

# Finding new therapeutic targets through genetics & sequencing

Judy H. Cho, M.D.

Yale University

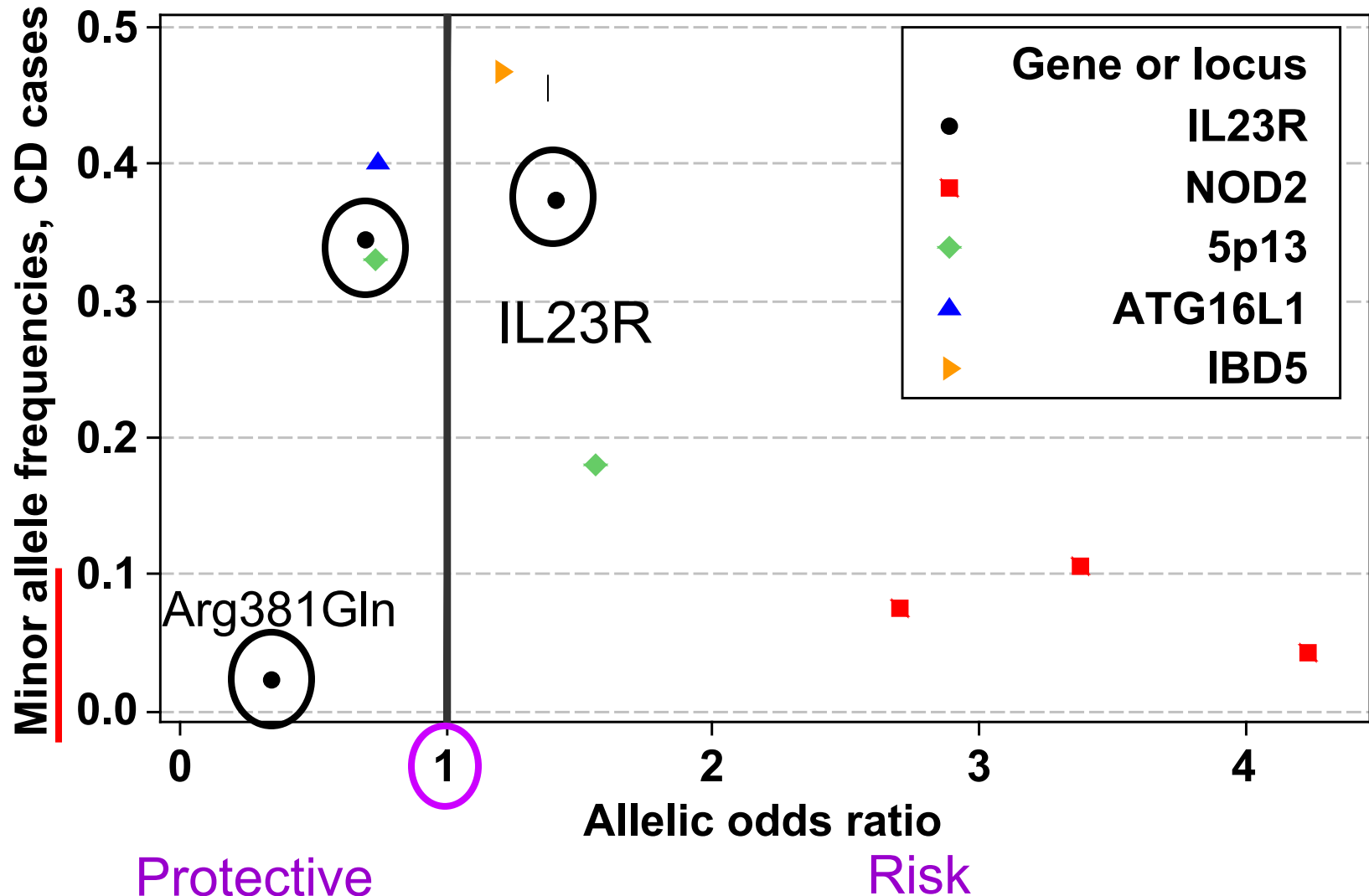
6.29.2012

# Overview

- ▣ Examples from inflammatory bowel disease
  - ▣ IL-23 pathway
  - ▣ NOD2, mycobacterial diseases, & innate immune cells
  - ▣ TNF pathway
- ▣ Systematically leveraging high throughput sequencing to prioritize new targets
  - ▣ Phenotype driven
  - ▣ Genotype (Encode data) driven

# The IL-23 pathway in immune-mediated diseases

# Multiple signals in IL23R gene region: uncommon protective Arg381Gln allele



# IL-23 signaling (Th17 cells)

5/7 members of the primary IL-23 pathway associated in IBD

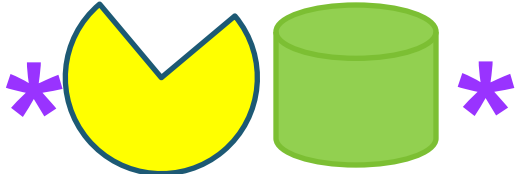
IL23A(p19) chr12q13  
IL12B(p40) chr5q33

Cytokine

Receptor

IL23R  
chr1p31

IL12RB1  
chr19p13



JAK2 chr9p24  
TYK2 chr19p13

STAT3  
chr17q21



- Th17 cells:
- Patrol mucosal surfaces
  - Fungal and bacterial defense

**\* IBD associated**

# Arg381Gln protective allele in IL23R is a loss-of-function allele

OPEN ACCESS Freely available online

PLoS one

## The IL23R R381Q Gene Variant Protects against Immune-Mediated Diseases by Impairing IL-12/23 Th17 Effector Response in Humans

Paola Di Meglio<sup>1</sup>, Antonella Di Cesare<sup>1,3</sup>, Ute Laan<sup>1</sup>,  
Villanova<sup>1</sup>, Isabella Tosi<sup>1</sup>, Francesca C...

<sup>1</sup> St. John's Institute of Dermatology, King's College London

## Inflammatory disease protective R381Q IL23 receptor polymorphism results in decreased primary CD4+ and CD8+ human T-cell functional responses

Ritu Sarin, Xingxin Wu, and Clara Abraham<sup>1</sup>

Department of Internal Medicine, Section of Digestive Diseases, Yale University, New Haven, CT 06520

Edited by Warren Leonard, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, and accepted by the Editorial Board April 27, 2011 (received for review November 29, 2010)

The SNP (c.1142G > A;p.R381Q) in the IL-23 receptor (IL23R) confers protection from multiple inflammatory diseases, representing one

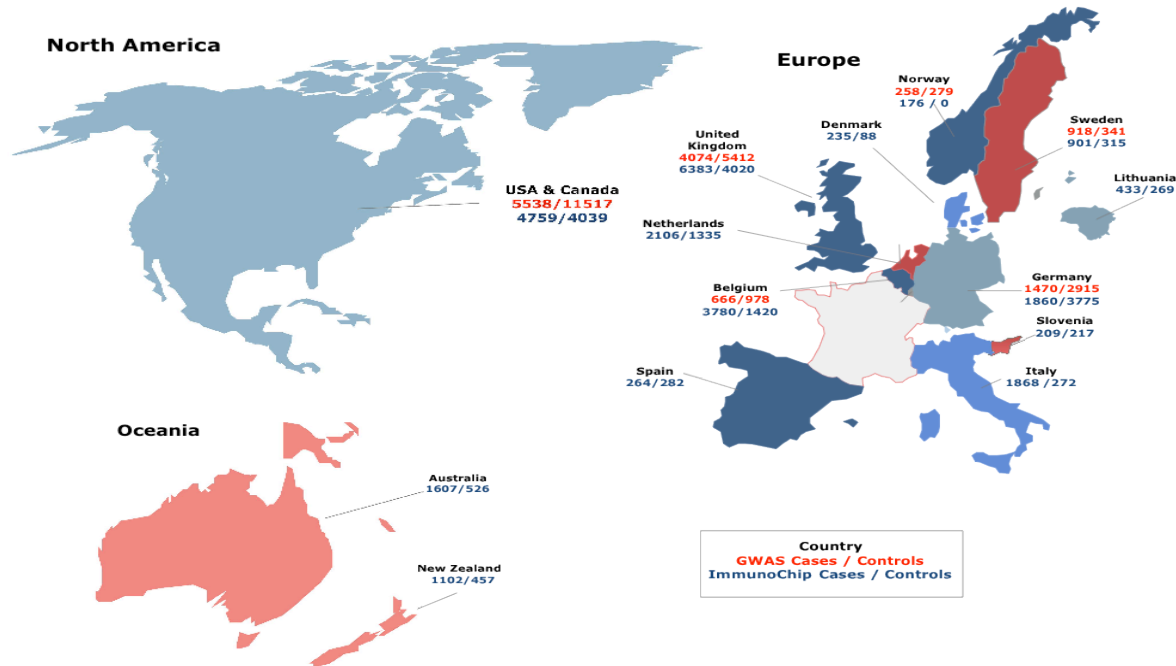
of immune response. Controls not carrying the R381Q IL23R allele. Because of increased IL-23 expression on memory CD4+ T cells (13

□ Anti-p40 treatment (blocks IL12/23):

- Approved in psoriasis
- IBD phase III studies ongoing
- Issues: How to block IL23 pathway?
  - Blocking IL-23 alone vs. IL12/23??
  - Blockade at what level? Receptor? JAK?

# NOD2, mycobacterial disease & innate immune cells

# The ImmunoChip effort in IBD: a large scale international collaboration

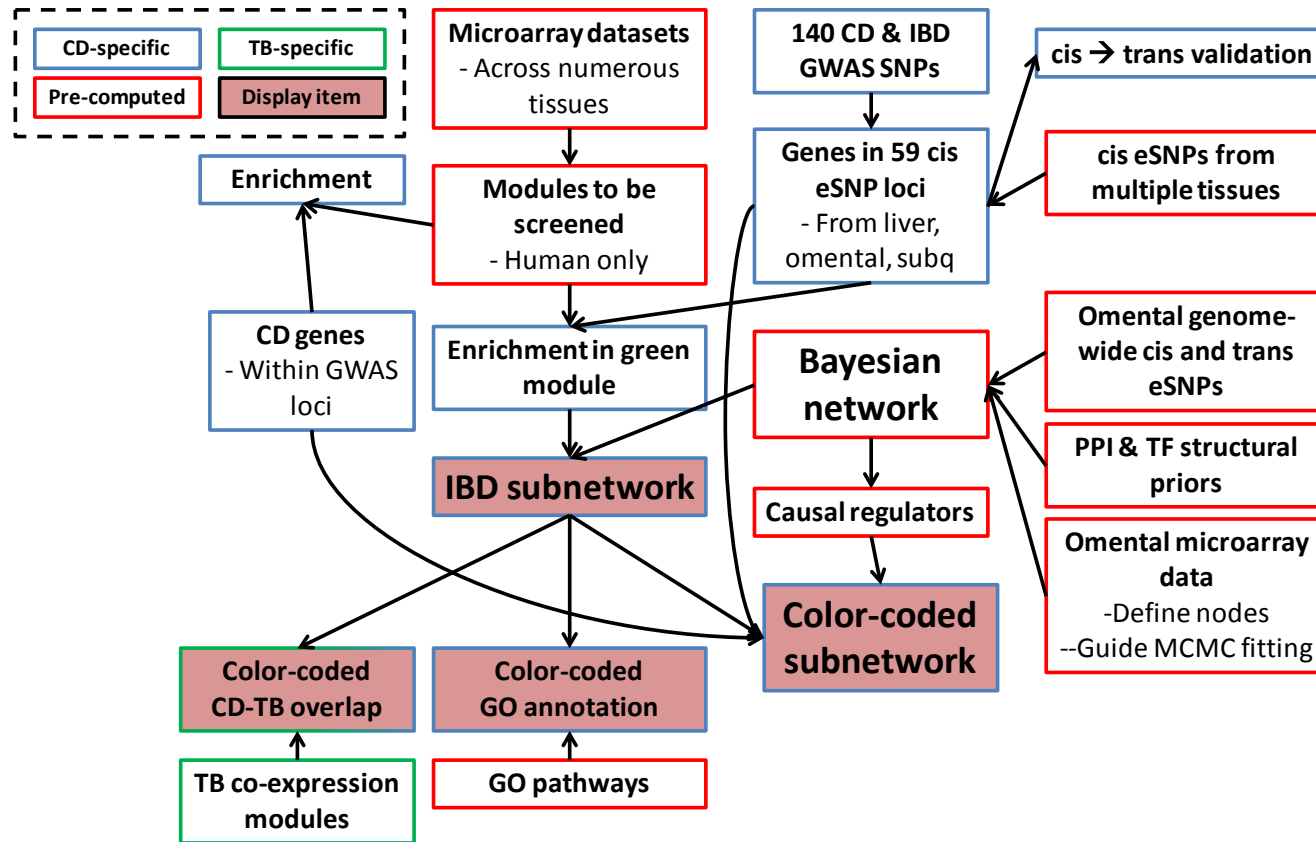


- 38,565 cases, 37,747 controls
- 71 new loci → 163 loci with genome-wide significant association (~1500 genes)

Jeff Barrett  
Luke Jostins



# 163 loci → improved network analysis—key role of *directionality*



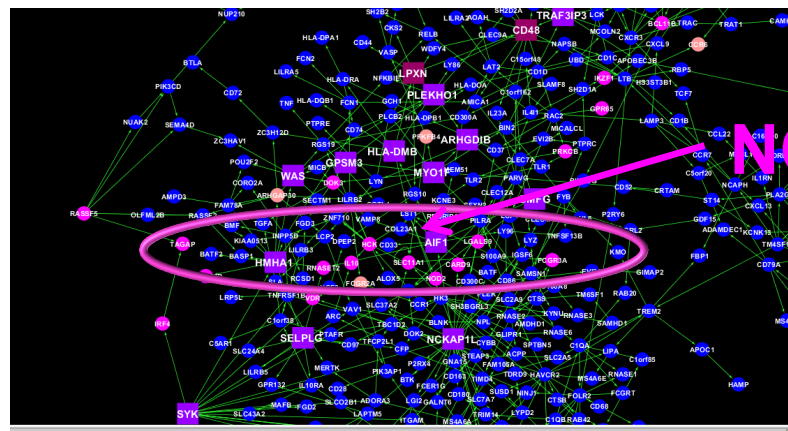
- Top module: omental adipose (macrophage enriched) from obese patients

Eric Schadt  
Ken Hui

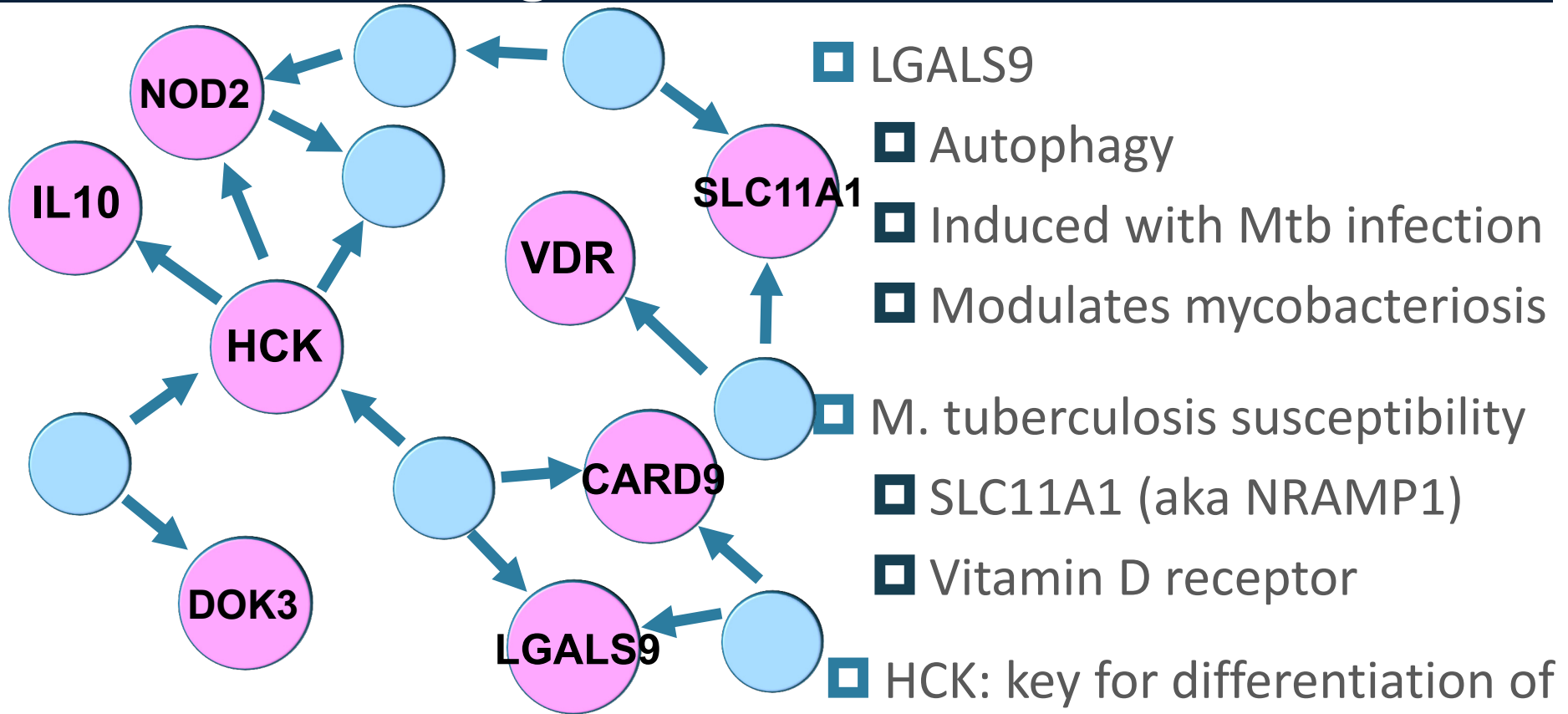
# 163 loci → improved network based analyses based on gene co-expression

- Co-expression modules: tracking similar gene expression based on large microarray datasets
- The co-expression module with the greatest enrichment of IBD-associated genes: 523 gene module in omental adipose tissue (macrophage-enriched gene expression)—value of direct ex-vivo tissue analysis

● *Gene in IBD-associated locus*



# NOD2-centric view of the submodule: 7 IBD-associated genes near NOD2



*Highly correlated RNA expression between NOD2, IL10 & HCK (hematopoietic cell kinase)*

# The TNF pathway

# IBD is a TNF-mediated disorder

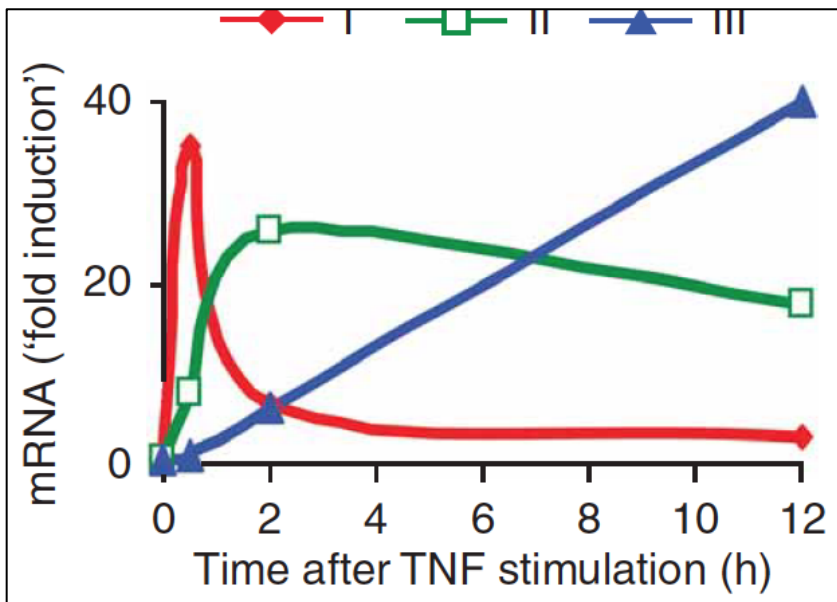
- ▣ TNF-overexpressing mice develop ileitis and arthritis
- ▣ Anti-TNF is a highly effective treatment for IBD
- ▣ GWAS: multiple TNF-mediated signals
  - ▣ NF- $\kappa$ B (NFKB1, REL, RELA, TNFAIP3)
- ▣ TNF: crucial in pathogen eradication—reactivation of tuberculosis a side effect of anti-TNF therapy

# Molecular integration of TNF and 3'UTRs: crucial role of kinetics/functional responses

nature  
immunology

The stability of mRNA influences the temporal order of the induction of genes encoding inflammatory molecules

Shengli Hao & David Baltimore



TNF  
A20 (TNFAIP3)

CCL2—max association in 3'UTR

Kinetics of gene expression: multiple ub/dub associations: NDFIP1, CPEB4, CUL2, UBE2L3, as well as TNFAIP3 (15 loci involved ( $p < 0.001$ ))

Few AREs (<2), very stable mRNA

Some AREs (2-4), moderately stable mRNA

Many AREs (4-10), unstable mRNA

## Systematically leveraging high throughput sequencing to prioritize new targets: phenotype to genotype (1)

- LOF, protective alleles as ideal therapeutic targets
  - PCSK9 & CAD
  - IL23R & psoriasis/IBD/ankylosing spondylitis
  - CCR5 & HIV
  - IFIH1 & T1DM?
- Value of sequencing
  - Targeted re-sequencing of GWAS signals: enormous structure-function data useful for improved targeting

## Systematically leveraging high throughput sequencing to prioritize new targets: phenotype to genotype (2)

- Early onset, severe cases: medical resequencing
  - LOF IL10 pathway genes → bone marrow transplantation
  - New biology: Nick Volker—young boy with early onset IBD → XIAP mutation (essential for NOD2-signaling)
- -omics data & systems biology
  - RNASeq: improved quantification should improve predictive models
  - Systematic interrogation of disease-associated transcription factors: ChIPSeq
- Cross-phenotype analyses: immune-mediated diseases & infectious diseases



# Striking overlap of loci between diseases: the genetics of infectious diseases

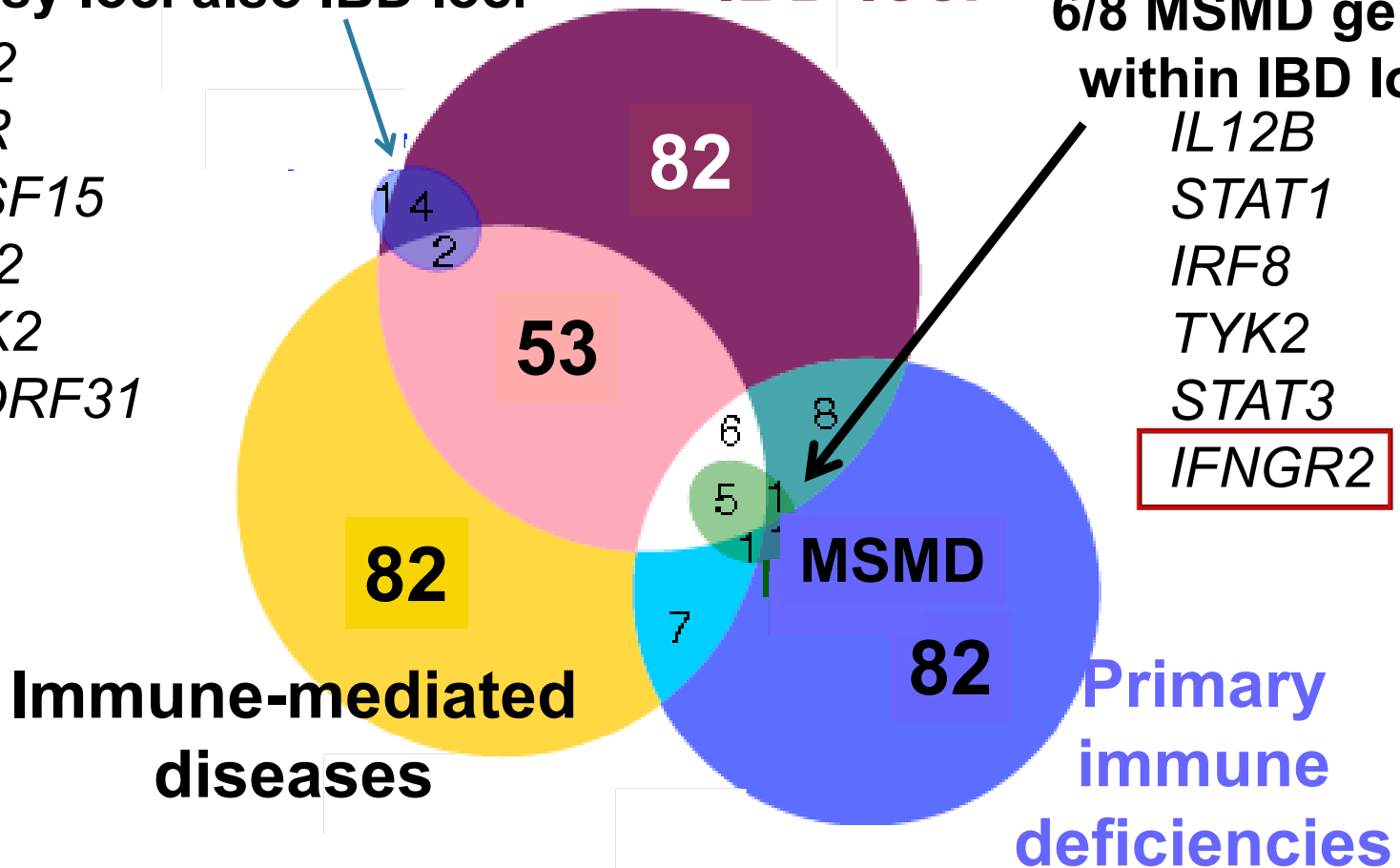
6/7 leprosy loci also IBD loci

*NOD2*  
*IL23R*  
*TNFSF15*  
*RIPK2*  
*LRRK2*  
*C13ORF31*

**IBD loci**

6/8 MSMD genes within IBD loci

*IL12B*  
*STAT1*  
*IRF8*  
*TYK2*  
*STAT3*  
*IFNGR2*



MSMD, Mendelian susceptibility to mycobacterial disease

# Genotype to phenotype: rare coding mutations and gains of functional moieties

## Gains of glycosylation comprise an unexpectedly large group of pathogenic mutations

Guillaume Vogt<sup>1</sup>, Ariane Chappier<sup>1</sup>, Kun Yang<sup>1,2</sup>, Nadia Chuzhanova<sup>3,4</sup>, Jacqueline Feinberg<sup>1</sup>, Claire Fieschi<sup>1,5</sup>, Stéphanie Boisson-Dupuis<sup>1</sup>, Alexandre Alcais<sup>1</sup>, Orchidée Filipe-Santos<sup>1</sup>, Jacinta Bustamante<sup>1</sup>, Ludovic de Beaucoudrey<sup>1</sup>, Ibrahim Al-Mohsen<sup>6</sup>, Sami Al-Hajjar<sup>6</sup>, Abdulaziz Al-Ghoniaim<sup>6</sup>, Parisa Adimi<sup>7</sup>, Mehdi Mirsaedi<sup>7</sup>, Soheila Khalilzadeh<sup>7</sup>, Sergio Rosenzweig<sup>8,17</sup>, Oscar de la Calle Martin<sup>9</sup>, Thomas R Bauer<sup>10</sup>, Jennifer M Puck<sup>11</sup>, Hans D Ochs<sup>12</sup>, Dieter Furthner<sup>13</sup>, Carolin Engelhorn<sup>14</sup>, Bernd Belohradsky<sup>14</sup>, Davood Mansouri<sup>7</sup>, Steven M Holland<sup>8</sup>, Robert D Schreiber<sup>15</sup>, Laurent Abel<sup>1</sup>, David N Cooper<sup>4</sup>, Claire Soudais<sup>1</sup> & Jean-Laurent Casanova<sup>1,2,16</sup>

Mutations involving gains of glycosylation have been considered rare, and the pathogenic role of the new carbohydrate chains has never been formally established. We identified three children with mendelian susceptibility to mycobacterial disease who were homozygous with respect to a missense mutation in *IFNGR2* creating a new N-glycosylation site in the IFN $\gamma$ R2 chain. The resulting additional carbohydrate moiety was both necessary and sufficient to abolish the cellular response to IFN $\gamma$ . We then searched the Human Gene Mutation Database for potential gain-of-N-glycosylation missense mutations; of 10,047 mutations in 577 genes encoding proteins trafficked through the secretory pathway, we identified 142 candidate mutations (~1.4%) in 77 genes (~13.3%). Six mutant proteins bore new N-linked carbohydrate moieties. Thus, an unexpectedly high proportion of mutations that cause human genetic disease might lead to the creation of new N-glycosylation sites. Their pathogenic effects may be a direct consequence of the addition of N-linked carbohydrate.

# Genotype to phenotype: the Encode approach

- Covalent modifications: missense mutations &
  - Glycosylation
  - Phosphorylation
  - Ubiquitination/sumoylation
- Regulation of expression
  - Conserved sequences
  - AU-rich elements: RNA-binding protein sites in 3'UTR
  - TF-binding sites, miRNA-binding sites, splice sites
- Analysis and information dissemination: validity & magnitude of effects
  - Bioinformatic probability vs. experimental validation
  - Frequency, population specificity
  - Distinguishing negative selection from drift

# Acknowledgements

- NIDDK IBD Genetics Consortium
  - Steven Brant, Richard Duerr, Dermot McGovern, John Rioux, Mark Silverberg, Mark Daly
  - DCC: Phil Schumm, Yashoda Sharma, Clarence Zhang, Kaida Ning
- International IBD Genetics Consortium

