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BRIEF ARTICLE

Susceptibility to ulcerative colitis in Hungarian patients determined by gene-gene interactions

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Abstract

AIM: To study the inflammatory bowel disease-5 locus (IBD5) and interleukin-23 receptor (*IL23R*) gene variants in UC patients and test for gene-gene interaction.

METHODS: The study population (n = 625) was comprised of 320 unrelated ulcerative colitis (UC) patients with Caucasian origin and 316 age- and gender-matched, healthy controls. Five variants in the IBD5 locus (*IGR2198a_1* rs11739135, *IGR2096a_1* rs12521868, *IGR2230a_1* rs17622208, *SLC22A4* rs1050152 and *SLC22A5* rs2631367) and two of the *IL23R* gene (rs1004819, rs2201841) were analysed. PCR and restriction fragment length polymorphism methods were used for genotyping, the *SLC22A4* rs1050152 genotypes were determined by direct sequencing. Interactions and specific genotype combinations of the seven variants were tested by binary logistic regression analysis. The *IL23R* genotypes were stratified by IBD5 genotypes for further interaction analyses.

RESULTS: For the *IL23R* rs1004819 A allele we found significantly higher allele frequency (P = 0.032) in UC patients compared to control subjects. The *SNP* rs1004819 showed significant association with UC risk for carriers (P = 0.004, OR = 1.606; 95%CI: 1.160-2.223) and the *SNP* rs2201841 for homozygotes (P = 0.030, OR = 1.983; 95%CI: 1.069-3.678). Individually none of the IBD5 markers conferred risk to UC development. There was no evidence for statistical interaction either between IBD5 loci and *IL23R* genes using logistic regression analysis. After genotype stratification, we could detect a positive association on the background of rs1004819 A allele for *SLC22A4* T, *SLC22A5* C, *IGR2198a_1* C or *IGR2096a_1* T allele, the highest OR was calculated in the presence of *SLC22A4* T allele (P = 0.005, OR = 2.015; 95%CI: 1.230-3.300). There was no association with UC for any combinations of rs1004819 and IGR2230a_1. The *IL23R* rs2201841 homozygous genotype and IBD5 carrier status together did not confer susceptibility for UC.

CONCLUSION: The present study has shown that UC susceptibility genes are likely to act in a complex interactive manner similar to CD.

Key words: Gene-gene interaction; Interleukin-23 receptor gene; Inflammatory bowel disease-5 locus; Ulcerative colitis; Inflammatory bowel disease

Core tip: Most of the identified inflammatory bowel disease genes individually have only modest effects on inflammatory bowel disease susceptibility, suggesting

that complex interactions are more important. The authors investigated the gene-gene interactions of the inflammatory bowel disease-5 loci and *IL23R* susceptibility alleles in a Hungarian ulcerative colitis cohort. The exploration of high risk genotype combinations could further add to our knowledge about the development of ulcerative colitis and could facilitate the diagnosis of high-risk patients.

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INTRODUCTION

Inflammatory bowel disease (IBD)-clinically classified as Crohn's disease (CD; MIM 26600) or ulcerative colitis (UC; MIM 191390)-is a common chronic, relapsing inflammatory disorder of the gastrointestinal tract[1]. According to the consensus hypothesis, in genetically predisposed individuals the commensal luminal flora trigger an inappropriate, overactive mucosal immune response causing intestinal tissue damage that is further modified by specific environmental factors (*e.g.*, smoking)[2]. Interestingly, the heritability of CD may be higher than that of UC[3].

Genome-wide association studies (GWAS) have resulted in the identification of many novel loci for CD initially and latterly for UC[4,5] which is thought to be more genetically heterogeneous than CD. To date, the number of known risk loci has expanded to 163[6]. The IBD-associated loci encode for genes involved in innate pattern recognition (*NOD2/CARD15*), autophagy (*ATG16L1*, *IRGM*), differentiation of Th17- T lymphocytes (*IL23R*), maintenance of epithelial barrier integrity (IBD5 locus), and coordination of adaptive immune responses (*HLA*-region)[7].

The most studied SNPs (*SLC22A4*, *SLC22A5*, *IGR2096_a*, *IGR2198_a*, *IGR2230_a*) on inflammatory bowel disease-5 (IBD5) locus (chromosome 5q31) have been reported to confer susceptibility to CD[8-11]. In some studies, including a GWAS meta-analysis[12], association with UC has also been established[13-16]. The interleukin-23 receptor (*IL23R*) gene was originally described as a CD susceptibility gene[17], but recently the association with UC has been also confirmed in three separate GWA studies[18-20].

Most of the identified genes individually have only modest effects on IBD susceptibility, suggesting that complex interactions are more important[21,22]. Epistasis, defined generally as gene-gene interactions, has become a hot topic in complex disease genetics in recent years[23] and can explain the lack of replication of single-locus results.

In previous single-locus association studies, the IBD5 loci[24,25] and *IL23R*[26,27] SNPs were examined in Hungarian IBD patients. The aim of our current work was to study the *IL23R* rs2201841 and rs1004819 SNPs in Hungarian UC population and to test for possible statistical interaction, stratifying the *IL23R* genotypes by IBD5 genotypes.

MATERIALS AND METHODS

Study subjects

The study population (n = 625) was comprised of 320 UC patients (men 42.8%, women 57.2%, age: 41.9 ± 14.3 years) and 316 healthy, unrelated controls (men 53.5%, women 46.5%, age: 46.52 ± 16.02 years). All patients and controls were Caucasian and of Hungarian origin. Sample collection started in 2003 in collaboration of the following participating Hungarian centers: 1st and 3rd Department of Internal Medicine, University of Pecs; Department of Medicine and Gastroenterology, Markusovszky Hospital, Szombathely; Department of Medicine and Gastroenterology, Rethy Pal Hospital, Bekescsaba; 2nd Department of Medicine, Semmelweis University, Budapest. The diagnosis of UC was determined according to established guidelines based on clinical, endoscopic, radiological and histopathological criteria[28]. Patients with indeterminate colitis were excluded from the study.

The control subjects were healthy blood donors and did not have any gastrointestinal or other autoimmune disorders. The origin of DNA samples was the central Biobank governed by the University of Pecs, as part of the National Biobank Network of Hungary (www.biobank.hu), which belongs also to the pan-European Biobanking and Biomolecular Resources Research Infrastructure (BBMRI) preparatory phase project (http://bbmri.eu/bbmri/).

Ethics statement

The study design was approved by the National Ethics Committee (ETT TUKEB) and adhered to the Ethical Principles for Medical Research Involving Human Subjects of the Helsinki Declaration (1975). Written, informed consent was obtained from all participants.

Genotyping

Genomic DNA was isolated from peripheral blood leukocytes with routine salting out method. For genotyping the variants of IBD5 locus [*IGR2198a_1* (rs11739135), *IGR2096a_1* (rs12521868), *IGR2230a_1* (rs17622208) and *SLC22A5* (rs2631367)] and *IL23R* (rs1004819, rs2201841) gene PCR-RFLP (restriction fragment length polymorphism) methods were applied, for the *SLC22A4* (rs1050152) direct DNA sequencing was used by BigDye Terminator labeling with ABI 3100 automatic sequencer (Foster City, CA, United States). The primers designed and used are given in Table 1.

The PCR amplifications were performed on MJ Research PTC-200 thermal cyclers (Bio-Rad, Hercules, CA, United States). Amplification included an initial denaturation step (96 ℃ for 2 min) followed by 35 cycles of

Table 1 Primer sequences for the analysed variants

Gene	SNP	Primers (5'-3')
IL23R	rs1004819	Forward: GCATTCTAGGACCGTTTTGG Reverse: ATCTGGTGGAAATATGTGAAACCTA
IL23R	rs2201841	Forward: GGCAAAAGGGAATTGAGAGG Reverse: GGCCTATGATTATGCTTTTTCCTG[1]
SLC22A4	rs1050152	Forward: AGAGAGTCCTCCTATCTGATTG Reverse: TCCTAGCTATTCTTCCATGC
SLC22A5	rs2631367	Forward: GCCGCTCTGCCTGCCAGC Reverse: GGTCGCTATCAGGAACACGGAGGA
IGR2230a_1	rs17622208	Forward: CAGAAGAATGCCCTTGATGTG Reverse: TCAGAAGCTGTCCATCCCAC
IGR2198a_1	rs11739135	Forward: AGACACTGGGACATCATCTGTCTG Reverse: GGGCAATTCTATGAGGACATTTAGA
IGR2096a_1	rs12521868	Forward: CAAGATTTCTGCCATAGCCTCCT Reverse: GGAGGGTGGTGTAGCCAGAGTAG

[1]Mismatch base is underlined.

denaturation (95 ℃ for 30 s), annealing for 45 s at 54 ℃ (rs1004819, rs17622208, rs1050152), 55 ℃ (rs2201841), 58 ℃ (rs11739135, rs12521868, rs2631367), primer extension at 72 ℃ for 45 s and final extension at 72 ℃ for 5 min. Each polymerase chain reaction contained 200 μmol/L of each dNTP, 1 unit of Taq polymerase, 5 μL of reaction buffer (100 mmol/L Tris HCl, pH = 9.0; containing 500 mmol/L KCl, 15 mM $MgCl_2$), 0.2 μmol/L of each primer and 1 μL DNA to be amplified in a final volume of 50 μL. The amplicons were digested by allele-specific restriction endonucleases *Hin1II* (rs11739135), *TruI I* (rs12521868), *DdeI* (rs17622208), *HpaII* (rs2631367), *TaaI* (rs1004819) and *HpyF3 I* (rs2201841). The amplicon contained an obligate cleavage site of the restriction enzyme for the suitable visual control of the efficacy of the digestion. The restriction fragments were separated by electrophoresis on 3% agarose gels containing ethidium bromide and visualized by UV transillumination.

Statistical analysis

Each genetic marker was tested for Hardy-Weinberg equilibrium in the control population. Statistical analysis was carried out using SPSS 19.0 package for Windows (SPSS Inc, Chicago, IL, United States). Genotype and allele frequency differences between cases and controls were evaluated using Pearson's χ^2-test. Haploview 4.1 was used to test linkage disequilibrium. The r^2 values for the tested IBD5 loci (*IGR2198a_1*, *IGR2096a_1*, *IGR2230a_1*, *SCL22A4, SCL22A5*) and for *IL23R* (rs1004819 and rs2201841) were below 0.8, for *SCL22A4* and IGR2096a_1 the r^2 value was 0.9.

Binary logistic regression analysis was applied to observe the individual contributions of IBD5 and *IL23R*, and to test for pairwise statistical interaction. An association was considered significant if a P value of < 0.05 was attained. The *IL23R* genotypes were stratified by IBD5 genotypes. The odds ratios and confidence intervals for these specific combinations of IBD5 and *IL23R* were derived from χ^2 in 2 × 2 contingency tables.

RESULTS

Single SNP marker association analysis of IBD5 and IL23R

We genotyped 5 candidate SNP variants for the IBD5 locus including the reported functional variants in the *SLC22A4* and *SLC22A5* transporter genes present on the risk haplotype and two SNP variants for *IL23R*. All of the investigated SNPs were in Hardy-Weinberg equilibrium in controls. Genotype distributions are shown in Table 2. For the *IL23R* rs1004819 A allele we found significantly higher allele frequency ($P = 0.032$) in UC patients compared to control subjects. The SNP rs1004819 showed significant association with UC risk for carriers (heterozygotes and homozygotes together, $P = 0.004$, OR = 1.606; 95%CI: 1.160-2.223) and the SNP rs2201841 for homozygotes ($P = 0.030$, OR = 1.983; 95%CI: 1.069-3.678). No significant association for any variants of IBD5 region and UC was observed.

Gene-gene interaction analysis

We analyzed the possible statistical interactions by pairs of *IL23R* variants and IBD5 with binary logistic regression. No evidence of interactions between these seven markers was found. None of the P values was significant; the lowest P value was 0.084 (Table 3).

Next, we stratified the *IL23R* genotypes by IBD5 genotypes and observed these specific genotype combinations of single markers by pairs, the combined odds ratios are shown in Table 4. The *IL23R* rs1004819 A variant did not show significant association with UC on the background of all wild type IBD5 genotypes, respectively. We could detect significantly elevated high odds ratios for rs1004819 A variant only in carriers of *SLC22A4* T allele, *SLC22A5* C, *IGR2198a_1* C or *IGR2096_a* T allele. The combined OR seen in rs1004819 A and *SLC22A5* C carriers ($P = 0.048$, OR = 1.691; 95%CI: 1.003-2.821) and the odds ratio for rs1004819 A in single gene analysis ($P = 0.004$, OR = 1.606; 95%CI: 1.160-2.223) were nearly equal while in combination with *IGR2198a_1* C ($P =$

Table 2 Case-control genotypes and allele frequencies of variants in *IL23R* and inflammatory bowel disease-5 locus *n* (%)

	UC (*n* = 320)	Controls (*n* = 316)	OR (95%CI)[1]	*P* value
IL23R (rs1004819)				
GG	126 (39.4)	158 (50.0)		
GA	168 (52.5)	134 (42.4)		
GA + AA	194 (60.6)	158 (50.0)	1.606 (1.160-2.223)[a]	0.004[a]
AA	26 (8.1)	24 (7.6)	1.254 (0.696-2.261)	0.452
RAF	0.343	0.287		0.032[a]
IL23R (rs2201841)				
TT	140 (43.8)	155 (49.1)		
TC	150 (46.9)	143 (45.3)		
TC + CC	180 (56.3)	161 (51.0)	1.268 (0.920-1.749)	0.147
CC	30 (9.4)	18 (5.7)	1.983 (1.069-3.678)[a]	0.030[a]
RAF	0.328	0.283		0.242
SLC22A4 (rs1050152)				
CC	93 (29.1)	110 (34.8)		
CT	159 (49.7)	148 (46.8)		
CT + TT	227 (71.0)	206 (65.2)	1.319 (0.935-1.86)	0.115
TT	68 (21.3)	58 (18.4)	1.150 (0.768-1.723)	0.498
RAF	0.46	0.417		0.120
SLC22A5 (rs2631367)				
GG	83 (25.9)	89 (28.2)		
GC	163 (50.9)	156 (49.4)		
GC + CC	237 (74.0)	227 (71.9)	1.138 (0.794-1.631)	0.481
CC	74 (23.1)	71 (22.5)	0.982 (0.669-1.440)	0.925
RAF	0.485	0.471		0.607
IGR2230a_1 (rs17622208)				
GG	87 (27.2)	90 (28.5)		
AG	160 (50.0)	157 (49.7)		
AG + AA	233 (72.8)	226 (71.5)	1.073 (0.751-1.532)	0.698
AA	73 (22.8)	69 (21.8)	0.990 (0.673-1.457)	0.960
RAF	0.478	0.466		0.685
IGR2198a_1 (rs11739135)				
GG	105 (32.8)	117 (37.0)		
GC	159 (49.7)	150 (47.5)		
GC + CC	215 (67.2)	199 (63.0)	1.260 (0.900-1.763)	0.179
CC	56 (17.5)	49 (15.5)	1.169 (0.760-1.797)	0.477
RAF	0.423	0.392		0.260
IGR2096a_1 (rs12521868)				
GG	101 (31.6)	117 (37.0)		
GT	164 (51.3)	147 (46.5)		
GT + TT	219 (68.5)	199 (63.0)	1.256 (0.897-1.760)	0.185
TT	55 (17.2)	52 (16.5)	1.045 (0.680-1.608)	0.840
RAF	0.428	0.397		0.262

[1]Adjusted for age and gender. [a]Associations significant at $P < 0.05$ *vs* controls. RAF: Risk allele frequency; UC: Ulcerative colitis.

0.020, OR = 1.803; 95%CI: 1.096-2.966) and *IGR2096_a* T ($P = 0.010$, OR = 1.911; 95%CI: 1.162-3.143) the rs1004819 A variant showed higher disease risk. The highest OR value was calculated in the presence of *SLC22A4* T allele ($P = 0.005$, OR = 2.015; 95%CI: 1.230-3.300). There was no association with UC for any combinations of rs1004819 and *IGR2230a_1*.

For the combinations of IBD5 loci and *IL23R* rs2201841 we could detect significantly elevated high odds ratios only in carriers of rs2201841 homozygotes and wild type IBD5 genotypes ($P = 0.018$, OR = 3.413; 95%CI: 1.169-9.965 for *SLC22A4*; $P = 0.014$, OR = 3.946; 95%CI: 1.232-12.645 for *SLC22A5*; $P = 0.018$, OR = 3.777; 95%CI: 1.181-12.084 for *IGR2230a_1*; $P = 0.027$, OR = 3.165; 95%CI: 1-088-9.206 for *IGR2198a_1*; $P = 0.026$, OR = 2.977; 95%CI: 1.099-8.066 for *IGR2096a_1* background). The *IL23R* rs2201841 homozygous genotype and IBD5 carrier status together did not confer susceptibility for UC.

DISCUSSION

Genetic components play an important role in the pathogenesis of IBD. GWAS have shown disease-associated loci on several chromosomes[4-6]. Some loci seem to be specific to either CD or UC, whereas others confer common susceptibility to IBD; approximately 30% of IBD-related genetic loci are shared[29,30]. Both the IBD5 and the *IL23R* genes have been identified originally as CD susceptibility genes, but their association with UC has also been confirmed[12-14,16,18-20].

Most of the identified IBD genes individually have only modest effects on IBD susceptibility, suggesting that gene-gene interactions as well as gene-environmental interactions play a key role in IBD pathogenesis[21]. Gene-gene interactions often referred to as epistasis, are ubiquitous

Table 3 Pairwise analysis of interactions of IBD5 and *IL23R* to risk of ulcerative colitis

Model	OR (95%CI)	*P* value
IL23R rs1004819 * *IGR2230a_1*	1.266 (0.627-2.553)	0.51
IL23R rs1004819 * *IGR2198a_1*	1.383 (0.711-2.690)	0.340
IL23R rs1004819 * *IGR2096a_1*	0.942 (0.625-1.420)	0.776
IL23R rs1004819 * *SLC22A4*	1.017 (0.516-2.002)	0.962
IL23R rs1004819 * *SLC22A5*	1.116 (0.549-2.270)	0.761
IL23R rs2201841 Ho* *IGR2230a_1*	0.316 (0.080-1.247)	0.100
IL23R rs2201841 Ho* *IGR2198a_1*	0.388 (0.105-1.430)	0.155
IL23R rs2201841 Ho* *IGR2096a_1*	0.413 (0.117-1.458)	0.169
IL23R rs2201841 Ho* *SLC22A4*	0.118 (0.095-1.301)	0.352
IL23R rs2201841 Ho* *SLC22A5*	0.298 (0.075-1.179)	0.084

Ho: Homozygotes.

among common human diseases and that complex interactions are more important than the independent main effects of any single susceptibility gene[21,31]. Therefore, attention of recent studies has focused on multi-locus analysis, especially involving the *CARD15*[32-34], *IL23R*[35-39], *ATG16L1*[40-42], *DLG5* genes and IBD5 locus[43, 44].

In CD the interactions between the main susceptibility genes are better characterized, than in UC. Multidimensionality reduction analysis suggested an interaction between IBD5, *ATG16L1*, and *IL23R* risk alleles in CD patients from Manitoba IBD Research Registry[32]. Weersma *et al*[38] observed multiple gene combinations. According to their results an association between the increase in the number of risk alleles (*ATG16L1*, *IL23R*, *CARD15,* IBD5 and *DLG5*) and an increase risk for the development of CD and a more severe disease course was found. Csongei *et al*[43] investigated the *IL23R*, *ATG16L1*, *CARD15* and IBD5 locus interactions. In almost all cases, the combined risk of susceptibility pairs was higher in patients carrying two different risk-associated gene variants together than individuals with just one polymorphism. In contrast with the single gene effects, after genotype stratification, the *IGR2198a_1* C and *IGR2096a_1* T variants were found to confer susceptibility only in subjects with *CTLA4* + 49 AA genotype[44]. The epistasis of the *IL23R* rs1004819 risk variant with IBD5 (and *CARD15*) was examined in a German CD population, but no gene-gene interaction was found[36]. In the study of Cummings *et al*[35] with the exception of rs11209026, *IL23R* risk polymorphisms showed significant CD association only in the subgroup of persons positive for the *IGR2060* variant in IBD5. This result may suggest that the *IL23R* gene influences CD in tandem with effects of the IBD5 haplotype. Two protective variations of the *IL23R* gene, the rs7517847 and rs11209026 were also weakly associated with UC, however the gene interaction test related to UC risk were not implied in these analyses[35].

In previous Hungarian single-locus association studies our research group found no significant differences in the allele frequencies of *SLC22A4* and *SLC22A5* genes either in CD in pediatric and adult patients[45] or in UC[24]. The TC haplotype was not associated with a higher risk of CD[46] and UC[24] in the Hungarian population. IGR2096_a and IGR2198_a on IBD5 locus were found to confer susceptibility to CD but not for UC[25]. The distribution of *IGR2230_a* was not significantly different in the CD or the UC group compared with the controls[47]. We observed an increased prevalence of the *IL23R* rs2201841 and rs1004819 in CD in previous Hungarian studies[43,48]. In our recent work, besides confirming the negative association for IBD5 loci in UC[24,25,47] we could detect significantly higher allele frequency for the *IL23R* rs1004819 and increased prevalence of the homozygous rs2201841 CC genotypes in UC patients compared to controls in Hungarian population.

In the next step, we analyzed the possible statistical interactions by pairs of risk-conferring *IL23R* variants and IBD5 using binary logistic regression. No evidence of interaction was found between these seven markers, suggesting that all the examined loci are independent factors.

Since the IBD5 loci were not found to confer risk for UC in the Hungarian population, we performed a combined genetic analysis, stratifying two other UC susceptibility gene variants, *IL23R* rs2201841 and rs10004819 by the IBD5 markers (*SLC22A4, SLC22A5*, *IGR 2096_a*, *IGR2198_a*, *IGR2230_a*). The IBD5 carrier status itself did not confer risk for UC in the presence of *IL23R* rs1004819 wild type but we could detect a positive association on the background of rs1004819 A allele for *SLC22A4* T allele, *SLC22A5* C, IGR2198a_1 C or IGR2096_a T allele. There was no association with UC for any combinations of rs1004819 and IGR2230a_1. The *IL23R* rs1004819 A variant did not show significant association with UC on the background of all wild type IBD5 genotypes, respectively. The IBD5 carrier status did not confer susceptibility for UC either in the presence of *IL23R* rs2201841 TT + TC or CC genotypes. For the combinations of IBD5 loci and *IL23R* rs2201841 we could detect significant association only in carriers of rs2201841 CC homozygote alleles and wild type IBD5 genotypes.

In summary, we identified the *IL23R* rs1004819 as susceptibility factor for UC in Hungarian patients and could detect increased prevalence of the homozygous rs2201841 CC genotypes in UC patients compared to controls in Hungarian population. The combined gene-gene analysis reveals that the *IL23R* rs2201841 CC variant confers risk for UC only on a wild-type IBD5 background and the rs1004819 A allele in combination with IBD5 carrier status except of *IGR2230_a*. We found no statistical evidence of interaction with the UC susceptibility genes *IL23R* and IBD5. However, this study has shown that UC susceptibility genes are likely to act in a complex interactive manner similar to CD. Our results play an important role in the understanding of the pathogenesis of UC and areas of overlap with CD but further studies are needed to confirm them.

Table 4 Genotype-specific ulcerative colitis odds ratios (with 95%CI) for combinations of variants in *IL23R* and IBD5

	SLC22A4		*SLC22A5*		*IGR2230a_1*		*IGR2198a_1*		*IGR2096a_1*	
	CC	CT + TT	GG	GC + CC	GG	AG + AA	GG	GC + CC	GG	GT + TT
IL23R rs1004819										
GG	1	1.298 (0.782-2.156) $P = 0.313$	1	1.064 (0.623-1.815) $P = 0.821$	1	0.941 (0.556-1.593) $P = 0.822$	1	1.033 (0.623-1.711) $P = 0.900$	1	1.104 (0.666-1.831) $P = 0.702$
GA + AA	1.527 (0.872-2.673) $P = 0.138$	[1]2.015 (1.230-3.300) $P = 0.005$	1.424 (0.776-2.614) $P = 0.253$	[1]1.691 (1.003-2.821) $P = 0.048$	1.300 (0.716-2.359) $P = 0.388$	1.549 (0.927-2.588) $P = 0.093$	1.263 (0.736-2.166) $P = 0.396$	[1]1.803 (1.096-2.966) $P = 0.020$	1.281 (0.744-2.206) $P = 0.372$	[1]1.911 (1.162-3.143) $P = 0.010$
IL23R rs2201841										
TT + TC	1	1.328 (0.989-1.927) $P = 0.058$	1	1.254 (0.868-1.812 $P = 0.228$	1	1.184 (0.823-1.704) $P = 0.363$	1	1.296 (0.922-1.822) $P = 0.136$	1	1.385 (0.982-1.953) $P = 0.063$
CC	[1]3.413 (1.169-9.965) $P = 0.018$	1.716 (0.788-3.739 $P = 0.171$	[1]3.946 (1.232-12.645) $P = 0.014$	1.474 (0.679-3.200) $P = 0.324$	[1]3.777 (1.181-12.084) $P = 0.018$	1.411 (0.652-3.056) $P = 0.381$	[1]3.165 (1.088-9.206) $P = 0.027$	1.592 (0.735-3.449) $P = 0.236$	[1]2.977 (1.099-8.066) $P = 0.026$	[1]1.701 (0.765-3.784) $P = 0.189$

[1]Associations significant at $P < 0.05$ *vs* controls.

COMMENTS

Background

Crohn's disease (CD) and ulcerative colitis (UC) are the two main types of inflammatory bowel diseases (IBD). Their precise etiology is still unknown but genetic factors play an important role in their pathogenesis. Genome-wide association studies have resulted in the identification of many novel susceptibility loci on several chromosomes for CD initially and latterly for UC, including the inflammatory bowel disease-5 (IBD5) locus and interleukin-23 receptor (*IL23R*) gene.

Research frontiers

An increasing number of studies suggested the association between UC susceptibility and the IBD5 SNPs and *IL23R* gene variants, individually. The lack of replication of single-locus results in IBD studies is presumed to be related to epistasic gene effects. Therefore multiple SNPs are required to be investigated simultaneously in UC patients to understand both the individual effect of single genes and gene-gene interactions. In the present study two SNPs of the *IL23R* gene and five variants in the IBD5 locus were genotyped and involved in interaction analysis in Hungarian UC population.

Innovations and breakthroughs

In the recent work, we could detect significantly higher allele frequency for the *IL23R* rs1004819 A variant and increased prevalence of the homozygous rs2201841 CC genotype in Hungarian UC patients relative to controls. All the analysed IBD5 variants were found to have neutral effect on UC pathogenesis. The statistical analysis of pairwise interactions between *IL23R* and IBD5 loci confirmed the independence of these genes, while specific combinations by pair showed further correlations. The *IL23R* rs1004819 A variant increased the risk of disease development only in the presence of IBD5 polymorphisms. Although the *IL23R* rs2201841 CC genotype conferred risk on wild type IBD5 background, in the presence of IBD5 polymorphisms lack of association was detected. The present study has shown that UC susceptibility genes are likely to act in a complex interactive manner.

Applications

The results play an important role in the understanding of the pathogenesis of UC but further studies investigating gene-gene and gene-environmental interactions are necessary as well. These studies are of high importance since the clarification of interactions between specific IBD genetic polymorphisms could facilitate the differential diagnosis and optimize treatment efficacy of high-risk patients.

Terminology

IBD is a common chronic, remitting-relapsing inflammatory disease of the gastrointestinal tract. UC causes continuous mucosal inflammation of the colon without granulomas, affecting the rectum and a variable extent of the colon, while CD can affect discontinuously and transmurally any part of the gastrointestinal tract. Gene-gene interactions often referred to as epistasis, may play role in the mechanisms of complex diseases such as IBD. Besides the independent main effects of any single susceptibility gene, complex interactions are of great importance as well.

Peer review

This study addresses the possible interactions between very popular genes associated with IBD in determining an increased risk ratio for ulcerative colitis in a relatively large of population of ulcerative colitis patients and controls from Hungary. The study is reasonably sized to possibly give some hints for the complex study of genetics in IBD.

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