the G908R and the 1007finsC were significantly more frequent in the pediatric CD group compared to the controls. We observed an association between these two CARD15 mutations and pediatric cases. The R702W variant showed no disease association. We concluded from these results, that in the Hungarian population the adult Crohn's profile differs from the pediatric Crohn's profile. It depends on the different populations studied, whether CARD15 mutations are susceptibility factors for Crohn's disease or not.

We examined the SLC22A4 1672T and the SLC22A5 -207C combination definied TC haplotype in Hungarian adult IBD and first in the literature in pediatric population. We found that the TC haplotype was not associated with IBD in the Hungarian population either in the adult or in the pediatric cohort. We concluded that these SNPs do not necessarily indicate susceptibility to IBD. Our findings show that it might depend on the population if this haplotype confers susceptibility to IBD.

We examined the CTLA4 gene +49A/G polymorphism and we found that the G variant does not represent an obligatory susceptibility factor for Crohn's disease or for ulcerative colitis. We could not demonstrate an association between this variant and the development of the disease in the Hungarian population. It most likely depends on the population studied, whether this variant is a risk factor for IBD or not.

For the first time we examined in Hungarian population the variants of IL23R, that Duerr and colleagues described in 2006. The rs10889677 and rs2201841 showed significant increased homozygous genotype and mutant allele frequency in the CD group compared to the controls. These variants are in strong association with Crohn's disease in the Hungarian population, while the neutral variant rs 1884444 showed no disease association. Our results support the data of Duerr and colleagues, as they have found that the rs10889677 and the rs2201841 are susceptibility factors for CD in Jewish and non-Jewish populations.

The results of this dissertation help us to understand the genetic background of inflammatory bowel disease. Identification of novel genetic variants and determination of genotype-phenotype associations might contribute to recognition of risk factors, to early diagnosis and efficient prevention of the disease.

6. CONCLUSION

I. We ascertained, that in our examined adult Crohn's disease patients, one of the CARD15 mutations, the 1007finsC was significantly associated with an increased risk for CD.

II. Examining the pediatric Crohn's disease patients, we concluded that two of the CARD15 mutations, the G908R and the 1007finsC confer risk for the development of CD.

III. SLC22A4/A5-TC haplotype showed no disease association comparing the results of the adult IBD populations to the controls.

IV. We concluded also the same in the pediatric group, the TC haplotype showed no disease association, when we compared the result of the pediatric CD cohort to the controls.

V. The A+49G variant of the CTLA4 gene was not an independent determinant to IBD.

VI. We found that the IL-23 receptor gene variants, rs10889677 and rs2201841 appear to increase susceptibility to CD in our Hungarian adult Crohn's disease population.

7. LIST OF PUBLICATIONS

7.1. The thesis is based on the following publications

1. **Magyari L**, Bene J, Komlosi K, Talian G, Farago B, Csongei V, Jaromi L, Safrany E, Sipeky C, Lakner L, Varga M, Gasztonyi B, Melegh B. Prevalence of SLC22A4 1672T and SLC22A5 -207C Combination Defined TC Haplotype in Hungarian Ulcerative Colitis Patients. Pathol Oncol Res. 2007;13(1):53-6. IF:1.241

2. **Magyari L**, Farago B, Bene J, Horvatovich K, Lakner L, Varga M, Figler M, Gasztonyi B, Mozsik G, Melegh B. No association of the cytotoxic T-lymphocyte associated gene CTLA4 +49A/G polymorphisms with Crohn's disease and ulcerative colitis in Hungarian population samples. World J Gastroenterol. 2007;13(15):2205-8.

3. Bene J, **Magyari L**, Talian G, Komlosi K, Gasztonyi B, Tari B, Varkonyi A, Mozsik G, Melegh B. Prevalence of SLC22A4, SLC22A5 and CARD15 gene mutations in Hungarian pediatric patients with Crohn's disease. World J Gastroenterol. 2006;12(34):5550-3.

4. Bene J, Komlosi K, **Magyari L**, Talian G, Horvath K, Gasztonyi B, Miheller P, Figler M, Mozsik G, Tulassay Z, Melegh B. Plasma carnitine ester profiles in Crohn's disease patients characterized for SLC22A4 C1672T and SLC22A5 G-207C genotypes. Br J Nutr. 2007;98(2):345-50. IF:2.708

5. Farago B, **Magyari L**, Safrany E, Csongei V, Jaromi L, Horvatovich K, Sipeky C, Maasz A, Radics J, Gyetvai A, Szekanecz Z, Czirjak L, Melegh B. Functional variants of interleukin-23 receptor gene confer risk for rheumatoid arthritis but not for systemic sclerosis. Ann Rheum Dis. 2008 Feb;67(2):248-50. Epub 2007 Jul 2. IF:5.767

7.2. Other articles

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2. Maasz A, Kisfali P, Horvatovich K, Mohas M, Marko L, Csongei V, Farago B, Jaromi L, **Magyari L**, Safrany E, Sipeky C, Wittmann I, Melegh B. Apolipoprotein A5 T-1131C variant confers risk for metabolic syndrome. Pathol Oncol Res. IF:1.241

3. Farago B, Talian G, Maasz A, **Magyari L**, Horvatovich K, Kovacs B, Cserep V, Kisfali P, Kiss G C, Czirjak L, Melegh B Prevalence of functional haplotypes of the peptidylarginine deiminase citrullinating enzyme gene in patients with rheumatoid arthritis: no influence of the presence of anti-citrullinated peptide antibodies. Clin Exp Rheumatol. 2007;25(4):523-8. IF:2.189

4. Szolnoki Z, Maasz A, **Magyari L**, Horvatovich K, Farago B, Somogyvari F, Kondacs A, Szabo M, Bodor A, Hadarits F, Melegh B. The combination of homozygous MTHFR 677T and angiotensin II type-1 receptor 1166C variants confers the risk of small-vessel-associated ischemic stroke. J Mol Neurosci. 2007;31(3):201-7. IF:2.965

5. Safrany E, Csongei V, Jaromi L, Maasz A, **Magyari L**, Sipeky C, Melegh B. Mitochondrial DNA and its mutations: novel fields in a new era. Orv Hetil. 2007;27;148(21):971-8. Hungarian.

6. Banyai K, Jiang B, Bogdan A, Horvath B, Jakab F, Meleg E, Martella V, **Magyari L**, Melegh B, Szucs G. Prevalence and molecular characterization of human group C rotaviruses in Hungary. J Clin Virol. 2006 Dec;37(4):317-22. Epub 2006 Sep 25. IF:2.630

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