**Name of Journal: *World Journal of Gastroenterology***

**ESPS Manuscript NO: 30153**

**Manuscript Type: ORIGINAL ARTICLE**

***Basic Study***

***IL23R* single nucleotide polymorphisms could be either beneficial or harmful in ulcerative colitis**

**Running title**: Influence of *IL23R* on the phenotype of UC

Sarah Fischer, Erzsébet Kövesdi, Lili Magyari, Veronika Csöngei, Kinga Hadzsiev, Béla Melegh, Péter Hegyi, Patrícia Sarlós

**Sarah Fischer, Patrícia Sarlós,** Division of Gastroenterology, First Department of Internal Medicine, University of Pécs, 7624 Pécs, Hungary

**Péter Hegyi,** Institute for Translational Medicine, University of Pécs, 7624 Pécs, Hungary

**Péter Hegyi, Patrícia Sarlós,** Division of Translational Medicine, First Department of Medicine, University of Pécs, 7624 Pécs, Hungary

**Erzsébet Kövesdi, Lili Magyari, Veronika Csöngei, Kinga Hadzsiev, Béla Melegh**, Department of Medical Genetics, University of Pécs, 7624 Pécs, Hungary

**Erzsébet Kövesdi, Lili Magyari, Veronika Csöngei, Kinga Hadzsiev, Béla Melegh**, Szentágothai Research Centre, 7624 Pécs, Hungary

**Author contributions**: Sarlós P, Fischer S, Melegh B and Hegyi P designed the research; Fischer S, Kövesdi E, Magyari L, Csöngei V and Hadzsiev K performed the research; Fischer S, Magyari L and Csöngei V analyzed and interpreted the data; Sarlós P, Fischer S, Kövesdi E and Magyari L wrote the article and made critical revisions related to important intellectual content of the manuscript; Sarlós P, Hegyi P and Melegh B gave final approval of the version of the article to be published.

**Supported by** Hungarian Science Foundation (OTKA), No. K103983 and No. K119540.

**Institutional review board statement:** The governance, maintenance and management principles of the Biobank had been approved by the National Scientific Research Ethics Committee (ETT TUKEB). Clinical data guidelines and regulations of the local Ethics Committee and Helsinki Declaration in 1975 were followed during collection and use of DNA samples. At blood collection patients gave their informed consent for the future use of their anonymized DNA.

**Conflict-of-interest statement:** To the best of our knowledge, no conflict of interest exists.

**Data sharing statement:** no additional data are available for sharing.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to**: **Patrícia Sarlós, MD, PhD,** Division of Gastroenterology, 1st Department of Medicine, University of Pécs, 12 Szigeti street, 7624 Pécs, Hungary. sarlos.patricia@pte.hu

**Telephone:** +36-72-536145

**Fax:** +36-72-536146

**Received:** September 14, 2016

**Peer-review started:** September 19, 2016

**First decision:** October 10, 2016

**Revised:** October 29, 2016

**Accepted:** December 8, 2016

**Article in press:**

**Published online:**

**Abstract**

## *AIM*

## to investigate the association of seven single nucleotide polymorphisms (SNPs) of the *IL23R* genewith the clinical picture of ulcerative colitis (UC).

***METHODS***

Genomic DNA samples of 131 patients (66 males, 65 females, mean age 55.4 ± 15.8 years) with Caucasian origin, diagnosed with UC were investigated. The diagnosis of UC was based on the established clinical, endoscopic, radiological, and histopathological guidelines. DNA was extracted from peripheral blood leukocytes by routine salting out method. Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) were used to identify the alleles of seven SNPs of *IL23R* gene (rs11209026, rs10889677, rs1004819, rs2201841, rs7517847, rs10489629, rs7530511).

## *RESULTS*

## Four out of seven analyzed SNPs had statistically significant influence on the clinical picture of UC. Two SNPs were associated with greater colonic extension (rs2201841 *P* = 0.0084; rs10489629 *P* = 0.0405). For two of the SNPs, there was more frequently need for operations (rs2201841 *P* = 0.0348, OR = 8.0; rs10889677 *P* = 0.0347, OR = 8.0). The rs2201841 showed to be a risk factor for the development of iron deficiency (*P* = 0.0388, OR = 6.1837). For patients with the rs10889677, a therapy with azathioprine was more frequently necessary (*P* = 0.0116, OR = 6.1707). Patients with rs10489629 SNP had a lower risk for weight loss (*P* = 0.0169, OR = 0.3394). Carriers of the heterozygous variant had a higher risk for an extended disease (*P* = 0.0284). The rs7517847 showed a protective character leading to mild bowel movements. Three SNPs demonstrated no statistically significant influence on any examined clinical features of UC.

## *CONCLUSION*

## We demonstrated susceptible or protective character of the investigated *IL23R* SNPs on the phenotype of UC, confirming the genetic association.

**Key words:** *IL23R* gene; ulcerative colitis; phenotype; polymorphism; Hungarian

**© The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** *IL23R* gene plays important role in the development and influences the phenotype of inflammatory bowel diseases. We investigated the association of seven single nucleotide polymorphisms (SNPs) of *IL23R* genewith the clinical picture of ulcerative colitis (UC). Two SNPs were associated with greater colonic extension. At two SNPs, there was more frequently need for operations. Rs2201841 was found as a risk factor for the development of iron deficiency. Patients with rs10889677, therapy with azathioprine was more frequently necessary. Patients with rs10489629 SNP had lower risk for weight loss. This study demonstrated the influence of the investigated SNPs of *IL23R* on the phenotype of UC, confirming genetic association.

Fischer S, Kövesdi E, Magyari L, Csöngei V, Hadzsiev K, Melegh B, Hegyi P, Sarlós P.*IL23R* single nucleotide polymorphisms could be either beneficial or harmful in ulcerative colitis. *World J Gastroenterol* 2016; In press

**INTRODUCTION**

Crohn’s disease (CD) and ulcerative colitis (UC) represent the two most common forms of inflammatory bowel diseases (IBD). IBDs are complex diseases with suspected genetic and environmental etiology. Several IBD-associated genes were identified. Interleukin-23 receptor (*IL23R*) is one of those genetic factors found by genome-wide association studies (GWAS)[[1](#_ENREF_1)]. Various studies suggest that the IL23-Th17 cell-axis plays an important role in the pathogenesis of IBDs.

*IL23R* is the human gene of the IL23-receptor located on chromosome 1p31[[2](#_ENREF_2)]. IL23R is expressed by CD4+ T cells, monocytes/ macrophages, and CD11c+ dendritic cells (DCs)[[3](#_ENREF_3)]. IL23 is a pro-inflammatory cytokine belonging to the Interleukin 12-family[[4](#_ENREF_4)]. It is a heterodimer consisting of subunit p19 (IL23A), and p40, a subunit of IL12B[[5](#_ENREF_5)]. IL23 is mainly produced by activated macrophages and DCs[[4](#_ENREF_4)].

Th17 cells differentiate from naive CD4+ Th cells under the influence of transforming growth factor beta (TGFβ) and IL6[[6](#_ENREF_6)]. This can happen in a concentration-dependent manner. In a low concentration, TGFβ has synergistic effects with IL6 and IL21, which results in the induction of retinoid orphan nuclear receptor gamma t (Rorγt) and an increase of IL23, as well as promoting the differentiation into Th17 cells. In a high concentration, as well as in combination with IL2, TGFβ decreases the IL23-level, and raises the forkhead box protein transcription factor 3- level (Foxp‑3), which is a master regulator in the differentiation of regulatory T cells (Treg). This leads to the conclusion that there might exist a fluid balance between Th17 and Treg cells[[7](#_ENREF_7)]. Pathogenic Th17 cells seem to play an important role in autoimmunity. Overexpression of Th17-associated cytokines in the bowel tissue of IBD patients was proven[[8](#_ENREF_8)].

IL17, a pro-inflammatory cytokine produced by Th17 cells, is produced under the influence of IL23[6,7]. It activates stromal and epithelial cells, which results in the excretion of cytokines and chemokines for chemotaxis of neutrophil leukocytes[[9](#_ENREF_9)].

These pathways make IL23R a potential target in the treatment of various autoimmune inflammatory diseases. Ustekinumab and briakinumab, antibodies against the p40 subunit (IL12 and IL23) already demonstrated therapeutic efficiency in psoriasis, as well as in CD[[10-12](#_ENREF_10)]. An IL12 antibody was able to induce remission in CD[[13](#_ENREF_13),[14](#_ENREF_14)].

Duerr *et al*[[1](#_ENREF_1)]were the first who identified *IL23R* as a gene associated with IBDs. They were able to provide evidence for an association in non-Jewish UC population.

In a big German cohort Glas *et al*[[4](#_ENREF_4)] investigated interactions of IBD genes and the influence of *IL23R* on the phenotype. All of observed *IL23R* gene variants, showed a strong association to CD and a weaker association to UC. Nevertheless, eight out of ten SNPs showed significant association with UC. The fact that these SNPs were either protective or susceptible in both CD and UC suggests similar disease-modifying effects. Rs7517847 was the SNP with the strongest association to, and the only independent risk factor of UC. The SNP with the strongest association to CD (rs1004819) was analyzed for phenotypic correlation. Though the TT homozygous carriers showed more frequently ileal involvement and stenosis, it did not reach significance. Rs7517847 did not show any specific influence on the phenotype of UC. There was no evidence for epistasis between the *IL23R,* and three other IBD genes *CARD15/NOD2*, *SLC22A4* and *SLC22A5*. Gene-gene interactions seemed to influence the phenotype but didn´t reach statistical significance[[4](#_ENREF_4)].

Hayatbakhsh *et al*[[15](#_ENREF_15)] were able to connect the presence of rs7517847 in UC patients with two main clinical manifestations: blood in the stool and bowel movements. In a Jiangsu Han population the SNP rs17375018 with the G allele was correlated with mild activity in UC[[16](#_ENREF_16)]. Recent studies also suggest the SNPs of *IL23R* to be a predictive factor of response to the therapy, *e.g.*, non-response to mesalazine and corticosteroids as well as higher response rate for azathioprine and infliximab[[17](#_ENREF_17),[18](#_ENREF_18)].

The aim of our present study was to investigate the association of seven SNPs of *IL23R* (rs11209026, rs10889677, rs1004819, rs2201841, rs7517847, rs10489629, rs7530511) with the clinical picture of UC in Hungarian patients diagnosed with UC.

# Materials and Methods

## *Patients*

We examined 131 Hungarian patients of Caucasian origin (66 males, 65 females, mean age 55.4 +/- 15.8 years) diagnosed with UC. The origin of the DNA samples was the central Biobank, governed by the University of Pecs, as part of the National Biobank Network of Hungary (www.biobanks.hu), which belongs to the pan-European Biobanking and Bio-molecular Resources Research Infrastructure preparatory phase project (<http://bbmri.eu/bbmri/>). The governance, maintenance and management principles of the Biobank had been approved by the National Scientific Research Ethics Committee (ETT TUKEB). Clinical data guidelines and regulations of the local Ethics Committee and Helsinki Declaration in 1975 were followed during collection and use of DNA samples. At blood collection patients gave their informed consent for the future use of their anonymized DNA.

The diagnosis of UC was based on the established clinical, endoscopic, radiological, and histopathological guidelines. Patients with indeterminate colitis were excluded from the study.

## *Genotyping*

DNA was extracted from peripheral blood leukocytes by routine salting out method. Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) were used to identify the alleles of the *IL23R* gene. The PCR amplifications were performed on MJ Research PTC-200 thermal cyclers (Bio-Rad LTD., Budapest, Hungary). Amplification included an initial denaturation step (96 °C for 2 min) followed by 35 cycles of denaturation (95 °C for 30 s), annealing for 30 s at 54 °C (rs1004819); 60°C for 45 s (rs10889677 and rs7530511); 55 °C for 45 s (rs7517847, rs11209026 and rs10489629); 72 °C for 30 s (rs2201841), primer 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 unit 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 allelespecific restriction endonucleases *TaaI* (rs1004819), *HpyF3I* (rs2201841), *MnlI* (rs10889677), *BseMII* (rs7517847), *HphI* (rs7530511), *Hpy188I* (rs11209026) and *SspI* (rs10489629). 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*

The investigated phenotypic parameters included severity (age of manifestation, extension, frequency of relapse, blood in stool, bowel movements), complications (fever, weight loss, anemia, iron deficiency, hypoalbuminemia, need for operation), extraintestinal manifestation (in eyes, joints, skin), medication (aminosalicylate, azathioprine, corticosteroids), as well as familial cases of IBDs and colorectal cancer.

For the age of manifestation, three groups were formed analogous to the Montreal classification of CD (group 1: younger than 17 years, group 2: age between 17 and 40 years, group 3: older than 40 years). The extension of the disease along the colon was defined accordingly to the E-stages of the Montreal classification for UC (E1: involvement limited to the rectum, E2: involvement limited to distal of splenic flexure, E3: involvement extends to proximal to the splenic flexure)[[19](#_ENREF_19)]. For analyzing the frequency of bowel movements, the patients were divided in three groups (group 1: one up to three a day, group 2: four up to ten a day, group 3: more than ten a day).

Data were examined for independence. Null hypothesis was formulated as follows: the phenotype of UC is independent of the genotype of *IL23R*.

2 × 3 or 3 ×3 contingency tables were created depending on the attribute. For characteristics that were tested for presence or lack of presence, 2 × 3 contingency tables were created. For characteristics divided in three subsets such as age of onset, Montreal-classification, frequency of relapse and bowel movements, 3 × 3 contingency tables were generated. Genotype was partitioned into wild type (Wt), heterozygous (Hz) and homozygous (Ho) susceptible SNP.

The distribution was tested in total (wild type + heterozygous + homozygous) as well as separated and regrouped. *χ*2-test was performed if the expected value was not lower than five in at least 80% of the cells. In any other case Fishers exact test was performed. For *χ2*-test SPSS Statistics 22.0 was used (SPSS Inc., Chicago, IL, United States). SPSS performs Fishers exact test for 2 × 2 contingency tables. Two other calculators were used, available on vassarstat.net and in-silico.net. Results were verified using Kruskal-Wallis-Test. Odds Ratios were determined only for 2 × 3 contingency tables.

Phenotypic parameters were compared using chi-squared test (expected value in 80% of the squares not lower than fife), and Fishers exact test (in all other cases). Chi-squared test was accomplished using SPSS Statistics 22.0 (SPSS Inc., Chicago, IL, United States). P values below 0.05 were considered statistically significant. In this case null hypothesis was rejected.

# RESULTS

The frequencies of the genotypes and alleles are shown in Table 1.

At the investigation of rs11209026, out of 114 subjects 109 (95.6%) carried the wild type (GG), while 5 (4.4%) were heterozygous (GA). The AA homozygous variant did not appear in this population. That is not surprising, considering a mean allele frequency (MAF) of A = 0.0219. Regarding the low number of the SNP no significant results could have been expected.

For rs1004819, out of 129 subjects, 52 (40.3%) were wild type carriers (GG), while 68 (52.7%) carried the heterozygous (GA) and 9 (7%) the homozygous (AA) variant. For this polymorphisms no significant results were observed.

Out of 129 patients, 58 (40.0%) were carrier of the wild type (TT) of rs2201841, 60 (46.5%) carried the heterozygous (TC) and 11 (8.5%) the homozygous (CC) variant. Three characteristics reached level of significance: Montreal-Classification, appearance of iron deficiency and need for surgery (Figure 1). The Montreal-Classification and iron deficiency reached significance for the total distribution (Wt + Hz + Ho). Heterozygous carriers had significant higher risk to require surgery compared to the wild type (*P* = 0.0348, OR = 8.0). The distribution of the Montreal-Classification is shifted to greater extension for carriers of the heterozygous variant. Total distribution reached level of significance (*P* = 0.0084). This was verified by comparison of the obtained and expected values. The significance was mainly caused by the heterozygous TC variant (*P* = 0.0429).

Carriers of the rs2201841 had significant higher risk for iron deficiency (*P* = 0.0299). When Hz and Ho SNPs were combined and compared with the Wt, no significance was observed (*P* = 0.7476). Statistically significance was found when Wt and Hz SNPs were merged and compared with the Ho variant (*P* = 0.0388, OR=6.1837). Surgery was more frequently needed for heterozygous carriers of rs2201841 (*P* = 0.0348, OR = 8.0). The homozygous form seemed to have no influence on the need for operations.

Out of 112 patients, 40 (35.7%) carried the wild type (TT), 56 (50%) the heterozygous (TG) and 16 (14.3%) the homozygous (GG) rs7517847 SNP. Frequency of bowel movements was significant (*P* = 0.0078). If instead of three (< 4, 4-10, > 10) just two groups were used (< 3 and > 3 defecations per day), the distribution was still significant (*P* = 0.0358). If homozygous and heterozygous SNP were added together and compared to the wild type, no significant difference was found (*P* = 0.0634). Significance was caused by the heterozygous variant compared to the wild type (*P* = 0.0050). The rs7517847 seems to have a protective character and prevent its carrier from severe bowel movements.

Analyzing the rs7530511 SNP, 94 (73.4%) of 129 patients carried the wild type (CC), 32 (25.0%) the heterozygous (CT) and 2 (1.6%) the homozygous (TT) variant. Any significant different was found at this SNP.

Out of 127 patients 44 (34.6%) carried the wild type (GG), 67 (52.8%) the heterozygous (GA) and 16 (12.6%) the homozygous (AA) variant of rs10489629 SNP. Total distribution (Wt + Hz + Ho) of the Montreal-Classification was significant (*P* = 0.0405). Carrying the Hz and Ho SNP alone didn´t reach level of significance. Carriers of the heterozygous variant had a higher risk for an extended disease (*P* = 0.0284). For weight loss significance was observed (*P* = 0.035). If wild type was compared with heterozygous and homozygous SNP together (Hz + Ho) significance was reached (*P* = 0.0169, OR = 0.3394). The homozygous variant showed significance (*P* = 0.0457, OR = 0.1244). Patients who carried the heterozygous susceptible SNP had numerous but statistically not significant lower risk of weight loss (*P* = 0.0652).

In the case of rs10889677 SNP, out of 112 patients, 51 (45.5%) carried the wild type (CC), 53 (47.3%) the heterozygous (CA) and 8 (7.1%) the homozygous (AA) variant. Carriers of the susceptible SNP needed more frequently azathioprine treatment (*P* = 0.0285). If wild type was compared with the heterozygous and homozygous SNP, level of significance was found (*P* = 0.0136, OR=5.8732). The heterozygous variant also leads to a higher risk for need for surgery (*P* = 0.0347, OR = 8.0).

Summarized four out of seven SNPs had a statistically significant influence on the phenotype of UC. The results are shown in Table 2.

The rs2201841 showed higher stage of extension along the colon (*P* = 0.0084). The CC homozygous carriers had a higher risk for iron deficiency (*P* = 0.0388, OR = 6.183). Patients with the heterozygous genotype needed more often operation (*P* = 0.0348, OR = 8.0).

Patients with the rs7517847 GT heterozygous variant suffered less from bowel movements (*P* = 0.005).

The rs10489629 SNP in heterozygous form was connected to greater colonic extension (*P* = 0.0405). Both the heterozygous and homozygous from this variant were associated with lower risk of weight loss (*P* = 0.0169, OR = 0.3394). In case of rs10889677, therapy with azathioprine was more often necessary for patients carrying the heterozygous and the homozygous SNP (*P* = 0.0116, OR = 6.1707). Heterozygous carriers showed higher risk for the need of operations (*P* = 0.0347, OR = 8.0).

The reason for the statistical significance only to appear for the heterozygous genotype in some cases can be attributable to low numbers of homozygous carriers.

The variants rs11209026, rs1004819, and rs7530511 had no statistical significant influence on the phenotype of UC.

# DISCUSSION

Several recent studies suggest *IL23R* to be a suspect in the pathogenesis of diverse autoimmune diseases such as IBDs[1,[4](#_ENREF_4),[20-25](#_ENREF_20)], psoriasis[[11](#_ENREF_11),[22](#_ENREF_22),[26](#_ENREF_26)], Graves disease[[27](#_ENREF_27)], ankylosing spondylitis[[28](#_ENREF_28),[29](#_ENREF_29)], and rheumatoid arthritis[[26](#_ENREF_26),[29-31](#_ENREF_29)]. Previous investigations in Hungarian populations verified *IL23R* to play a role in the development of UC[[25](#_ENREF_25),[32](#_ENREF_32)]. The present study demonstrated the correlation between the SNPs of *IL23R,* and the phenotype of UC.

Two of the susceptible SNPs for the development of UC (rs2201841, rs10889677) seem to shift the clinical picture from mild into more severe. The rs10889677 was associated with azathioprine-therapy, suggesting the patients to be refractory to 5-aminosalicylic-acid (5-ASA). These results are analogous to the findings of Cravo *et al*[18]. Interestingly one risk-polymorphism (rs10489629) had risk-conferring (greater extension), and protective features (lower risk of weight loss). The reason for the protective and harming character of rs10489629 remains unclear. A possible connection could be a more frequent need for steroids because of the greater extension of the disease along the colon, leading to a lower risk for weight loss. This study did investigate the need for corticosteroids but not the administration frequency. No significant higher need for steroids could been shown. The rs7517847 variant showed a protective character (less bowel movements). Rs7517847 has shown to protect the individual from acquiring the disease[[4](#_ENREF_4)], assuming a general protective character of this polymorphism.

An Iranian study (Hayatbakhsh *et al*[[15](#_ENREF_15)],2012) demonstrated the protective influence of rs7517847 on bowel movements, and blood in stool, the two important features of UC[[15](#_ENREF_15)]. We were partly able to reproduce these results for the examined Hungarian population. The rs1004819 showed no influence on these characteristics, neither in the Iranian nor the present study. Chinese research detected similar results suggesting the rs17375018 SNP to have a protective character[[20](#_ENREF_20)]. There are studies that suggest IL23R to be a risk factor for the development of extraintestinal manifestations[[18](#_ENREF_18)]. This was not reproducible in our study with Hungarian UC patients.

The data of the present study are in contrast to the results of Duerr *et al*[[1](#_ENREF_1)] and Glas *et al*[[4](#_ENREF_4)], who did find an association between *IL23R* and inflammatory bowel diseases but no influence on the phenotype of ulcerative colitis.

More studies are necessary to clarify the exact role of *IL23R* in the development of IBDs, as well as other autoimmune diseases, to improve the knowledge about their pathogenesis and pathophysiology. This may allow individual risk stratification, individual pharmacotherapy, and new approaches for medication with targeted therapy.

# Acknowledgments

The authors would like to thank the to Dr. Junker U, Dr. Beck A and Sandhu S for their technical support. The present scientific contribution is dedicated to the 650th anniversary of the foundation of the University of Pécs, Hungary.

**COMMENTS**

***Background***

Inflammatory bowel diseases (IBD) are complex diseases with suspected genetic and environmental etiology. Interleukin-23 receptor (*IL23R*) is one of those genetic factors found by genome-wide association studies (GWAS). IL23R is expressed by CD4+ T cells, monocytes/macrophages, and CD11c+ dendritic cells (DCs). IL23 is a pro-inflammatory cytokine which has potential target in the treatment of various autoimmune inflammatory diseases. Duerr and coworkerswere the first who identified *IL23R* as a gene associated with IBDs. They were able to provide evidence for an association in non-Jewish ulcerative colitis (UC) population. In a big German cohort Glas *et al* (2007) investigated interactions of IBD genes and the influence of *IL23R* on the phenotype. Eight out of ten SNPs showed significant association with UC. The fact that these SNPs were either protective or susceptible in both CD and UC suggests similar disease-modifying effects. Recent studies also suggest the SNPs of *IL23R* to be a predictive factor of response to the therapy, *e.g.* non-response to mesalazine and corticosteroids as well as higher response rate for azathioprine and infliximab.

***Research frontiers***

Previous investigations in Hungarian populations verified *IL23R* to play a role in the development of UC. The present study demonstrated the correlation between the SNPs of *IL23R,* and the phenotype of UC.

***Innovations and breakthroughs***

Two of the susceptible SNPs for the development of UC (rs2201841, rs10889677) seem to shift the clinical picture from mild into more severe. The rs10889677 was associated with azathioprine-therapy, suggesting the patients to be refractory to 5-aminosalicylic-acid (5-ASA). The rs7517847 variant showed a protective character (less bowel movements). Rs7517847 has shown to protect the individual from acquiring the disease, assuming a general protective character of this polymorphism.

***Applications***

These results may allow individual risk stratification, individual pharmacotherapy, and new approaches for medication with targeted therapy. However, more studies are necessary to clarify the exact role of *IL23R* in the development of IBDs.

***Peer-review***

The study is well conducted, the results are interesting but, as the number of patients is not very high, the data should be validated in another cohort in a future study.

**REFERENCES**

1 **Duerr RH**, Taylor KD, Brant SR, Rioux JD, Silverberg MS, Daly MJ, Steinhart AH, Abraham C, Regueiro M, Griffiths A, Dassopoulos T, Bitton A, Yang H, Targan S, Datta LW, Kistner EO, Schumm LP, Lee AT, Gregersen PK, Barmada MM, Rotter JI, Nicolae DL, Cho JH. A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. *Science* 2006; **314**: 1461-1463 [PMID: 17068223 DOI: 10.1126/science.1135245]

2 **OMIM**. Interleukin 23 receptor.

3 **Awasthi A**, Riol-Blanco L, Jäger A, Korn T, Pot C, Galileos G, Bettelli E, Kuchroo VK, Oukka M. Cutting edge: IL-23 receptor gfp reporter mice reveal distinct populations of IL-17-producing cells. *J Immunol* 2009; **182**: 5904-5908 [PMID: 19414740 DOI: 10.4049/jimmunol.0900732]

4 **Glas J**, Seiderer J, Wetzke M, Konrad A, Török HP, Schmechel S, Tonenchi L, Grassl C, Dambacher J, Pfennig S, Maier K, Griga T, Klein W, Epplen JT, Schiemann U, Folwaczny C, Lohse P, Göke B, Ochsenkühn T, Müller-Myhsok B, Folwaczny M, Mussack T, Brand S. rs1004819 is the main disease-associated IL23R variant in German Crohn's disease patients: combined analysis of IL23R, CARD15, and OCTN1/2 variants. *PLoS One* 2007; **2**: e819 [PMID: 17786191 DOI: 10.1371/journal.pone.0000819]

5 **OMIM**. Interleukin 23-alpha.

6 **Neumann J**. Immunbiologie. Berlin Heidelberg Springer-Verlag, 2008

7 **O'Shea JJ**, Steward-Tharp SM, Laurence A, Watford WT, Wei L, Adamson AS, Fan S. Signal transduction and Th17 cell differentiation. *Microbes Infect* 2009; **11**: 599-611 [PMID: 19379825 DOI: 10.1016/j.micinf.2009.04.007]

8 **Geremia A**, Arancibia-Cárcamo CV, Fleming MP, Rust N, Singh B, Mortensen NJ, Travis SP, Powrie F. IL-23-responsive innate lymphoid cells are increased in inflammatory bowel disease. *J Exp Med* 2011; **208**: 1127-1133 [PMID: 21576383 DOI: 10.1084/jem.20101712]

9 **Kolls JK**, Lindén A. Interleukin-17 family members and inflammation. *Immunity* 2004; **21**: 467-476 [PMID: 15485625 DOI: 10.1016/j.immuni.2004.08.018]

10 **Janssen Research & Development L.** A Study to Evaluate the Safety and Efficacy of Ustekinumab Induction and Maintenance Therapy in Participants With Moderately to Severely Active Ulcerative Colitis (UNIFI). In: Janssen Research & Development L, 2016

11 **Krueger GG**, Langley RG, Leonardi C, Yeilding N, Guzzo C, Wang Y, Dooley LT, Lebwohl M. A human interleukin-12/23 monoclonal antibody for the treatment of psoriasis. *N Engl J Med* 2007; **356**: 580-592 [PMID: 17287478 DOI: 10.1056/NEJMoa062382]

12 **Panaccione R**, Sandborn WJ, Gordon GL, Lee SD, Safdi A, Sedghi S, Feagan BG, Hanauer S, Reinisch W, Valentine JF, Huang B, Carcereri R. Briakinumab for treatment of Crohn's disease: results of a randomized trial. *Inflamm Bowel Dis* 2015; **21**: 1329-1340 [PMID: 25989338 DOI: 10.1097/MIB.0000000000000366]

13 **Zhang Z**, Hinrichs DJ, Lu H, Chen H, Zhong W, Kolls JK. After interleukin-12p40, are interleukin-23 and interleukin-17 the next therapeutic targets for inflammatory bowel disease? *Int Immunopharmacol* 2007; **7**: 409-416 [PMID: 17321463 DOI: 10.1016/j.intimp.2006.09.024]

14 **Mannon PJ**, Fuss IJ, Mayer L, Elson CO, Sandborn WJ, Present D, Dolin B, Goodman N, Groden C, Hornung RL, Quezado M, Yang Z, Neurath MF, Salfeld J, Veldman GM, Schwertschlag U, Strober W. Anti-interleukin-12 antibody for active Crohn's disease. *N Engl J Med* 2004; **351**: 2069-2079 [PMID: 15537905 DOI: 10.1056/NEJMoa033402]

15 **Hayatbakhsh MM**, Zahedi MJ, Shafiepour M, Nikpoor AR, Mohammadi M. IL-23 receptor gene rs7517847 and rs1004819 SNPs in ulcerative colitis. *Iran J Immunol* 2012; **9**: 128-135 [PMID: 22735800 DOI: IJIv9i2A6]

16 **Zhao XD**, Shen FC, Zhang HJ, Shen XY, Wang YM, Yang XZ, Tu HM, Tai YH, Shi RH. [Association of interleukin-23 receptor gene polymorphisms with susceptibility and phenotypes of inflammatory bowel diseases in Jiangsu Han population]. *Zhonghua Nei Ke Za Zhi* 2011; **50**: 935-941 [PMID: 22333126]

17 **Jürgens M**, Laubender RP, Hartl F, Weidinger M, Seiderer J, Wagner J, Wetzke M, Beigel F, Pfennig S, Stallhofer J, Schnitzler F, Tillack C, Lohse P, Göke B, Glas J, Ochsenkühn T, Brand S. Disease activity, ANCA, and IL23R genotype status determine early response to infliximab in patients with ulcerative colitis. *Am J Gastroenterol* 2010; **105**: 1811-1819 [PMID: 20197757 DOI: 10.1038/ajg.2010.95]

18 **Cravo ML**, Ferreira PA, Sousa P, Moura-Santos P, Velho S, Tavares L, de Deus JR, Ministro P, Peixe P, Correia LA, Velosa JF, Maio RF, Brito M. IL23R polymorphisms influence phenotype and response to therapy in patients with ulcerative colitis. *Eur J Gastroenterol Hepatol* 2014; **26**: 26-32 [PMID: 24168842 DOI: 10.1097/MEG.0000000000000004]

19 **Dignass A**, Eliakim R, Magro F, Maaser C, Chowers Y, Geboes K, Mantzaris G, Reinisch W, Colombel JF, Vermeire S, Travis S, Lindsay JO, Van Assche G. Second European evidence-based consensus on the diagnosis and management of ulcerative colitis part 1: definitions and diagnosis. *J Crohns Colitis* 2012; **6**: 965-990 [PMID: 23040452 DOI: 10.1016/j.crohns.2012.09.003]

20 **Yu P**, Shen F, Zhang X, Cao R, Zhao X, Liu P, Tu H, Yang X, Shi R, Zhang H. Association of single nucleotide polymorphisms of IL23R and IL17 with ulcerative colitis risk in a Chinese Han population. *PLoS One* 2012; **7**: e44380 [PMID: 22984500 DOI: 10.1371/journal.pone.0044380]

21 **Mihaljević S**, Kibel A, Stefanić M, Glavas-Obrovac L, Takac B, Krznarić Z, Samardiija M, Pinotić L, Milas J, Segec I. Polymorphisms of interleukin-23 receptor in patients with inflammatory bowel disease in a Croatian tertiary center. *Coll Antropol* 2013; **37**: 1171-1177 [PMID: 24611330]

22 **Safrany E**, Szabo M, Szell M, Kemeny L, Sumegi K, Melegh BI, Magyari L, Matyas P, Figler M, Weber A, Tulassay Z, Melegh B. Difference of interleukin-23 receptor gene haplotype variants in ulcerative colitis compared to Crohn's disease and psoriasis. *Inflamm Res* 2013; **62**: 195-200 [PMID: 23093364 DOI: 10.1007/s00011-012-0566-z]

23 **Silverberg MS**, Cho JH, Rioux JD, McGovern DP, Wu J, Annese V, Achkar JP, Goyette P, Scott R, Xu W, Barmada MM, Klei L, Daly MJ, Abraham C, Bayless TM, Bossa F, Griffiths AM, Ippoliti AF, Lahaie RG, Latiano A, Paré P, Proctor DD, Regueiro MD, Steinhart AH, Targan SR, Schumm LP, Kistner EO, Lee AT, Gregersen PK, Rotter JI, Brant SR, Taylor KD, Roeder K, Duerr RH. Ulcerative colitis-risk loci on chromosomes 1p36 and 12q15 found by genome-wide association study. *Nat Genet* 2009; **41**: 216-220 [PMID: 19122664 DOI: 10.1038/ng.275]

24 **McGovern DP**, Gardet A, Törkvist L, Goyette P, Essers J, Taylor KD, Neale BM, Ong RT, Lagacé C, Li C, Green T, Stevens CR, Beauchamp C, Fleshner PR, Carlson M, D'Amato M, Halfvarson J, Hibberd ML, Lördal M, Padyukov L, Andriulli A, Colombo E, Latiano A, Palmieri O, Bernard EJ, Deslandres C, Hommes DW, de Jong DJ, Stokkers PC, Weersma RK, Sharma Y, Silverberg MS, Cho JH, Wu J, Roeder K, Brant SR, Schumm LP, Duerr RH, Dubinsky MC, Glazer NL, Haritunians T, Ippoliti A, Melmed GY, Siscovick DS, Vasiliauskas EA, Targan SR, Annese V, Wijmenga C, Pettersson S, Rotter JI, Xavier RJ, Daly MJ, Rioux JD, Seielstad M. Genome-wide association identifies multiple ulcerative colitis susceptibility loci. *Nat Genet* 2010; **42**: 332-337 [PMID: 20228799 DOI: 10.1038/ng.549]

25 **Magyari L**, Melegh B. [Susceptibility genetic variants in Hungarian morbus Crohn and ulcerative colitis patients]. *Orv Hetil* 2009; **150**: 81-88 [PMID: 19103559 DOI: 10.1556/OH.2009.28445]

26 **Zhu KJ**, Zhu CY, Shi G, Fan YM. Association of IL23R polymorphisms with psoriasis and psoriatic arthritis: a meta-analysis. *Inflamm Res* 2012; **61**: 1149-1154 [PMID: 22706445 DOI: 10.1007/s00011-012-0509-8]

27 **Huber AK**, Jacobson EM, Jazdzewski K, Concepcion ES, Tomer Y. Interleukin (IL)-23 receptor is a major susceptibility gene for Graves' ophthalmopathy: the IL-23/T-helper 17 axis extends to thyroid autoimmunity. *J Clin Endocrinol Metab* 2008; **93**: 1077-1081 [PMID: 18073300 DOI: 10.1210/jc.2007-2190]

28 **Lee YH**, Choi SJ, Ji JD, Song GG. Associations between interleukin-23R polymorphisms and ankylosing spondylitis susceptibility: a meta-analysis. *Inflamm Res* 2012; **61**: 143-149 [PMID: 22089529 DOI: 10.1007/s00011-011-0398-2]

29 **Szabo M**, Safrany E, Pazar B, Melegh BI, Kisfali P, Poor G, Figler M, Szekanecz Z, Czirjak L, Melegh B. Marked diversity of IL23R gene haplotype variants in rheumatoid arthritis comparing with Crohn's disease and ankylosing spondylitis. *Mol Biol Rep* 2013; **40**: 359-363 [PMID: 23054009 DOI: 10.1007/s11033-012-2068-z]

30 **Song GG**, Bae SC, Choi SJ, Ji JD, Lee YH. Associations between interleukin-23 receptor polymorphisms and susceptibility to rheumatoid arthritis: a meta-analysis. *Mol Biol Rep* 2012; **39**: 10655-10663 [PMID: 23053963 DOI: 10.1007/s11033-012-1955-7]

31 **Zhai Y**, Xu K, Huang F, Peng H, Feng CC, Zhu KK, Leng RX, Pan HF, Ye DQ. Association of interleukin 23 receptor gene polymorphisms (rs10489629, rs7517847) with rheumatoid arthritis in European population: a meta-analysis. *Mol Biol Rep* 2012; **39**: 8987-8994 [PMID: 22718508 DOI: 10.1007/s11033-012-1768-8]

32 **Sarlos P**, Varszegi D, Csongei V, Magyari L, Jaromi L, Nagy L, Melegh B. Susceptibility to ulcerative colitis in Hungarian patients determined by gene-gene interactions. *World J Gastroenterol* 2014; **20**: 219-227 [PMID: 24415875 DOI: 10.3748/wjg.v20.i1.219]

**P-Reviewer:** Cravo M, Daniel F **S-Editor:** Gong ZM

**L-Editor:** **E-Editor:**

**Specialty type:** Gastroenterology and hepatology

**Country of origin:** Hungary

**Peer-review report classification**

Grade A (Excellent): 0

Grade B (Very good): B

Grade C (Good): C

Grade D (Fair): 0

Grade E (Poor): 0





**Figure 1 Distribution of the phenotype characteristics for the *IL23R* single nucleotide polymorphisms.** A: Distribution of Montreal-classification (E1-3) for rs2201841; B: Summary of the occurrence and non-occurrence of iron deficiency for rs2201841; C: Representation of the number of operations regarding to rs2201841; D: Representation of stool frequency in three categories for rs7517847; E: Distribution of Montreal-classification (E1-3) on the alleles of rs10489629; F: Distribution of patients with and without weight loss to the alleles of rs10489629; G: Need to azathioprine therapy in respect to rs10889677; H: Representation of the number of operations regarding to rs10889677.

**Table 1 Genotypes of the investigated *IL23R* single nucleotide polymorphisms in Hungarian population**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **SNP** | **Genotype** | **UC (*n*)** | **%** | **Allele** | **UC (*n*)** | **%** |
| rs11209026  | GG | 109 | 95.6 | G | 223 | 97.8 |
| AG | 5 | 4.4 | A | 5 | 2.2 |
| AA | 0 | 0 |  |  |  |
| total | 114 |  |  |  |  |
| RAF | 0.0219 |
| rs10889677 | CC | 51 | 45.5 | C | 155 | 69.2 |
| AC | 53 | 47.3 | A | 69 | 30.8 |
| AA | 8 | 7.1 |  |  |  |
| total | 112 |  |  |  |  |
| RAF | 0.308 |
| rs1004819 | GG | 52 | 40.3 | G | 172 | 66.7 |
| AG | 68 | 52.7 | A | 86 | 33.3 |
| AA | 9 | 7 |  |  |  |
| total | 129 |  |  |  |  |
| RAF | 0.333 |
| rs2201841 | TT | 58 | 45 | T | 176 | 68.2 |
| CT | 60 | 46.5 | C | 82 | 31.8 |
| CC | 11 | 8.5 |  |  |  |
| Total | 129 |  |  |  |  |
| RAF | 0.317 |
| rs7517847 | TT | 40 | 35.7 | T | 136 | 60.7 |
| GT | 56 | 50 | G | 88 | 39.3 |
| GG | 16 | 14.3 |  |  |  |
| Total | 112 |  |  |  |  |
| RAF | 0.392 |
| rs10489629 | GG | 44 | 34.6 | G | 155 | 61 |
| AG | 67 | 52.8 | A | 99 | 39 |
| AA | 16 | 12.6 |  |  |  |
| Total | 127 |  |  |  |  |
| RAF | 0.389 |
| rs7530511 | CC | 94 | 73.4 | C | 220 | 85.9 |
| TC | 32 | 25 | T | 36 | 14.1 |
| TT | 2 | 1.6 |  |  |  |
| Total | 128 |  |  |  |  |
| RAF | 0.140 |

RAF: Risk allele frequency; SNP: single nucleotide polymorphism.

**Table 2 *IL23R* single nucleotide polymorphisms with statistically significant influence on the clinical picture of ulcerative colitis**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **SNP** | **Wt *vs* Hz** | **Wt *vs* Ho** | **Wt *vs* Hz + Ho** | **Wt + Hz *vs* Ho** | **Total distribution** |
|
| rs2201841 |  |  |  |  |  |
| Montreal- classification | 0.0429a | 0.2270 | 0.1921 | 0.0217a | 0.0084a |
| Iron deficiency | 0.1892 | 0.1108 | 0.7476 | 0.0388a | 0.0299a |
| Need for operation | 0.0348a | 1.0 | 0.0766 | 0.6017 | 0.0564 |
| rs7517847 |  |  |  |  |  |
| Bowel movements | 0.0050a | 0.8183 | 0.0634 | 0.2979 | 0.0078a |
| rs10489629 |  |  |  |  |  |
| Montreal classification | 0.1017 | 0.3906 | 0.4445 | 0.1170 | 0.0405a |
| Weight loss | 0.0652 | 0.0457a | 0.0169a | 0.1191 | 0.0350a |
| rs10889677 |  |  |  |  |  |
| Azathioprine | 0.0116a | 0.3407 | 0.0136a | 0.6113 | 0.0285a |
| Need for operation | 0.0347a | 0.8491 | 0.0746 | 0.6153 | 0.0595 |

Indicates significant difference (a*P* < 0.05). Hz: heterozygous; Ho: homozygous; Wt: wild type; Total distribution: Wt + Hz + Ho; SNP: single nucleotide polymorphism.