Early-Onset IBD: Genetic Testing and Clinical Applications

Presenters

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What Causes Inflammatory Bowel Diseases (IBD)?

- Microbiome
- Immune Response
- Genetic Susceptibility
- Environmental Triggers
Do All Causes Contribute Equally

- Genetics
- Immune System
- Environment

Diagram shows the interconnections between Genetics, Immune System, and Environment.
Early-Onset IBD Is Different

- **More severe phenotype**
  - Pancolitis is more common in children with ulcerative colitis (UC)
    - 80–90 % vs. 60 %
  - More aggressive
    - More difficulty in achieving steroid independence

- **Colon-only involvement more common with Crohn’s disease (CD)**
  - 2/3 of children with CD vs. 30% of adults

- **Male-to-female ratio in CD**
  - 1.6:1 vs. 1:1 in adults

# Classification of Pediatric IBD

## Age of Onset
- Younger than 17
- Younger than 10
- Younger than 6
- Younger than 2
- First 28 days of life

## Group/Classification
- Montreal A1/Paris A1b
- Paris A1a
- VEOIBD
- Infantile IBD
- Neonatal IBD


What Causes Inflammatory Bowel Diseases (IBD)?

- Microbiome
- Immune Response
- Genetic Susceptibility
- Environmental Triggers
What Causes Very Early-Onset IBD in Children?

- Genetic Susceptibility
- Microbiome
- Environmental Triggers
- Immune Response
How Does This Apply to Adult Patients?

- These kids grow up
- Variable penetrance
- Variation in age of onset
- Familial
FOXP3

- IPEX
  - Immunodeficiency
  - Polyendocrinopathy
  - Enteropathy
  - X-linked

The immunological and genetic basis of immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome

Khalid Bin Dhuban and Ciriaco A. Piccirillo

Representative Case

• 24-year-old male
• Disseminated varicella age 5 PICU
• Severe Crohn ileocolitis
• Immunoglobulin deficiencies (IVIG)
• Periodic fevers
Whole Exome Sequencing


Heterozygous pathogenic variant in *MEFV* gene, p.E148Q associated with periodic fever (FMF; familial Mediterranean fever). Patient has reported episodes of unexplained fever.

Also, had heterozygous variants (VUS) in *SLC37A4* and *TTC37* genes.

*SLC37A4* gene mutations are associated with glycogen storage diseases 1b and 1c.

*TTC37* gene mutations are associated with trichohepatoenteric syndrome (THE).

Both the above are autosomal recessive conditions.
Patient Data Compared to Healthy Control
Percent Positive Cells for Each Subset Listed on X Axis

% Gated

Regulatory T cell

Percentage of positive cells

Patient data
Healthy control data
How Did This Change the Patient’s Care?

- Sirolimus
- Referral for bone marrow transplantation
“In a routine clinical setting, TNGS is the method to prefer as it is cost- and time-effective while providing optimal coverage of the genes of interest.”
Accurate Diagnosis of Early-Onset IBD

Clinical Presentation
- Clinical Course
- Personal History
- Family History

Immunologic/Functional Assays
- Protein Expression
- Protein Function
- Other Immunologic Assays

Genetic analysis
- Identification of Pathogenic Genetic Variants
- Family Studies
Inflammatory Bowel Disease Primary Immunodeficiency Panel (IBDGP)

• Designed to detect variants in genes associated with monogenic inflammatory bowel disease (IBD) or IBD-like conditions
  • Patients typically have early-onset or very early-onset disease

• May allow for:
  • A more specific treatment plan
  • Predictive testing of at-risk family members
Inflammatory Bowel Disease Primary Immunodeficiency Panel (IBDGP)

• Next-generation sequencing (NGS) panel with supplemental Sanger sequencing
• 51 genes included
• Specimen types: blood, blood spot, peripheral blood mononuclear cells (PBMCs), cultured fibroblasts, skin biopsy, and DNA
### IBDGP: 51 Genes

<table>
<thead>
<tr>
<th>ADA</th>
<th>DOCK8</th>
<th>IL2RG</th>
<th>PLCG2</th>
<th>TNFAIP3</th>
</tr>
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<tbody>
<tr>
<td>ADAM17</td>
<td>FOXP3</td>
<td>ITGB2</td>
<td>RAG1</td>
<td>TTC37</td>
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<tr>
<td>AICDA</td>
<td>G6PC3</td>
<td>LIG4</td>
<td>RAG2</td>
<td>TTC7A</td>
</tr>
<tr>
<td>BTK</td>
<td>ICOS</td>
<td>LRBA</td>
<td>RTEL1</td>
<td>WAS</td>
</tr>
<tr>
<td>CD3G</td>
<td>IKBKG</td>
<td>MEFV</td>
<td>SH2D1A</td>
<td>WIPF1</td>
</tr>
<tr>
<td>CD40LG</td>
<td>IL10</td>
<td>MVK</td>
<td>SKIV2L</td>
<td>XIAP</td>
</tr>
<tr>
<td>CTLA4</td>
<td>IL10RA</td>
<td>NCF2</td>
<td>SLC37A4</td>
<td>ZAP70</td>
</tr>
<tr>
<td>CYBA</td>
<td>IL10RB</td>
<td>NCF4</td>
<td>STAT1</td>
<td></td>
</tr>
<tr>
<td>CYBB</td>
<td>IL21</td>
<td>NLRC4</td>
<td>STAT3</td>
<td></td>
</tr>
<tr>
<td>DCLRE1C</td>
<td>IL21R</td>
<td>PIK3CD</td>
<td>STIM1</td>
<td></td>
</tr>
<tr>
<td>DKC1</td>
<td>IL2RA</td>
<td>PIK3R1</td>
<td>STXBP2</td>
<td></td>
</tr>
</tbody>
</table>
Epithelial Barrier

- X-linked ectodermal immunodeficiency (IKBKG)
- TTC7A deficiency (TTC7A)
- ADAM17 deficiency (ADAM17)

Hyperinflammatory/Autoinflammatory Disorders

- Mevalonate kinase deficiency (MVK)
- Phospholipase C-γ2 defects (PLCG2)
- Familial Mediterranean fever (MEFV)
- Familial hemophagocytic lymphohistiocytosis (STXBP2)
- X-linked lymphoproliferative syndrome (XIAP, SH2D1A)

Immune Dysregulation

- IPEX/IPEX-like (FOXP3, IL2RA, STAT1)
- IL10-signaling defects (IL10RA, IL10RB, IL10)
- CTLA4 haploinsufficiency with autoimmune infiltration (CTLA4)
### Phagocyte Defects

- Chronic granulomatous disease (*CYBB, CYBA, NCF2, NCF4*)
- Glycogen storage disease (*SLC37A4*)
- Congenital neutropenia (*G6PC3*)
- Leukocyte adhesion deficiency (*ITGB2*)

### T- and B-cell Defects

- Common variable immunodeficiency (*LRBA, ICOS*)
- IL-21 deficiency (*IL21*)
- Agammaglobulinemia (*BTK, PIK3R1*)
- Hyper IgM syndrome (*CD40LG, AICDA*)
- Hyper IgE syndrome (*DOCK8*)
- Wiskott-Aldrich syndrome (*WAS, WIPF1*)
- Omenn syndrome/SCID (*DCLRE1C, ZAP70, RAG2, IL2RG, LIG4, ADA, CD3γ*)
- Hoyeraal-Hreidarsson syndrome (*DKC1, RTEL1*)

### Other

- MASP deficiency (*MASP2*)
- Trichohepatoenteric syndrome (*SKIV2L, TTC37*)
A Behind-the-Scenes Look at the Lab
Genetic Testing at Mayo Clinic Laboratories

• **Sample arrives in lab**
  • Patient information sheet is reviewed (if provided) to ensure appropriate testing
  • Genetic counselors will contact ordering provider if a misorder is suspected

• **DNA is extracted and sample is run on our targeted panel**
  • NGS with Sanger sequencing to fill in gaps in coverage and for homologous genes
<table>
<thead>
<tr>
<th></th>
<th>Targeted Panels</th>
<th>Exomes</th>
<th>Genomes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of Genes</strong></td>
<td>&lt;500</td>
<td>4,000–20,000</td>
<td>&gt;20,000 plus intergenic</td>
</tr>
<tr>
<td><strong>Inclusion of Genes for Disease of Interest</strong></td>
<td>Yes (gaps in coverage typically filled)</td>
<td>Possibly (may miss key genes)</td>
<td>Generally yes (not optimized for specific disease)</td>
</tr>
<tr>
<td><strong>Inclusion of Important Non-Coding Regions</strong></td>
<td>Often included in test design</td>
<td>Typically not</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Depth of Coverage</strong></td>
<td>High</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td><strong>Incidental findings</strong></td>
<td>Usually none</td>
<td>Possible</td>
<td>Possible</td>
</tr>
<tr>
<td><strong>Variants of Uncertain Significance</strong></td>
<td>+ (proportional to size of panel)</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td><strong>Genes of Uncertain Significance</strong></td>
<td>No (with optimal panel design)</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td><strong>Turn-around time</strong></td>
<td>~1–2 weeks</td>
<td>~3–4 weeks or longer</td>
<td>Long (~12 weeks); rapid genomes (24 hours) available</td>
</tr>
<tr>
<td><strong>Pros</strong></td>
<td>Higher sensitivity for specific phenotypes</td>
<td>Useful for non-specific/overlapping phenotypes</td>
<td>Useful for non-specific/overlapping phenotype</td>
</tr>
<tr>
<td><strong>Cons</strong></td>
<td>Limited by what is on the panel</td>
<td>Generally lower sensitivity</td>
<td>Generally lower sensitivity</td>
</tr>
<tr>
<td><strong>Cost</strong></td>
<td>$</td>
<td>$$</td>
<td>$$$$$</td>
</tr>
</tbody>
</table>
NGS: Depth of Coverage (Read Depth)

Individual reads

- How many times each base has been sequenced/read
- NGS has relatively high error rate
- Each base needs to be read many times to make a confident base call (i.e., >30x)

237 reads = 237x coverage
Exome/Genome Sequencing Does Not Include All Genes

**IKBKG**
- X-linked ectodermal immunodeficiency
- Crohn’s disease-like enterocolitis; increased epithelial apoptosis
- Skin lesions

[Diagram with exome and genome sequencing data]

https://gnomad.broadinstitute.org/gene/ENSG00000073009

Targeted NGS vs Exome Coverage

Exome

• Most ES platforms cover only 85–90% of exons
• 50% of exons are <30x average coverage in 60,000 samples from ExAC*
• 60% of low-coverage reads in ES occur in highly repetitive stretches of DNA**
• Thus, exome sequencing can miss critically important regions/variants

Targeted panels

• Coverage issues are usually easy to overcome
• Regions that still have poor coverage after boosting can be analyzed by supplementary methods

Genetic Testing at Mayo Clinic Laboratories

- Sample arrives in lab
- DNA is extracted and sample is run on our targeted panel
- Data is analyzed
  - A team of genetic counselors and laboratory directors reviews each case
  - We use the American College of Medical Genetics and Genomics (ACMG) criteria
Classification of Variants and Results Interpretation

- **Pathogenic**
- **Likely Pathogenic**
- **Uncertain Significance**
- **Likely Benign**
- **Benign**

- **Amino Acid & Nucleotide Conservation**
- **Functional Studies**
- **In silico Predictions of Splicing & Amino Acid Changes**
- **Previous Reports of Variant/ Cases; Segregation of Variant with Disease**
- **Disease-Specific and Population Databases**
- **Patient Clinical Features & Family History**
- **Minor Allele Frequency**
Genetic Variant Classification and Interpretation

- Genetic testing is probabilistic in nature
  - Variants are classified along a continuum of estimated likelihood that a variant causes disease based on the weight of current evidence
  - Variant classification and interpretation is the most challenging portion of genetic testing
    - Although guidelines with specific criteria are used, professional judgement is required
    - Is a detected variant the cause of the patient’s phenotype?
  - A variant’s classification may change over time as more evidence becomes available
Genetic Test Results Should Be Used in the Context of the Patient’s Clinical Presentation

<table>
<thead>
<tr>
<th>Classification</th>
<th>Criteria</th>
<th>Recommendations</th>
</tr>
</thead>
</table>
| Pathogenic              | • Variant has met criteria such that provider may use molecular testing information in clinical decision-making  
                         | • Use in conjunction with other clinical information when possible       |                                                     |
| Likely Pathogenic       | • Sufficient evidence that the provider may use molecular testing information in clinical decision-making when combined with other evidence of the disease in question  
                         | • Additional follow-up testing is recommended to support decision-making |                                                     |
| Uncertain Significance  | • Should not be used in clinical decision-making  
                         | • Efforts to resolve the classification as pathogenic or benign should be undertaken  
                         | • Additional monitoring of the patient for the disorder in question should be considered |                                                     |
| Likely Benign           | • Sufficient evidence that the provider may conclude the variant is not the cause of the patient’s disorder when combined with other information  
                         | • Typically not reported clinically                                      |                                                     |
| Benign                  | • Sufficient evidence that the provider may conclude the variant is not the cause of the patient’s disorder  
                         | • Typically not reported clinically                                      |                                                     |
Limitations of Mayo Clinic’s Inflammatory Bowel Disease Primary Immunodeficiency Panel (IBDGP)

• Unlikely to be helpful for patients who present in late adolescence or adulthood, particularly those who respond to conventional therapy
  • Patients less likely to have a monogenic cause of IBD

• Detection of copy number variation is not included in the current version (update expected soon)

• If chronic granulomatous disease (CGD) is suspected, consider adding dihydorhodamine flow test (DHR)
  • Variants in $NCF1$ ($p47^{phox}$) account for 25% of CGD in the Western world and a higher percentage elsewhere
  • $NCF1$ has a common GT deletion that is difficult to detect by NGS and is not included in our panel
Benefits of Performing Genetic Testing for Early-Onset IBD through Mayo Clinic Laboratories

• **Expert curation and interpretation of each variant identified**
  - Genetic counselors and laboratory directors are only a phone call (or email) away and happy to discuss appropriateness of testing and/or results with ordering providers

• **Testing for family members is available when a clinically significant variant is identified (known variant [KVAR])**

• **In addition to genetic testing, many functional tests are available through Mayo Clinic Laboratories**
  - Helpful to confirm diagnosis and/or obtain additional information when a variant of unknown significance (VUS) is identified

• **Easy-to-read report with detailed information on findings**
Inflammatory Bowel Disease Primary Immunodeficiency (PIDD) Panel

Result Summary

Pathogenic Variant(s) Detected

Result

The following variant was detected:

Gene (Transcript): LIPA (NM_0005735.4)
Genomic position: 20:4390387g > 20:4390387t
cDNA change: c.3267delC
Amino acid change: p.S1064fsX14

The patient is homozygous for this variant.

Classification: Pathogenic

The results for the remaining genes on this panel are negative.
Interpretation

This individual is homozygous for the c.2267dupC (p.Glu757Argfs*14) variant in the LRBA gene. This variant results in a premature termination codon and is therefore predicted to be pathogenic.

The LRBA gene encodes lipopolysaccharide-responsive beige-like anchor protein. Biallelic pathogenic loss-of-function variants in LRBA result in LRBA deficiency and autosomal recessive LATAIE (LRBA deficiency with autoantibodies, regulatory T cell defects, autoimmune infiltration, and enteropathy), which often includes inflammatory bowel disease-like mucosal inflammation and severe diarrhea. Although this truncating variant (p.Glu757Argfs*14) has not been described previously, other truncating variants in the LRBA gene have been reported in association with autosomal recessive LRBA deficiency. This variant has not been observed in sequencing data gathered from large, multi-ethnic cohorts, suggesting that it is a rare variant (1–2). Taken together, this evidence supports a pathogenic classification for this LRBA gene variant.

The finding of a homozygous pathogenic variant in the LRBA gene is supportive of a diagnosis of LRBA deficiency for this individual, but should be interpreted in the context of clinical findings, family history, and other laboratory testing. Consultation with a genetics professional may be of benefit for interpretation of this result and to determine whether familial testing may be of benefit to this family. Genetic testing for family members is available by ordering Known Variant Analysis (KVAR) for the specific variant detected. Please contact the laboratory at 1-800-533-1710 or the online test catalog at www.mayomedicallaboratories.com for information about the test codos available for Known Variant Analysis. Please refer to family number 8675309 if ordering testing on family members of this individual.

Some of the genes tested by this panel may have more than one associated phenotype and/or inheritance pattern. Additionally, some genetic variants may have reduced penetrance and/or variable expressivity in some individuals. For information regarding the phenotypic spectrum which may be involved, see OMIM (www.ncbi.nlm.nih.gov/omim) and/or GeneReviews (www.genereviews.org) for this specific gene/disorder.

Next generation sequencing may not detect all types of genetic variants. If results do not match clinical findings, alternative testing methods could be considered.

REFERENCES:
Benefits of Performing Genetic Testing for Early-Onset IBD through Mayo Clinic Laboratories

• We recognize that there is a patient behind every sample that comes into our laboratory

• We offer high-quality testing to help you care for your patients
Thank You!
Questions?