GENETICS in IBD

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NEW GENOMIC ERA in IBD
DNA sequence is formed from a chain of four nucleotide bases: A, C, G, and T.

A single nucleotide polymorphism, or SNP, is a variation at a single position of nucleotide in a DNA sequence.

If more than 1% of a population does not carry the same nucleotide at a specific position in the DNA sequence, then this variation can be classified as a SNP.
GENE is a region of DNA (deoxyribonucleic acid) coding for the **messenger RNA** encoding the amino acid sequence in a polypeptide chain.

An **Allele** is a variant form of a gene. Some genes have a variety of different forms, which are located at the same position, or genetic locus, on a chromosome.

Humans are called **diploid organisms** because they have two alleles at each genetic locus, with one allele inherited from each parent. Each pair of alleles represents the **genotype** of a specific gene.
• A **haplotype** is a group of genes that was inherited together from a single parent.

• A **haplotype** can also refer to the inheritance of a cluster of single nucleotide polymorphisms (SNPs).

• **PENETRANCE** measures the proportion of individuals in a population who carry a specific gene and express the related trait. It is a measurement of the relationship between a genotype and phenotype.
GENOMIC ERA IN IBD MANAGEMENT

1. WHAT DO WE NEED?

   GENETIC TESTING

1. WHAT DO WE KNOW?

2. WHAT SHOULD WE DO?
GENETIC TESTING

Pathways and processes involved in disease pathogenesis

1. **To Predict:**
   - The Risk of biological behavior
   - The Outcome of the disease
   - The Survival

2. **To better approach and use** of therapeutics either through novel development or for re-purposing of existing drugs.
GENOMIC ERA IN IBD MANAGEMENT

1. WHAT DO WE NEED?

2. WHAT DO WE KNOW?

GENETIC VARIATIONS
PATHOGENIC MECHANISMS

1. WHAT WE SHOULD DO?
Gene Mapping Approaches

I. LINKAGE MAPPING

II. GENOME-WIDE ASSOCIATION STUDY (GWAS)

III. WHOLE GENOME SEQUENCING
I- Linkage mapping

Linkage mapping studies identified segments of human chromosomes shared among affected relatives, greater than expected by chance.
NOD2 PATHOGENESIS/INNATE IMMUNITY

INNATE IMMUNITY

NOD2 is a positive regulator of immune defense.
NOD2 is expressed by many leukocytes, including antigen presenting cells, macrophages, and lymphocytes, as well as ileal Paneth cells.

Activation of NOD2 by microbial ligands activates the transcription factor nuclear factor B (NF-κB) and mitogen-activated protein kinase signaling.
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NOD2 GENOTYPE MUTATION

THREE most common mutations in NOD2 that are associated with CD:

- Arg702Trp [rs2066844],
- Gly908Arg [rs2066845], and
- Leu1007fsinsC [rs41450053]

RESULT = EXCESSIVE ACTIVATION OF ADAPTIVE IMMUNITY
NOD2 PHENOTYPE

• NOD2 mutations are consistently associated with:
  – ileal location
  – stricturing disease
  – mainly present in individuals of European ancestry
  (heterozygous carriage confers a 2.4-fold increase in risk for CD; Homozygous confers a 17.1-fold increase in risk for CD.)
II - GENOME-WIDE ASSOCIATION STUDY (GWAS)

- Disease type: Mendelian → Complex
- Genes: Monogenic → Polygenic
- Variant: Frequency
- Technology: Whole exome/whole genome → GWAS
- Size of cohort: Family → Populations
CHROMOSOME

MULTIPLE HAPLOTYPES
(containing GWAS SNPs)

Other SNPs in linkage disequilibrium (LD)
including causal variant

GWAS SNP

Causal variant

Cases

Controls

Minor allele frequency

Cases

Controls
GWAS

• A meta-analysis identified 163 loci associated with IBD:
  – 110 conferred risk to both IBD subtypes,
  – 30 were unique to CD and
  – 23 loci to UC.
  – More recently, a trans-ethnic analysis identified an additional 38 new IBD loci.

• It explained **13.6% of CD and 7.5% of UC** total disease variances, respectively.
PATHWAYS IN PATHOGENESIS

A. Cytokine signaling
B. Microbial recognition
C. Lymphocyte activation,
D. Endoplasmic reticulum stress responses
E. Epithelial barrier function
PATHWAYS IN PATHOGENESIS

A. Cytokine signaling
B. Microbial recognition
C. Epithelial barrier function
D. Leucocyte trafficking
• APC (macrophage, dendritic cells) lead to activation of T cells.

• **ADAPTIVE IMMUNITY** may be deranged along IL12/IL23 pathway toward the spectrum of TH 17, leading to defect in regulatory T cells (T reg)
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• **ADAPTIVE IMMUNITY** may be deranged along IL12/IL23 pathway toward the spectrum of TH 17, leading to defect in regulatory T cells (T reg)
It is the most significant variant associated with CD after variants in NOD2.

The mutation encodes the amino acid change **Arg381Gln** in the IL-23 receptor (IL23R).
IL23R PHENOTYPE

IL-23 signaling (Th17 cells)
- IL23A chr12q13
- IL12B (p40) chr5q33
- IL12RB1 chr19p13
- IL23R chr1p31
- JAK2 chr9p24
- TYK2 chr19p13
- STAT3 chr17q21

IL-12 signaling (Th1 cells)
- IL12B (p40) chr5q33
- IL12RB1 chr19p13
- IL12A (p35) chr3q25
- TYK2 chr19p13
- JAK2 chr9p24
- STAT4 chr2q32
PATHWAYS IN PATHOGENESIS

A. Cytokine signaling
B. Microbial recognition
C. Epithelial barrier function
D. Leucocyte trafficking
A- AUTOPHAGY ROLE

1. Degrades damaged organelles and proteins.

2. It is important for the clearance of pathogens.

3. It is required for immunity to multiple different types of bacteria.
GENES RELATED TO AUTOPHAGY

• Autophagy 16-like 1 (ATG16L1) encodes a protein component of the autophagy complex.

• Genes that encode immunity-related guanosine triphosphatase M (IRGM) and leucine-rich repeat kinase 2 (LRRK2) also regulate autophagy.
• A single **Thr300Ala substitution** in ATG16L1 has been associated with CD. Thr300Ala has reduced ability to capture bacteria.

• **Genetic polymorphisms** in IRGM include a 20-kb deletion polymorphism 1.6 kb upstream of the IRGM promoter and a recently described tetra-nucleotide insertion.
PATHWAYS IN PATHOGENESIS

A. Cytokine signaling,
B. Microbial recognition
C. Epithelial barrier function
D. Leucocyte trafficking
Defects in the barrier function of the intestinal mucosa through **PTGER 4 variant** can lead to increased microbial and Ag penetration of the mucosa and resulting in immune activation.
A. Cytokine signaling,
B. Microbial recognition
C. Epithelial barrier function
D. Leucocyte trafficking
Leucocyte trafficking is a necessary element in amplification of the mucosal immune response. Integrins facilitate adhesion of leucocytes to endothelium and recruitment into tissue.
• GWAS found common, human genetic variants associated with disease.

• it is possible that uncommon polymorphisms also contribute to IBD; these may be identified through whole-genome sequencing approaches.
VERY EARLY ONSET (VEO IB)

A substantial proportion of patients with monogenic diseases present with very early onset intestinal inflammation (at less than 10 years of age)
VEO IB GENOTYPE MUTATIONS

– To date, as many as 50 or more single genes causing IBD (monogenic forms of IBD) are implicated in VEO IBD, and the number of genes is expected to increase.

– A mutation in the IL10R gene that underlined an association of IBD with primary immunodeficiency.

– A rare mutation affecting the regulatory function of the X-linked inhibitor of apoptosis (XIAP) gene.
VEO IB PHENOTYPE

• A more severe disease course and

• More frequently shows a positive family history for IBD
GENOMIC ERA IN IBD MANAGEMENT

1. WHAT DO WE NEED?
2. WHAT DO WE KNOW?
3. WHAT SHOULD WE DO?
GENETIC TESTING

for incorporating genetic parameters into everyday clinical practice.
NEW GENOMIC ERA

• **Screen** family members for carrier detection and genetic counseling.

• **Early detection** of patients with:
  - more complicated disease
  - extra-intestinal manifestations

• Early intervention

• Identify individuals:
  - likely to respond to, or
  - have adverse effects or.
  - resistance to **therapy**.

• Inclusion of genotyping in clinical trials of novel therapeutic agents.
This study suggests that a combination of genetic, serological, and inflammatory markers was superior to a serological panel alone for discriminating non-IBD from IBD.

Prognostics Studies

• A variant **IL12B** was identified with **disease severity** in CD.

• A noncoding **FOXO3A SNP** was associated with a milder clinical **natural history** in both Crohn’s disease and rheumatoid arthritis.

• **Ribonuclease T2** Gene Polymorphisms is associated with decreased expression of severity in CD

• **RNASET2** disease risk variants were associated in CD patients with more complicated disease or resistance to therapy.
The ability to use a combination of gene expression profiles from 5 innate immunity genes to generate a “score” with 95% sensitivity and 85% specificity for response to infliximab in UC.


**VEO IBD** in a young child with severe IBD resistant to conventional therapy, we identified a homozygous IL10R variant with a hematopoietic source for the functional defect and a bone marrow transplant resolved the IBD.

SIDE EVENTS OF TREATMENT

- Yang et al performed an unbiased GWAS identifying a non-synonymous (basic arginine to polar cysteine at codon 139) NUDT15 polymorphism that was associated with both early and late leucopenia after thiopurine therapy.

- A similarly designed study from Heap et al identified HLA-DQA1-HLA-DRB1 variants associated with developing pancreatitis after thiopurine exposure in Europeans.
CONCLUSION
GENETIC TESTING TO GO FURTHER