Finding new therapeutic targets through genetics & sequencing

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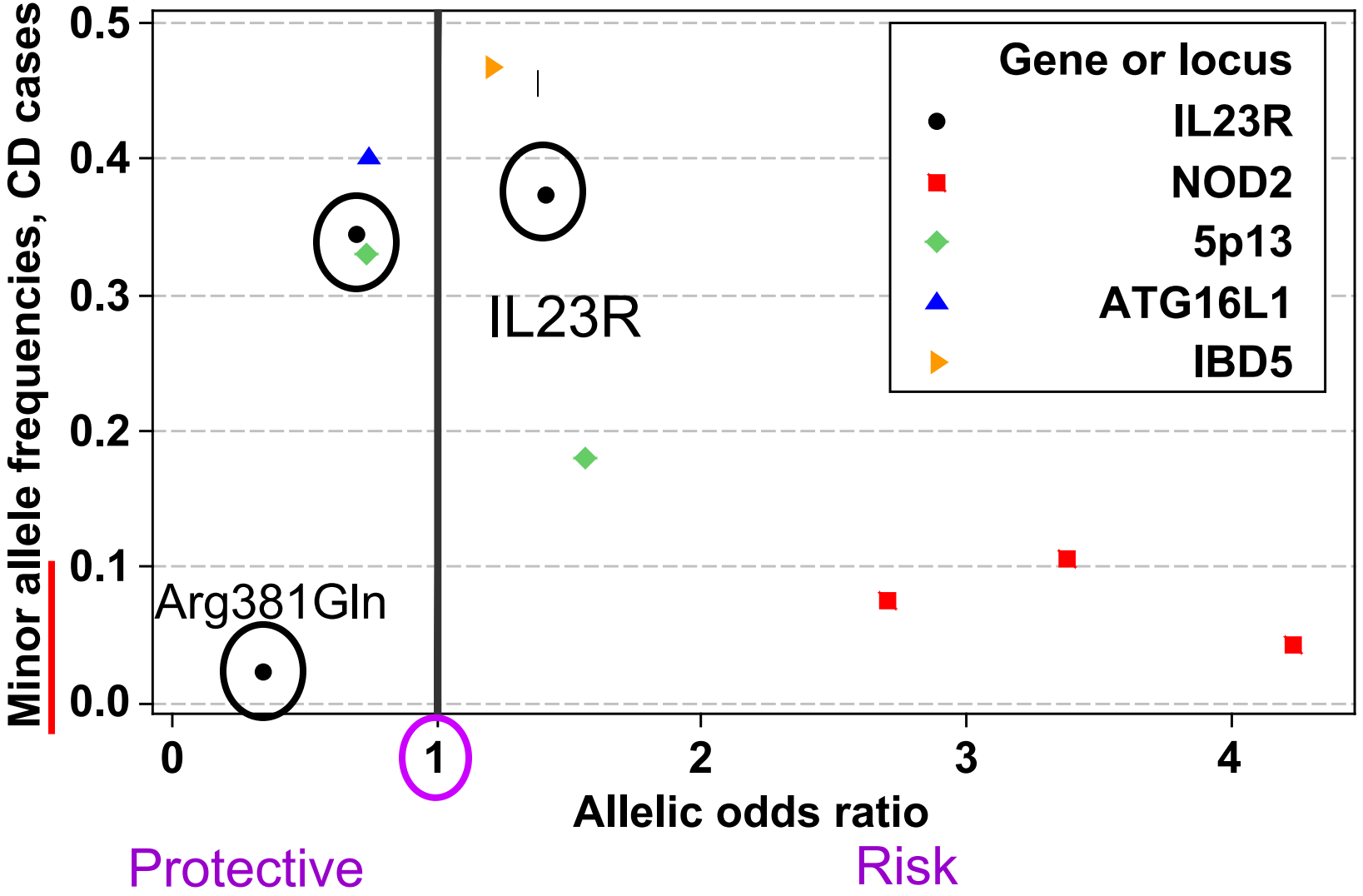
Overview

- 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

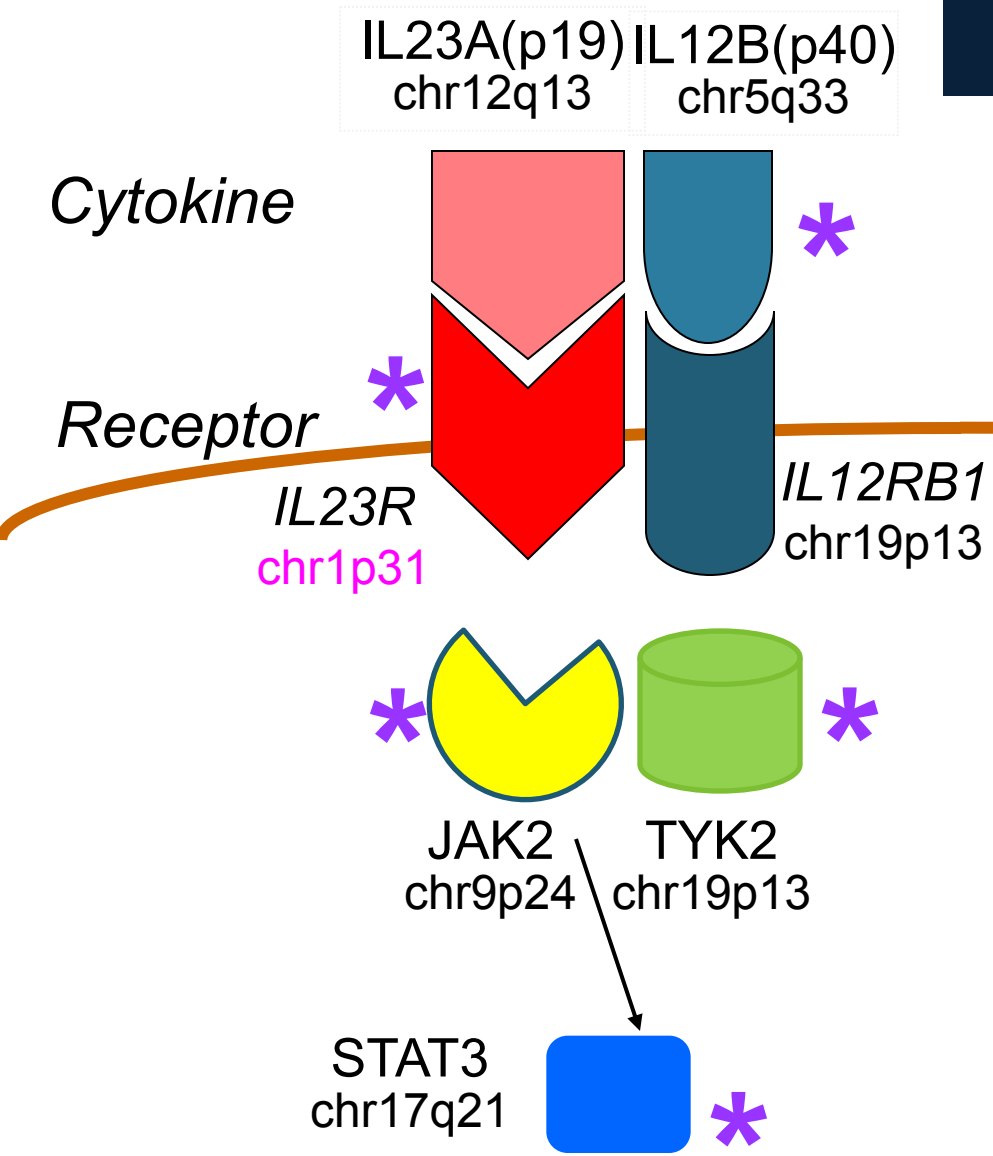
The IL-23 pathway in immune-mediated diseases

Multiple signals in IL23R gene region: uncommon protective Arg381Gln allele



IL-23 signaling
(Th17 cells)

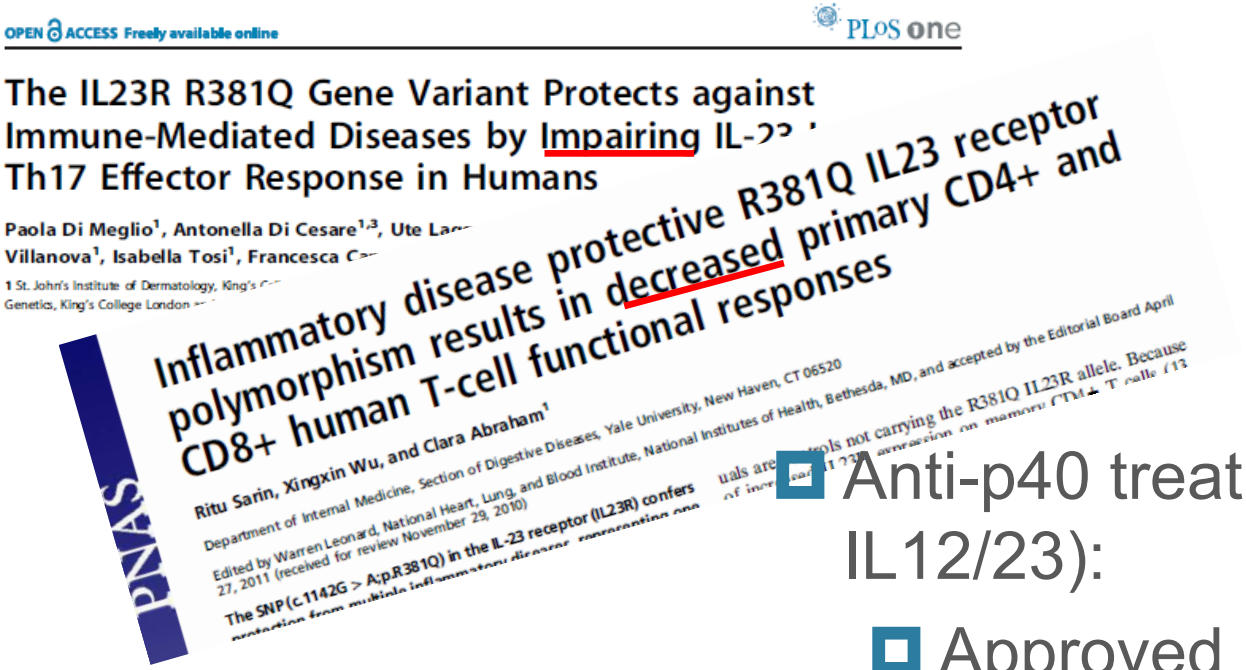
5/7 members of the primary IL-23 pathway associated in IBD



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-23-Dependent Th17 Effector Response in Humans

Paola Di Meglio[1], Antonella Di Cesare[1,3], Ute La... Villanova[1], Isabella Tosi[1], Francesca C...

1 St. John's Institute of Dermatology, King's C... Genetics, King's College London ...

PNAS

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

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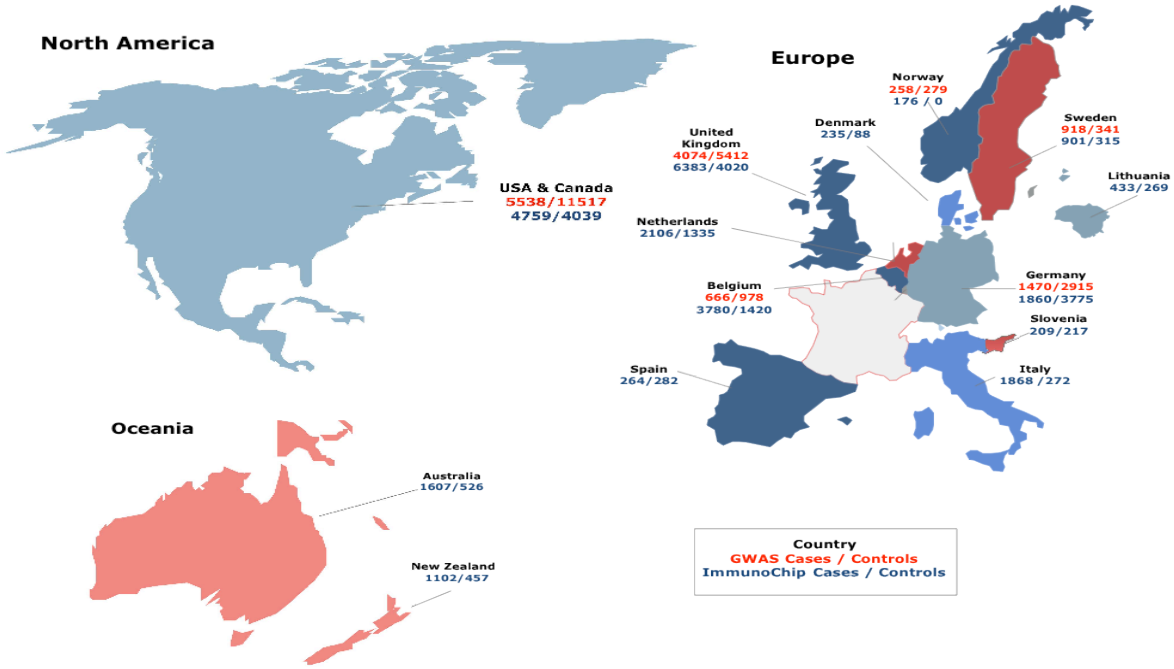
Edited by Warren Leonard, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, and accepted by the Editorial Board April 27, 2011 (received for review November 29, 2010)

The SNP (c.1142G > A;p.R381Q) in the IL-23 receptor (IL23R) confers ... uals are ...pols not carrying the R381Q IL23R allele. Because ... 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

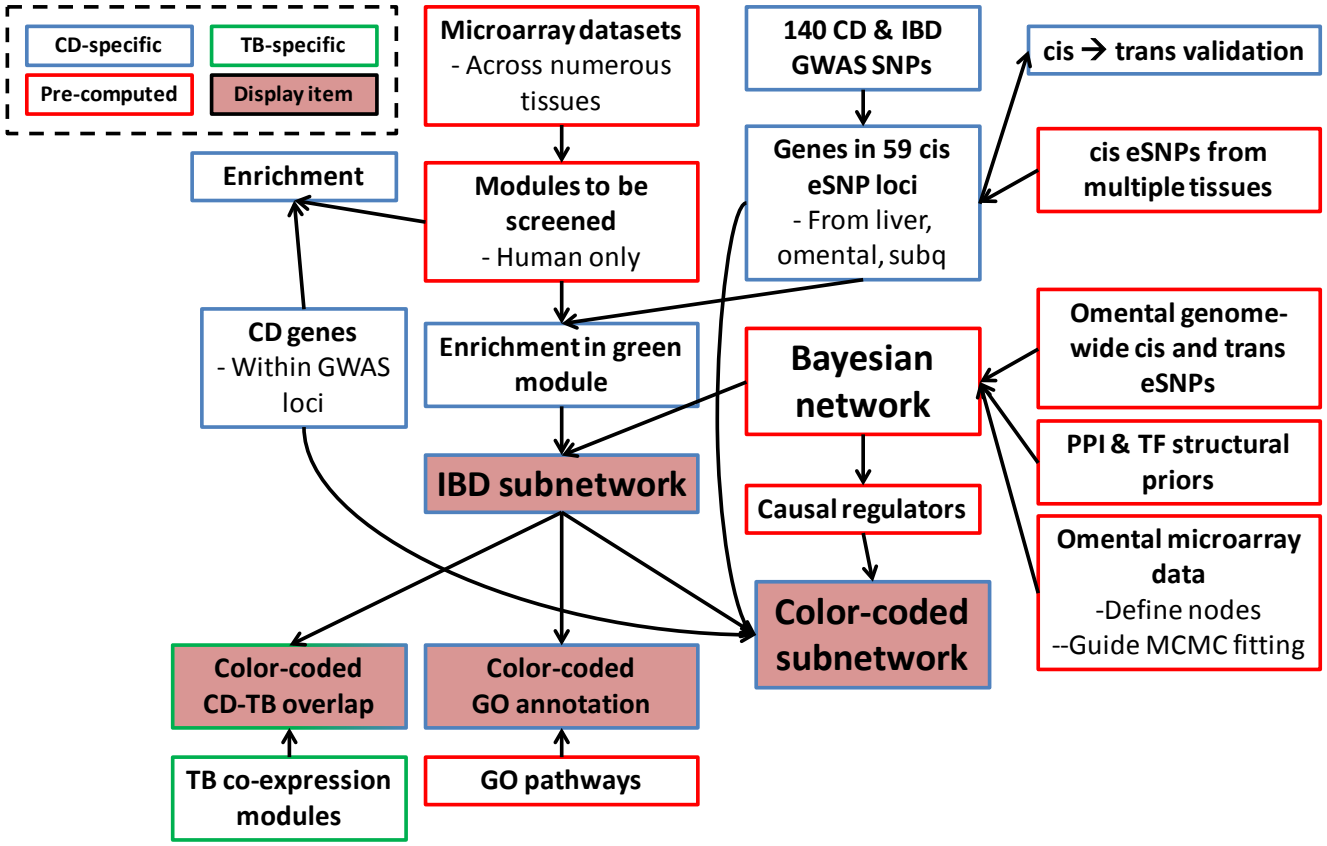
The Immunochip effort in IBD: a large scale international collaboration



- 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*



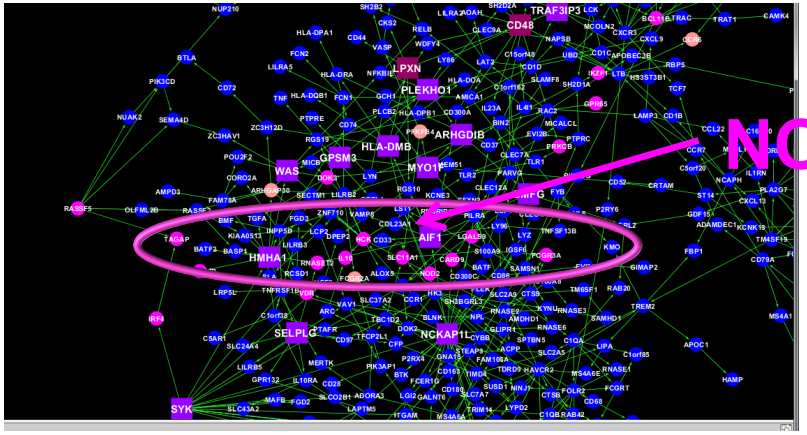
- Top module: omental adipose (macrophage enriched) from obese patients

Eric Schadt
Ken Hui

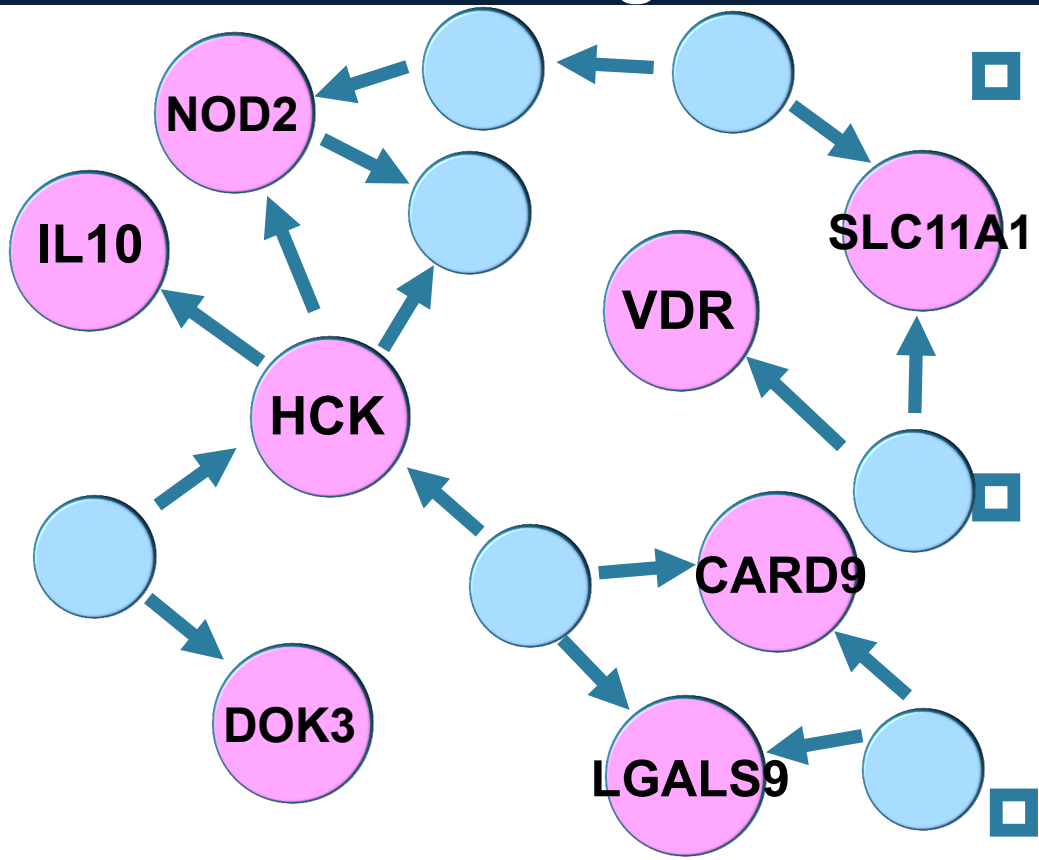
163 loci→ improved network based analyses based on gene co-expression

- 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)

- LGALS9
  - Autophagy
  - Induced with Mtb infection
  - Modulates mycobacteriosis
- M. tuberculosis susceptibility
  - SLC11A1 (aka NRAMP1)
  - Vitamin D receptor
- HCK: key for differentiation of M2 macrophages (anti-inflammatory→IL10)

The TNF pathway

IBD is a TNF-mediated disorder

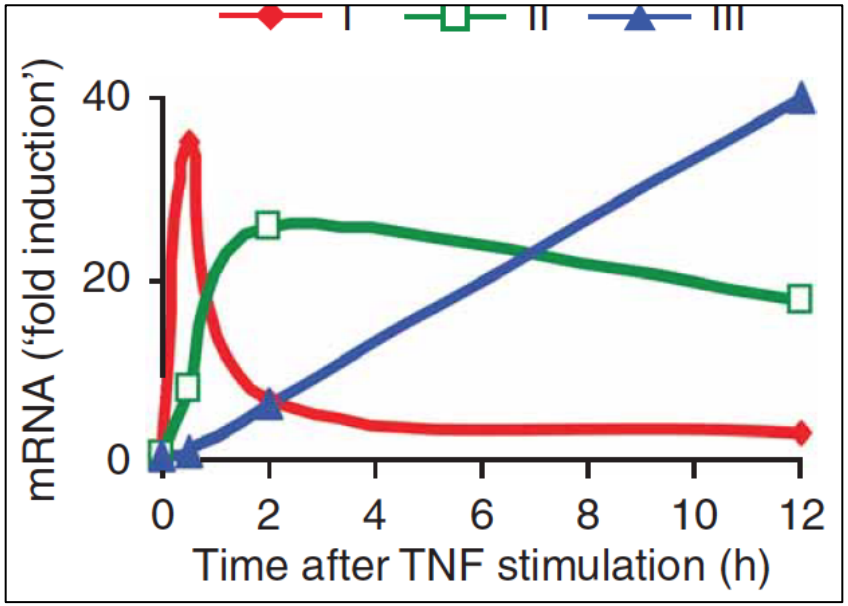
- TNF-overexpressing mice develop ileitis and arthritis
- Anti-TNF is a highly effective treatment for IBD
- GWAS: multiple TNF-mediated signals
  - NF-kB (NFKB1, REL, RELA, TNFAIP3)
- TNF: crucial in pathogen eradication—reactivation of tuberculosis a side effect of anti-TNF therapy

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 inolved ($p < 0.001$)

Few AREs (<2), very stable mRNA

Some AREs (2-4), moderately stable mRNA

Many AREs (4-10), unstable mRNA

Hao 2009

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

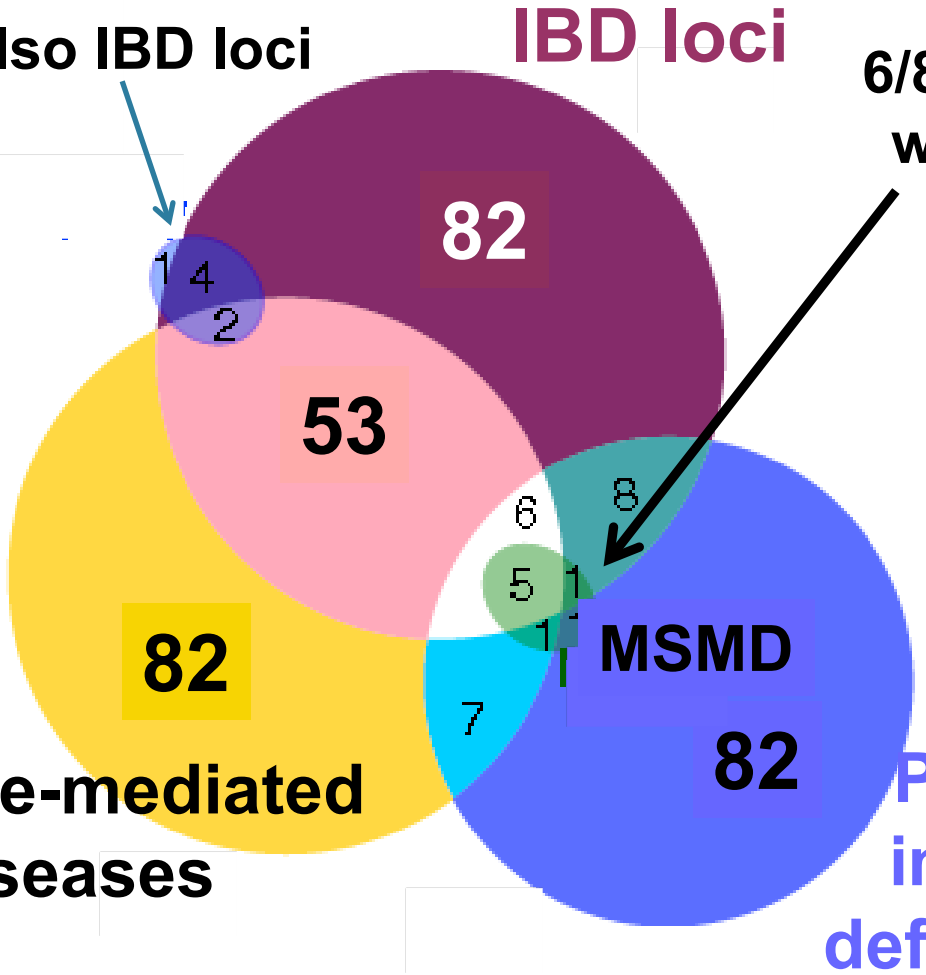
Systematically leveraging high throughput sequencing to prioritize new targets: phenotype to genotype (2)

- 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



6/8 MSMD genes within IBD loci

IL12B
STAT1
IRF8
TYK2
STAT3
IFNGR2

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

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 IFNγR2 chain. The resulting additional carbohydrate moiety was both necessary and sufficient to abolish the cellular response to IFNγ. 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

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- International IBD Genetics Consortium