Genetics of Pediatric Inflammatory Bowel Disease

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IBD Education Day 2/9/2014
Objectives

• Brief overview of role of genetics and IBD
• Brief review of DNA
• Evolution of genetic technology
• How we can apply these findings to disease
• Specific considerations
  – Very early-onset IBD
  – Pediatrics: burden of disease
IBD Susceptibility

Adapted from Inflamm Bowel Dis 2010:16;152
Genetics of Complex Disease

- Several genes involved in the expression of the clinical trait
- Gene-gene interactions and gene-environment interactions
- Incomplete penetrance – age-dependent
Genetic Susceptibility

• There is strong link between genetics and IBD
  – Identical twins studies show concordance rates of 36 – 58% in CD
  – Non-identical twins studies show much lower concordance rates of about 4% in both CD and UC
DNA
Chromosome, DNA and Genes

Genes contain instructions for making proteins.

Proteins act alone or in complexes to perform many cellular functions.

U.S. DEPARTMENT OF ENERGY
Progression of Genomic Technology
Battle For the Human Genome
Beginning.....
First Phase: Sanger Sequencing
GWAS studies in IBD

- As of the beginning of 2011, 99 IBD susceptibility gene loci had been identified
  - 71 associated with Crohn’s disease
  - 47 associated with ulcerative colitis
  - 28 associated with both
Next Generation Sequencing
And Beyond...

Graphical User Interfaces for Alignment Tools
Marina Sirota, Michael Brudno, Serafim Batzoglou

Biologists need efficient and accurate alignment tools to compare long DNA sequences and find the conserved biological features between distant species. LAGAN is a parametrizable system for rapid global alignment of two homologous genomic sequences. It uses previously generated pairwise local alignments as anchors to limit the search area of the Needleman-Wunsch algorithm.

LAGAN Application Interface
- Allows the user to:
  - Import and view DNA sequences in MultiFAS format
  - Align two sequences and display the alignment
  - Open and browse an existing alignment
  - Create a visualization for an alignment
  - Save visualizations as images (implemented in Java)

LAGAN GUI
Biologists rely on alignment and visualization tools in their analysis of genomic data. However, most available aligners allow little or no interaction between the user and the program; moreover, most alignment visualizers are also static, permitting the user little flexibility in generating output. Developing a GUI for an alignment tool, such as LAGAN, provides an easy and effective method of interaction between the user and the program. The GUI uses graphical images and widgets (such as menus, buttons, radio boxes, and icons) to accomplish its goals and thus frees the user from learning complex command languages, creating an extra wall of abstraction between the user and the implementor. Combining the ability to actually align DNA sequences and visualize the results, the LAGAN GUI is one of the first stand-alone applications of its kind.

LAGAN Web Interface
- Contains information about the toolkit and the authors
- Allows the user to perform pairwise alignments with email notification using LAGAN or ShufR-LAGAN and multiple alignments using Multi-LAGAN (implemented in HTML and Perl)

References:
Michael Brudno, Chunyan He, Gregory Cooper, Michael F. Sam, Eugene Bravkin, NSB Sequencing Consortium, Eric D. Green, Xerol Sidow and Serafim Batzoglou
Timeline of confirmed IBD Loci / genes in children and adults with IBD

Confirmed in both adults & children:
- ATG16L1
- PTPN2
- NKX2-3
- IRGM
- DGKD
- IL12B
- CDKAL1
- ATG16L1
- IL23R
- TNFSF15

To date only confirmed in adults:
- 3p13
- 10q21
- 5p13
- 1q13
- 1q23
- 1q24
- 1q24
- 1q32
- 6q27
- 1q32
- 13q14
- 17q21
- 17q21
- 12q12
- 17q21
- 21q21
- c11orf30
- MTMR3, LIF
- HORMAD2
- ZMIZ1
- TLR cluster

Year of gene discovery in chronological order:
- NOD2 (2001)
- OCTN1/2 (2004)
- TNFSF15 (2005)
- IL23R (2006)
- 5p13 (2007)
- 7p12 (2007)
- ICOSLG (2007)
- 6p21 (2009)
- 9q32 (2009)
- IL27 (2010)
- Many New Loci from meta analysis
Genetics of IBD: 
163 confirmed loci

Common pathways:
- Leprosy
- Mycobacterial susceptibility
- Other immune-mediated disease

30 CD specific loci
23 UC specific loci

Genes in common

Identification of Disease Associated Pathways

IBD-related processes

Epithelial barrier
- GNA12*, HNF4A, CDH1, ERRF1, MUC19, ITLN1*

Restitution
- REL, PIGR4, NKX2-3, STAT3, ERRF11, HNF4A, PLA2G2A/E

Solute transport
- SLC9A4, SLC22A5, SLC22A4*, AQP12A/B, SLC9A3, SLC26A3

Paneth cells
- ITLN1*, NOD2*, ATG16L1*, XBPI*

Innate mucosal defence
- NOD2*, ITLN1*, CARD9*, REL, SLC11A1, FCGR2A/B

Immune cell recruitment
- CCL11/CCL2/CCL7/CCL8, CCR6, IL8RA/IL8RB, MST1*

Antigen presentation
- ERAP2*, LNP, DENND1B

IL-23/T-helper 17
- IL23R*, JAK2, TYK2*, STAT3, ICOSLG, IL21, TNFSF15*

T-cell regulation
- NDFIP1, TNFSF8, TAGAP, IL2, IL2R*, TNFRSF9, PIM3, IL7R*, IL12B, IL23, PRDM1, ICOSLG, TNFSF8, IFNG, IL2

B-cell regulation
- IL5, IKZF1, BACH2, IL7R*, IRF5

Immune tolerance
- IL10, IL27*, SBN02, CREM, IL1R1/IL1R2, NOD2*

Cellular responses

Autophagy
- ATG16L1*, IRGM, NOD2*, LRRK2, CUL2, PARK7, DAP*

Apoptosis/necroptosis
- FASLG, THADA*, DAP, PUS10, MST1*

ER stress
- CPEB4, ORMDL3, SERINC3, XBPI*

Carbohydrate metabolism
- GCKR*, SLC2A4RG

Intracellular logistics
- VAMP3, KIF21B, TTL8, FGFR1OP, CEP72, TPPP

Oxidative stress
- PRDX5, BACH2, ADO, GPX4, GPX1*, SLC22A4, LRRK2, NOD2*, CARD9*, HSPA6, DLD, PARK7, UTS2*, PEX13

Cell migration
- ARPC2, LSP1, AAMP

Microbial sensors

Recruitment of mediators

Signal amplification

Transducers and effectors

Microbiota, diet

P

P

G

ILC

B cell

T-helper 17

T-reg cell

Plasma cell

IgA

Xavier 2011

UC

CD

UC/CD

crs-eQTL

*Coding mutation
“Bacterially”-Generated Phenotypes

IL-10⁻/⁻ → Germ-Free

IL-10⁻/⁻ → Commensal Bacteria

IL-10⁻/⁻ → E. faecalis

IL-10⁻/⁻ → E. coli

Genetic Differences and Host Response to Bacteria

- IL-10 deficient
- B. vulgatus
- Minimally inflamed

- HLA-B27 β2 transgenic
- B. vulgatus
- Inflamed
Innate and Adaptive Immunity are Linked

**Threat” Detection**

Pattern Recognition Receptors

**TLRs, NODs**

0-6 Hours

Immediate Responses

Innate Immunity Broad Action

Cytokines And Chemokines

- Release of anti-microbials
- Recruitment of cells
- Localized Inflammation

1-5 Days

Longer-Term Mobilization

Adaptive Immunity Antigen-Specific

KILL PATHOGENS CLEAR INFECTION

B cell or T cell

A frameshift mutation in NOD2 associated with susceptibility to Crohn’s disease

NOD2 Contributes to Normal Mucosal Defenses

NOD2 expression increased with inflammatory mediators.
Innate Immunity: NOD2 Signaling Activates Proinflammatory Cytokines

INTRACELLULAR BACTERIA

NOD2 SENSES PEPTIDOGLYCANS (MDP)

NF-κB

PROINFLAMMATORY RESPONSE
Innate Immunity: Failure of NOD2 Signaling alters cell function

- Intracellular bacteria
- NOD2 senses peptidoglycans (MDP)
- Persistence of intracellular bacteria
- Defensin deficiency (loss of sterile crypts)
- Altered epithelial cell function (increased permeability)
ATGL1 and Autophagy: More Defense

**Functional autophagy**
- Bacterial entry
- Niche establishment
- Niche failure
- Autophagosome capture and degradation
- Slow replication

**Absence of autophagy**
- Bacterial entry
- Niche establishment
- Niche failure
- Escape and rapid cytosolic replication
- Slow replication
Adaptive Immunity

Th1: IFNγ, T-bet

Th2: IL-4, IL-5, IL-13, GATA3

Th17: IL-17, IL-21, IL-22, IL-26, RORγt

Th22: IL-22, AHR

Treg: IL-10, TGF-β, FOXP3

DC: Lymphoid Precursor

ILC1: E4BP4

ILC2: GATA3, RORγt, Notch

NCR+ ILC3: RORγt

NCR- ILC3: RORγt

LTI CELL: RORγt
Model for Crohn’s Disease Genotype-Phenotype associations
Model for Crohn’s Disease
Genotype-Phenotype associations

Inflammation

NOD2 and other genes (location)
Model for Crohn’s Disease Genotype-Phenotype associations

Fibrostenosis

NOD2/CARD15

Earlier surgery
Model for Crohn’s Disease Genotype-Phenotype associations

- NOD2
- Atg16L1
- Smoking
- ASCA/I2/OmpC expression

Aggressive disease – more surgeries
## Therapies Directed at These Pathways

### Table 1  Current and emerging biological inflammatory bowel disease (IBD) treatments targeting the mucosal immune cells and pathways described in this review

<table>
<thead>
<tr>
<th>Biological target</th>
<th>Drugs</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Innate immune cell signalling</strong></td>
<td></td>
<td>The most well established biological treatment for IBD likely acts by neutralisation of inflammatory macrophage derived TNF. However there is also evidence that these agents also promote T cell apoptosis and may induce regulatory macrophages.</td>
</tr>
<tr>
<td>TNFα</td>
<td>Infliximab, adalimumab, certolizumab pegol</td>
<td>TLR activation in response to bacterial recognition such as TLR9 binding to unmethylated CpG motifs activate downstream inflammatory cascades that may contribute to disease and perpetuate inflammation in response to commensals. TLR signalling is also required for maintenance of a healthy epithelium, and Myd88 deficient mice have increased susceptibility to experimental colitis.</td>
</tr>
<tr>
<td>TLR</td>
<td>DAIM0150 (Kappaproct), BL-7040 RDP58</td>
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<tr>
<td>TLR9 MyD88</td>
<td></td>
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<tr>
<td><strong>T cell activity</strong></td>
<td></td>
<td>An exaggerated T cell response is a fundamental hallmark of IBD, and these treatments are therefore aimed at limiting T cell proliferation and expansion, but will also inhibit protective and regulatory T cell functions.</td>
</tr>
<tr>
<td><strong>T cell proliferation</strong></td>
<td>CD3 Visilizumab</td>
<td>Inhibits the CCR9 chemokine receptor and intestinal homing of T cells in response to CCL25 chemokine ligand.</td>
</tr>
<tr>
<td>CD25</td>
<td>Basiliximab, daclizumab</td>
<td>Blocks the α4β7 integrin, which interacts with MAdCAM-1 on intestinal endothelial cells and mediates gut T cell homing.</td>
</tr>
<tr>
<td>Protein kinase C inhibitor</td>
<td>Sotastaurin</td>
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</tr>
<tr>
<td><strong>Chemotaxis</strong></td>
<td>CCR9 CCX-025, CCX282-B</td>
<td>Blocks interferon inducible protein-10 (IP-10), also known as CXCL10 (high levels seen in UC), which binds to CXCR3 and recruits activated T cells, NK cells and eosinophils.</td>
</tr>
<tr>
<td>α4β7 integrin</td>
<td>Vedolizumab, natalizumab, ELND-004, AJM-300, etrolizumab</td>
<td></td>
</tr>
<tr>
<td>MAdCAM-1</td>
<td>PF-547659 MDX-1100</td>
<td>Injection of autologous, OVA-expanded regulatory T cells.</td>
</tr>
<tr>
<td>IP-10</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Regulatory T cells</strong></td>
<td>OvaSave</td>
<td></td>
</tr>
<tr>
<td><strong>Pro-inflammatory cytokines</strong></td>
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<tr>
<td>These have traditionally been considered in the context of T cells; many of the following cytokines are also produced and/or act on other immune cells such as innate lymphoid cells (ILCs)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>JAK</strong></td>
<td>Tafocitinib</td>
<td>Inhibits JAK-STAT signalling pathway and thereby blocks subsequent inflammatory cytokine production; including IL-2, 4, 7, 9, 15, and 21.</td>
</tr>
<tr>
<td><strong>IL-12/23</strong></td>
<td>Ustekinumab, SCH900222, briakinumab</td>
<td>Produced by myeloid cells, IL-12 and IL-23 share their p40 subunit and promote pro-inflammatory Th1 and Th17 differentiation; but more recently have been also been shown to act on ILCs.</td>
</tr>
<tr>
<td><strong>IL-17</strong></td>
<td>Secukinumab, brodalumab, vidofludimus</td>
<td>Produced by Th17 and ILCs, IL-17 is considered pro-inflammatory but also has important homeostatic function. Notably secukinumab (anti-IL-17A) appears to worsen CD.</td>
</tr>
<tr>
<td>IFNγ</td>
<td>Fontolizumab</td>
<td>IFNγ is a classical Th1 cytokine; also produced by inflammatory ILCs.</td>
</tr>
<tr>
<td><strong>IL-13</strong></td>
<td>QAX576, anruckinumab, tralokinumab</td>
<td>IL-13 is a cytokine associated with UC, whose production by NKT cells appears essential in murine oxazolone colitis.</td>
</tr>
<tr>
<td><strong>IL-6 and IL-6R</strong></td>
<td>Tocilizumab, PF04236921, C326</td>
<td>IL-6 produced by myeloid cells upregulates anti-apoptotic genes in T cell and promotes inflammatory for example, Th17 cell expansion.</td>
</tr>
</tbody>
</table>
Biology of Interleukins 12 and 23

Stimulus TLR?

Antigen Presenting Cell

CD4

MHCII

TCR

IL-12

Anti-IL-12/23

p35

p40

Anti-IL-12/23

IL-12Rβ1

IFNg (Th1)

IL-17 (Th17)

Anti-IL-12/23

p40

p19

IL-23

Anti-IL-12/23

IL-12Rβ1

p40

p35
Are there novel genes that confer susceptibility to pediatric onset IBD?

International Pediatric IBD GWAS
Most genes identified in adult GWAS show association with pediatric IBD

“Novel” genes subsequently confirmed in adult cohorts

Shows similarity in pathways in both adult and pediatric onset IBD

Included patients were predominantly older children or adolescents at time of diagnosis (A1b or A2)

Common variants at five new loci associated with early-onset inflammatory bowel disease

Marcin Imielinski1,2,6, Robert N Baldassano2,6, Anne Griffiths3,6, Richard K Russell4,2,6, Vito Annese5,6, Marla Dubinsky6,2,6, Subra Kugathasan7,6, Jonathan Bradfield1, Thomas Walters3, Patrick Sleiman1, Cecilia Kim1, Alexo Muise2, Kai Wang1, Joseph Glossner1, Shehzad Saeed3, Haitao Zhang1, Edward Frackelton1, Cuiping Hou1, James Flory1, George Otieno3, Rosetta Chiavacci3, Robert Grundmeier2,8, Massimo Castro10, Anna Latiano10, Bruno Dallapiccola11, Joanne Stempak12, Debra J Abrams9, Kent Taylor6, Dermot McGovern6, Western Regional Research Alliance for Pediatric IBD, International IBD Genetics Consortium18, Melvin B Heyman13, George D Ferry7, Barbara Kirschner14, Jessica Lee15, Jonah Essers15, Richard Grand15, Michael Stephens16, Arie Levine5,17, David Piccoli2,9, Johan Van Limbergen18, Salvatore Cucchiara19, Dimitri S Monos20, Stephen L Guthery21, Lee Denson22, David C Wilson23, Struan F A Grant1,2,24, Mark Daly25, Mark Silverberg12,27, Jack Satsang18,27 & Hakon Hakonarson1,2,24,27

The inflammatory bowel diseases (IBD) Crohn's disease and ulcerative colitis are common causes of morbidity in children and young adults in the western world. Here we report the results of a genome-wide association study in early-onset IBD involving 3,426 affected individuals and 11,963 genetically matched controls recruited through international collaborations in Europe and North America, thereby extending the results from a previous study of 1,011 individuals with early-onset IBD. We have identified five new regions associated with early-onset IBD susceptibility, including 16p11 near the cytokine gene IL27 (rs8049439, P = 2.41 × 10−8, 2q12 (rs2412973, P = 1.55 × 10−8), 10q22 (rs1250550, P = 5.63 × 10−8), 2q37 (rs4676410, P = 3.64 × 10−8) and 19q13.11 (rs10500264, P = 4.26 × 10−10). Our scan also detected associations at 23 of 32 loci previously implicated in adult-onset Crohn’s disease and at 8 of 17 loci implicated in adult-onset ulcerative colitis, highlighting the close pathogenetic relationship between early- and adult-onset IBD.

Most genetic analyses in IBD have been performed in adult-onset disease. Early-onset IBD, however, has unique characteristics of phenotype, severity and familiarity, features that provide support for the search for loci that may be specific to early-onset disease. In addition, because early-onset IBD has a stronger familial component than the adult disease, studies targeting this subgroup potentially provide additional power to identify genes that contribute modest effects, as illustrated by the success of our previous scan in identifying 20q13 and 21q22 as IBD loci.

We now report results from the largest GWAS conducted so far in early-onset IBD (Fig. 1). Our IBD discovery cohort (DC-IBD) comprised 2,413 individuals of European ancestry with IBD (cases), including 1,636 with Crohn’s disease (DC-CD), 724 with ulcerative colitis (DC-UC) and 53 with IBD of unclassified type (IBD-U), and 6,158 genetically matched controls, and was genotyped on the Illumina HumanHap550 platform. Affected individuals were recruited from multiple centers from four geographically discrete countries, were diagnosed before their nineteenth birthday and fulfilled standard diagnostic criteria for IBD (Supplementary Table 1). Our study extends a
Inflammatory Bowel Disease (IBD)

- We identified and replicated significantly associated, previously unreported loci on chromosomes 20q13 and 21q22 located close to the **TNFRSF6B** and **PSMG1** genes, respectively.

- Gene discovery studies in childhood-onset disease have unveiled genetic factors that are less likely to surface in adult studies.

- We have identified six other novel IBD loci and replicated the vast majority of loci discovered in adult onset IBD.
Very Early-Onset IBD: The Challenge

• Diagnosed ≤5 years of age
• Frequently different phenotype and more severe disease presentation
• Often unresponsive to conventional therapy
Genomics and VEO-IBD

• GWAS approach
  – Primarily included adults and children > 10
  – Frequently misses rare variants, MAF < 5%

• Role of primary immunodeficiency

• Are we missing important pathways in this cohort?
WES: The Low-Hanging Fruit?

Common variants of small effect, non-coding variants of small to moderate effect (no currently available technology is capable of finding them)

Rare and uncommon coding variants with large effect (targeted and exome sequencing)

Common variants of moderate effect (fine mapping and immunochip)

Common variants of large effect (GWAS)
# Identified Rare Variants in Candidate Genes

<table>
<thead>
<tr>
<th>Chr</th>
<th>Position</th>
<th>dbSNP ID</th>
<th>Ref</th>
<th>Alt</th>
<th>DNA Alteration</th>
<th>Gene</th>
<th>Immunodeficiency</th>
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<tr>
<td>1</td>
<td>114699</td>
<td>rs1146666761</td>
<td>C</td>
<td>G</td>
<td>p.T43R</td>
<td>TNFRSF18</td>
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<tr>
<td>6</td>
<td>31727677</td>
<td>Novel</td>
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<td>p.S554T</td>
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<td>28950051</td>
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<td>151793903</td>
<td>rs72719663</td>
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<td>G</td>
<td>p. T1724V</td>
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<td>rs2066844</td>
<td>C</td>
<td>T</td>
<td>p. R702W</td>
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<td>IBD risk loci</td>
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<td>1</td>
<td>183532364</td>
<td>rs35012521</td>
<td>T</td>
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<td>p.N419I</td>
<td>NCF2</td>
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<td>rs11466045</td>
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<td>p.1591T</td>
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<td>11</td>
<td>117869853</td>
<td>rs143538561</td>
<td>C</td>
<td>T</td>
<td>p.R412W</td>
<td>IL10RA</td>
<td>IL10R (see IL10)</td>
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<tr>
<td>19</td>
<td>17945695</td>
<td>rs5577834</td>
<td>C</td>
<td>T</td>
<td>p.v7221</td>
<td>JAK3</td>
<td>Primary immunodeficiency; Multiple immunodeficiency pathways</td>
</tr>
<tr>
<td></td>
<td>17953949</td>
<td>rs3213409</td>
<td>G</td>
<td>C</td>
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</tr>
</tbody>
</table>
IBD pathogenesis

Crohn disease-like

- Colonization (bacteria, viruses, fungi, worms)
- Crohn activating infections
- Crohn specific genes (Nod2)

Ulcerative colitis-like

- Core genes Regulating Inflammation, Epithelial barrier, autophagy, etc
- Loss of protective flora
- UC specific genes
The microbiome shapes the innate immune response and vice versa
Conclusions

• Genomics of IBD is evolving
• Newer technology has allowed us to identify pathways critical in the development of IBD
• Candidate causative mutations in early-onset IBD can be identified by exome sequencing
• Goal is combine our findings in genomics and microbiome and target therapy for the individual patient
Thank You