Genetic Variation In Very Early Onset Inflammatory Bowel Disease

CJ Moran; JR Kelsen; J Kaplan; H Huang; M Rivas; N Dawany; MB Heyman; B Kirschner; T Mangatu; K Benkov; JE Teitelbaum; S Cohen; BD Gold; M Devoto; R Xavier; R Baldassano; MJ Daly; HS Winter

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Disclosure

• I have no financial relationships to disclose.

Common Variants in IBD

• 163 Adult IBD Risk SNPs from GWAS Meta-analysis
  – Many play role in pediatric IBD
  – Limited individual effect (OR <2.0)
  – Adult CD: High SNP burden associated with ileal involvement and “early onset”
• Much heritability remains unexplained (~75%)
  – Rare variants are one possible source
  – GWAS may miss rare variation (MAF<1% vs. 5%)

Joslin L. Nature 2012
Essers JB Inflamm Bowel Dis 2009
Ananthakrishnan AN Am J Gastro 2014
Studying Very Early Onset IBD

- Mendelian Immunodeficiency presents as VEO-IBD
- Earlier onset = Less environmental triggers
- VEO-IBD subset may be enriched for highly penetrant (if not causative) variants
- LoF mutations can cause IBD
  - IL-10 defects (IL10, IL10RA, IL10RB) by linkage
- Whole exome sequencing identifies Mendelian IBD
  - XIAP, LRBA, TTC7A

Project Aim

To determine the role that IBD risk SNPs AND rare variants play in VEO-IBD

Methods

- Patients (and parents) diagnosed with IBD at <6yo were recruited
  - Patients with severe phenotype diagnosed just after 6yo also included
- Recruitment across US and beyond
  - Children’s Hospital of Philadelphia
  - University of Chicago
  - UCSF
  - Monmouth Medical Center
  - Mount Sinai Hospital
  - Children’s Healthcare of Atlanta
- DNA collected from blood or saliva
Proband Cohort

- 95 Probands
  - Mean age at Diagnosis: 2.6yo [IQR 1.3-4.0]
- 89.0% Caucasian, 1.4% Asian, 1.4% Hispanic, 8.2% Middle-Eastern
- 20% had 1st degree relative with IBD
- 35 Crohn’s disease
  - L1 5.9% L2 64.7% L3 29.4%
- 36 Ulcerative Colitis (76.9% E4)
- 24 IBD-Unclassified

Hypothesis 1

The age of onset of VEO-IBD is due to a large burden of known IBD risk SNPs.

Common SNP Genetic Burden

- Genotyping performed using Immunochip
- Genetic Risk Score was calculated
  - Cumulative score based on 110 risk alleles
  - Component score (at each locus) based on log OR (for IBD) from Jostins et al., & number of risk alleles (0-2)
  - Normalized based on “IBD Risk” of Healthy Controls
  - Compared to adult-onset UC and adult-onset CD
VEO-IBD Genetic Risk Score

- VEO-IBD GRS higher than controls ($p=1\times10^{-7}$)
- VEO-IBD similar to adult-onset UC ($p=0.2$)
- VEO-IBD lower than adult-onset CD ($p=0.02$)
- Linear Regression found no association between VEO-IBD Age of Onset and Risk Score ($p>0.3$)

Burden of "known IBD SNPs" does not explain early onset of VEO-IBD

Hypothesis 2

The age of onset of VEO-IBD is due to excessive rare variation in IBD risk genes.

WES Methods

- Exome sequencing performed at the Broad Institute (Cambridge, MA)
- Exome capture was performed using Agilent Whole Exome SureSelect kit
- Sequencing performed on Illumina HiSeq
- Variant calling was done with GATK toolkit
- In silico modeling incorporated into analysis
  - SIFT, Polyphen-2, FATHMM, Mutation Taster
VEO-IBD Cohort Analysis

- Analysis filtered on:
  - Extended splice site, nonsense, & missense variants
  - Variants with 2+ deleterious predictors
  - Found in Mendelian and IBD GWAS genes
  - MAF < 0.5% in ExAC Controls (n=~60,000)
- Binary outcome of presence/absence of deleterious variant in a gene for filtered genes
- Compared to ExAC controls

Gene List Filter

- Mendelian Gene List:
  - IL-10 defects (IL10, IL10RA, IL10RB)
  - CGD (CYBB, CYBA, RAC2, NCF1, NCF2, NCF4)
  - Hermansky Pudlak (HPS1-8)
  - Familial Mediterranean fever (MEFV)
  - Wiskott Aldrich syndrome (WASP)
  - TTCT7A, PIK3R1, XIAP, LRBA
  - SKIV2L, PLCG2
- Risk Genes from IBD GWAS

Rare Variation Analysis

<table>
<thead>
<tr>
<th>Genes</th>
<th>OR</th>
<th>Rare Variation Rate</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCDC888</td>
<td>2.6</td>
<td>5.4% vs. 2.2%</td>
<td>0.051</td>
</tr>
<tr>
<td>NFIL3</td>
<td>4.0</td>
<td>2.2% vs. 0.6%</td>
<td>0.053</td>
</tr>
<tr>
<td>IL6ST</td>
<td>2.8</td>
<td>4.3% vs. 1.6%</td>
<td>0.058</td>
</tr>
<tr>
<td>NOD2</td>
<td>0.8</td>
<td>4.3% vs. 5.3%</td>
<td>0.667</td>
</tr>
</tbody>
</table>

No individual gene is overly burdened with very rare LoF mutations.
Summary

• In our cohort of VEO-IBD patients, there was not an excessive burden of known IBD Risk SNPs

• Although rare genetic variants occur in VEO-IBD, no individual gene drives the disease

• Rare genetic variants still may play a strong role in VEO-IBD

Future Directions

• Focus on outlier probands in the GRS distribution to identify causative variants

• Broaden gene filter to include candidate genes related to Mendelian genes (nox1, duox2)

• Functional studies in specific variants

• Ultimate goal to identify key pathways to select a novel therapy or find a new target for drug development

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Any Questions?