IL-10R Polymorphisms Are Associated with Very-early-onset Ulcerative Colitis

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IL-10R Polymorphisms are Associated with Very Early-Onset Ulcerative Colitis

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*NEOPICS – Canadian National Early Onset Pediatric IBD Cohort Study: see Supplemental Material for details

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AMM has nothing to disclose.

Author Contribution: AMM, and SBS conceived and designed all experiments. MSS, AMM, AMG, TDW, SK provided study samples. CJM and TDW analyzed the data. CJM, VMW, CHG performed functional analysis under supervision of AMM, and SBS. CJM, SBS and AMM wrote the manuscript with contributions from all authors.
Abstract

**Background and Aims**—Interleukin-10 (IL-10) signaling genes are attractive inflammatory bowel disease (IBD) candidate genes as IL-10 restricts intestinal inflammation, IL-10 polymorphisms have been associated with IBD in genome-wide association studies, and mutations in IL-10 and IL-10 receptor (IL-10R) genes have been reported in immunodeficient children with severe infantile-onset IBD. Our objective was to determine if IL-10R polymorphisms were associated with early-onset IBD (EO-IBD) and very early-onset IBD (VEO-IBD).

**Methods**—Candidate-gene analysis of IL10RA and IL10RB was performed after initial sequencing of an infantile onset-IBD patient identified a novel homozygous mutation. The discovery cohort included 188 EO-IBD subjects and 188 healthy subjects. Polymorphisms associated with IBD in the discovery cohort were genotyped in an independent validation cohort of 422 EO-IBD subjects and 480 healthy subjects.

**Results**—We identified a homozygous, splice-site point mutation in IL10RA in an infantile-onset IBD patient causing a premature stop codon (P206X) and IL-10 insensitivity. IL10RA and IL10RB sequencing in the discovery cohort identified five IL10RA polymorphisms associated with ulcerative colitis (UC) and two IL10RB polymorphisms associated with Crohn’s disease (CD). Of these polymorphisms, two IL10RA SNPs, rs2228054 and rs2228055 were associated with very early-onset UC in the discovery cohort and replicated in an independent validation cohort (OR 3.08, combined p=2×10\(^{-4}\); and OR 2.93, p=6×10\(^{-4}\), respectively).

**Conclusions**—We identified IL10RA polymorphisms that confer risk for developing VEO-UC. Additionally, we identified the first splice site mutation in IL10RA resulting in infantile-onset IBD. This study expands the phenotype of IL10RA polymorphisms to include both severe arthritis and VEO-UC.

**Keywords**

IL-10; IL-10 Receptor; inflammatory bowel disease; immunodeficiency

Introduction

Interleukin-10 (IL-10) is an anti-inflammatory cytokine secreted by a variety of cell types and is critical for maintaining immune homeostasis in the gastrointestinal (GI) tract. These immunosuppressive effects include restricting T cell proliferation, down-regulating co-stimulatory protein expression on antigen-presenting cells, and limiting pro-inflammatory cytokine production. IL-10 activates downstream signaling by binding to the IL-10 receptor (IL-10R), comprised of two α subunits (encoded by IL10RA) and two β subunits (encoded by IL10RB). This activates JAK1 and TYK2, leading to phosphorylation and nuclear translocation of STAT3 and gene transcription. Intact IL-10 signaling is required for restricting inappropriate Th17 cell expansion – an effector cell type that has been associated...
with Crohn’s disease (CD).\textsuperscript{2,3} Consistent with the anti-inflammatory role of IL-10, mice deficient in either \textit{IL-10} or \textit{IL-10R} develop enterocolitis.\textsuperscript{3,5}

Recent studies have shown that loss-of-function mutations in \textit{IL10RA} and \textit{IL10RB}\textsuperscript{6,7} and \textit{IL10}\textsuperscript{8} in immunodeficient patients are associated with severe, infantile-onset IBD. This has stimulated renewed interest in studying IL-10 pathway genes in the pathogenesis of inflammatory bowel disease (IBD). Genome-wide association studies (GWAS) in IBD populations (including exclusively pediatric-onset disease) have associated single nucleotide polymorphisms (SNPs) in \textit{IL10} and \textit{STAT3} loci with ulcerative colitis (UC) and CD.\textsuperscript{9-12}

Given the severe phenotype of infantile-onset IBD observed with IL-10R mutations as well as that of an infant described in this paper who was found to have a homozygous, splice site mutation in \textit{IL-10RA},\textsuperscript{6,7} we hypothesized that \textit{IL10RA} and \textit{IL10RB} polymorphisms contribute to the risk of IBD developing in the very young. To date, SNPs within \textit{IL10RA} and \textit{IL10RB} loci have not been identified by GWAS of IBD. However, the contribution of IL-10R polymorphisms to IBD susceptibility in very young patients may be underestimated in GWAS as this age group represents a very select population that is poorly represented even in GWAS focusing on pediatric IBD.\textsuperscript{13} Rather than studying only the overall population of patients that constitute “pediatric IBD”, we subdivided patients so that we could concentrate our analyses on extremely young patients (that may be most likely to have the strongest genetic component to IBD risk). Although the Paris classification for IBD stratifies pediatric IBD into those diagnosed younger than 10 years of age and those diagnosed between 10 and 18 years of age,\textsuperscript{14} we focused on patients diagnosed prior to their 6th birthday - a cohort that comprises ~15% of pediatric IBD and represents a greater extreme of the disease - since the average age at diagnosis of “pediatric IBD patients” is ~10 years old.\textsuperscript{15} Therefore, we undertook a candidate gene study by deep sequencing of \textit{IL10RA} and \textit{IL10RB} in children with early onset IBD (EO-IBD, defined as age of onset <18yo) and subsequently focused on the very early-onset IBD (VEO-IBD, defined as age of onset <6yo).

**Methods**

**Identification of Novel \textit{IL-10RA} Mutation in Patient with Infantile Colitis**

We identified an IL-10RA mutation in a Caucasian female presenting with infantile colitis who was not recognized \textit{a priori} to be the product of a consanguineous marriage, but whose parents came from the same geographically-isolated region of Canada. Her first year of life was complicated by recurrent fevers (initially at six days of life). At three months old, the patient developed cellulitis and scalp eczema and generalized erythroderma (Figure 1A). Bloody diarrhea and a perianal skin tag developed at five months of age (Figure 1B) that progressed to perianal and recto-vaginal fistulae. Further complications included urinary tract infections, scalp pustules, and severe, effusive, large joint polyarthritis (Figure 1C). Joint aspirations demonstrated high neutrophil counts (>100,000/mm$^3$) despite sterile cultures. The patient did not manifest thyroid involvement. Endoscopic evaluation revealed patchy colonic ulcerations (Figure 1D) and biopsies showed chronic colitis (Figure 1E) with the absence of small bowel inflammation. Gastrointestinal symptoms were refractory to antibiotics, intravenous immunoglobulins, corticosteroids, anakinra, sulfasalazine, and azathioprine, and the patient underwent a loop ileostomy at 23 months old.

An extensive immunological evaluation included normal immunoglobulin levels and lymphocyte subsets, normal NADPH oxidase function, a normal thymic biopsy, normal karyotyping and negative FISH for 22q11. Screening for \textit{JAK3}, \textit{IPEX}, \textit{RMRP}, \textit{IL2RA}, \textit{IRAK4}, and \textit{TRAPS} mutations and mutations associated with chronic granulomatous disease were negative.
Genomic DNA was purified from whole blood using Puregene Blood Kit (Qiagen, USA). *IL10RA* and *IL10RB* were amplified using intronic primers flanking each exon and sequenced with the ABI3730 DNA analyzer (Applied Biosystems, Melbourne, Australia). *IL10RA* and *IL10RB* variants are numbered according to GeneBank accession numbers NM_001588 and NM_00628, respectively. Amino acid numbering refers to position in the immature protein (including signal peptide).

RNA was isolated from whole blood by PAXgene Blood RNA kit (Qiagen, USA) according to the manufacturer’s instructions. cDNA was synthesized using SuperScript III Reverse Transcriptase (Life Technologies, Carlsbad, California, USA). Primers for full-length *IL10RA*, full-length *IL10RB*, and for Exon 4 to Exon 7 of *IL10RA* were designed and synthesized at The Centre for Applied Genomics, Toronto. PCR was performed according to standard protocol, and the purified PCR product was cloned into pJET cloning vector (Fermentas, USA) and sequenced by ABI 3730 DNA analyzer (Applied Biosystems, Melbourne, Australia).

**Functional IL-10 Testing in IL-10R-Deficient Patient**

PBMCs from the patient and a healthy control were isolated using Polymorphoprep (Nycomed Pharma AS, Norway). The PBMCs were stimulated with IL-10 (40ng/mL) or IL-6 (20ng/mL) for 10 and 30 minutes. Cellular extracts were prepared following standard protocol. The protein concentration was measured by protein assay kit (Bio-Rad, Hercules, Canada), and 50μg protein was separated by 8% SDS-PAGE gel and transferred to membrane. The membrane was incubated with antibodies to STAT3 and phosphorylated (Tyr705) STAT3 (p-STAT3) (Cell Signaling, USA) followed by HRP-conjugated secondary antibodies. The membrane was read using a chemiluminescence system (GE Healthcare, UK). β-actin was used as a loading control.

**Flow Cytometry**

Thawed PBMC were stained in 2% FBS with CD4-PerCP (eBioscience, USA) and CD25-PE (Miltenyi Biotech, USA) followed by intracellular staining with FoxP3-FITC (eBioscience, USA). Flow cytometry was performed using a FACSCalibur (BD Biosciences, USA). Co-expression of CD25 and intracellular FoxP3 was assessed in cells within CD4⁺ lymphocyte gate.

**IL-10R Sequencing in Discovery Cohort**

The discovery cohort included DNA samples from 188 EO-IBD patients from the Hospital for Sick Children (Toronto, Canada) (49 patients) including some subjects as part of the NEOPICS consortium (Supplemental Table 1) and Children’s Hospital of Wisconsin (Milwaukee, Wisconsin) (139 patients). Control DNA samples were obtained from The Centre for Applied Genomics (Ontario Population Genomics Platform, plates used: 1-4); 95% of this cohort are Caucasians of European ancestry. Complete description can be found at [http://www.tcag.ca/facilities/cyto_population_control_DNA.html](http://www.tcag.ca/facilities/cyto_population_control_DNA.html).

The coding sequences (and intervening sequences and 3’-untranslated regions) of *IL10RA* and *IL10RB* were amplified. PCR products were sequenced by Sanger method (Beckman-Coulter Genomics, Danvers, MA). SNPs were detected using Polyphred software and all chromatograms with reliability score <99 were manually reviewed.

Allelic frequencies were determined for SNPs identified in discovery cohort. Testing for Hardy-Weinberg equilibrium was done by χ² analysis. Odds ratios (OR) were calculated by comparing the ratio of individuals with two copies of the major allele to those with one or two copies of a variant allele (DD vs. Dd/dd model) between EO-IBD and control groups.
OR were determined for UC and CD and specific age groups: children diagnosed before their 18th birthday (<18yo) and children diagnosed before their 6th birthday (<6yo). p values were calculated (but not adjusted for multiple testing in discovery cohort). SNPs with p value <0.05 were considered to be associated with IBD risk in the discovery cohort. Linkage between SNPs was assessed by χ² analysis using a 3×3 table of genotypes. Linkage disequilibrium (LD) plots were created with Golden Helix SVS 7.0 (Golden Helix, Montana) using expectation-maximization (EM) algorithm to calculate r² and D’ values.

**Genotyping of IL10RA and IL10RB of Replication Cohort**

DNA samples were collected from 422 pediatric IBD patients at the Hospital for Sick Children and genotyped using Taqman probes for SNPs associated with IBD from the discovery cohort. Control DNA samples (distinct from discovery cohort controls) were obtained from The Centre for Applied Genomics. Allelic frequencies were calculated and Hardy-Weinberg equilibrium was assessed by χ² analysis. OR were calculated (DD vs. Dd/dd model) and analyzed by χ² analysis. p values were adjusted for multiple testing by Bonferroni method (based on the number of SNPs associated with IBD in discovery cohort), and an adjusted p value <0.05 was considered statistically significant. As quality control, allele frequencies in discovery and validation cohort were compared by χ² analysis. LD analysis and haplotype testing was performed as above.

All probands had a confirmed diagnosis of IBD fulfilling standard diagnostic criteria. Phenotypic characterization was based on Montreal classification. Age categories reflect age at diagnosis: early-onset IBD (EO-IBD) defined as diagnosis before 18th birthday; very early-onset IBD (VEO-IBD) defined diagnosis before 6th birthday; infantile-onset IBD defined as diagnosis before 1st birthday. Institutional review boards at Hospital of Sick Children, Children’s Hospital of Wisconsin, Mount Sinai Hospital (Toronto, Canada), and Massachusetts General Hospital approved these studies.

**Results**

**Novel, Aberrant IL10RA Splice Site Mutation Identified in Patient with Infantile Colitis**

We identified a patient with severe, infantile-onset IBD with significant arthritis and folliculitis (Figure 1). Sequencing of IL10RA and IL10RB in this patient identified a homozygous mutation in the intervening sequence of intron 5 at the second base of the conserved GT splice donor site (g.IVS5+2T>C) (Figure 2A). This novel mutation was confirmed by sequencing in an independent laboratory (data not shown) and has not been identified in the 1000 Genomes Project.

This mutation was located in the 5’ splice donor site - a critical position for RNA splicing. Mutation of the invariant dinucleotide GT at the 5’ splice donor site impairs normal splicing and allows activation of cryptic splice site(s). We identified a cryptic splice donor site (CAGgtgaaa) in Exon 5 of IL10RA using Human Splicing Finder (Figure 2A). To confirm aberrant IL10RA splicing, RNA was isolated from whole blood and IL10RA transcripts were analyzed by RT-PCR. The expected 433bp PCR fragment was detected in 5 healthy donors. However, only a smaller fragment was observed in the patient (Figure 2B) and sequencing of this fragment revealed a 76bp deletion (c.690_765del) from the 3’ end of Exon 5 (Figure 2C). The resulting reading frame-shift mutation led to incorporation of 3 aberrant amino acids of Exon 6 (p.204ISP206) and a premature stop codon at P206X (Figure 2C). The truncated protein contained only the extracellular domain of IL10RA (Figure 3A). The patient’s asymptomatic parents were g.IVS5+2T>C carriers displaying wild-type and altered splicing variants (Figure 2B).
IL10RA Splice Mutation Prevents IL-10-Dependent STAT3 Phosphorylation

In order to determine the functional effects of P206X IL10R1, we assessed in vitro STAT3 phosphorylation, a required step in the IL-10 signaling cascade for downstream immunosuppressive effects. PBMC were stimulated with IL-10, and STAT3 phosphorylation was measured by Western blot analysis. IL-10-induced STAT3 phosphorylation was defective in the patient. IL-6-induced STAT3 phosphorylation was intact (Figure 3B). Thus, P206X IL-10R1 leads to defective IL-10 downstream signaling.

FoxP3+ Regulatory T cells are Present in the Absence of IL-10 Signaling

Regulatory T cells (Tregs) that express the transcription factor FoxP3 possess suppressive ability, and defects in the FOXP3 gene (in mice and humans) cause systemic autoimmunity. As some but not all studies have suggested that IL-10 signaling is critical for the stability of FoxP3 expression, we sought to determine whether FoxP3+ Tregs were present in the absence of IL-10 signaling. FoxP3+ Tregs were identified in the peripheral blood of the affected patient (Figure 3C). This provides evidence in humans that FoxP3+ Tregs can develop in the absence of intact IL-10 signaling.

IL10RA and IL10RB Polymorphisms are Associated with IBD

In order to determine whether IL10RA and IL10RB mutations are associated more generally with IBD and extend beyond the rare patients with infantile-onset IBD that have homozygous, loss-of-function mutations, we performed deep sequencing of coding and flanking intronic regions of IL10RA and IL10RB in a cohort of 188 EO-IBD patients and 188 controls (Supplementary Table 2). The EO-IBD group included 121 children with CD, 2 with IBD-unclassified, and 65 with UC. The ages of children ranged from 6.5 months to 11 years old, with 68 VEO-IBD subjects (including two infants).

In this discovery cohort, 59 SNPs (28 novel) were found in IL10RA and 39 SNPs (15 novel) in IL10RB (Supplemental Table 3). There were 14 (6 novel) non-synonymous SNPs (12 in IL10RA and 2 in IL10RB). Each novel, non-synonymous SNP was identified only in the heterozygous state, and none of the resulting amino acid changes were predicted to be deleterious by SIFT or Polyphen. Additionally, we found 5 SNPs in IL10RA were associated with EO-UC and 2 SNPs in IL10RB were associated with EO-CD (Table 1 and Supplemental Table 3). Three IL10RA SNPs (rs10892202, rs4252249, and rs4252270) were protective for EO-UC while the remaining two IL10RA SNPs (rs2228054 and rs2228055) were associated with increased risk for EO-UC and VEO-IBD. In subgroup analysis of these five IL10RA SNPs, four of the SNPs were found to be associated with very early-onset UC (VEO-UC) while there was a positive trend for VEO-UC in rs2228055 (Table 2). Of the IL10RB SNPs that were associated with EO-CD, rs1058867 was associated with increased CD risk and rs8178561 was protective for EO-CD. The minor allelic frequencies of the SNPs described above did not differ between the IBD cohorts from Wisconsin and Toronto (data not shown).

Linkage analysis of these SNPs showed that rs2228054 and rs2228055 were in LD (p<0.0001) (Haplotype Block 1 AG) as were rs10892202, rs4252249, and rs4252270 (Haplotype Block 2 CAT) (p<0.0001) (LD plot in Supplementary Figure 1). Haplotype Block 1 AG was associated with VEO-IBD (OR 2.35, p=0.020), UC (OR 2.41, p=0.016) and VEO-UC (OR 2.48, p=0.025) but not for EO-IBD or EO-CD (Supplementary Table 4). Haplotype Block 2 had a protective effect for VEO-IBD (OR 0.40, p=0.025), UC (OR 0.29, p=0.007) and VEO-UC (OR 0.28, p=0.025), EO-IBD (OR 0.57, p=0.030) but had no significant effect in EO-CD.
Validation Study Confirms *IL10RA* SNP Associations with VEO-UC

We determined the frequency of the SNPs (found in the discovery cohort) in an independent cohort of 422 children with EO-IBD and 480 healthy controls (validation cohort) by Taqman genotyping. This EO-IBD cohort was comprised of 122 children with UC and 300 children with CD (including 18 VEO-UC and 10 VEO-CD). $\chi^2$ analysis of discovery and validation controls showed that they were similar with respect to the studied SNPs (data not shown).

Although the association with the 7 IL10R SNPs and EO-IBD did not replicate in the validation cohort, combined analysis of the discovery and showed nominal association with all the *IL10RA* SNPs (Table 1) and EO-UC. Furthermore, the association with VEO-UC with both rs2228054 and rs2228055 was replicated in the validation VEO-UC cohort, (rs2228054 OR 3.86 (95% CI 1.39-10.71, p=0.005, adjusted p=0.044) and rs2228055 OR 4.21 (95% CI 1.51-11.69, p=0.003, adjusted p=0.024) (Table 2)) and remained significant in a combined analysis of the discovery and validation cohorts (OR 3.08, combined p=2×10$^{-4}$; and OR 2.93, p=6×10$^{-4}$, respectively).

Linkage analysis of Haplotype Block 1 AG and Haplotype Block 2 CAT showed that these SNPs were also in LD in the validation cohort (p<0.0001). The Haplotype Block 1 AG was also associated with VEO-IBD (OR 2.43, p=0.023), and VEO-UC (OR 3.40, p=0.006) but not for overall IBD or EO-UC (Supplementary Table 4). Haplotype Block 2 CAT was not associated with VEO-IBD (p=0.89) or VEO-UC (p=0.51).

Discussion

Homozygous, loss-of-function mutations in *IL10RA* and *IL10RB* have been reported to cause severe, infantile-onset IBD.\(^6\,7\) In this report, we identify a novel splice site mutation in *IL-10RA* resulting in defective IL-10 signaling. In addition to the colitis, severe perianal disease, and recurrent infections that have been previously described in patients with IL-10R mutations, this patient also suffered from severe arthritis.

The contribution of IL10R variants to IBD susceptibility more generally in children was heretofore unclear. Our study is the first to specifically examine by deep sequencing the role of IL-10R SNPs in EO-IBD and VEO-IBD. We have identified two *IL10RA* SNPs associated with VEO-UC and these results have been validated in an independent replication cohort with OR of 2.9 and 3.1. These two SNPs, rs2228054 and rs2228055, were frequently found in the heterozygous state among IBD patients and inherited as a haplotype. The conferred risk may be due to one or both SNPs. Alternatively, the increased risk may reside in a regulatory region (e.g., promoter) in LD with these SNPs. We propose that this risk haplotype exerts a mild phenotype in the general population resulting in disease only in the presence of another genetic variant(s) or environmental trigger. In contrast to the complete loss-of-function IL-10R mutations (described here and previously\(^6\,7\)) which present with symptoms more typical of Crohn’s disease, these SNPs are associated with UC, which is consistent with the association of SNPs in IL-10 signaling genes and UC.\(^10\,12\) Although this study points to an important role of the IL-10 pathway in VEO-UC, due to the limited number of VEO-IBD patients, this association with VEO-UC will require further functional investigations and replication.

Although GWAS of adult and pediatric IBD have identified novel pathways important in the pathogenesis of IBD, the SNPs detected by GWAS only account for ~25% of the assumed genetic heritability.\(^11\) The risk for IBD associated with SNPs in *IL10*, *TYK2*, and *STAT3* loci demonstrate the importance of IL-10 in IBD pathogenesis.\(^9\,12\) However, GWAS have not identified an association between SNPs in the *IL10RA* or *IL10RB* loci and IBD risk in the pediatric population.\(^13\,25\) Similarly, the recently published meta-analysis of GWAS of...
UC patients failed to show a statistically significant increase in risk for rs2228054.\textsuperscript{26} This is most likely due to the unique subset of patients examined in our study, and highlights the importance of candidate gene studies examining rare presentations of IBD. GWAS evaluating exclusively-pediatric IBD focus primarily on EO-IBD patients which has been shown to be phenotypically more similar to adult-onset IBD than VEO-IBD.\textsuperscript{14} Therefore, the contribution of IL-10R SNPs to the susceptibility of IBD in very young children has been underestimated in recent adult and late-onset pediatric GWAS as VEO-IBD represents a very unique population that is poorly represented even in GWAS focusing on pediatric IBD.\textsuperscript{13}

Although defective IL-10 signaling causes a strong inflammatory phenotype in the GI tracts of both humans and mice, it is still unclear which is the most critical cell types(s) that require IL-10 signaling. While Murai \textit{et al} suggested that an IL-10-dependent signal was required for the maintenance of FoxP3\textsuperscript{+} Tregs,\textsuperscript{22} Chaudhry \textit{et al} demonstrated in mice with a Treg lineage-specific deletion of IL-10R that IL-10-dependent signals were required for the function but not the maintenance of Tregs.\textsuperscript{3} Peripheral blood FoxP3\textsuperscript{+} Tregs were present in the patient studied in this report with defective IL-10 signaling demonstrating that this signal is not required for the development of FoxP3\textsuperscript{+} Tregs.

In summary, we have described a novel splice site mutation in \textit{IL10RA} that broadens the existing phenotype of IL-10R deficiency to include severe arthritis. The discovery of this patient in a cohort of VEO-IBD patients reinforces that IL-10R deficiency should be considered in all infantile-onset IBD cases, especially those with severe perianal disease and now arthritis, and that this diagnosis should prompt consideration of stem cell transplantation. Additionally, the association of SNPs in \textit{IL10RA} with VEO-UC implicates a role for IL-10R in the pathogenesis of IBD in a broader population of patients than recently appreciated. Such information may permit stratification of patients for clinical trials that target the IL-10 pathway.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

**Acknowledgments**

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**Abbreviations**

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<td>CD</td>
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<td>IBD</td>
<td>inflammatory bowel disease</td>
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IL-10  Interleukin-10
IL10RA  IL-10 Receptor A
IL10RB  IL-10 Receptor B
PBMC  peripheral blood mononuclear cells
PBS  phosphate-buffered saline
PMN  polymorphonuclear neutrophils
SNP  single nucleotide polymorphism
STAT3  signal transducer and activator of transcription 3
p-STAT3  STAT3 phosphorylated at tyrosine 705
UC  ulcerative colitis

References


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Figure 1. Clinical Features of Patient with Severe, Infantile-Onset IBD
The patient had skin folliculitis (A), perianal disease with fistulae (B), joint effusions (C), and colonic erosions (D). Histology showed colitis with gland branching and mixed lamina propria cell infiltrate with fibropurulent exudate (arrow) (E).
Figure 2. Identification of IL10RA g.IVS5+2T>C Mutation in Genomic DNA

(A) Sequencing of Exon 5 of IL10RA revealed a homozygous g.IVS5+2T>C mutation (Bottom), compared to wild-type (Top). The splice donor site, putative cryptic splice donor site, and T>C mutation are underlined. (B) RT-PCR of IL10RA (Exon 4-7) from RNA isolated from blood and ileum. Lane 1 and Lane 1-ileum is from the patient. Lanes 2-6 are controls. Right: RT-PCR of Exon 4-7 of IL10RA from RNA from the patient and her parents. (C) Sequence of abnormal PCR product shows 76bp deletion (c.690_765del) leading to a premature stop codon (underlined).
Figure 3. Altered IL-10 Signaling in Patient with IL-10RA Mutation

(A) Truncated IL10RA protein product found in the patient compared to full-length IL10RA in parents/controls. Extracellular (ECD), intracellular (ICD), signaling peptide (SP), and transmembrane (TM) domains are shown. (B) Western blot analysis of IL-10-induced STAT3 phosphorylation in PBMCs from patient and control. (C) Flow cytometric analysis of peripheral blood FoxP3+ Tregs of patient and mother. Cells depicted are CD4+ in small lymphocyte gate and are plotted CD25 vs. intracellular FoxP3.
Table 1

IL-10R Polymorphisms Associated with EO-IBD

Odds ratios using DD vs. Dd/dd model. Discovery Cohort: EO-IBD (n=188), UC (n=65), CD (n=121), and controls (n=188). Validation Cohort: EO-IBD (n=422), UC (n=122), CD (n=300), and controls (n=480). Threshold for significance in Validation cohort was 0.007 to correct for multiple testing.

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<th>Validation Cohort</th>
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Table 2

IL-10R Polymorphisms Associated with VEO-IBD

Odds ratios using DD vs. Dd/dd model. Discovery Cohort: VEO-IBD (n=65), VEO-UC (n=41), VEO-CD (n=24), and controls (n=188). Validation Cohort: VEO-IBD (n=28), VEO-UC (n=18), VEO-CD (n=10), and controls (n=480). Threshold for significance in Validation cohort was 0.007 to correct for multiple testing.

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