Interaction of the major inflammatory bowel disease susceptibility alleles in Crohn’s disease patients

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Abstract

AIM: To investigate the interaction of interleukin-23 receptor (IL23R) (rs1004819 and rs2201841), autophagy-related 16-like 1 (ATG16L1) (rs2241880), caspase recruitment domain-containing protein 15 (CARD15) genes, and IBD5 locus in Crohn’s disease (CD) patients.

METHODS: A total of 315 unrelated subjects with CD and 314 healthy controls were genotyped. Interactions and specific genotype combinations of a total of eight variants were tested. The variants of IBD5 locus (IGR2198a_1 rs11739135 and IGR2096a_1 rs12521868), CARD15 (R702W rs2066845 and L1007fs rs2066847), ATG16L1 (rs2241880) and IL23R (rs1004819, rs2201841) genes were genotyped by PCR-RFLP, the G908R (rs2066844) in CARD15 was determined by direct sequencing.

RESULTS: The association of ATG16L1 T300A with CD was confirmed [P = 0.004, odds ratio (OR) = 1.69, 95% CI: 1.19-2.41], and both IL23R variants were found to represent significant risk for the disease (P = 0.008, OR = 2.05, 95% CI: 1.20-3.50 for rs1004819 AA; P < 0.001, OR = 2.97, 95% CI: 1.65-5.33 for rs2201841 CC). Logistic regression analysis of pairwise interaction of the inflammatory bowel disease (IBD) loci indicated that IL23R, ATG16L1, CARD15 and IBD5 (IGR2198a_1) contribute independently to disease risk. We also analysed the specific combinations by pair of individual ATG16L1, IL23R, G908R, CARD15 and IBD5 genotypes for disease risk influence. 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. The highest OR was found for IL23R rs2201841 homozygous genotype with combination of positive CARD15 status (P < 0.001, OR = 9.15, 95% CI: 2.05-40.74).

CONCLUSION: The present study suggests a cumulative effect of individual IBD susceptibility loci.

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Key words: Gene interaction; Interleukin-23 receptor; Autophagy-related 16-like 1; IBD5; Caspase recruitment domain-containing protein 15; Crohn’s disease; Inflammatory bowel disease

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INTRODUCTION

Two main clinical presentations of inflammatory bowel disease (IBD) are Crohn’s disease (CD) and ulcerative colitis (UC)[5]. IBD is now widely believed to originate from an uncontrolled mucosal immunity of the gastrointestinal tract[5]. Twin and family studies have reported that besides environmental factors genetic susceptibility is essential in IBD development[6]. Up to now, many novel candidate genes have been found to confer increased risk for the disease, some loci seem to be specific to CD or UC, others have been reported to confer susceptibility to IBD overall; at the moment the most replicated loci are interleukin-23 receptor (IL23R) and autophagy-related 16-like 1 (ATG16L1). Duerr et al[6] identified IL23R gene as an IBD-associated gene in a genome-wide association study. Subsequent genome-wide association studies provided replication and confirmed the role of IL23R in CD[5-7].

In a European genome-wide association study the coding variant T300A (rs2241880) within the ATG16L1 gene was reported to be highly associated with CD, and to carry the whole disease risk exerted by this locus[8]. The association of ATG16L1 gene and CD was replicated in numerous studies[9-12]. IL23R and ATG16L1 T300A were also proved to be risk variants in the Hungarian CD population[9].

The rising number of CD candidate genes gives us the possibility to evaluate gene-gene interactions among susceptibility genes. Playing a role in biomolecular mechanisms, these interactions, or epistases are ubiquitous features of the genetic architecture of common human diseases[13], their existence has been proved by several studies[14-16]. Since gene-gene interactions cannot only enhance but also weaken the individual gene effects, which can explain the lack of replication of single-locus results[14-17], complex gene-gene interactions may be considered more important than independent effects of single susceptibility genes.

Therefore our aim was to join the major susceptibility genes into a gene-gene interaction analysis in the Hungarian CD population: two IL23R gene risk variants, namely the intronic rs2201841 and rs1004819, the ATG16L1 gene variant T300A, the three well-known SNPs (R702W, L1007fs and G908R) of caspase recruitment domain-containing protein 15 (CARD15) gene and two markers located in IBD5 (IGR2198a_1 and IGR2096a_1) were tested for statistical interaction.

MATERIALS AND METHODS

Patients

We examined 315 unrelated patients with CD (151 males, 164 females, mean age 38.65 ± 0.79 years). The CD group included mixed Caucasian patients who had typical symptoms and diagnosis. A group of 314 clinically healthy subjects (170 males, 144 females, mean age 40.8 ± 0.80 years) with no IBD or other autoimmune disease were collected for the study. 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 preparatory phase project (http://bbmiri.eu/bbmiri/). The governance, maintenance and management principles of the Biobank had been approved by the national Scientific Research Ethics Committee, Budapest (ETT TUKEB).

During the entire investigation period the guidelines and regulations of the 1975 Helsinki Declaration and the currently operative national laws were followed; the patients gave their informed consent for use of their collected, anonymized DNA samples for research purposes.

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes with a routine salting out method. For genotyping the variants of IBD5 locus (IGR2198a_1 rs11739135 and IGR2096a_1 rs12521868), CARD15 (R702W rs2066845 and L1007fs rs2066847), ATG16L1 (rs2241880) and IL23R (rs1004819, rs2201841) genes PCR-RFLP methods were applied, 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, USA) using the following conditions: initial denaturation at 96°C for 2 min followed by 35 cycles of denaturation at 95°C for 30 s, annealing for 45 s at 54°C (rs1004819), 55°C (rs2066845 and rs2201841), 58°C (rs11739135, rs12521868), 60°C (rs2066847), and 62°C (rs2066845), extension at 72°C for 45 s and final extension at 72°C for 5 min. Each polymerase chain reaction contained 200 μmol/L of each dNTP, 1 U of Taq polymerase, 5 μL of reaction buffer (100 mmol/L Tris HCl, pH = 9.0; containing 500 mmol/L KCl, 15 mmol/L MgCl2), 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 Hind II (rs11739135), Trol I (rs12521868), HincP I (rs2066845), BspJ I (rs2066847), Lave I (rs2241880), Taq I (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. The genotyping of G908R (rs2066844) in CARD15 was carried out by direct sequencing by BigDye Terminator labelling with ABI 3100 automatic sequencer (Foster City, CA, USA).

**Statistical analysis**

Statistical analysis was carried out using the SPSS 15.0, package for Windows (SPSS Inc., Chicago, IL, USA). The allele frequencies were compared with Pearson's χ² test. Haploview 4.1 was used to test linkage disequilibrium. The χ² values for IGR2198a_1 and IGR2096a_1; and for IL23R rs1004189 and rs2201841 were below 0.8 (χ² = 0.63 and χ² = 0.62, respectively). Binary logistic regression analysis was applied to observe the individual contributions of IBD5, CARD15, IL23R, and for to test for pairwise statistical interaction. An association was considered significant if a P value of < 0.05 was attained. CARD15 status was classified as - (wild type) or + (at least one mutation in any of the three SNPs). The odds ratios (ORs) and confidence intervals for these specific combinations of IBD5, CARD15, and IL23R were derived from χ² in 2x2 contingency tables.

**RESULTS**

All analysed allele frequencies and genotype distributions were in Hardy-Weinberg equilibrium in both patients and controls; results are shown in Table 2.

**Table 1** Primer sequences for the analysed variants

| Gene      | SNP      | Primers (5’-3’)                     | F: TGGCTAACTCCTGCAGTCTC | 16 (5.1) | 0.004 | 0.62 (0.06-6.98) | F: GCATTCTAGGACCGTTTTGG | 131 (41.6) | 9 (2.9) | 139 (44.1) | 23 (7.3) | 2.05 (1.20-3.50) | 0.0088  |
|------------|----------|-------------------------------------|--------------------------|----------|-------|-----------------|--------------------------|-----------|-------|-----------|---------|-----------------|---------|
| CARD15     | rs2066844| F: GAGCCACCAACCTCAGATC           | R: ACTTGAGGTGCCACATTC    | 259 (82.2) | 2.57 (1.51-4.36) |
| IL23R      | rs1004189| F: AGACACTGGGACATCATCTGTCTG       | R: GGAGGGTGGTGTAGCCAGAGTAG | 72 (22.9) | 0.363 |
| ATG16L1    | rs2241880| F: TCTGTCACCATATACAGCCTGG         | R: TCTAGAAGGACAGGCTATACAGATG | 144 (44.0) | 1.69 (1.19-2.41) |
| CARD15     | rs2201841| F: TGCGTAATCCCTGCCAGTCTC         | R: GATCTCTAAAAATCTCGGACTTC | 220 (69.8) | 0.381  |

1Mismatch bases are underlined. F: Forward; R: Reverse; IL23R: Interleukin-23 receptor; ATG16L1: Autophagy-related 16-like 1; CARD15: Caspase recruitment domain-containing protein 15.

**Table 2** Case-control genotypes and allele frequencies of variants in IL23R, ATG16L1, CARD15 and IBD5 n (%)  

| CD (n = 315) | Controls (n = 314) | OR (95% CI) | P |
|--------------|-------------------|-------------|---|
| IL23R (rs1004189) |                    |             |    |
| GG           | 119 (37.8)        | 151 (48.1)  |    |
| GA           | 152 (48.3)        | 140 (44.6)  |    |
| GG+AA        | 196 (62.2)        | 163 (51.9)  | 1.50 (1.09-2.80) | 0.013* |
| AA           | 44 (14.0)         | 27 (8.7)    | 2.05 (1.20-3.50) | 0.0088 |
| RAF          | 0.381             | 0.296       | 0.001* |
| IL23R (rs2201841) |                  |             |    |
| TT           | 131 (41.6)        | 152 (48.4)  |    |
| TC           | 139 (44.1)        | 145 (46.2)  |    |
| TT+TC        | 250 (80.4)        | 272 (87.0)  | 0.8 (0.62-1.04) | 0.07 |

The risk allele frequencies of IL23R rs1004819 and rs2201841, ATG16L1 rs2241880, IGR2198a_1, (rs11739135), IGR2096a_1 (rs12521868), CARD15 R702W (rs2066844) and CARD15 L1007fs (rs2066847) were significantly higher in patients compared to controls (Table 2). After adjusting for age and gender, logistic regression analysis showed that IL23R rs1004819 A and
We found that bearing the G16L1 variant did not detect a similar effect for rs1004189 A carriers. Subjects together) background significantly; we could increase the susceptibility for the disease on the OR = 9.15, 95% CI: 2.05-40.74). To far the highest OR (P < 0.001, OR = 3.82, 95% CI: 1.86-7.86 for ATG16L1 GG genotype and + CARD15 status together showed by far the highest OR (P < 0.001, OR = 9.15, 95% CI: 2.05-40.74). The IL23R rs2201841 C variant in homozygous form increased the susceptibility for the disease on the ATG16L1 nonhomozygous (i.e. wild type and heterozygous subjects together) background significantly; we could not detect a similar effect for rs1004189 A carriers. ATG16L1 was associated with CD in the absence of IL23R rs2201841 CC genotype, but not in rs1004189 noncarriers. We found that bearing the ATG16L1 GG genotype together with one of the IL23R susceptibility variants enhanced the risk for CD (P < 0.001, OR = 2.51, 95% CI: 1.55-4.08 for rs1004189; P = 0.001, OR = 4.68, 95% CI: 1.72-12.78 for rs2201841 homozygous genotype).

The ORs calculated for specific combinations of IBD5 with IL23R and ATG16L1 genotypes are shown in Table 5. We detected significantly increased risk in patients carrying the IL23R rs2201841 CC genotype on a wild type IBD5 background. The IGRs showed significant association with the disease on an IL23R rs2201841 nonhomozygous or ATG16L1 nonhomozygous background. ATG16L1 did not significantly influence the susceptibility of CD except in the presence of IGR variants (P < 0.001, OR = 2.38, 95% CI: 1.46-3.87 for IGR2198a_1 C; and P = 0.001, OR = 2.32, 95% CI: 1.42-3.79 for IGR2096a_1 T background). Moreover, for the combinations of IGRs and IL23R rs1004189 we could detect significantly elevated high ORs only in carriers of IGR2198a_1 C and rs1004189 A, or in patients with IGR2096a_1 T and rs1004189 A variants (P = 0.001, OR = 2.44, 95% CI: 1.43-4.15 for IGR2198a_1 C; and P = 0.001, OR = 2.41, 95% CI: 1.42-4.09 for IGR2096a_1 T). The IGRs and IL23R rs2201841 CC genotypes together resulted in higher risk than the IGRs in themselves, but this OR value was lower than the OR calculated for the rs2201841 CC genotype alone (P < 0.001, OR = 3.66, 95% CI: 1.81-7.41 for IGR2198a_1 C; and P < 0.001, OR = 3.71, 95% CI: 1.80-7.67 for IGR2096a_1 T in patients carrying IL23R rs2201841 homozygous genotype).

The ORs for individual CARD15 genotypes stratified by IBD5 IGR2196a_1 and IGR2096a_1 genotypes are summarized in Table 6. Both the IBD5 markers significantly increased the risk of CD in the absence of CARD15 mutations; the CARD15 variants were confirmed to be stronger risk factors for CD than IGRs. The IBD5 variants showed higher significant risk together with + CARD15 status (P < 0.001, OR = 3.19, 95% CI: 1.90-5.37 for IGR2198a_1; and P < 0.001, OR = 3.18, 95% CI: 1.87-5.39 for IGR2096a_1 on the background of CARD15 + status).

**DISCUSSION**

Since the identification of NOD2/CARD15 as the first susceptibility gene for CD in 2001[19,20], several additional loci have been implicated in CD and confirmed by replication, among others the IBD5, IL23R and ATG16L1[16,18,19,21,22] loci.

Recently the idea was raised that exploring gene-gene interactions might lead to a better understanding of disease cause and might help the prediction of disease risk. So far numerous studies have assessed the risk for the development of CD by combining information from the known genetic risk variants associated with the disease. Though Hampe et al[1] found a modest but significant association between ATG16L1 and CARD15 in their pioneer study, no interaction was demonstrated between the two loci in the majority of
Table 4  Genotype-specific CD odds ratios\(^1\) (with 95% CI) for combinations of variants in *IL23R, ATG16L1* and *CARD15*

| CARD15 R702W | CARD15 G908R | CARD15 L1007fs | CARD15 status | ATG16L1 |
|--------------|-------------|----------------|--------------|----------|
| **CC** | **CT+TT** | **GG** | **GC+CC** | **-** | **-** | **-** | **+** | **AA+AG** | **GG** |
| **IL23R rs1004189** |
| GG | 1 | 2.35 | (1.03-5.34)\(^a\) | 1 | 1.55 | 1 | 2.85 | 1 | 2.100 | 1 | 1.16 |
| GA+AA | 1.56 | 3.18 | (1.45-6.97)\(^a\) | 1.51 | 3.23 | 1.57 | 3.40 | 1.50 | 3.33 | 1.34 | 2.51 |
| **IL23R rs2201841** |
| TT+TC | 1 | 2.25 | (1.26-4.03)\(^a\) | 1 | 1.49 | 1 | 2.43 | 1 | 2.12 | 1 | 1.48 |
| CC | 3.04 | 4.75 | (0.53-42.81) | 2.69 | (1.49-4.86)\(^a\) | 2.89 | 8.52 | 2.70 | 9.15 | 2.67 | 4.68 |
| **ATG16L1** |
| AA+AG | 1 | 2.20 | (1.12-2.20)\(^a\) | 1 | 1.44 | 1 | 2.37 | 1 | 2.12 | - | - |
| GG | 1.57 | 3.18 | (1.11-9.08) | 1.51 | 6.91 | 1.54 | 4.27 | 1.54 | 3.82 | - | - |

\(^1\)CARD15 R702W, G908R and L1007fs: OR relative to wild type genotype; CARD15 status: OR relative to - (wild type) group; ATG16L1: OR relative to wild and heterozygous genotypes together. *P < 0.05 vs controls.

Table 5  Genotype-specific CD odds ratios\(^1\) (with 95% CI) for combinations of variants in *IBDS, IL23R* and *ATG16L1*

| **IL23R rs1004189** | **IL23R rs2201841** | **ATG16L1** |
|---------------------|---------------------|--------------|
| **GG** | **GA+AA** | **TT+TC** | **CC** | **AA+AG** | **GG** |
| **IGR2198a_1** |
| GG | 1 | 1.43 (0.80-2.53) | 1 | 4.83 (1.52-15.37) | 1 | 1.71 (0.94-3.09) |
| GC+CC | 1.45 (0.84-2.48) | 2.44 (1.43-4.15)\(^a\) | 1.58 (1.11-2.24)\(^a\) | 3.66 (1.81-7.41)\(^a\) | 1.60 (1.07-2.38)\(^a\) | 2.38 (1.46-3.87)\(^a\) |
| **IGR2096a_1** |
| GG | 1 | 1.55 (0.88-2.73) | 1 | 4.03 (1.39-11.62) | 1 | 1.63 (0.91-2.92) |
| GC+CC | 1.49 (0.87-2.56) | 2.41 (1.42-4.09)\(^a\) | 1.50 (1.06-2.13)\(^a\) | 3.71 (1.80-7.67)\(^a\) | 1.50 (1.01-2.24) | 2.32 (1.42-3.79)\(^a\) |

\(^1\)IGR2198a_1, IGR2096a_1 and IGR2096a_1 OR relative to wild type genotype; ATG16L1 and IL23R rs2201841: OR relative to wild and heterozygous genotypes together. *P < 0.05 vs controls.

Table 6  Genotype-specific CD odds ratios\(^1\) (with 95% CI) for combinations of variants in *IBDS* and *CARD15*

| **CARD15 R702W** | **CARD15 G908R** | **CARD15 L1007fs** | **CARD15 status** |
|------------------|------------------|------------------|------------------|
| **CC** | **CT+TT** | **GG** | **GC+CC** | **-** | **-** | **-** | **+** |
| **IGR2198a_1** |
| GG | 1 | 3.17 (1.15-8.69)\(^a\) | 1 | 1.79 (0.39-8.22) | 1 | 3.04 (1.37-7.14) | 1 | 2.65 (1.36-5.17)\(^a\) |
| GC+CC | 1.60 (1.13-2.27)\(^a\) | 2.81 (1.39-5.72)\(^a\) | 1.52 (1.08-2.13)\(^a\) | 2.69 (0.97-7.45) | 1.61 (1.13-2.31)\(^a\) | 3.58 (1.80-7.16)\(^a\) | 1.63 (1.11-2.39)\(^a\) | 3.19 (1.90-5.37)\(^a\) |
| **IGR2096a_1** |
| GG | 1 | 3.07 (1.20-7.86)\(^a\) | 1 | 2.19 (0.51-9.41) | 1 | 2.43 (1.06-5.99) | 1 | 2.46 (1.29-4.69) |
| GC+CC | 1.55 (1.09-2.20)\(^a\) | 2.75 (1.32-5.70)\(^a\) | 1.47 (1.05-2.06)\(^a\) | 2.41 (0.86-6.77) | 1.48 (1.03-2.11)\(^a\) | 3.74 (1.85-7.54)\(^a\) | 1.54 (1.05-2.26) | 3.18 (1.87-5.39) |

\(^1\)IGR2198a_1, IGR2096a_1, CARD15 R702W, G908R and L1007fs: OR relative to wild type genotype; CARD15 status: OR relative to - (wild type) group. *P < 0.05 vs controls.

Subsequent Caucasian studies\(^{15,19,24}\) also demonstrated that *ATG16L1* increased the susceptibility for CD irrespective of *CARD15* status; at the same time they detected increased *ATG16L1* G allele frequency in *CARD15* carriers compared with noncarriers, which may indicate a weak interaction between these candidate CD susceptibility genes. Moreover, an additive effect was reported between *ATG16L1* and *CARD15*\(^{25}\). The independence of *ATG16L1* and *IBD5* variants was also confirmed in IBD\(^{12,23}\).

Besides the individual risk of *IL23R* variants several studies examined their epistatic interaction with other IBD genes like *CARD15*\(^{24-29}\). Mostly the well-replicated *IL23R R381Q* protecting variant was implied in gene-gene interaction analyses and was reported to act independently of *CARD15*. In one study an additive effect was found between this *IL23R* variant and *CARD15* gene\(^{25}\). The epistasis of the intronic rs1004189 risk variant with *CARD15* and *IBD5* was examined in a German CD population, but no gene-gene interaction was found\(^{29}\). No evidence of epistatic interaction between *CARD15* and *IBD5* was demonstrated in an Italian IBD study either\(^{25}\).
Besides combining the most associated CD susceptibility variants by pair, multilocus analyses with three or more loci were also performed. Latiano et al.\(^24\) did not find any significant interaction between \(ATG16L1\), \(IL23R\), \(CARD15\) and \(IBD5\) by triplets. Prescott et al.\(^13\) established a combined additive risk for all high risk genotypes in \(ATG16L1\), \(CARD15\) and \(IBD5\), which was 20 fold that of the baseline risk for individuals carrying none of the risk alleles. Weersma et al.\(^26\) found an association between the increase in the number of risk alleles (\(ATG16L1\), \(IL23R\), \(CARD15\), \(IBD3\) and \(DLG5\)) and an increased risk for the development of CD.

Here we selected two risk-conferring variants of \(IL23R\) and \(ATG16L1\) T300A mutation, and studied them together with the well-replicated \(CARD15\) and \(IBD5\) loci in Hungarian CD patients. Besides confirming our previous results with respect to \(IL23R\) rs2201841\(^29\), we found a significant positive association between \(IL23R\) rs1004819 variant and CD observed in the Hungarian population for the first time. For \(ATG16L1\) rs2241880 the estimated risk derived from the 315 CD patients showed a significant 1.7-fold increase in risk for the homozygous genotype. Our data are in line with most previous reports in Hungarian and other Caucasian populations\(^12,18\). Our results also verify that the previously identified R702W and L1007fs alterations in \(CARD15\) gene act as CD susceptibility factors in the Hungarian population\(^16,19\). Since neither \(SLC22A4\) nor \(SLC22A5\) variants seem to confer risk for CD in the Hungarian population\(^32\), we tested two other disease-associated IBD5 markers, IGR2198a_1 (rs11739135), IGR2096a_1 (rs12521868) for gene-gene interactions\(^22,33,34\).

First we observed the statistical pairwise interactions between \(IL23R\) (rs1004189, rs2201841), \(ATG16L1\) (rs2241880), \(CARD15\) status and IGR2198a_1 (rs11739135). No evidence of epistatic interaction was found by logistic regression suggesting that all examined loci contribute independently to disease risk.

Next, we analysed the specific combinations of individual genotypes by pairs with respect to CD risk. We detected a significant association with CD for IGR2198a_1 and IGR2096a_1, respectively, on the background of wild type \(-\) \(CARD15\) status, indicating that these two IBD5 markers and \(CARD15\) are independent determinants of disease risk. Also high, significant ORs were found for \(ATG16L1\) and \(IL23R\) variants (rs1004189, rs2201841), respectively, both in the presence and absence of \(CARD15\) mutations, suggesting that these genes also act independently on CD risk. We detected a significant association in patients bearing \(IL23R\) rs2201841 CC genotype and wild type \(IBD5\) background together, and \textit{vice versa}. In carriers of IGR variants a significantly high risk was detected on \(IL23R\) rs2201841 nonhomozygous background; accordingly they may play an independent role in CD susceptibility. Similar to the results of previous studies\(^16,22\), \(ATG16L1\) increased the disease risk both in the presence and absence of \(CARD15\), moreover in our study it was independent from \(IL23R\) rs2201841 genotype, but not from rs1004189 and \(IBD5\) status. For combinations of \(IL23R\) rs1004189 and \(IBD5\) markers, significant association was seen only in individuals carrying together the rs1004189 mutation and one of the two IGR variants.

In almost all specific pairwise combinations, the highest OR was found in patients with two different risk-associated gene variants, this cumulative OR was by far the highest in individuals with \(IL23R\) rs2201841 CC genotype and + \(CARD15\) status. However, we cannot detect significant statistical interaction between the analysed \(IL23R\), \(ATG16L1\), \(CARD15\) and \(IBD5\) risk alleles. The results of the present study suggest that these susceptibility factors may have a possible cumulative effect in the Hungarian CD population. By combining information from the known common risk polymorphisms significant predictive value from genetic markers might be gained; accordingly further large, well-powered studies should be performed to clarify the exact nature of these possible correlations.

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**COMMENTS**

**Background**

Up to now, strong evidence has been provided that genetic factors play a significant role in determining the susceptibility of individuals to inflammatory bowel disease (IBD), especially for Crohn’s disease (CD). After the identification of the disease-associated \(NOD2\) [caspase recruitment domain-containing protein 15 (\(CARD15\))] gene, huge genome-wide linkage-analyses and meta-analyses have reported several CD susceptibility regions like \(IBDS\) locus, \(DLG5\), interleukin-23 receptor (\(IL23R\)), and autophagy-related 16-like 1 (\(ATG16L1\)) gene.

**Research frontiers**

Besides establishing the risk of carrying single variants, numerous studies have performed gene-gene interaction analyses for the major well-replicated susceptibility genes (\(CARD15\), \(ATG16L1\), \(IL23R\) genes and \(IBD5\) locus). Mostly the independence of these main loci has been reported; nevertheless some studies have found an increased disease risk for carrying two or more certain risk variants together compared to non-carriers or individuals with only one susceptibility variant. In the present study two SNPs of \(IL23R\), one of the \(ATG16L1\), three of the \(CARD15\) genes and two of \(IBDS\) locus were genotyped and involved in interaction analysis in the Hungarian CD population.

**Innovations and breakthroughs**

The present study confirms the reported association between \(IL23R\) rs2201841 and \(ATG16L1\) rs2241880 variants and CD susceptibility. The authors examined the \(IL23R\) rs1004189 in the Hungarian CD population for the first time, and found it significantly more frequent in patients compared to healthy controls. The analysis of statistical pairwise interactions between \(IL23R\), \(ATG16L1\), \(CARD15\) status and \(IBD5\) confirmed the independence of these susceptibility genes, while the specific combinations by pair showed the highest odds ratio in patients with two different risk-associated gene variants, suggesting that they may have a cumulative effect in this Hungarian CD population.

**Applications**

The exploration of epistatic interactions between the major susceptibility genes and the specification of high risk genotype combinations could support the better understanding of the development of CD and could facilitate the diagnosis of high-risk patients.
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