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IL-23 Receptor (*IL-23R*) Gene Protects Against Pediatric Crohn's Disease

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Abstract

Background— The IL-23 receptor (*IL-23R*) has been found to be associated with small bowel Crohn's disease (CD) in a whole genome association study. Specifically, the rare allele of the R381Q single nucleotide polymorphism (SNP) conferred protection against CD. It is unknown whether *IL-23R* is associated with IBD in children. The aim was to examine the association of *IL-23R* with susceptibility to IBD in pediatric patients.

Methods— DNA was collected from 609 subjects (151 CD and 52 ulcerative colitis [UC] trios). Trios were genotyped for the R381Q SNP of the *IL-23R* gene and SNP8, SNP12, SNP13, of the *CARD15* gene using Taqman. The transmission disequilibrium test (TDT) was used for association to disease using GENEHUNTER 2.0.

Results— The rare allele of R381Q SNP was present in 2.7% of CD and 2.9% UC probands. The *CARD15* frequency was 31.5% (CD) and 18% (UC). The *IL-23R* allele was negatively associated with inflammatory bowel disease (IBD): the R381Q SNP was undertransmitted in children with IBD (8 transmitted [T] versus 27 untransmitted [UT]; $P = 0.001$). This association was significant for all CD patients (6 T versus 19 UT; $P = 0.009$), especially for non-Jewish CD patients (2 T versus 17 UT; $P = 0.0006$). TDT showed a borderline association for UC (2 T versus 8 UT; $P = 0.06$). As expected, *CARD15* was associated with CD in children by the TDT (58 T versus 22 UT $P = 0.00006$), but not with UC.

Conclusions— The protective *IL-23R* R381Q variant was particularly associated with CD in non-Jewish children. Thus, the initial whole genome association study based on ileal CD in adults has been extended to the pediatric population and beyond small bowel CD.

Keywords

pediatrics; Crohn's disease; genetics

The currently accepted etiopathogenic hypothesis suggests that chronic intestinal inflammation and related systemic manifestations characteristic of inflammatory bowel disease (IBD) are

due to an overly aggressive or pathologic immune response to resident luminal bacterial constituents. Predisposing factors are genetic dysregulation of mucosal immune responses and/or barrier function, with onset triggered by environmental stimuli. The increased level of concordance between identical twins, increased rates of IBD among the Ashkenazi Jewish population, and the familial risk of IBD provides strong evidence that genetic factors play an important role in the pathogenesis of IBD.¹

Genetic advances have led to the discovery that variants of *CARD15* located at 16q12 and the 5q31 (IBD5) haplotype confer susceptibility to Crohn's disease (CD). However, the risks associated with these 2 genes account for only a portion of the overall genetic risk. This has led to additional gene findings efforts, the most recent being the recent genome-wide association study that identified the association of the interleukin 23 receptor gene (*IL-23R*) with small bowel CD. In that study the rare glutamine allele of Arg381Gln (R381Q single nucleotide polymorphism, SNP) conferred protection against CD.² Distortion of allele transmission was also observed for non-Jewish ulcerative colitis (UC)-affected offspring. This study was conducted in an adult IBD cohort and it remains unknown whether *IL-23R* is associated with IBD in children.

The *IL-23R*, consisting of an IL-12 β 1 and an IL-23R chain, is highly expressed on memory T cells.³ IL-23 is a novel cytokine formed via the binding of IL-12p40 to a p19 protein.⁴ After binding to the IL-23 receptor, IL-23 preferentially activates memory T cells. IL-23 does exhibit some similar biological activities to IL-12, however, IL-12 is more involved in the differentiation of naïve T cells into T_H1 lymphocytes and subsequent IFN γ production. IL-23, on the other hand, mediates proinflammatory activities in part by the production of IL-17 through activation of T_H17 lymphocytes.⁵ Other research has shown that IL-23 increases IL-6 production that may account for the tissue injury characteristic of IBD.⁶ It has also been shown that IL-23, not IL-12, is an important promoter of chronic joint inflammation.⁵ Becker et al⁷ suggested that there is a predisposition of IL-23-induced chronic inflammation in the terminal ileum, which strengthens the evidence for the recent association between *IL-23R* in small bowel CD patients.² The elevation of IL-17 levels in the colonic mucosa of both CD and UC patients, combined with the recent association of *IL-23R* with UC,² suggests that IL-23 may also play an important role in UC.⁸

In this study the association of *IL-23R* in a pediatric IBD population was tested using the robust method of transmission disequilibrium test (TDT) analysis. We specifically tested R381Q SNP, since this was the most significant result in the genome-wide association study.

MATERIALS AND METHODS

Patients and Families

IBD patients and families were enrolled from participating sites of the Western Regional Research Alliance for Pediatric IBD (WRRAPID). A total of 203 families (609 subjects) were enrolled. In order to be eligible all CD patients must have undergone complete colonoscopy with ileal intubation or complete colonoscopy and small bowel follow-through. A diagnosis of CD for this study required at least 2 of the following: 1) a history of abdominal pain, weight loss, short stature, malaise, rectal bleeding, or diarrhea; 2) characteristic endoscopic findings of discontinuous ulcerations, cobblestoning, fistula, or severe perianal disease; 3) radiologic features of stricture, fistula, or evidence of cobblestoning or ulceration of the mucosa; 4) macroscopic appearance at laparotomy of typical bowel wall induration, mesenteric lymphadenopathy, or serosal involvement showing creeping fat or other inflammatory changes; or 5) histopathology showing transmural inflammatory cell infiltrate or epithelial granulomas and absence of identifiable infectious agents.⁹ A diagnosis of UC for this study required at least 3 of the following after exclusion of infectious or neoplastic disease¹⁰: 1)

typical case history with diarrhea and/or blood in stools for more than a week or recurrent episodes; 2) typical sigmoidoscopic or colonoscopic findings of granulated, friable mucosa with or without superficial ulcerations; 3) characteristic histopathologic and/or cytologic findings of inflammation; or 4) radiologic findings of inflammation, with spiculated, granulated inner surface of the colon proximal to the rectum and/or frank ulcerations.

Parents of the IBD probands were also included as part of the TDT analysis. Indeterminate colitis patients were excluded from the study. Informed consent was obtained from all study participants and this study was approved by the Ethics Review Board at each participating site.

Genotyping

Whole blood samples were drawn on all IBD trios, each consisting of 1 child with IBD (CD or UC) and their 2 parents at each of the participating sites, and sent via overnight FedEx to the Genotyping Core Facility of the Medical Genetics Institute General Clinical Research Center at Cedars-Sinai Medical Center (CSMC). Both parents and the affected child were genotyped for the reported protective R381Q SNP (rs11209026) of the *IL-23R* gene and the well-established predisposing 3 variants of the *CARD15* gene (SNP8, SNP12, SNP13). R381Q was specifically examined as it had the greatest significance, exceeding genome-wide level significance, in the prior genome-wide association.² Genotyping methods were adapted to the TaqMan MGB genotyping platform following the manufacturer's instructions and using PrimerExpress design software (Applied Biosystems, Foster City, CA) as previously described.^{11,12}

Statistical Analysis

The TDT statistic was used to test the association with disease using GENEHUNTER 2.0.¹³ TDT tests whether a gene is under- or overtransmitted to the affected child. Since the genotyping and analysis was done within a family, it avoided the possibility of spurious associations due to unrecognized population stratification. *CARD15* TDT was used as an expected positive control for the study population.

RESULTS

Study Population

A total of 609 subjects were enrolled. Of the 203 trios, 151 were from CD probands and 52 from UC probands. The median age at diagnosis for all IBD patients was 11 years (range, 0.8–18 years). Of the 151 children with CD, 67% had both small and large bowel involvement, 30% large bowel disease only, and the remaining 13% had isolated small bowel disease. Our study population consisted of 62 Jewish ancestry probands; 37% (53/151) and 17% (9/52) were from CD and UC, respectively. The majority of the families (86%) were of the Caucasian ethnic group.

Genetic Analyses

Allele Frequencies—The rare glutamine allele of the R381Q SNP was present in only 2.67% of CD and 2.94% of UC probands (Table 1). *CARD15* allele frequency (any variant) was 31.5% (CD) and 18% (UC). Similar frequencies were observed for the parents for both genes.

Transmission Disequilibrium Test (TDT)—TDT in all IBD trios showed a highly significant association between IBD and undertransmission of the rare glutamine allele of R381Q; 8 transmitted (T) versus 27 untransmitted (UT); $P = 0.001$ (Table 2). This association was significant for all CD patients (6 T versus 19 UT; $P = 0.009$), especially for non-Jewish CD patients (2 T versus 17 UT; $P = 0.0006$) (Table 3). TDT showed a borderline association

for UC (2 T versus 8 UT; $P = 0.06$). As expected, *CARD15* was associated with CD in children by the TDT: (58 T versus 22 UT $P = 0.00006$), but not with UC (Table 4).

DISCUSSION

This is the first study to demonstrate a significant association between *IL-23R* and IBD in a pediatric cohort. The protective allele coding for glutamine of the *IL-23R* R381Q (Arg381Gln) variant was associated with CD in non-Jewish children. Thus, the initial whole genome association (GWA) study based on ileal CD has been extended to the pediatric population and beyond small bowel CD.² In this study the *CARD15* association acted as a positive control, and the observed association with *CARD15* demonstrated that applying the TDT to this pediatric cohort will be useful in further gene finding efforts for IBD.

The *IL-23R* protein is comprised of an extracellular domain, a single transmembrane domain, and a 252 amino acid cytoplasmic domain.³ The Arg381Gln variant identified in the genome-wide association encodes for a subunit of the *IL-23R* located on chromosome 1p31. The glutamine allele is the rare allele and the arginine is the common allele. The frequency of the rare allele in our IBD probands was 2.67% for CD and 2.94% for UC. In the prior case-control association study, the glutamine allele was shown to protect against CD in both the Jewish (odds ratio [OR]: 0.26) and non-Jewish populations (OR: 0.45). TDT analysis herein demonstrated that the glutamine allele was undertransmitted in all CD-affected offspring. Our study confirms the negative association of R381Q with CD using TDT, most significantly observed herein in non-Jewish families. Further studies need to be done to confirm an association between *IL-23R* and UC, as some susceptibility was observed in the prior GWA and a trend was seen in our pediatric patients using TDT.

Further research is certainly needed to examine other variants on the *IL-23R* gene and its adjacent region. Of interest is the proximity of the *IL-23R* gene to the *IL-12RB1* gene, which encodes the second subunit of the *IL-23R*. Research suggests that both IL-23 and IL-12 promote a Th1 proinflammatory response. Based on more recent gene targeted animal model studies it appears that IL-23 may be a more significant driver of inflammation. Murphy et al⁵ demonstrated that the targeted absence of IL-23 was protective, whereas loss of IL-12 exacerbated collagen-induced arthritis. In these studies the *IL-23* gene knockout mice did not develop clinical signs of disease or suffer any joint and bone pathology. In contrast, IL-12 deficiency appeared to exacerbate the signs of inflammation. A resistance to joint inflammation and bone pathology was found to be correlated with an absence of IL-17–producing CD4+T cells. This is in contrast to the IL-12–deficient mice who paradoxically developed more IL-17–producing CD4+ lymphocytes with increased expression of the proinflammatory cytokines; tumor necrosis factor, IL-1, IL-6, and IL-17. Such gene-targeted models are lacking in models of gut inflammation but recent evidence suggests that IL-23 does play an important role in the inflammatory cascade characteristic of IBD.^{6,7,14} Becker et al⁷ demonstrated that the small bowel, more specifically the terminal ileum, is predisposed to develop chronic inflammation through IL-23. The blockade of the proinflammatory cytokine TNF α has set the stage for an era of monoclonal antibody blockage of key cytokines involved in autoimmune diseases.¹⁵ Anti-TNF α therapies serve as a model for development of other therapies that will block the formation of or signaling pathway for proinflammatory cytokines. The IL-23 pathway has also been a target of antibody blockade. A small pilot study demonstrated that the administration of anti-IL-12p40 resulted in the downregulation of both IL-23 and IL-12, as well as their downstream proinflammatory mediators, given that both cytokines share an identical p40 chain that is bound to a p19 chain in the case of IL-23 and a p35 chain in IL12.⁶ Further research is needed to understand how variants of the *IL-23R* gene contribute to disease susceptibility in IBD. This will also help guide the future direction of therapeutic interventions.

The results of our study demonstrate that the allele coding for glutamine at the *IL-23R* R381Q (Arg381Gln) variable site protects against pediatric CD. Studies are planned to examine the association between *IL-23R* and phenotypic expression in IBD patients. It is hoped that the identification of other susceptibility variants and an understanding of the contribution of the *IL-23R* pathway to the susceptibility of chronic intestinal inflammation will lead to improved therapeutic interventions and outcomes for patients with IBD.

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References

1. Taylor, KD.; Yand, HY.; Rotter, JI. Inflammatory bowel disease. In: Rimoïn, DL.; Connor, JM.; Pyeritz, RE.; Korf, B., editors. Emery and Rimoïn's principles and practice of medical genetics. 5. London: Churchill Livingstone; 2007. p. 1549-1582.
2. Duerr RH, Taylor KD, Brant SR, et al. A genome-wide association study identifies *IL-23R* as an inflammatory bowel disease gene. *Science* 2006;314:1461–1463. [PubMed: 17068223]
3. Parham C, Chirica M, Timans J, et al. A receptor for the heterodimeric cytokine *IL-23* is composed of *IL-12Rbeta1* and a novel cytokine receptor subunit, *IL-23R*. *J Immunol* 2002;168:5699–5708. [PubMed: 12023369]
4. Oppmann B, Lesley R, Blom B, et al. Novel p19 protein engages *IL-12p40* to form a cytokine, *IL-23*, with biological activities similar as well as distinct from *IL-12*. *Immunity* 2000;13:715–725. [PubMed: 11114383]
5. Murphy CA, Langrish CL, Chen Y, et al. Divergent pro- and anti-inflammatory roles for *IL-23* and *IL-12* in joint autoimmune inflammation. *J Exp Med* 2003;198:1951–1957. [PubMed: 14662908]
6. Fuss IJ, Becker C, Yang Z, et al. Both *IL-12p70* and *IL-23* are synthesized during active Crohn's disease and are down-regulated by treatment with anti-*IL-12 p40* monoclonal antibody. *Inflamm Bowel Dis* 2006;12:9–15. [PubMed: 16374252]
7. Becker C, Wirtz S, Blessing M, et al. Constitutive p40 promoter activation and *IL-23* production in the terminal ileum mediated by dendritic cells. *J Clin Invest* 2003;112:693–706. [PubMed: 12952918]
8. Fujino S, Andoh A, Bamba S, et al. Increased expression of interleukin 17 in inflammatory bowel disease. *Gut* 2003;52:65–70. [PubMed: 12477762]
9. Loftus EV, Silverstein MD, Sandborn WJ, et al. Crohn's disease in Olmsted County, Minnesota, 1940–1993: incidence, prevalence, and survival. *Gastroenterology* 1998;114:1161–1168. [PubMed: 9609752]
10. Langholz E, Munkholm P, Davidsen M, Binder V. Course of ulcerative colitis: analysis of changes in disease activity over years. *Gastroenterology* 1994;107:300–302. [PubMed: 8020675]
11. ABI: Assays on demand SNP genotyping products. Publication 127PB04 – 01. 2002. Available from www.appliedbiosystems.com
12. Morin PA, Saiz R, Monjanez A. High-throughput single nucleotide polymorphism genotyping by fluorescent 5' exonuclease assay. *Biotechniques* 1999;27:538–552. [PubMed: 10489614]
13. Spielman RS, McGinnis RE, Ewens WJ. Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). *Am J Hum Genet* 1993;52:506–516. [PubMed: 8447318]
14. Schmidt C, Giese T, Ludwig B, et al. Expression of interleukin-12-related cytokine transcripts in inflammatory bowel disease: elevated interleukin-23p19 and interleukin-27p28 in Crohn's disease but not in ulcerative colitis. *Inflamm Bowel Dis* 2005;11:16–23. [PubMed: 15674109]
15. Targan SR, Hanauer SB, van Deventer SJ, et al. A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor alpha for Crohn's disease. Crohn's Disease cA2 Study Group. *N Engl J Med* 1997;337:1029–1035. [PubMed: 9321530]

TABLE 1

Allele Frequencies for *CARD 15* SNPs and *IL23R* R381Q SNP

SNP	Allele	CD Families		UC Families	
		Parent	Proband	Parent	Proband
<i>IL23R</i> R381Q SNP	R	95.48%	97.33%	95.05%	97.06%
	Q	4.52%	2.67%	4.95%	2.94%
<i>CARD15</i> SNP 8	C	93.77%	92.33%	97.03%	98.04%
	T	6.23%	7.67%	2.97%	1.96%
<i>CARD15</i> SNP12	G	96.59%	95.24%	98.04%	97.06%
	C	3.41%	4.76%	1.96%	2.94%
<i>CARD15</i> SNP13	—	94.97%	92.25%	97.06%	95.1%
	C	5.03%	7.95%	2.94%	4.9%
<i>CARD 15</i> Any SNP	Any rare allele	27.7%	31.5	15	18

SNP, single nucleotide polymorphism; CD, Crohn's disease; UC ulcerative colitis.

TABLE 2

IL23R TDT for IBD, CD, and UC

<i>IL23R</i> Variant	IBD			CD			UC		
	T	UT	<i>P</i> value	T	UT	<i>P</i> value	T	UT	<i>P</i> value
Glutamine allele	8	27	0.001	6	19	0.009	2	8	0.06

TDT, transmission disequilibrium test; IBD, inflammatory bowel disease; CD, Crohn's disease; UC ulcerative colitis; T, transmitted; UT, untransmitted.

TABLE 3*IL23R* TDT in Jewish and Non-Jewish IBD Families

Ancestry	IBD Subtype	Glutamine Allele T	Glutamine Allele UT	<i>P</i> value
Non-Jewish	CD	2	17	0.0006
	UC	1	6	0.06
Jewish	CD	4	2	0.41
	UC	1	2	0.56

TDT, transmission disequilibrium test; IBD, inflammatory bowel disease; CD, Crohn's disease; UC ulcerative colitis; T, transmitted; UT, untransmitted.

TABLE 4

CARD 15 TDT for IBD, CD, and UC

CARD 15 Allelic Variants	IBD			CD			UC		
	T	UT	P value	T	UT	P value	T	UT	P value
702Trp (SNP 8)	25	18	0.29	23	14	0.14	2	4	0.41
908Arg (SNP 12)	17	6	0.02	14	5	0.04	3	1	0.32
Leu1007fsinsC (SNP13)	28	6	0.0001	23	5	0.0007	5	1	0.1
Any variant	58	22	0.00006	50	17	0.00006	8	5	0.41

TDT, transmission disequilibrium test; IBD, inflammatory bowel disease; CD, Crohn's disease; UC ulcerative colitis; T, transmitted; UT, untransmitted.