Role of Genetics in Understanding Common Diseases: The Inflammatory Bowel Disease Example

CTSI Training Program
Module 6: The Role of Genetics in Clinical Research
March 13, 2012

Jerome I. Rotter, M.D.
Director of Research and Co-Director
Medical Genetics Institute, Cedars-Sinai
Board of Governors’ Chair in Medical Genetics, Cedars-Sinai
Director, Division of Medical Genetics, Cedars-Sinai
Professor of Medicine and Biomedical Sciences, Cedars-Sinai
Professor of Medicine, Pediatrics & Human Genetics, UCLA
Examples at CSMC
Common Disease Genetics Research

Coronary artery disease
Subclinical atherosclerosis
Blood pressure and hypertension
  Lipid disorders
  Type 2 diabetes
Insulin resistance
Insulin secretion
  Obesity
  Insulin Clearance
Diabetic nephropathy
Diabetic retinopathy
Crohn’s disease
Ulcerative colitis

Keratoconus
Polycystic ovarian syndrome
Type 1 diabetes
Ovarian cancer
Pituitary adenomas
Macular degeneration
Osteoporosis
Depression
Osteoarthritis
Bicuspid aortic valves
Cardiac arrhythmias
Stroke
Sudden cardiac death
IBD as Paradigms for Genetics of Complex Disease

• Introduction to IBD
• Genetics without genes
• Candidate genes
• Linkage and positional cloning
• Genome wide association
Inflammatory Bowel Disease

Crohn’s Disease

Ulcerative Colitis
Features of Inflammatory Bowel Disease

Small Bowel – Crohn’s

- Abscess
  - Fever
  - Mass
- Fistula

Stricture
- Pain/Cramps
- Nausea and vomiting
- Distension
- Loud bowel sounds

Widespread Inflammation
- Weight loss
- Diarrhea
- Fever
- Loss of appetite
Features of Inflammatory Bowel Disease

Colon - Crohn’s and Ulcerative Colitis

**Toxic Disease**
- High fever
- Distension
- Pain

**Rectal Disease**
- Urgency
- Pain
- Bleeding

**Diffuse Disease**
- Diarrhea
- Bleeding
- Fever
- Malaise

**Stricture**
- Distension
- Pain/Cramps
- Loud bowel sounds
- Changes in bowel habits
Inflammatory Bowel Disease

Systemic Complications

- Eye inflammation
- Liver and Bile Duct inflammation
- Gallstones
- Skin lesions
- Kidney Stones
- Arthritis and Joint pains
- Growth failure in children
Problem:

• What Causes IBD?
Most Common Diseases are Genetically Complex and Etiologically Idiopathic
Why does finding genes help?

- Gene identification
- Understanding mechanisms
- Developing specific therapies and prevention approaches
- Genetic screening to identify high risk individuals
IBD as Paradigms for Genetics of Complex Disease

- Introduction to IBD
- Genetics without genes
- Candidate genes
- Linkage and positional cloning
- Genome wide association
Genetics of IBD

- Evidence that *suggests* that genetic factors are important:
  - Ethnic variation
  - Familial aggregation
IBD: Ethnic Differences and Familial Aggregation

- First Degree Relative: > 10 times
- Jews: ~ 4 times
- Non-Jewish Caucasians: ~ 4 times
- Blacks: ~ 4 times
Genes vs. Environment
Genetics of IBD

Evidence that *proves* that genetic factors are important:

– Spouses at no increased risk
– Twins, MZ risk > DZ risk
– Familial aggregation remains when separated by space and time
– A feature of a number of genetic syndromes
How do we know IBD has environmental causes?

- Asian immigrants to UK have a higher incidence than Asians in Asia
- Ashkenazi Jews in LA have a higher incidence than in Tel Aviv
- Increased in Europe as industrialization proceeded country by country
- Increasing in Puerto Rico as public hygiene and health standards improve
Major etiologic risk factors for inflammatory bowel diseases -- ulcerative colitis and Crohn’s disease -- are Genetic Susceptibilities
Natural History of IBD

Clinical Expression Threshold

Conception
- Genetically Susceptible

Birth
- Exposure To Environmental Factors

Underlying Immunologic Changes?
- ANCA?

Histological Changes?
- Intestinal Permeability?

Clinical Disease
- IBD
Definition:

Subclinical markers are traits used to detect the abnormal genotype in the absence of the full phenotype. They represent abnormalities having a direct role in the pathogenesis of the disease.
Prevalence of ANCA in UC patients and relatives from three sets

**Frequency of positive ANCA**

- **UC patients**
  - Los Angeles: 68%
  - Calgary: 86%
  - Germany: 70%

- **Relatives**
  - Los Angeles: 16%
  - Calgary: 21%
  - Germany: 30%
IBD as Paradigms for Genetics of Complex Disease

• Introduction to IBD
• Genetics without genes
• Candidate genes
• Linkage and positional cloning
• Genome wide association
IBD is due to failure to down regulate normally self limited inflammation.

- Infection? Bacterial products?
- Acute moderate inflammation
- Failure to down regulate
- Chronic inflammation = IBD
- Down regulated
- N.I. gut

N.I. gut mildly inflamed
## Tentative Candidate Genes for IBD

<table>
<thead>
<tr>
<th><strong>Immunospecific Genes</strong></th>
<th><strong>Chromosome</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA class II region (DR, DQ, DP)</td>
<td>6</td>
</tr>
<tr>
<td>GM</td>
<td>14</td>
</tr>
<tr>
<td>KM</td>
<td>2</td>
</tr>
<tr>
<td>T-cell receptor alpha, delta chains</td>
<td>12</td>
</tr>
<tr>
<td>T-cell receptor beta, gamma chains</td>
<td>7</td>
</tr>
</tbody>
</table>

| **Immunoregulatory Genes**                                                             |               |
| **Complement cascade**                                                                 |               |
| Complement C3                                                                          | 19            |
| Complement C2, C4, Bf                                                                   | 6             |
| **Cytokines**                                                                          |               |
| Tumor necrosis factor                                                                  | 6             |
| IL-1 receptor antagonist                                                                | 2             |
| **Cell adhesion molecules**                                                             |               |
| Intercellular adhesion molecule-1                                                       | 19            |

| **Other Genes**                                                                        |               |
| Mucin genes                                                                            | multiple      |
HLA DR-2 and UC association

- UC Cases (93): 38.7%
- Controls (77): 20.8%
- CD cases (95): 23.2%

Significance:
- p < 0.018 (between UC Cases and Controls)
- p > 0.85 (between Controls and CD cases)

Source: Toyoda et al. Gastro 1993
Relationships between UC, ANCA, and DR2
Which Markers?

**Candidate Genes**: “round up the usual suspects”, the Casablanca approach
Testing genes which have been hypothesized to lie in the pathophysiologic pathway of disease; e.g. IBD – HLA

**Genome Scan**: “Needle in a haystack” approach
Screening random markers evenly distributed along the whole genome; e.g. CD – *IBD1*
Localization of a gene

To identify a person
To identify a gene

Entire Genome

Which city
Which chromosome

Which block
Which region

Exact address
Exact location

Here is the person
Here is the mutation

AGCACA CTAGGA
IBD as Paradigms for Genetics of Complex Disease

- Introduction to IBD
- Genetics without genes
- Candidate genes
- Linkage and positional cloning
- Genome wide association
Linkage Studies: Does the genetic marker travel together with disease in families?

- Requires families with multiple affected
- Uses genotype information (in the recent past, typically micro-satellite markers)
- For genome scan, markers should be evenly spaced across the genome
- Analysis can be model-based (specify mode of inheritance) or model-free (nonparametric)
Mapping of a Susceptibility Locus for Crohn’s Disease on Chromosome 16.


• Genotyped 41 sibpairs for 270 markers
• Two regions on Ch. 1 and 16 with p-value < 0.01
• Both of these regions typed in 70 additional sibpairs
• Chr. 16 region again p-value of 0.01 in additional sibpairs
CD Linkage Results: Chromosome 16
(*IBD1* locus)

Overlapping markers, Overlapping peaks

Hugot, 1996

Ohmen, 1996
Add’n Linkage & Positional Cloning

A. Original Results

B. More dense genotyping of a smaller region in addition families

C. BAC clones used to identify previously unknown SNPs which were then genotyped
Allelic variants of NOD2 are associated with Crohn’s disease

- Carriage of NOD2 allelic variants:\n  - Crohn’s disease: 27-39\%\(^6\) (49\% all DCM’s\(^4\))
  - Control population: 14-16\%\(^6\)
  - Ulcerative colitis population: 12+14\%\(^5\)

- 27 rare mutations account for 19\% of disease-causing mutations\(^4\)

2) Ogura, Y et al. (2001). Nature 411: 603
Outline of the innate-immune response to bacterial components

Bacterial components/LPS → CD14 → TLR4 → NOD2 → RICK → NF-κB/IκB → Degradation → NF-κB

Corticosteroids

Monocyte → Nucleus

IκB = Inhibitor of NF-κB; LPS = lipopolysaccharide; NF-κB = Nuclear factor-κB; RICK = RIP-like interacting CLARP kinase; TRAF6 = TNF receptor-associated factor 6

Inflammatory response
How do NOD2 variants lead to CD?

- Impaired response to ‘LPS’(!) in NF-kB activation assays

- Lack of ‘brake’ on TLR2 effect?
Pathophysiology of Crohn’s Disease

Innate Immune Response

Adaptive Immune Response

ASCA

Anti-I2

Anti-OmpC

NOD2/CARD15

TLR 4/TLR5

Commensal bacteria
Quantitative Response Can Be Represented by Quartiles for Each Microbial Antigen

**Quartiles**

**I2**

0 50 100 150 200 250 300 350

**OmpC**

0 25 50 75 100 125 150 200

**ASCA**

-0.75 -0.25 0.25 0.75 1.00 1.25

**CBir1**

0 60 120 180 240 300

ELISA Units (EU/ml)

**Quartile Sum**

(n=732)

Low reactivity → High reactivity

<table>
<thead>
<tr>
<th>No. of Individuals</th>
<th>Low reactivity</th>
<th>High reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>10</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>13</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(n=732)
Results: NOD2/CARD15 Variant is Associated With Increased Sero-reactivity to Microbial Antigens in Unaffected Relatives of CD Patients

\[ P = 0.02 \]

\[ P = 0.0003 \]

*Cohort specific quartiles; Student’s t-test*
IBD as Paradigms for Genetics of Complex Disease

- Introduction to IBD
- Genetics without genes
- Candidate genes
- Linkage and positional cloning
- Genome wide association
What is GWA?

- Genome Wide Association—A way of looking at the entire genome at one time with fine resolution, VERY fast
- Only recently has become technologically feasible
- Technology makes available the ability to genotype 700,000 to 2,500,000 SNPs per subject at 250 to 500 $US
- Computer methods to analyze a dataset of this size have become available
The Genomewide Association Study.

Meta-Analysis of Genomewide Association Studies.

Cedars-Sinai GWAS Publications vs. International GWAS Publications

Available at: www.genome.gov/gwastudies. Accessed 3/1/12
Genes Identified and Confirmed for Association with Crohn’s Disease (CD)

Positional Cloning and Candidate Gene Methods

- NOD2
- 5q31
- MHC
First GWAS in IBD, Yamazaki et. al., 2005

- 72,000 markers in 94 Japanese CD subjects
- Followed by 143 SNPs in 484 CD
- Identified TNFSF15 (TL1A) haplotype A with increased risk
- Confirmed in 2 British cohorts

TNFSF15 is a trans-ethnic IBD gene

- Identified haplotype B with decreased risk in a Jewish population (Picornell, et. al. 2007)
Genes Identified and Confirmed for Association with Crohn’s Disease (CD)

- NOD2
- 5q31
- MHC
- TNFSF15

Yamazaki, 2005
Picornell, 2007
GWA Single SNP Results

<table>
<thead>
<tr>
<th>SNP</th>
<th>p</th>
<th>p_{corrected}</th>
<th>GENE</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2066843</td>
<td>2.9e-09</td>
<td>0.00088</td>
<td>CARD15</td>
</tr>
<tr>
<td>rs2076756</td>
<td>5.1e-10</td>
<td>0.00016</td>
<td>CARD15</td>
</tr>
<tr>
<td>rs11209026</td>
<td>5.1e-09</td>
<td>0.0016</td>
<td>IL23R</td>
</tr>
</tbody>
</table>

- Freq of rs11209026: 2% CD, 7% control
- OR = 0.26, 95%CI 0.15-0.43 “Protective”
- rs11209026 is Arg381Gln
- 9 SNPs in IL23R had p < 0.0001

IL23R Gene on Chromosome 2

IL23R in Model System

Important for colitis in the IL10 knockout mouse model

IL10ko x IL23rko

IL10ko

Multiple genes in the IL23/IL17 pathway contribute to CD

- IL12RB1
- IL-23R
- IL23 (p40, p19)
- IL17A
- IL17RA

Activation of transcription, anti-apoptotic proteins, and pro-inflammatory cytokines lead to NFκB and MAPK pathway activation. Extracellular space is shown.
IL23R Pathways Genes Associated with CD

<table>
<thead>
<tr>
<th>Gene</th>
<th>P-values for Risk Haplotype</th>
<th>P-value for Protective Haplotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL23R B2</td>
<td>0.015</td>
<td>0.005</td>
</tr>
<tr>
<td>IL23R B3</td>
<td>0.015</td>
<td>0.003</td>
</tr>
<tr>
<td>IL17A</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>IL17RA</td>
<td>0.01</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IL12B</td>
<td>NS</td>
<td>0.007</td>
</tr>
<tr>
<td>IL12RB1</td>
<td>0.004</td>
<td>---</td>
</tr>
</tbody>
</table>
Cumulative Effect of Pathway Genes on CD Risk: IL23R, IL17A, IL17RA, IL12RB1

IL23 (P19)
- Immunoglobulin-like domain
- Fibronectin-like domain
- Cytokine-receptor homology

IL12B, p40

IL12RB1 → IL23R → T<sub>17</sub> cell

IL17A → IL17RA

Diagram:
- IL12RB1 (purple)
- IL23R (purple)
- T<sub>17</sub> cell
- IL17A (purple)
- IL17RA (purple)
Cumulative Effect of Pathway Genes on CD Risk: IL23R, IL17A, IL17RA, IL12RB1

Odds Ratio for CD

Number of “risk” haplotypes present

$p_{MH} < 0.0001$

Genes Identified and Confirmed for Association with CD

Positional Cloning and Candidate Gene Methods

- NOD2
- 5q31
- MHC
- TNFSF15
- IL23R
- ATG16L1
Genes **Confirmed** in Subsequent Samples

Int’l Crohn’s Disease GWAS Meta-analysis Working Group

Wellcome Trust Case Control Consortium

NIDDK IBD Genetics Consortium

Belgian-French IBD consortium
Genes Identified and Confirmed for Association with CD

GWAS N=29

Positional Cloning and Candidate Gene Methods
- NOD2
- 5q31
- MHC
- TNFSF15
- IL23R
- ATG16L1
- NIKX2-3
- PTPN2
- ZNF365
- IRGM
- PTGER4
- MST1
- ICOSLG

Prior 2005 2006 2007 2008

Supplementary Figure 2 – Results of association analysis ("Manhattan Plot"). The negative common logarithm of the P-values for the test statistic using single-SNP Z scores of the genome-wide association study are shown according to chromosome. Only markers that passed the quality criteria were used for plotting (n=953,241). Marker positions are in NCBI’s build 36 (hg18).
CD Genetics 2011 (71 Loci)

- Linkage
- Initial GWAS
- 2008 meta-analysis
- Individual GWAS
- 2010 meta-analysis (Franke, A, McGovern, DPB, et al.)

Prior

<table>
<thead>
<tr>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOD2</td>
<td>5q31</td>
<td>MHC</td>
<td>TNFSF15</td>
</tr>
<tr>
<td>1q24</td>
<td>1q32</td>
<td>6q21</td>
<td>7p12</td>
</tr>
<tr>
<td>8q24</td>
<td>10p11</td>
<td>13q14</td>
<td>19p13</td>
</tr>
<tr>
<td>21q21</td>
<td>ITLN1</td>
<td>PTPN22</td>
<td>IL12B</td>
</tr>
<tr>
<td>CDKAL1</td>
<td>CCR6</td>
<td>NKX2-3</td>
<td>JAK2</td>
</tr>
<tr>
<td>PTPN2</td>
<td>C11orf30</td>
<td>ZNF365</td>
<td>IRGM</td>
</tr>
<tr>
<td>ORMDL3</td>
<td>LRRK2/MUC19</td>
<td>IL23R</td>
<td>IL27</td>
</tr>
<tr>
<td>ATG16L1</td>
<td>MST1</td>
<td>PTGER4</td>
<td>STAT3</td>
</tr>
<tr>
<td>ICOSLG</td>
<td>CARD9</td>
<td>IL18RAP</td>
<td>FUT2</td>
</tr>
<tr>
<td>FADS1</td>
<td>UBE2D1</td>
<td>PRDX5/ESRRA</td>
<td>IL2RA</td>
</tr>
<tr>
<td>MTMR3</td>
<td>TNFRSF6B</td>
<td>ICAM1/3</td>
<td></td>
</tr>
<tr>
<td>DNMT3A</td>
<td>THADA</td>
<td>ERAP2</td>
<td></td>
</tr>
<tr>
<td>GCKR</td>
<td>EIF3C/CD19</td>
<td>CCL2/7</td>
<td></td>
</tr>
<tr>
<td>SMAD3</td>
<td>VAMP3</td>
<td>CLC2/7</td>
<td></td>
</tr>
<tr>
<td>GALC</td>
<td>PLCL1</td>
<td>SP140</td>
<td></td>
</tr>
<tr>
<td>LRRK2/MUC19</td>
<td>ITLN1</td>
<td>PTPN22</td>
<td></td>
</tr>
<tr>
<td>IL12B</td>
<td>CDKAL1</td>
<td>CCR6</td>
<td></td>
</tr>
<tr>
<td>NKX2-3</td>
<td>PTPN2</td>
<td>ZNF365</td>
<td></td>
</tr>
<tr>
<td>IRGM</td>
<td>ORMDL3</td>
<td>LRRK2/MUC19</td>
<td></td>
</tr>
<tr>
<td>IL23R</td>
<td>PTGER4</td>
<td>ATG16L1</td>
<td></td>
</tr>
</tbody>
</table>

Linkage

Initial GWAS

2008 meta-analysis

Individual GWAS

2010 meta-analysis (Franke, A, McGovern, DPB, et al.)
What proportion of genetic variance now explained?

~25%
UC Genetics 2011 (49 Loci)


Genes that overlap with CD

MHC  IL10  IL23R  IL17REL

TYK2  STAT3  RTEL1  IL1R2/1  1q32  CREM
CARD9  KIF21B  TNFSF15  KIF1A  CDKAL1  JAK2
ORMDL3  ZNF365  C11orf30  NKX2-3  SMAD3  PRDM1
REL  ORMDL3  PTPN2  ICOSLG  SMAD3  5q31
PTPN2  ICOSLG  SMAD3  PRDM1  IL12B  PTGER4

2008  2009  2010-2011
71 Loci for CD
47 Loci for UC

Genome-wide meta-analysis increases to 71 the number of confirmed Crohn’s disease susceptibility loci


Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47


Franke et al, Nat Genet, 42:1118-25, 2010

Anderson et al, Nat Genet, 43:246-252, 2011

### Cellular responses

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

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

**Carbohydrate metabolism**
- GCKR*, SLC2A4RG

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

**ER stress**
- CPEB4, ORMDL3, SERINC3, XBP1*

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

**Cell migration**
- ARPC2, LSP1, AAMP

### IBD-related processes

**Epithelial barrier**
- GNA12*, HNF4A, CDH1, ERRF11, MUC19, ITLN1*

**Restitution**
- REL, PTGER4, NKX2-3, STAT3, ERRF11, HNF4A, PLA2G2A/E

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

**Paneth cells**
- ITLN1*, NOD2*, ATG16L1*, XBP1*

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

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

**Antigen presentation**
- ERAP2*, LNPEP, DENND1B

**IL-23/TH17**
- IL23R*, JAK2, TYK2*, STAT3, ICOSLG, IL21, TNFSF15*

**T-cell regulation**
- NDFIP1, TNFSF8, TAGAP, IL2, IL2RA, TNFRSF9, PIM3, IL7R*, IL12B, IL23R*, PRDM1, ICOSLG, TNFSF8, IFNG, IL21

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

**Immune tolerance**
- IL10, IL27*, SBTN2, CREM, IL1R1/IL1R2, NOD2*

*Coding mutation
GOOD NEWS!

71 Genes Found!
GOOD NEWS???

Have list of associated genes/loci...
BUT NOW WHAT?
Characterize Associations

• Test known coding SNPs in linkage disequilibrium with associated SNPs
### Non-Synonymous SNPs: CD

<table>
<thead>
<tr>
<th>No.</th>
<th>Table 1, 2 &amp;</th>
<th>lead SNP</th>
<th>coding SNP</th>
<th>Risk allele - Allele frequency in control population</th>
<th>dbSNP ID</th>
<th>pos (dbSNP 130)</th>
<th>distance to lead SNP [kb]</th>
<th>Refgene</th>
<th>amino acid substitution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>rs780093</td>
<td>rs1260326</td>
<td>T - 0.418</td>
<td>2p23</td>
<td>27,742,603</td>
<td>11.66</td>
<td>GCGR</td>
<td>L446P</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>rs10495903</td>
<td>rs35720761; rs7578597</td>
<td>T - 0.129</td>
<td>2p21</td>
<td>43,660,422</td>
<td>286.94; 74.10</td>
<td>THADA</td>
<td>C1605Y; T1187A</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>rs6738825</td>
<td>rs1064213</td>
<td>A - 0.473</td>
<td>2q33</td>
<td>198,605,140</td>
<td>53.35</td>
<td>PLCL1</td>
<td>V667I</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>rs2549794Q</td>
<td>rs2549782</td>
<td>C - 0.409</td>
<td>5q15</td>
<td>96,270,305</td>
<td>13.55</td>
<td>ERAP2</td>
<td>K392N</td>
</tr>
<tr>
<td>5</td>
<td>21</td>
<td>rs4077515</td>
<td>rs3812571</td>
<td>T - 0.411</td>
<td>9q34</td>
<td>138,386,317</td>
<td>8.80</td>
<td>CARD9</td>
<td>S12N</td>
</tr>
<tr>
<td>6</td>
<td>29</td>
<td>rs8005161</td>
<td>rs1805078</td>
<td>T - 0.119</td>
<td>14q35</td>
<td>87,542,348</td>
<td>21.83</td>
<td>GALC</td>
<td>R184C</td>
</tr>
<tr>
<td>7</td>
<td>31</td>
<td>rs1511181</td>
<td>rs180743</td>
<td>G - 0.386</td>
<td>16p11</td>
<td>28,398,018</td>
<td>17.13</td>
<td>APOB48R</td>
<td>P419A</td>
</tr>
<tr>
<td>8</td>
<td>33</td>
<td>rs12720356</td>
<td>rs181206</td>
<td>G - 0.848</td>
<td>19q13</td>
<td>10,330,975</td>
<td>22.89</td>
<td>IL27</td>
<td>L119P</td>
</tr>
<tr>
<td>9</td>
<td>35</td>
<td>rs281379</td>
<td>rs601338; rs602662</td>
<td>A - 0.487</td>
<td>19q13</td>
<td>53,906,086</td>
<td>7.60; 7.29</td>
<td>FUT2</td>
<td>W154X; G258S</td>
</tr>
<tr>
<td>10</td>
<td>36</td>
<td>rs4809330</td>
<td>rs3208008</td>
<td>G - 0.709</td>
<td>20q13</td>
<td>61,820,030</td>
<td>23.48</td>
<td>RTEL1</td>
<td>Q1042H</td>
</tr>
<tr>
<td>11</td>
<td>37</td>
<td>rs181359</td>
<td>rs2298428</td>
<td>T - 0.203</td>
<td>22q11</td>
<td>20,258,641</td>
<td>54.25</td>
<td>YDJC</td>
<td>A263T</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>rs1120926</td>
<td>rs11479091</td>
<td>G - 0.907</td>
<td>1p31</td>
<td>67,705,958</td>
<td>3.42</td>
<td>IL23R</td>
<td>R381Q</td>
</tr>
<tr>
<td>13</td>
<td>2</td>
<td>rs2476601</td>
<td>same</td>
<td>G - 0.907</td>
<td>1p13</td>
<td>114,179,091</td>
<td></td>
<td>PTPN22</td>
<td>W602R</td>
</tr>
<tr>
<td>14</td>
<td>5</td>
<td>rs7554511</td>
<td>rs296520</td>
<td>C - 0.726</td>
<td>1q32</td>
<td>199,144,185</td>
<td>3.42</td>
<td>CIORF106</td>
<td>R453C</td>
</tr>
<tr>
<td>15</td>
<td>6</td>
<td>rs3792109</td>
<td>rs2241880</td>
<td>A - 0.529</td>
<td>2q37</td>
<td>233,849,156</td>
<td>1.05</td>
<td>ATG16L1</td>
<td>T300A</td>
</tr>
<tr>
<td>16</td>
<td>7</td>
<td>rs3197999</td>
<td>same</td>
<td>A - 0.297</td>
<td>3p21</td>
<td>49,696,536</td>
<td>17.95</td>
<td>MST1</td>
<td>R703C</td>
</tr>
<tr>
<td>17</td>
<td>9</td>
<td>rs12521868</td>
<td>rs1050152</td>
<td>T - 0.422</td>
<td>5q31</td>
<td>131,812,292</td>
<td>108.07</td>
<td>BSN</td>
<td>A741T</td>
</tr>
<tr>
<td>18</td>
<td>13</td>
<td>rs1799964</td>
<td>rs2259435</td>
<td>C - 0.209</td>
<td>6p21</td>
<td>31,650,287</td>
<td>45.39</td>
<td>MCCC1</td>
<td>E42K</td>
</tr>
<tr>
<td>19</td>
<td>24</td>
<td>rs11564258</td>
<td>rs2229094</td>
<td>same</td>
<td>12q12</td>
<td>40,792,300</td>
<td>1.75</td>
<td>LTA</td>
<td>C13R</td>
</tr>
<tr>
<td>20</td>
<td>25</td>
<td>rs3764147</td>
<td>rs11557467</td>
<td>A - 0.025</td>
<td>13q14</td>
<td>43,355,925</td>
<td>12.13</td>
<td>MUC19</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>27</td>
<td>rs2872507</td>
<td>rs2345480; rs2305479</td>
<td>A - 0.458</td>
<td>17q21</td>
<td>35,294,289</td>
<td>21.43; 21.45</td>
<td>GMSDL</td>
<td>P2895; G282R</td>
</tr>
<tr>
<td>22</td>
<td>28</td>
<td>rs11871801</td>
<td>rs11557467</td>
<td>A - 0.756</td>
<td>17q21</td>
<td>37,824,298</td>
<td>151.26</td>
<td>MLX</td>
<td>Q233R</td>
</tr>
</tbody>
</table>

Characterize Associations

- Test known coding SNPs in linkage disequilibrium with associated SNPs
- Fine-map genomic regions \((ZNF365)\)
Crohn’s Disease ~2008

- IL23R
- ATG16L1
- MST1
- IBD5 locus (5q31-33)
- NOD2
- TNFSF15
- PTPN2
- 5q21
- IRGM
- 10q21
- NKX2-3
- MHC

GFO Rail Co.
CD, 10q21, and ZNF365

- **rs224136** (NIDDK Consortium; Rioux *et al* 2007)
  - Intergenic
  - $p = 7.9 \times 10^{-6}$ discovery, $n = \sim 1000$ CD, $\sim 1000$ controls
  - $p = 2.9 \times 10^{-7}$ replication, $n = 530$ trios + $\sim 350$ CD, $\sim 200$ controls
  - Ileal CD

- **rs10761659** (The Wellcome Trust Case Control Consortium 2007; IIBDGC Consortium 2010)
  - Intergenic
  - $p = 2.7 \times 10^{-7}$, $n = \sim 2000$ CD, $\sim 3000$ controls
  - $p = 4.4 \times 10^{-22}$, $n = \sim 6000$ CD, $\sim 15,000$ controls

- **rs10995271** (International meta-analysis; Barrett *et al* 2008)
  - Intergenic
  - $p = 1.9 \times 10^{-11}$ discovery, $n = \sim 3000$ CD, $\sim 4800$ controls
  - $p = 1.6 \times 10^{-10}$ replication, $n = \sim 3600$ CD
Chr10 Fine-Mapping SNP Design

ZNF365 isoform D

A → B → C → D

63,798,139 → 64,219,617

10q21
rs7076156 Explains Observed Association

<table>
<thead>
<tr>
<th>SNP</th>
<th>Position</th>
<th>Minor</th>
<th>P-value</th>
<th>OR</th>
<th>Cond. P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs10740085</td>
<td>64065801</td>
<td>A</td>
<td>4.33E-06</td>
<td>0.72</td>
<td>0.91</td>
</tr>
<tr>
<td>rs12768538</td>
<td>64076364</td>
<td>G</td>
<td>4.21E-04</td>
<td>1.24</td>
<td>0.18</td>
</tr>
<tr>
<td>rs7068361</td>
<td>64078373</td>
<td>G</td>
<td>2.04E-04</td>
<td>0.80</td>
<td>0.58</td>
</tr>
<tr>
<td>rs7071642</td>
<td>64084066</td>
<td>G</td>
<td>9.38E-07</td>
<td>0.72</td>
<td>NA</td>
</tr>
<tr>
<td>rs7076156</td>
<td>64085190</td>
<td>A</td>
<td>5.23E-07</td>
<td>0.72</td>
<td>NA</td>
</tr>
<tr>
<td>rs729739</td>
<td>64100308</td>
<td>A</td>
<td>7.38E-05</td>
<td>0.77</td>
<td>0.19</td>
</tr>
<tr>
<td>*rs10995271</td>
<td>64108492</td>
<td>G</td>
<td>2.06E-04</td>
<td>1.25</td>
<td>0.08</td>
</tr>
<tr>
<td>rs12766391</td>
<td>64111210</td>
<td>A</td>
<td>1.17E-04</td>
<td>1.26</td>
<td>0.06</td>
</tr>
<tr>
<td>*rs10761659</td>
<td>64115570</td>
<td>A</td>
<td>3.56E-04</td>
<td>0.81</td>
<td>0.48</td>
</tr>
<tr>
<td>rs224120</td>
<td>64115766</td>
<td>A</td>
<td>5.84E-05</td>
<td>0.77</td>
<td>0.35</td>
</tr>
<tr>
<td>rs224123</td>
<td>64121239</td>
<td>G</td>
<td>6.38E-04</td>
<td>1.22</td>
<td>0.01</td>
</tr>
</tbody>
</table>

rs7076156 Resides in an Exon Specific to ZNF365 Isoform D

- Nonsynonymous SNP (G-A; Ala62Thr) in exon 4 of ZNF365 isoform D
- Minor allele (Threonine) of Ala62Thr protects against CD
  - Odds Ratio = 0.72 (95%CI 0.63-0.82)
  - Frequency (Thr): 24.0% CD (allele A) and 30.3% controls

Reduced ZNF365D expression in CD with risk genotype

- ELCLs from CD patients demonstrated lower levels of ZNF365D expression compared to non-IBD controls
- ELCLs from CD patients with risk GG genotype further exhibited lower levels of expression compared to CD subjects with protective genotype
- Genome-wide expression study suggests that this variant, along with disease status, is associated with significant differences in the expression of other genes, including ZNF148
- Mice homozygous for ZNF148-delN exhibit increased susceptibility to induced colitis, highlighting a potential role for ZNF148 in GI homeostasis

Collaborators in IBD Genetics

Cedars-Sinai Medical Center

Medical Genetics
Kent D. Taylor
Debbie Duttridge
Talin Haritunuians
Michelle Jones
Ling Mei
Yoanna Picornell
Sheila Pressman
Lily King
Emebet Mengesha
Nikki Nguyen
Kazu Sugimura
Hiroo Toyoda
Sheena Lin
Shannon Rhodes
Huiying Yang
Jun Xu
YD Ida Chen
Xiaofei Yan
Soonil Kwon
Dalin Li
Xiuqing Guo
Jerome I. Rotter

Gastroenterology/Immunology
Dermot McGovern
Marla Dubinsky
Eric Vasiliauskas
William Mow
Phillip Fleshner
Lore Karp
Kostas Papadakis
Katherine Michelsen
Andrew Ippoliti
Carol Landers
John Prehn
Richard Deem
Rivkah Gonsky
Maria Abreu
Scott Plevy
Masayuki Saruta
Hide Takedatsu
Gil Y. Melmed
Shane M. Devlin
David Shih
Stephan R. Targan

IBD Genetics Consortium

Cardiovascular Health Research Unit, Seattle
Josh Bis
Kristin Marcian
David Siscovick
Bruce Psaty

University of Alabama, Birmingham
Charles O. Elson

La Jolla Institute for Allergy and Immunology
Mitchell Kronenberg

Children’s Hospital of Oakland Research Institute
Beth Trachtenberg
Jill Hollenbach
Henry Erlich

UCLA Pathology
Bo Wei
David Ziring
Jonathan Braun

University of Puerto Rico
Nelly Rabel
Federico Gregory
Roberto Mera
Roberto Vendrell
Esther A. Torres