# Genetic Variation In Very Early Onset Inflammatory Bowel Disease

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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%)

Jostins 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*

Glocker EO *NEJM* 2009
Worthey EA *Gen Med* 2011
Alangari A *JACI* 2012
Avitzur Y *Gastroenterology* 2014

## 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

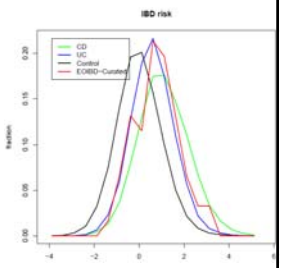
The age of onset of VEO-IBD is due to a large burden of known IBD risk SNPs.

## 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 ($p=1x10^{-7}$)
- 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

http://exac.broadinstitute.org [date (October 2015) accessed]

## 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*)
  - *TTC7A, PIK3R1*, *XIAP*, *LRBA*
  - *SKIV2L, PLCG2*
- Risk Genes from IBD GWAS

## Rare Variation Analysis

| Genes | OR | Rare Variation Rate | *p value* |
|---|---|---|---|
| *CCDC88B* | 2.6 | 5.4% vs. 2.2% | 0.051 |
| *NFIL3* | 4.0 | 2.2% vs. 0.6% | 0.053 |
| *IL6ST* | 2.8 | 4.3% vs. 1.6% | 0.058 |
| *NOD2* | 0.8 | 4.3% vs. 5.3% | 0.667 |

rs369059647 p=0.004 rs145020363 p=0.00016

**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?