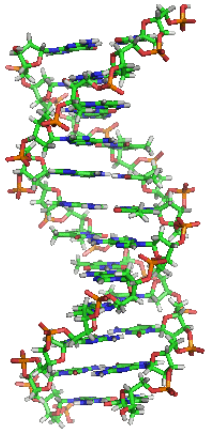
European Crohn's and Colitis Organisation

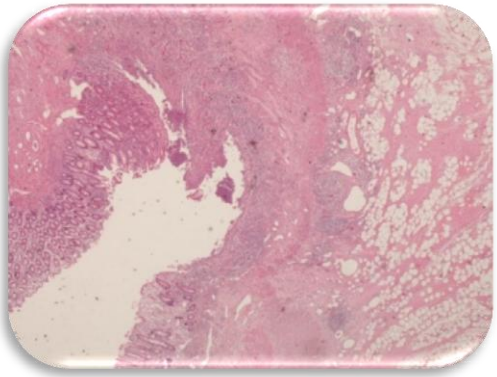
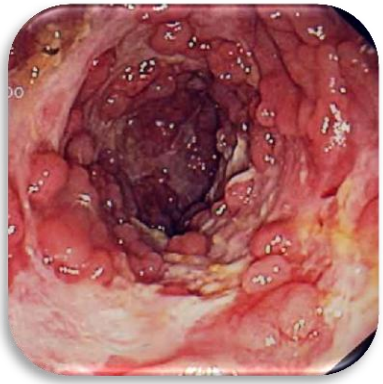
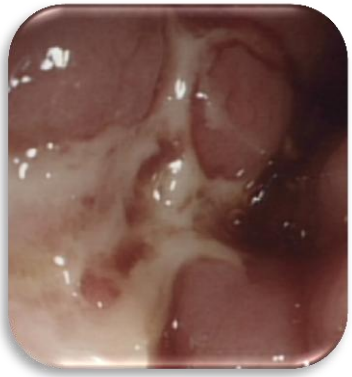
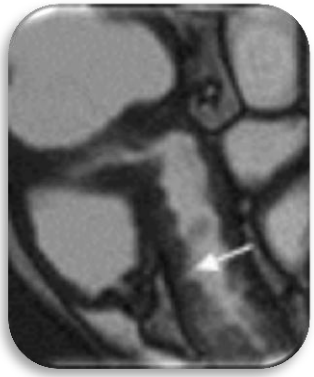
The Genetics of IBD

Charlie Lees
www.ibdgenetics.org



IBD Genetics

Barcelona, February 2012

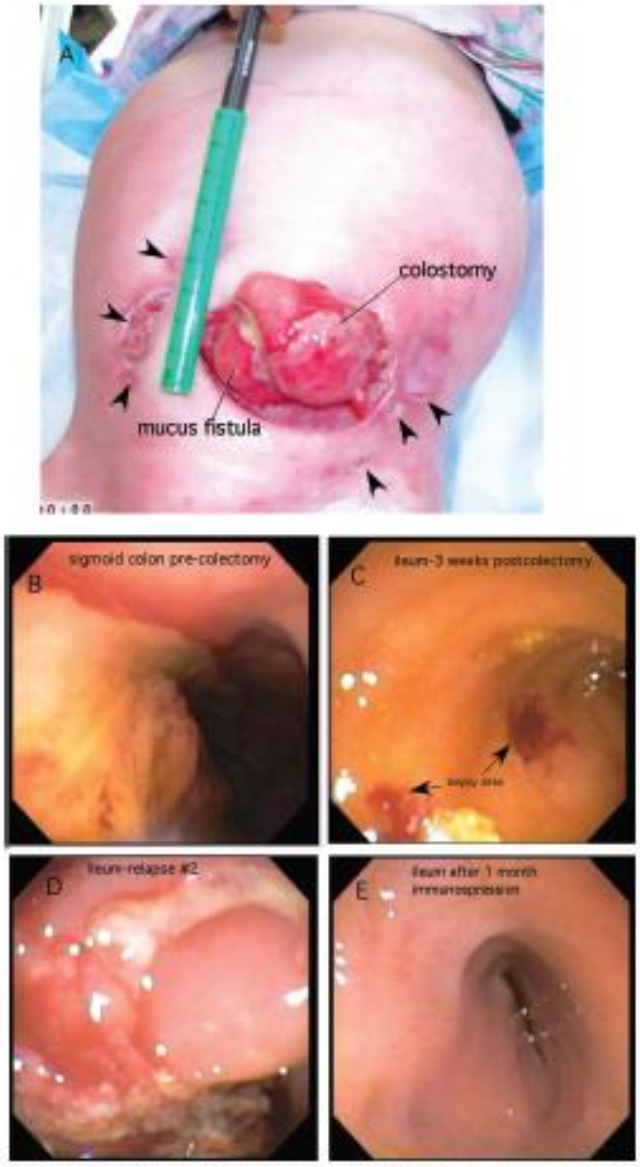


Fig. 1. Clinical presentation. A, Anterior abdomen showing the expanded abdominal wall defect containing colostomy and mucus fistula (arrow). Note formation of multiple new enterocutaneous fistulae (arrowheads). B, Endoscopic appearance of the colon before colectomy showing a large ulcer occupying approximately one third of the luminal surface. C, Terminal ileum 3 weeks postcolectomy, normal mucosa. D, Terminal ileum during second postcolectomy flare. E, Terminal ileum 3 weeks after panel D, after initiation of bowel rest, nonabsorbed antibiotics, and tacrolimus.

Making a definitive diagnosis: Successful clinical application of whole exome sequencing in a child with intractable inflammatory bowel disease

Elizabeth A. Worthey, PhD[1,2], Alan N. Mayer, MD, PhD[2,3], Grant D. Syverson, MD[2], Daniel Helbling, BSc[1], Benedetta B. Bonacci, MSc[2], Brennan Decker, BSc[1], Jaime M. Serpe, BSc[2], Trivikram Dasu, PhD[2], Michael R. Tschannen, BSc[1], Regan L. Veith, MSc[2], Monica J. Basehore, PhD[4], Ulrich Broeckel, MD, PhD[1,2,3], Aoy Tomita-Mitchell, PhD[1,2,3], Marjorie J. Arca, MD[3,5], James T. Casper, MD[2,3], David A. Margolis, MD[2,3], David P. Bick, MD[1,2,3], Martin J. Hessner, PhD[1,2], John M. Routes, MD[2,3], James W. Verbsky, MD, PhD[2,3], Howard J. Jacob, PhD[1,2,3,6], and David P. Dimmock, MD[1,2,3]

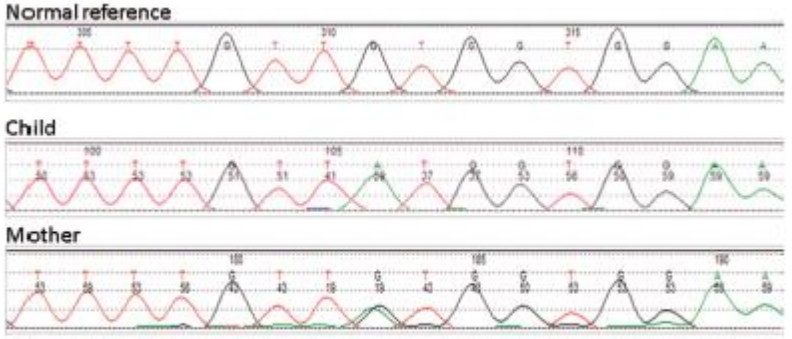
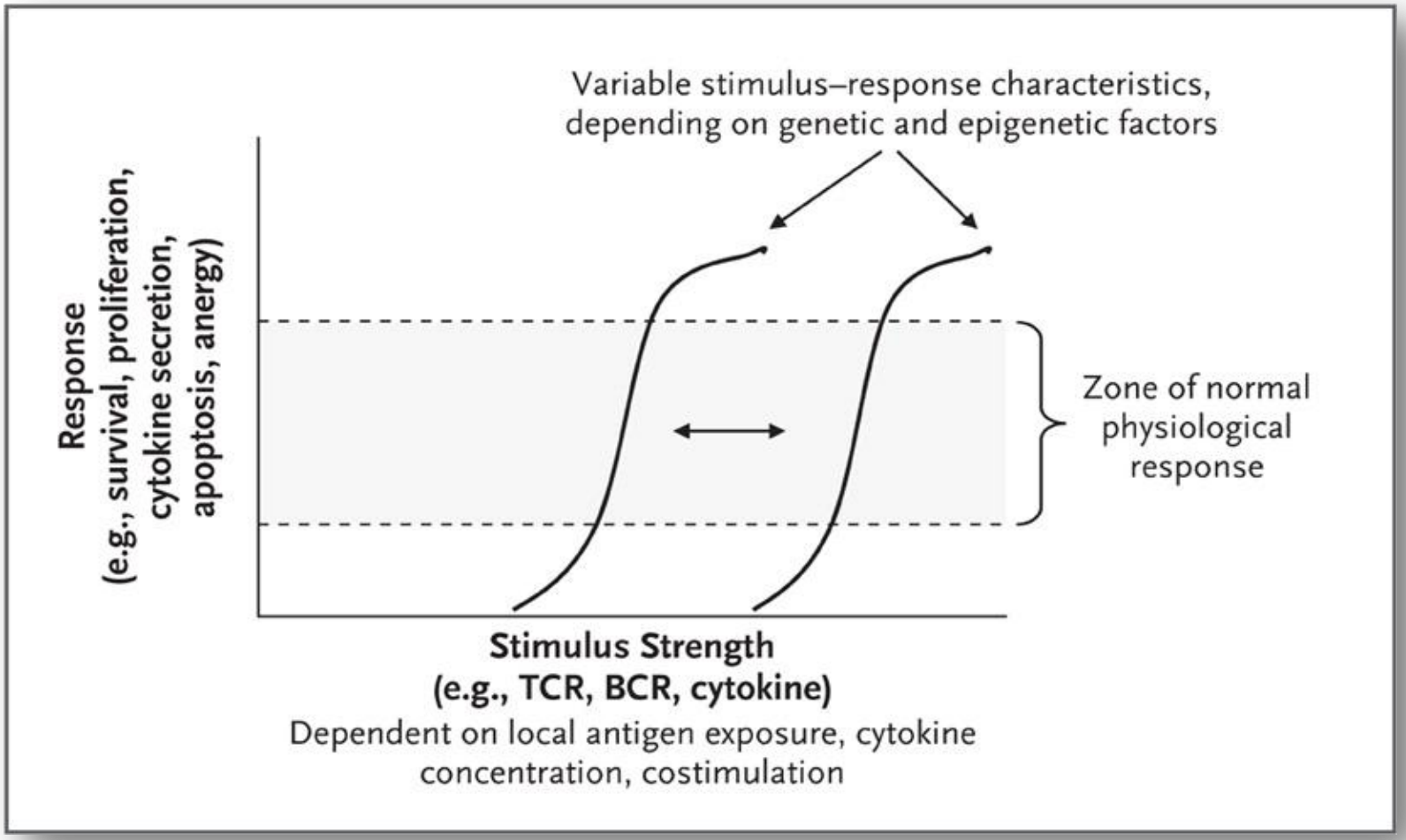


Fig. 3. Clinical confirmation in the child and mother. The region of the *XIAP* gene surrounding the mutation in both the child and the mother was sequenced using the BigDye Terminator Cycle Sequencing kit and analyzed on an ABI3730XL automated DNA sequencer. The Sanger sequence trace from a normal human control is shown at the top. Hemizygosity at the candidate locus is confirmed in the child (middle panel). The mother is heterozygous at this locus (bottom panel).

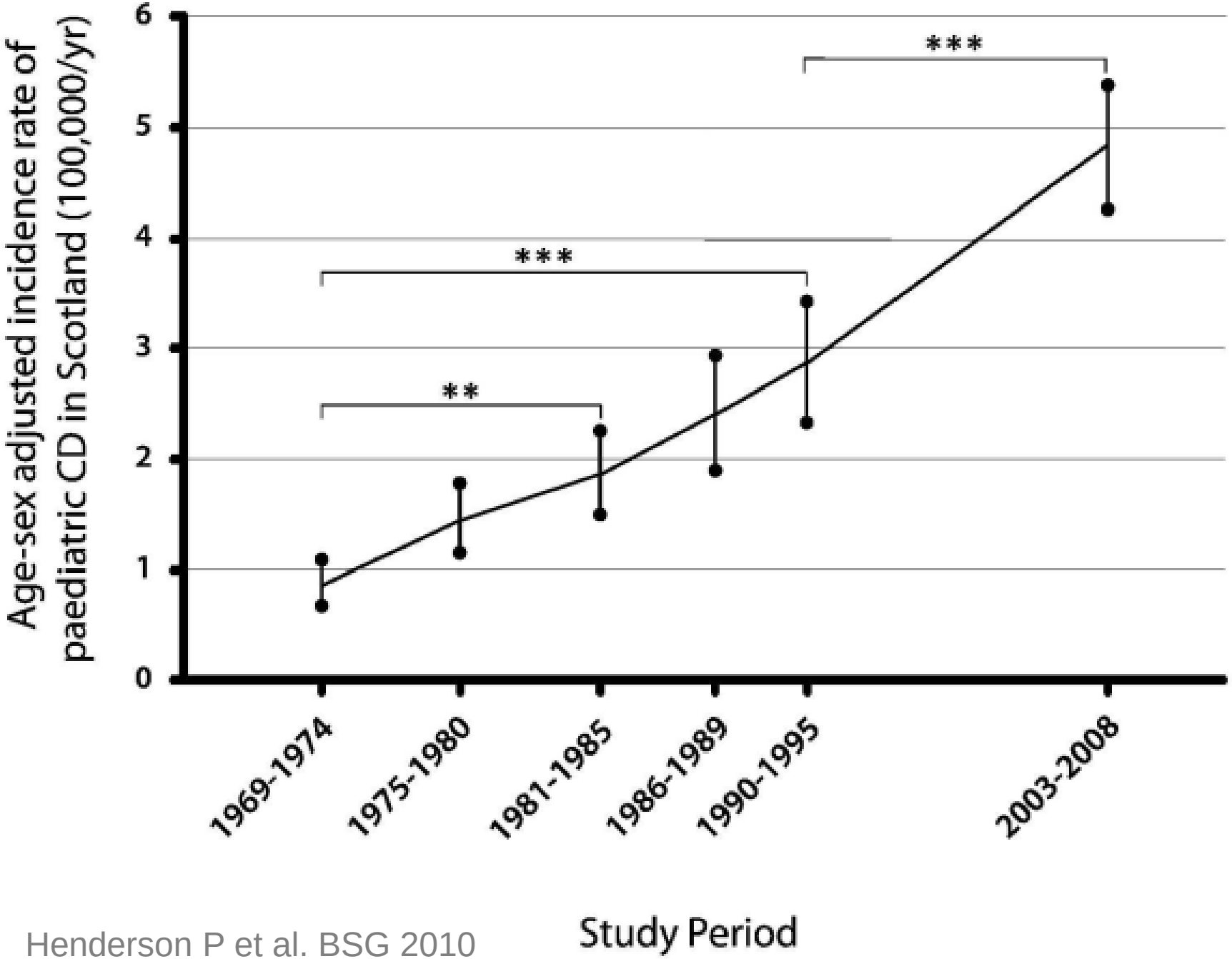
Stimulus–Response Thresholds & Immune Recognition as a Quantitative Trait.

Regulation of T and B cells controlled by cell signalling events:

- Normal variation in range of potency
- Varies between individuals & within different cells types in same individual



Cho JH, Gregersen PK. N Engl J Med 2011;365:1612-1623.



Henderson P et al. BSG 2010

Developed world:

* Massive increase in IBD in last century

Developing world:

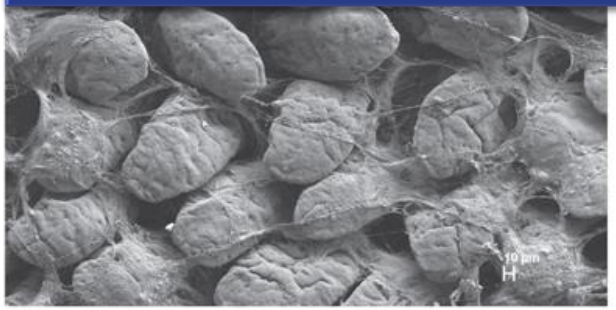
* Increase in recent years as countries adopt Western lifestyle

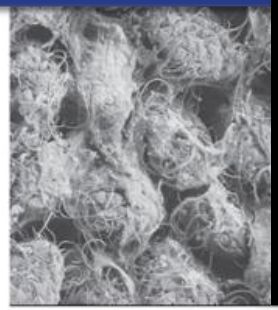
* Cannot be explained by genetics …

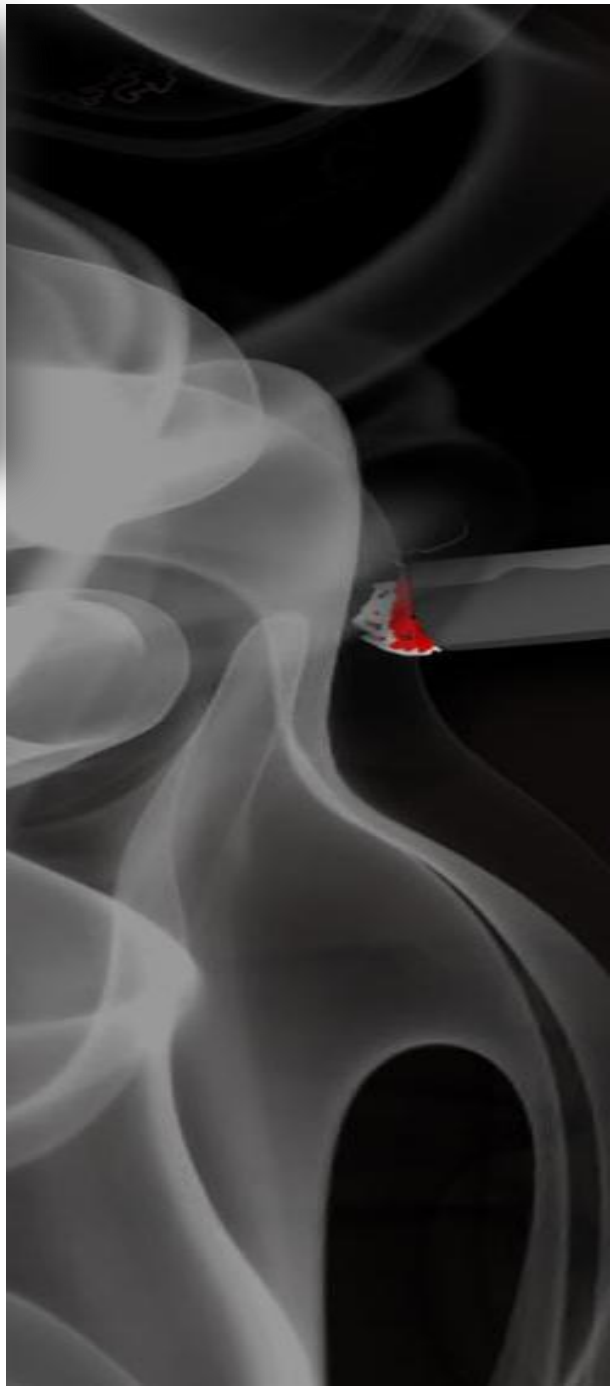
… rather, must be related to drastic environmental changes of last century and the not yet adapted (predisposing) genetic background of a sub-fraction of population

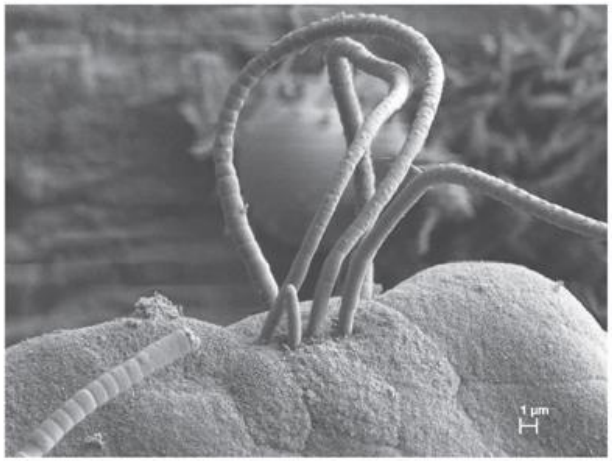
* IBD pathogenesis: insights from epidemiology

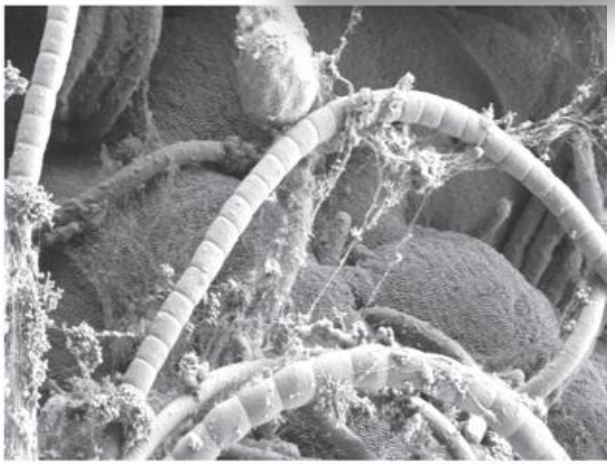
Environment & IBD

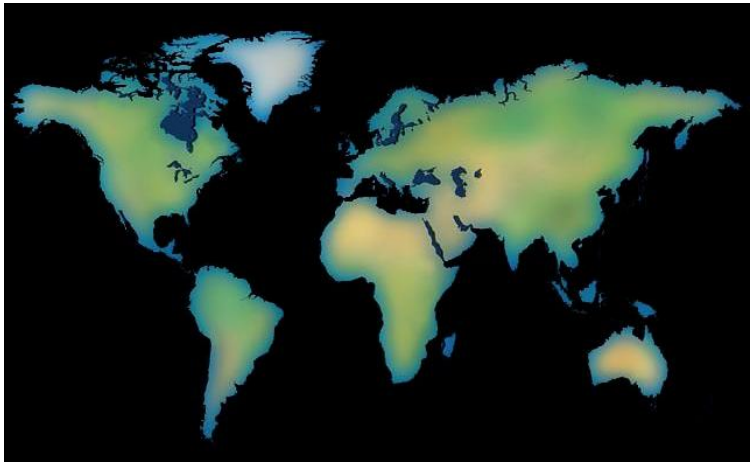












A positive family history is the strongest known risk factor in the development of Crohn's disease

Sibling relative risk (λs): 25-35

Monozygotic twin concordance: 45%

RELATIVE RISK OF CROHN'S DISEASE

MZ	1st degree	sibling	parent	offspring
667	5-35	25-42	12-16	2-30

Russell RK et al: *IBD: a Family Affair Best Practice Clinical Gastro* 2004;18:525-39

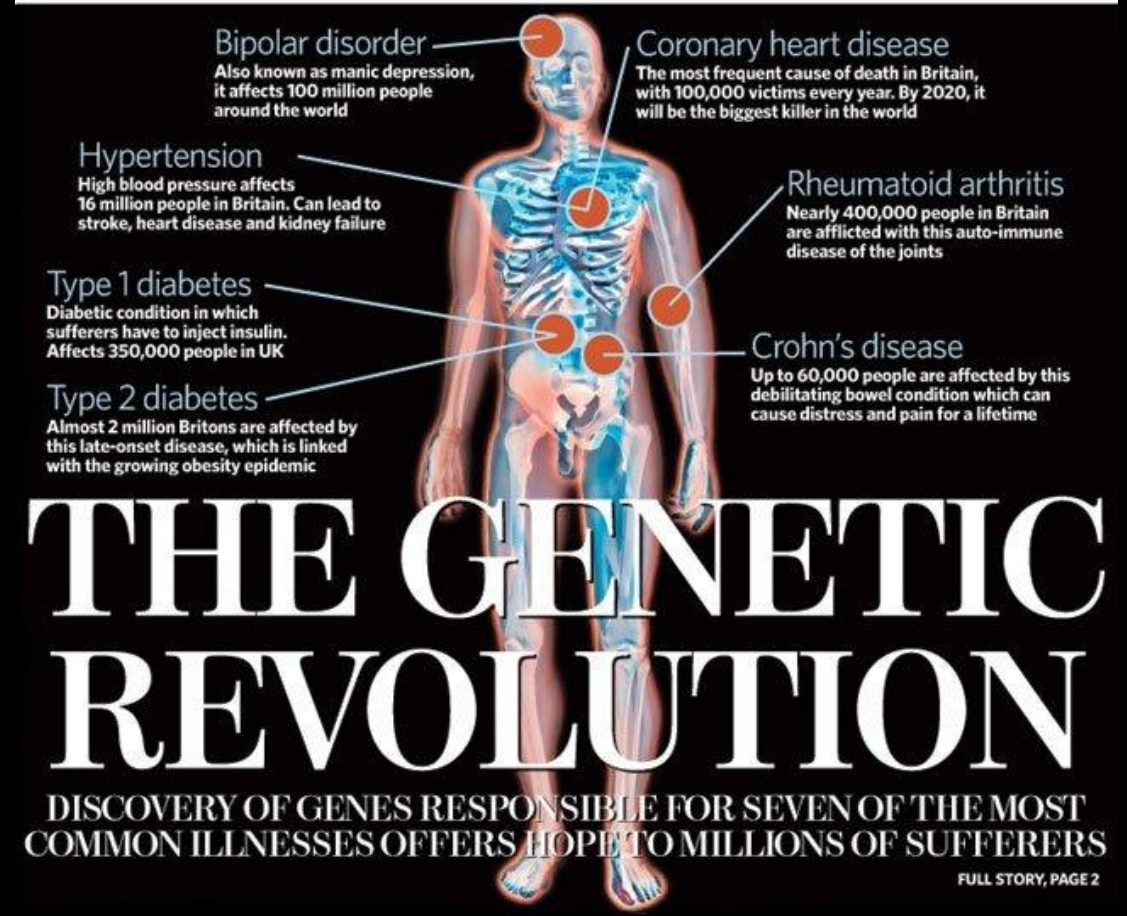
(Ireland, €1) 70p
Thursday 7 June 2007
www.independent.co.uk

THE INDEPENDENT

Tracey Emin

Exclusive: How I created the show of my life

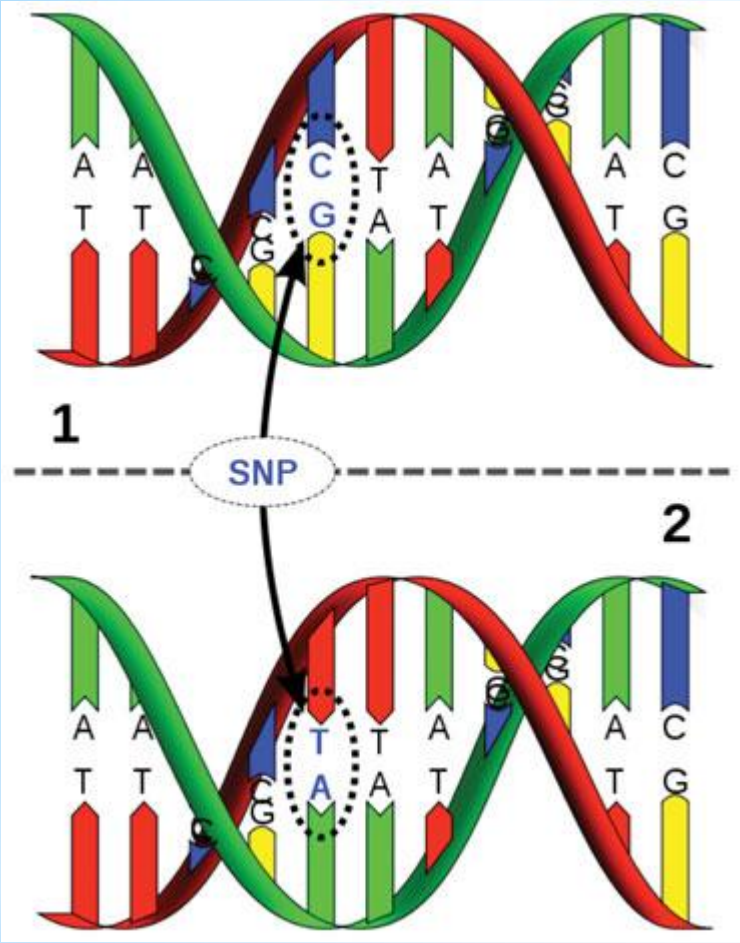
PLUS YOUR CHANCE TO OWN A LIMITED-EDITION ARTWORK IN EXTRA



THE GENETIC REVOLUTION

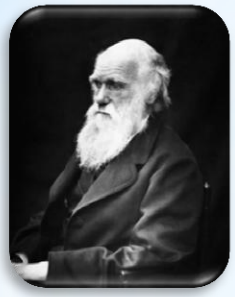
DISCOVERY OF GENES RESPONSIBLE FOR SEVEN OF THE MOST COMMON ILLNESSES OFFERS HOPE TO MILLIONS OF SUFFERERS

FULL STORY, PAGE 2



* 11 million SNPs in dbSNPv.129
* 3.5 million SNPs characterised in HapMap project
* ~3 million short insertions and deletions

* Single nucleotide polymorphisms (SNPs)



1859

1913

1932

1953

2000

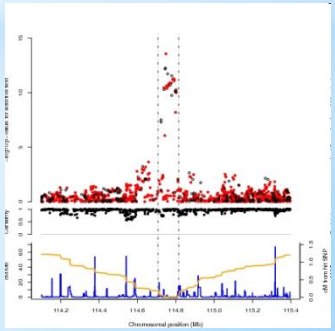
2005

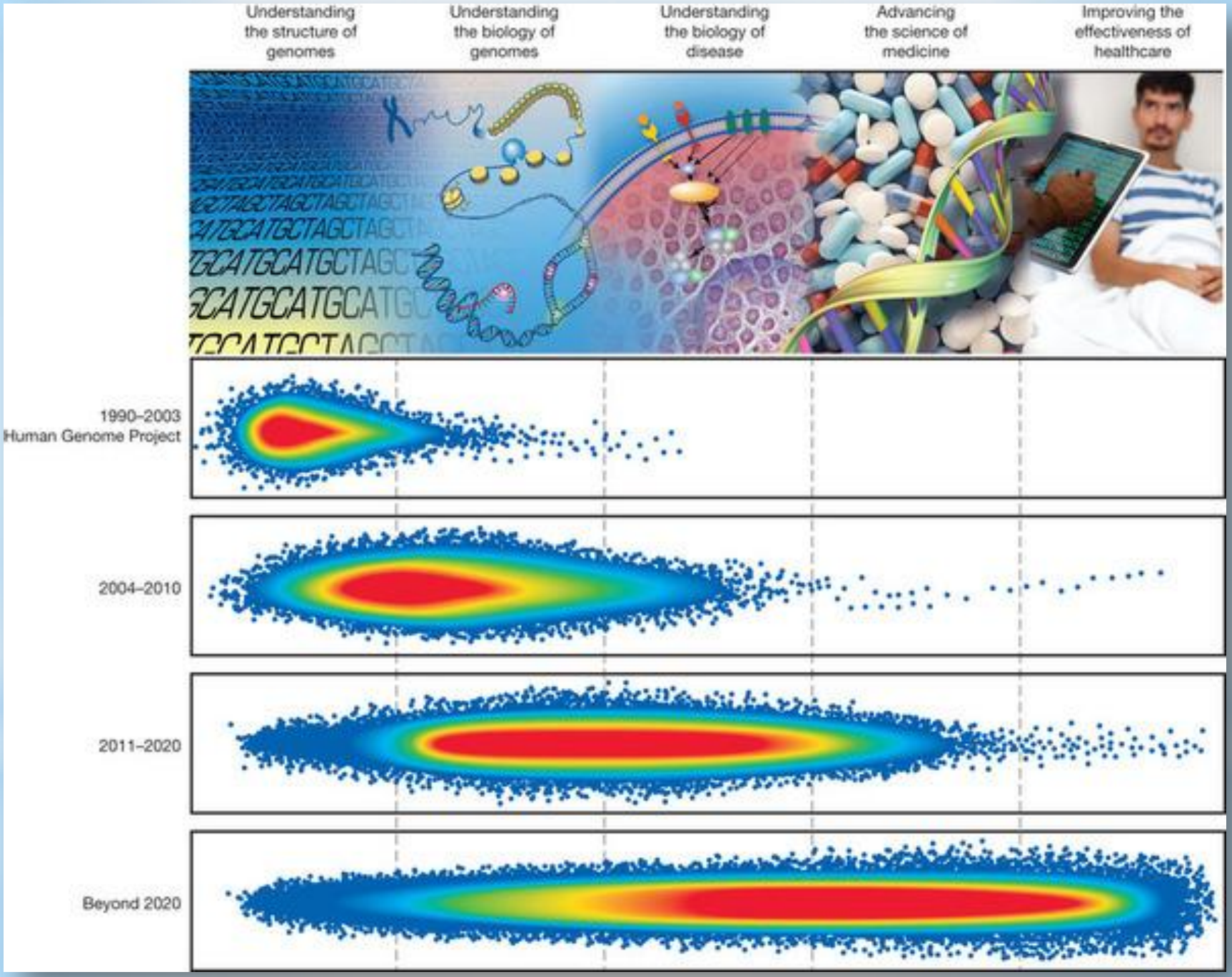
2006

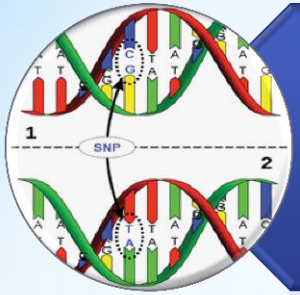
2010

GWAS

WGS

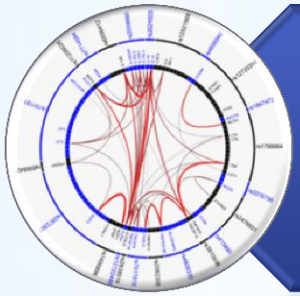



Understanding the structure of genomes
Understanding the biology of genomes
Understanding the biology of disease
Advancing the science of medicine
Improving the effectiveness of healthcare
1990–2003 Human Genome Project
2004–2010
2011–2020
Beyond 2020



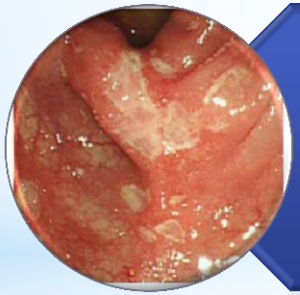
Identify susceptibility alleles

- Genome-wide linkage studies
- Genome-wide association scans (GWAS)
- Whole-genome sequencing



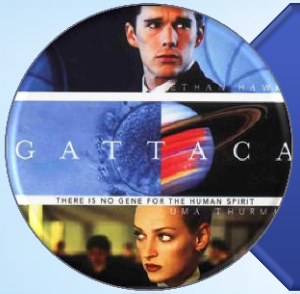
Novel biological insight

- Autophagy
- Th17 signalling
- Barrier function



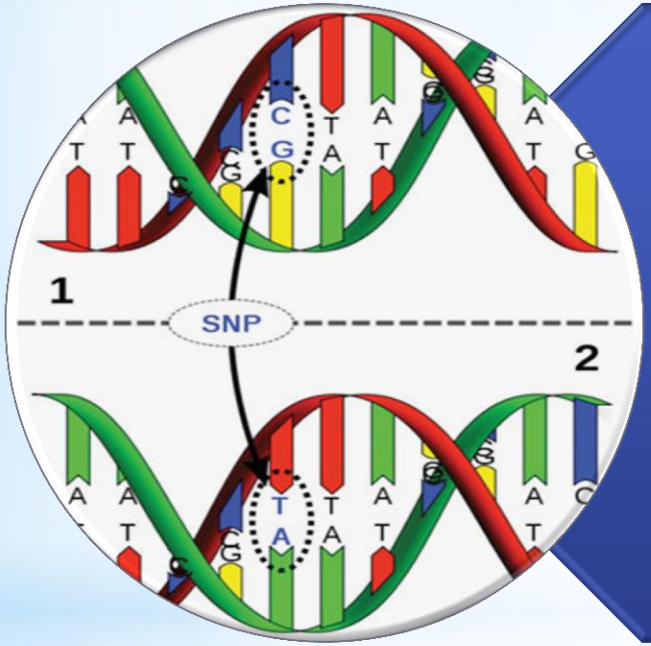
Clinical advance

- Therapeutics
- Prevention
- Biomarkers



Personalised medicine

- Diagnostics
- Prognostics
- Therapeutic optimisation



Identify susceptibility alleles

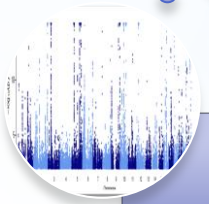
- Genome-wide linkage studies
- Genome-wide association scans (GWAS)
- Whole-genome sequencing

IBD Genetics

>160 independent IBD susceptibility loci

Whole-genome sequencing

Immunochip



Meta-GWAS

GWAS

Genome-wide linkage

Twin studies

2012

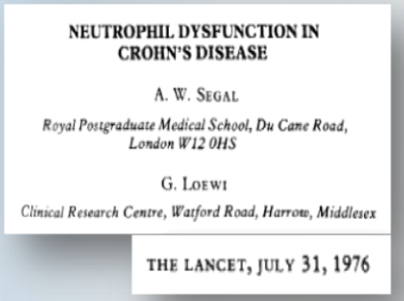
2011

2008

2007

2001

1980's



NEUTROPHIL DYSFUNCTION IN CROHN'S DISEASE

A. W. SEGAL

Royal Postgraduate Medical School, Du Cane Road, London W12 0HS

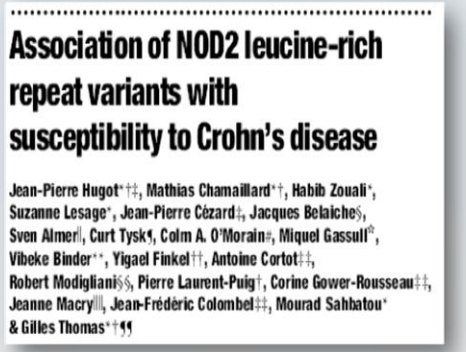
G. LOEWI

Clinical Research Centre, Watford Road, Harrow, Middlesex

THE LANCET, JULY 31, 1976

Neutrophil dysfunction

1976

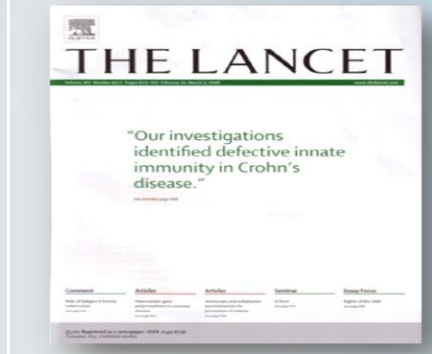


Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease

Jean-Pierre Hugot*†‡, Mathias Chamaillard*†, Habib Zouali*, Suzanne Lesage*, Jean-Pierre Cézard‡, Jacques Belaiche§, Sven Almer‖, Curt Tysk¶, Colm A. O'Morain#, Miquel Gassull**, Vibeke Binder**, Yigael Finkel††, Antoine Cortot‡‡, Robert Modigliani§§, Pierre Laurent-Puig†, Corine Gower-Rousseau‡‡, Jeanne Macry‖‖, Jean-Frédéric Colombel‡‡, Mourad Sahbatou* & Gilles Thomas*†¶¶

NOD2

2001



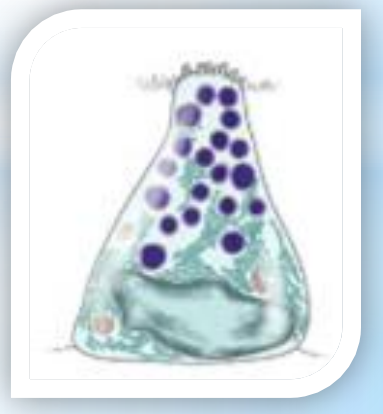
THE LANCET

"Our investigations identified defective innate immunity in Crohn's disease."

Innate immunity

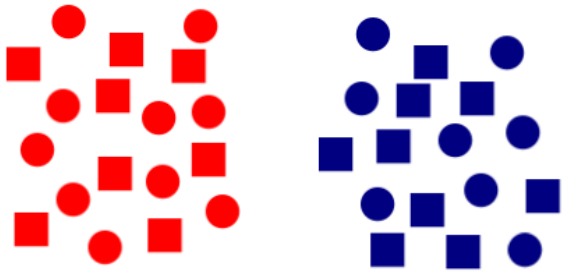
2006

Innate immunity

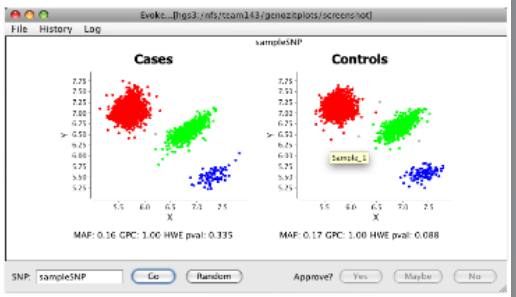


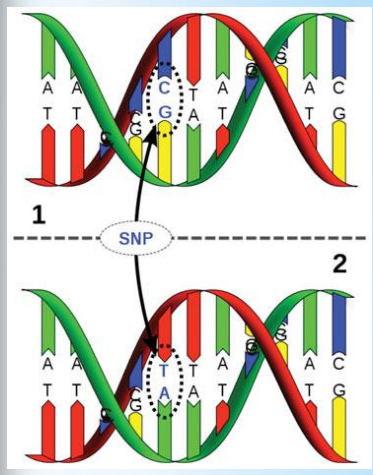
GWAS

A

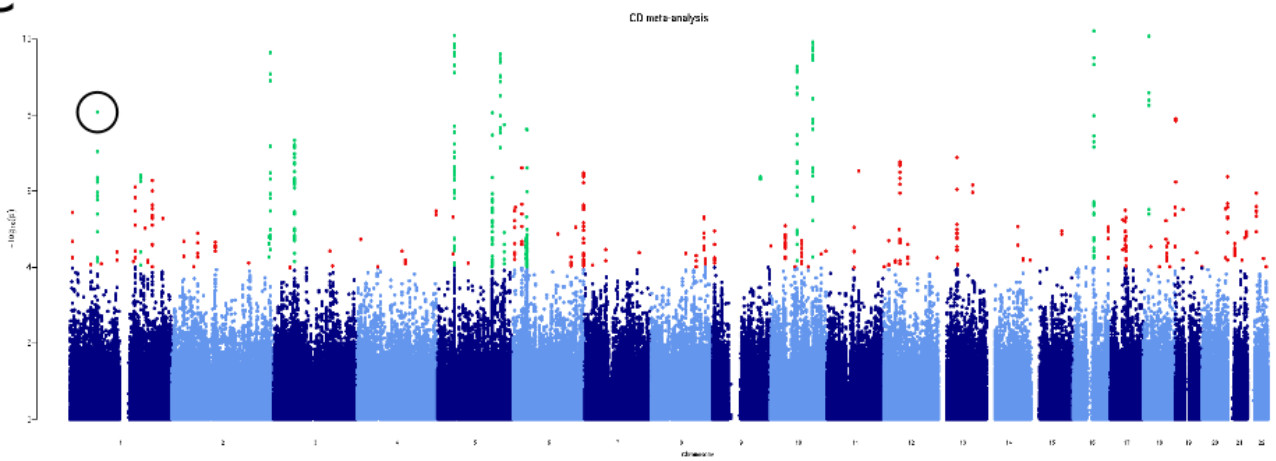


B


File History Log
sampleSNP
Cases
Controls
MAF: 0.16 GPC: 1.00 HWE pval: 0.335
MAF: 0.17 GPC: 1.00 HWE pval: 0.088
SNP: sampleSNP
Go
Random
Approve?

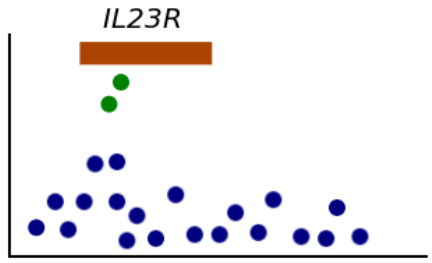

1
SNP
2

C

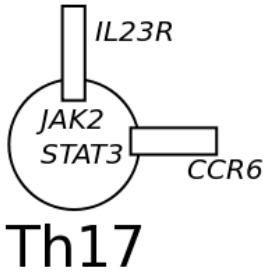

CD meta-analysis

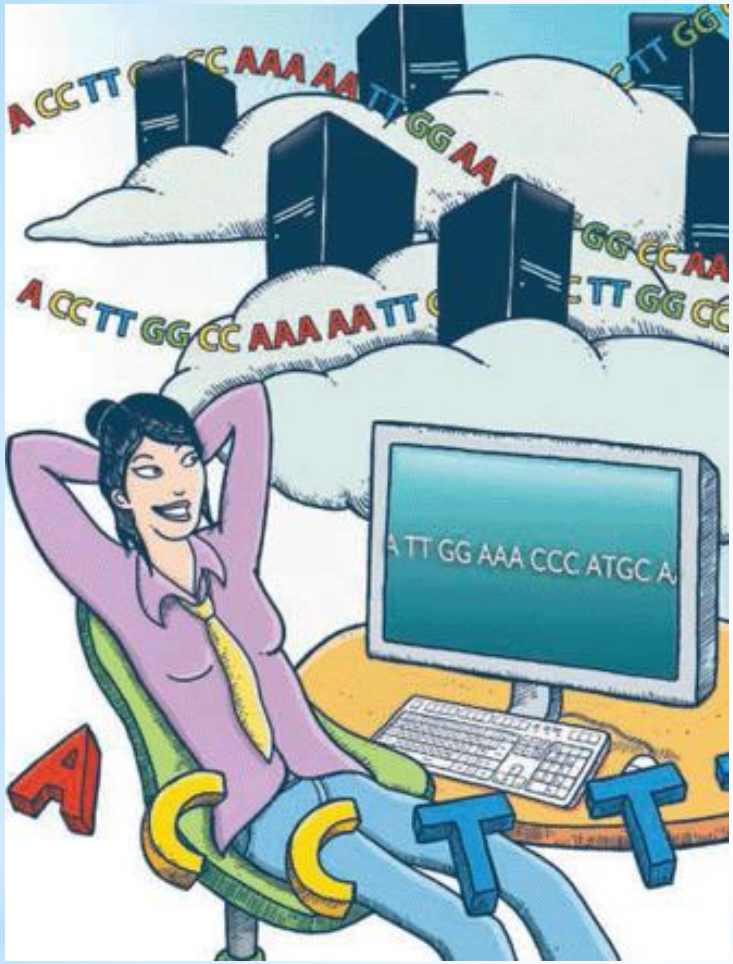

AFFYMETRIX
GeneChip
Genome-Wide Human
SNP Array 6.0

D


IL23R

E


IL23R
JAK2
STAT3
CCR6
Th17



- $P_{discovery} < 5 \times 10^{-5}$
- $P_{replication} < 0.05$
- $P_{combined} < 5 \times 10^{-8}$

Statistical rules of thumb

PHENOTYPE	No. of GWAS loci	Proportion of heritability explained (%)
Type 1 diabetes	41	~60
Foetal Hb levels	3	~50
Macular degeneration	3	~50
Type 2 diabetes	39	20-25
Lipid levels	95	20-25
Crohn's disease	71	23
Ulcerative colitis	47	16
Height	180	~12

* GWAS for common diseases & traits

UK IBD GENETICS CONSORTIUM

Understanding the genetics of Crohn's & Colitis

www.ibdresearch.co.uk

Crohn's disease

Vol 447|7 June 2007|doi:10.1038/nature05911 nature

ARTICLES

Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls

The Wellcome Trust Case Control Consortium*

NKX2.3

PTPN2

IL12B

3p21 [MST1]

nature genetics

Sequence variants in the autophagy gene *IRGM* and multiple other replicating loci contribute to Crohn's disease susceptibility

Ulcerative colitis

Genome-wide association study of ulcerative colitis identifies three new susceptibility loci, including the *HNF4A* region

The UK IBD Genetics Consortium & the Wellcome Trust Case Control Consortium 2*

Barrett J, Lee J, Lees CW *et al*

nature genetics

CDH1

LAMB1

HNF4A

IBD Genetics

www.ibdgenetics.org

International IBD Genetics Consortium (IIBDGC)

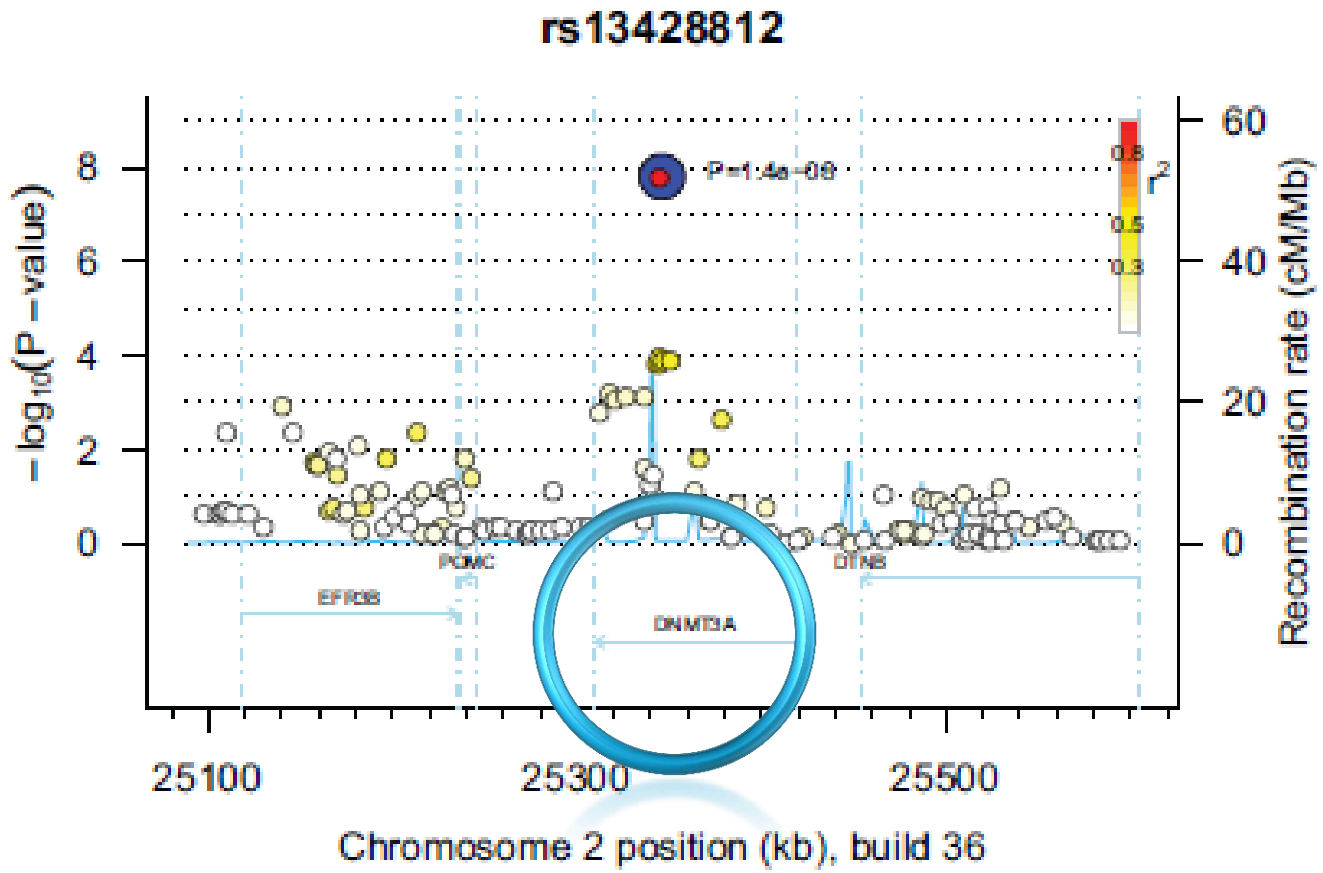
IBD Genetics

Crohn's disease meta-GWAS *Franke A, McGovern D, Barrett et al* *Nature Genetics 2010; 42(12):1118-25*	Crohn's Cases	Controls
Discovery cohort (6 GWAS)	6,333	15,056
Replication cohort	14,934	13,647
Crohn's disease total	**21,267**	**28,703**

Ulcerative colitis meta-GWAS *Anderson C, Boucher G, Lees CW et al* *Nature Genetics 2011 [Epub]*	UC Cases	Controls
Discovery cohort (6 GWAS)	6,782	20,099
Replication cohort	9,628	12,917
Ulcerative colitis total	**16,410**	**33,016**

Genetics

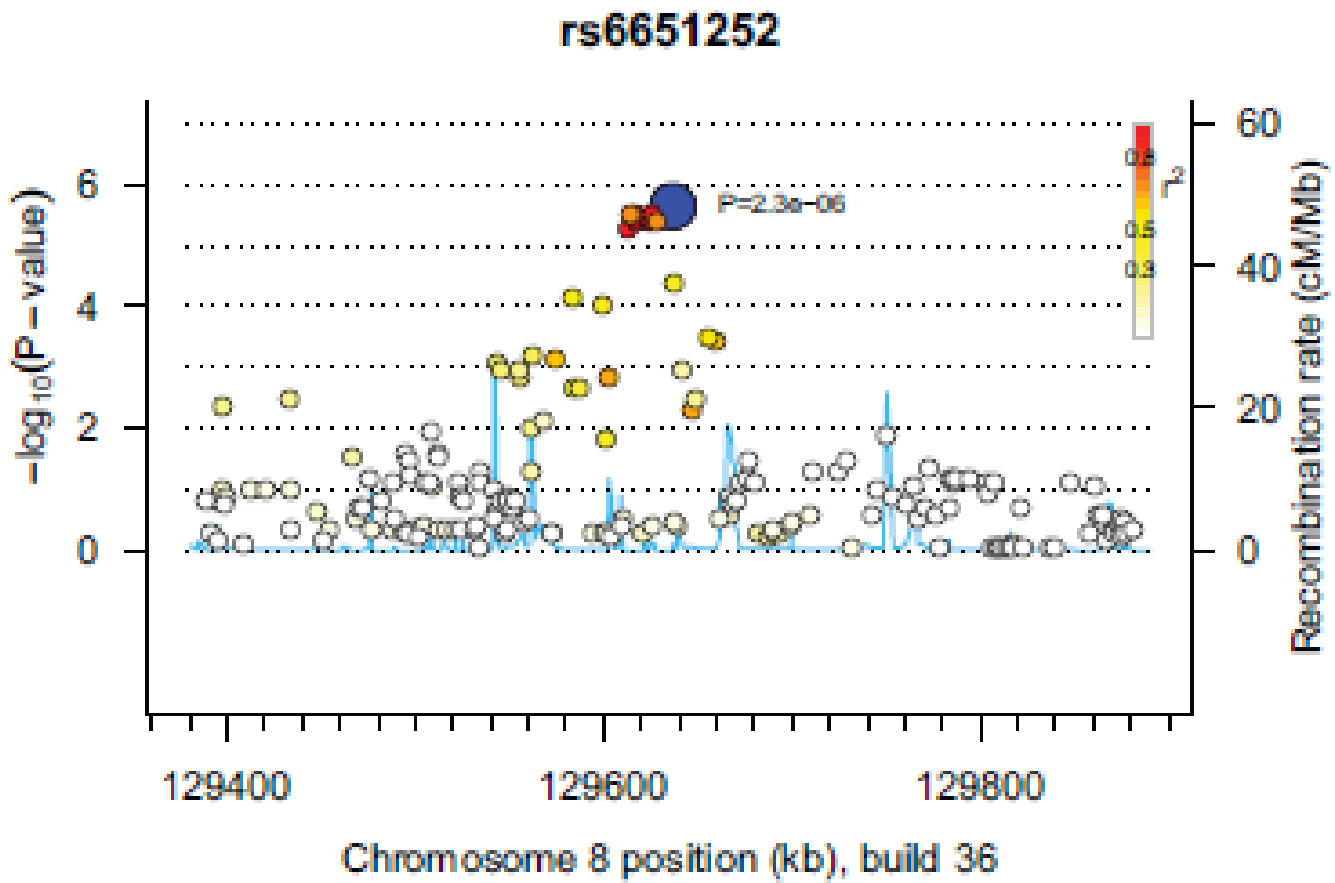
*‘one gene’ locus



DNMT3A

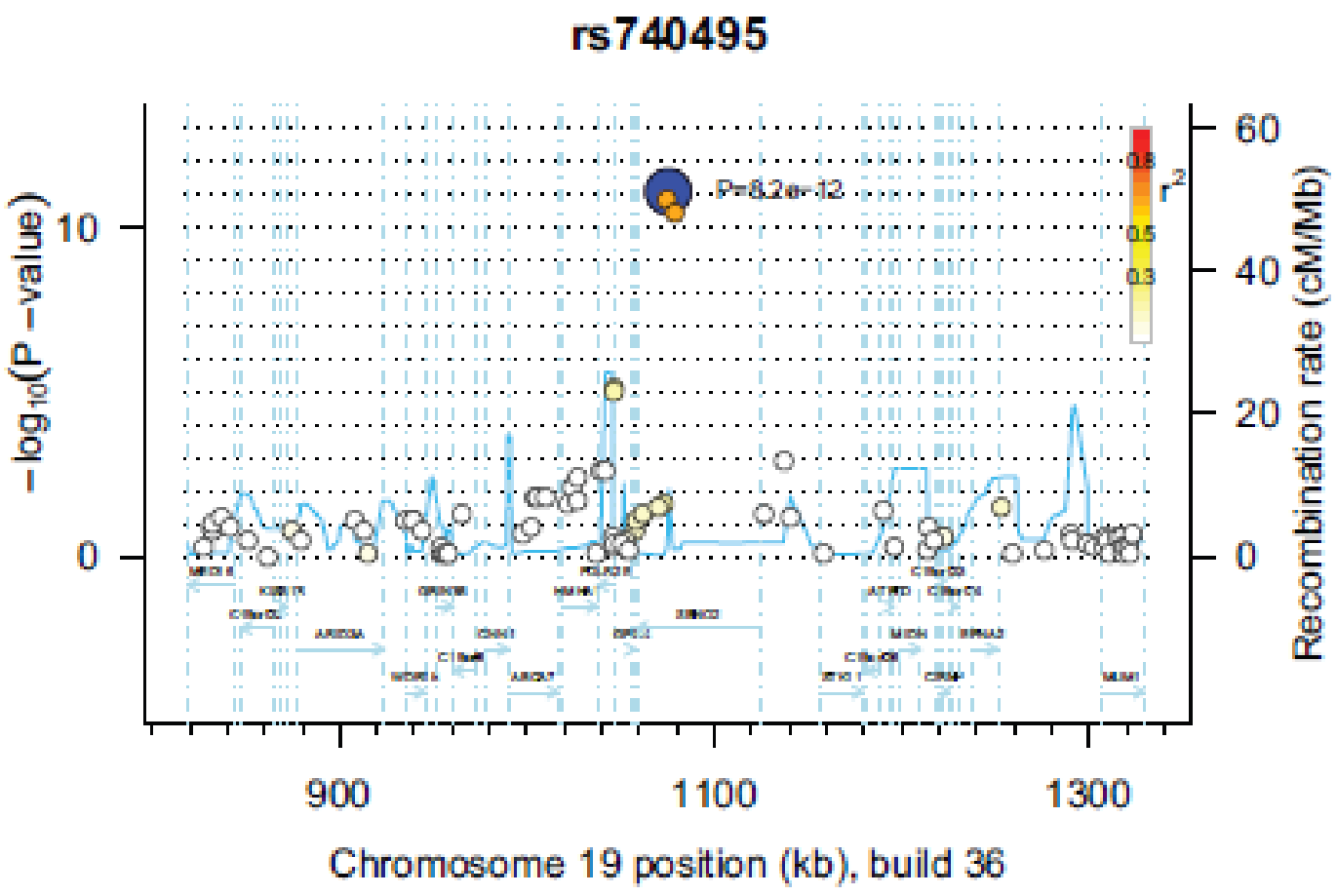
Genetics

*Gene 'desert'

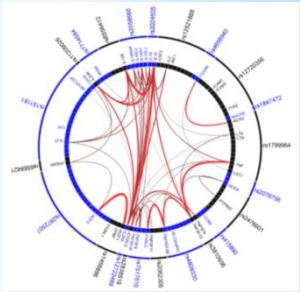


Genetics

*'Multi-gene' locus



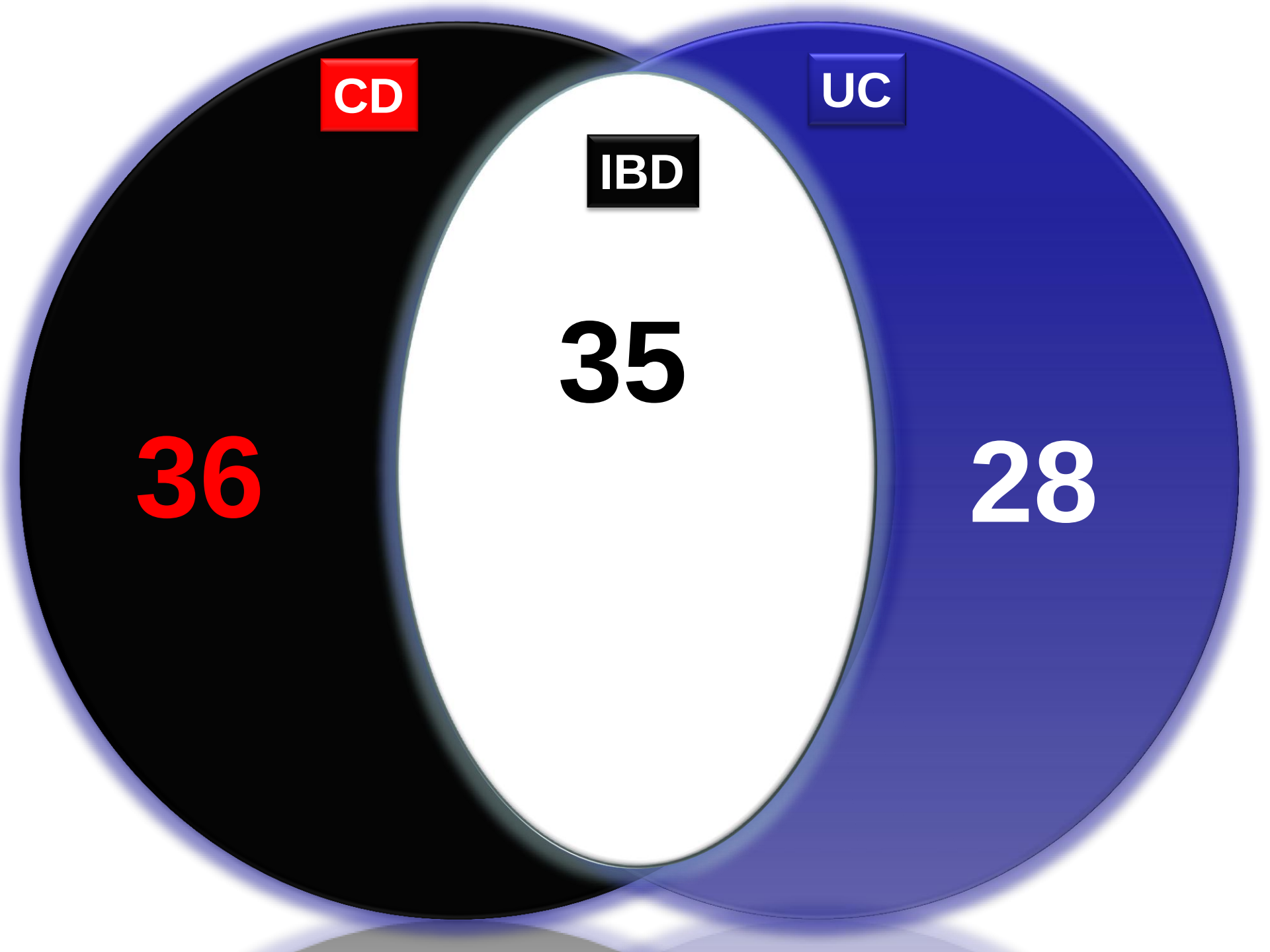
Genetics

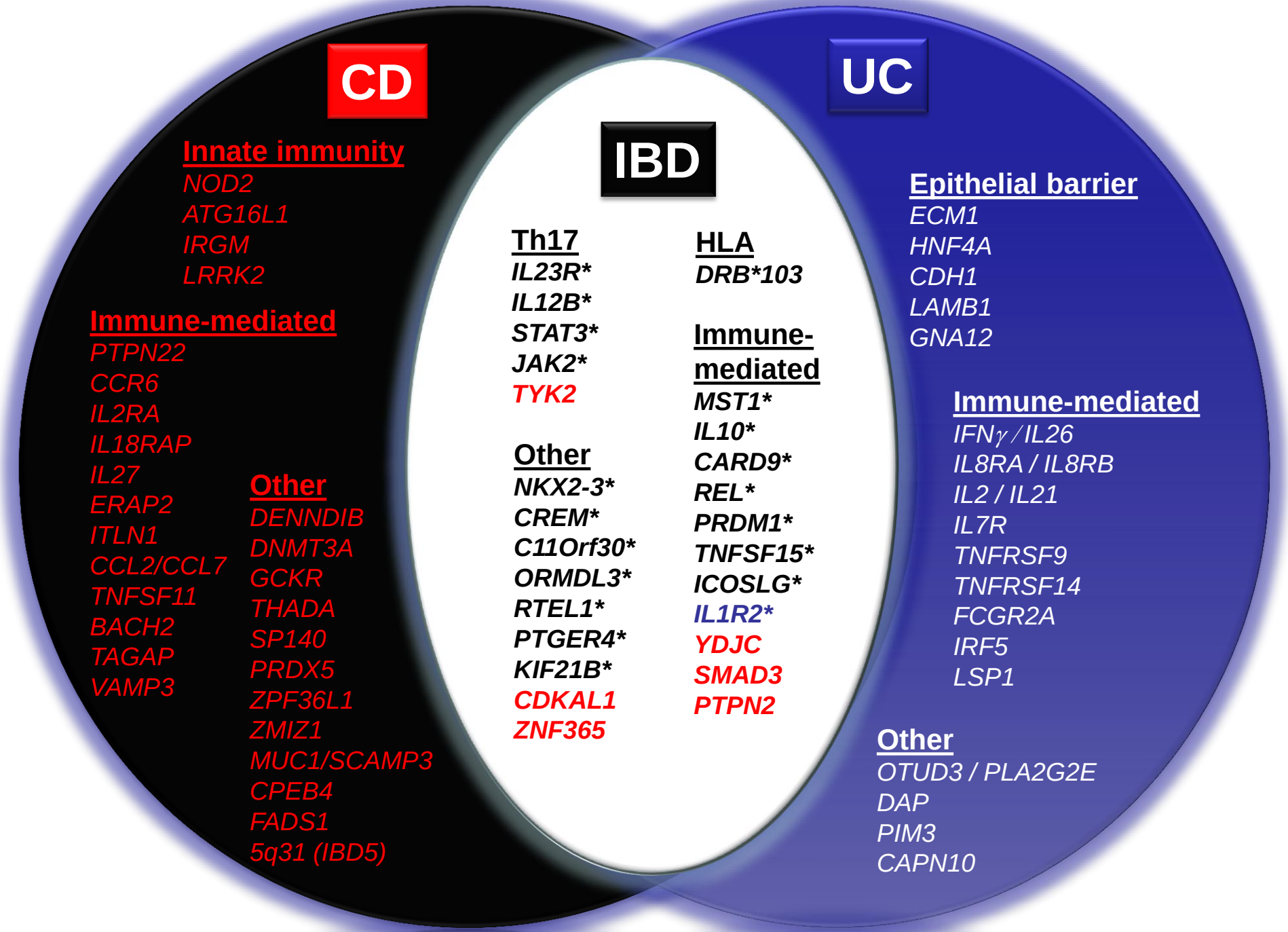


* GRAIL – literature-mining tool
  * Gene-Relationships Amongst Implicated Loci
* eQTLs from existing databases
  * Correlate focal SNP expression with gene expression
* nsSNP – 1000 genomes & HapMap3 data
* Position

Ultimately requires detailed fine-mapping of loci

* **How can we dissect multi-genic loci?**


CD
UC
IBD
35
36
28


CD
Innate immunity
NOD2
ATG16L1
IRGM
LRRK2
Immune-mediated
PTPN22
CCR6
IL2RA
IL18RAP
IL27
ERAP2
ITLN1
CCL2/CCL7
TNFSF11
BACH2
TAGAP
VAMP3
Other
DENNDIB
DNMT3A
GCKR
THADA
SP140
PRDX5
ZPF36L1
ZMIZ1
MUC1/SCAMP3
CPEB4
FADS1
5q31 (IBD5)
IBD
Th17
IL23R*
IL12B*
STAT3*
JAK2*
TYK2
Other
NKX2-3*
CREM*
C11Orf30*
ORMDL3*
RTEL1*
PTGER4*
KIF21B*
CDKAL1
ZNF365
HLA
DRB*103
Immune-mediated
MST1*
IL10*
CARD9*
REL*
PRDM1*
TNFSF15*
ICOSLG*
IL1R2*
YDJC
SMAD3
PTPN2
UC
Epithelial barrier
ECM1
HNF4A
CDH1
LAMB1
GNA12
Immune-mediated
IFNγ / IL26
IL8RA / IL8RB
IL2 / IL21
IL7R
TNFRSF9
TNFRSF14
FCGR2A
IRF5
LSP1
Other
OTUD3 / PLA2G2E
DAP
PIM3
CAPN10

CD genetics: proportion of variance explained

- 98 IBD genes contribute small amount to genetic variance:
- 23% (from 20%) in Crohn's
- 16% (from 11%) in UC

- Typing 50k CD cases would:
  - Yield 140 loci but only ↑ proportion explained by 2-3%

- NOD2 lead SNP accounts 0.8%
- All 3 common NOD2 SNPs together account for ~5%

* **Immunochip**
  * Customized Illumina GWA Chip
  * ~200.000 SNPs
  * Content focused on 9 immune-mediated diseases

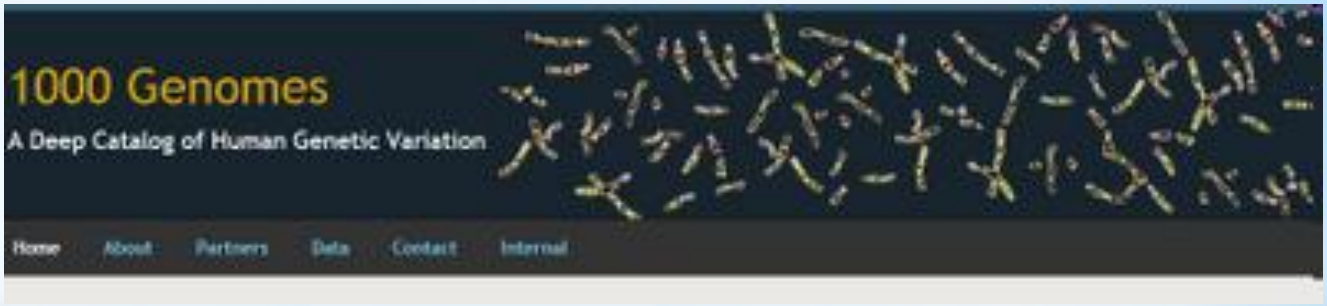
* **Two main aims**
  1) Locus finding:
    * Deep replication of the UC and CD meta-analyses
    * Shared genetics immune-mediated diseases
  2) Extensive fine-mapping of IBD associated loci

* Immunochip

* **Crohn's disease**
  * 43 Novel loci
    * 7 previously associated with UC (e.g. *IL2/IL21*, *FCGR2A)*
    * 23 associated with other immune-mediated diseases

* **Ulcerative Colitis**
  * 39 Novel loci
    * 13 previously associated with CD (e.g. *STAT3*, *LRRK2*, *IBD5*)
    * 17 associated with other immune-mediated diseases

* Interim I-chip analysis



ARTICLE

doi:10.1038/nature09534

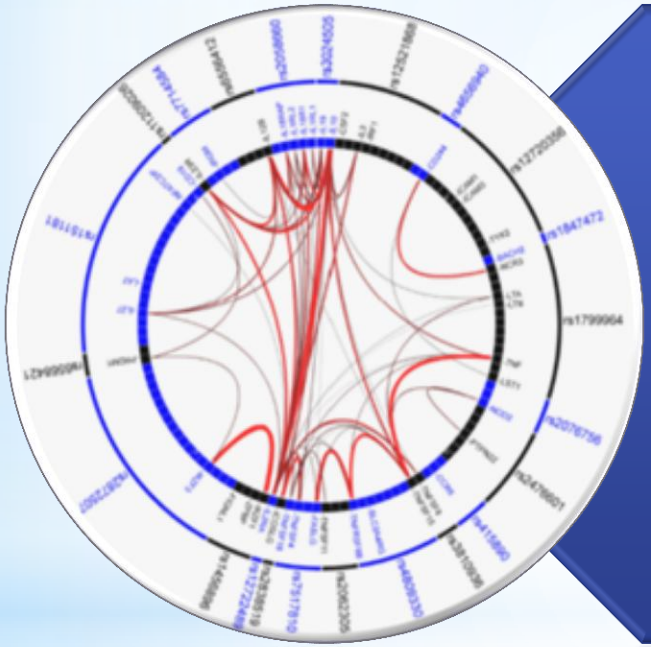
A map of human genome variation from population-scale sequencing

The 1000 Genomes Project Consortium*

Worldwide :

* 2,700 human genomes sequenced by the end of Nov 2010
* 30,000 human genomes sequenced by the end of 2011

* Whole-genome sequencing

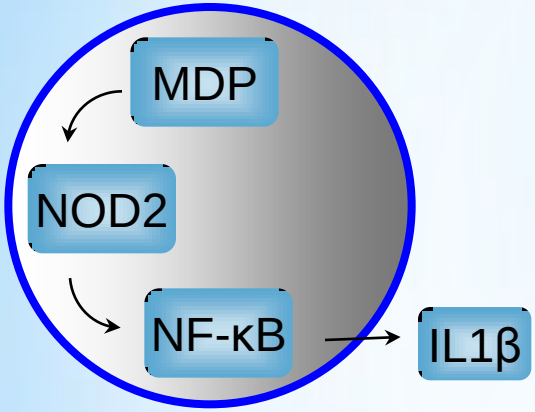


Novel biological insight

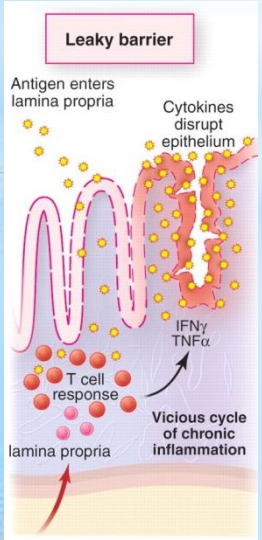
- Autophagy
- Th17 signalling
- Barrier function

INNATE IMMUNITY

NOD2



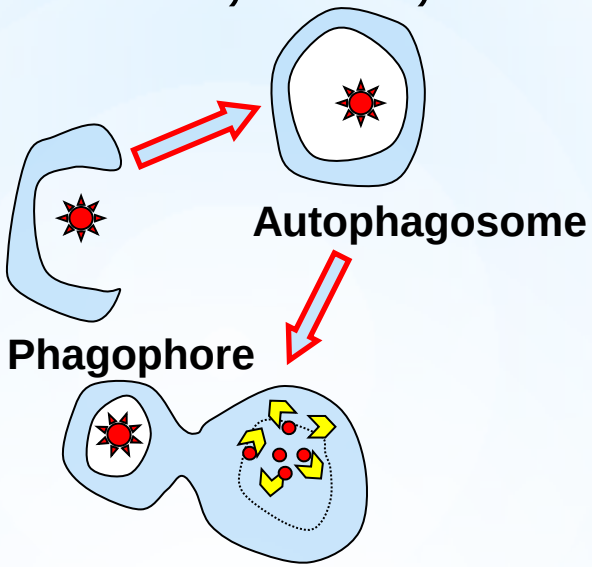
DEFECTIVE BARRIER



ECM1
CDH1
LAMB1
HNF4α

Autophagy

ATG16L1; IRGM; ATG5



IL10 signalling

IL22, IL26, IFNγ

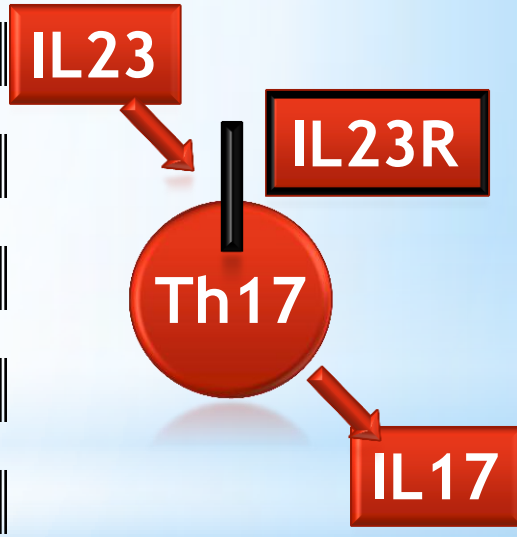
IL10RB ← ***IL10*** → ***STAT3***

NOD2

ATG16L1

ADAPTIVE IMMUNITY

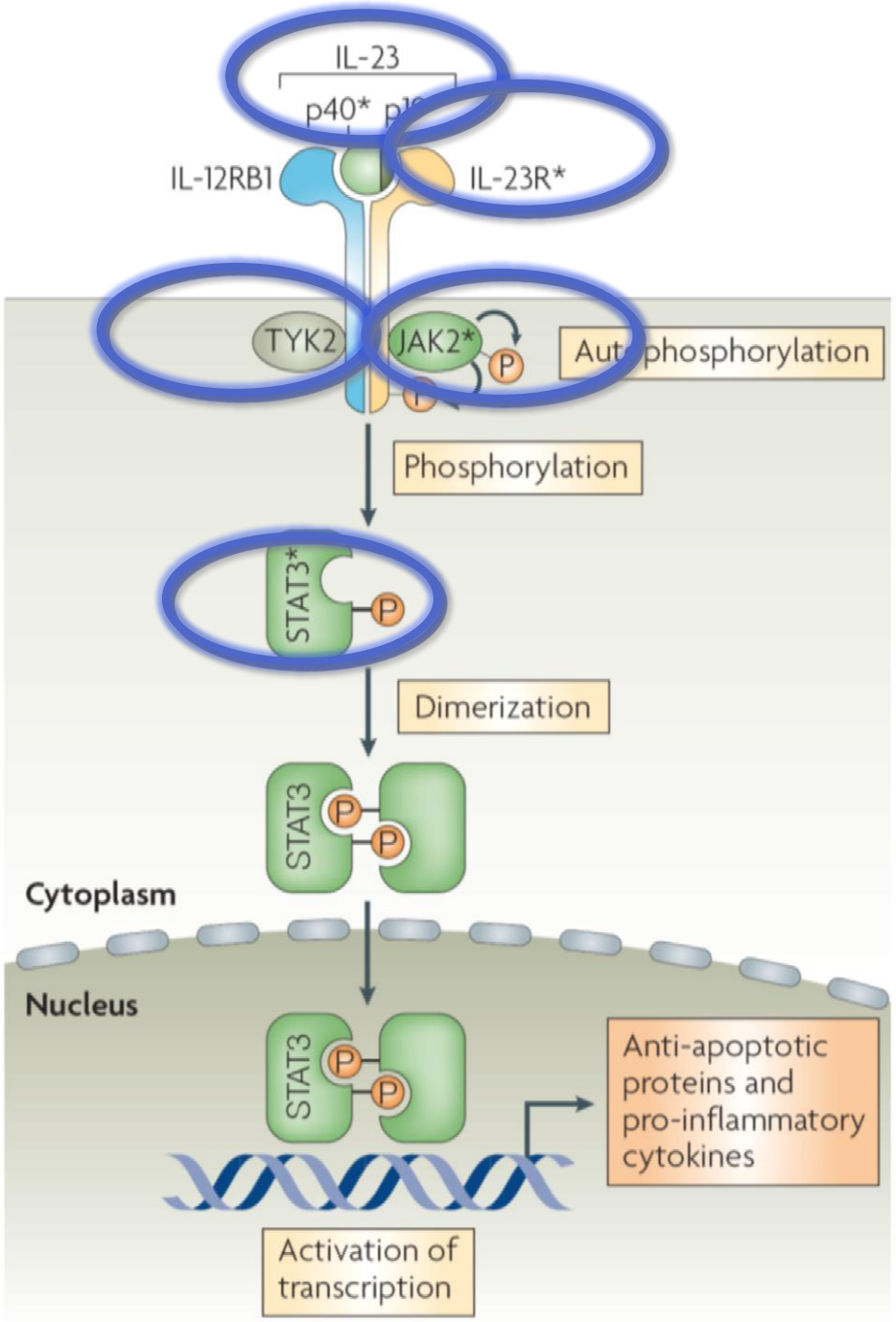
IL23R; IL12B
JAK2; STAT3
TNFSF15; CCR6
IL27; REL

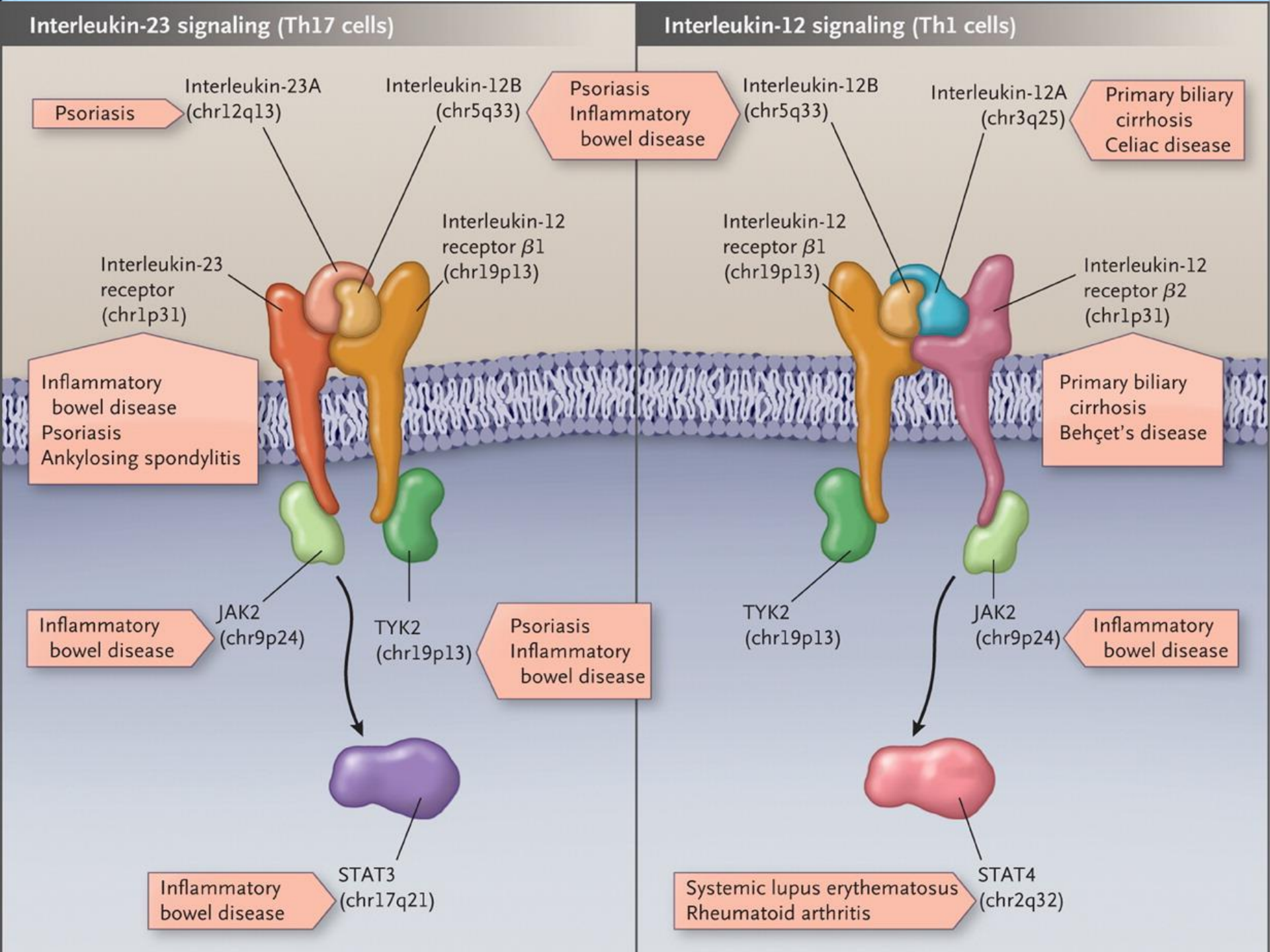


Genetics

A series of studies in animal models suggest IL23, rather than IL12, is a key regulator of Th1 response and colitis.

*IL23




Interleukin-23 signaling (Th17 cells)
Interleukin-12 signaling (Th1 cells)
Psoriasis
Interleukin-23A (chr12q13)
Interleukin-12B (chr5q33)
Psoriasis
Inflammatory bowel disease
Interleukin-12B (chr5q33)
Interleukin-12A (chr3q25)
Primary biliary cirrhosis
Celiac disease
Interleukin-12 receptor β1 (chr19p13)
Interleukin-23 receptor (chr1p31)
Interleukin-12 receptor β1 (chr19p13)
Interleukin-12 receptor β2 (chr1p31)
Inflammatory bowel disease
Psoriasis
Ankylosing spondylitis
Primary biliary cirrhosis
Behçet's disease
Inflammatory bowel disease
JAK2 (chr9p24)
TYK2 (chr19p13)
Psoriasis
Inflammatory bowel disease
TYK2 (chr19p13)
JAK2 (chr9p24)
Inflammatory bowel disease
Inflammatory bowel disease
STAT3 (chr17q21)
Systemic lupus erythematosus
Rheumatoid arthritis
STAT4 (chr2q32)

PTPN22
an intracellular phosphatase

- **Causal variant is almost certainly *R620W***
- Associated with T1DM, RA, A-I thyroid disease, myasthenia gravis, systemic sclerosis, vitilitgo, Addison's and alopecia
- And infectious disease (invasive bacterial infection & ?M.tb)
- BUT … in Crohn's disease it is protective
- R620W risk allele is 'gain-of-function'
  - Phosphatase with higher catalytic activity
  - More potent –ve regulation of T-cell activation
- *Lyp*$^{-/-}$ mice have incr T-cell activation with incr production of antibodies
- Alterations in PTPN22 change the thresholds for receptor signalling by T and B cells

Paneth cells

Cigarette Smoke

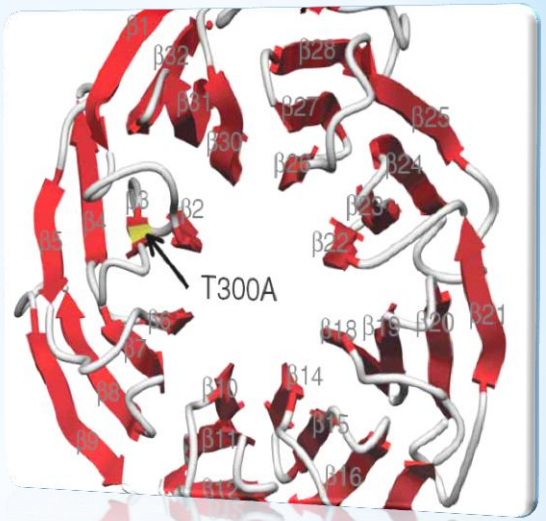
NOD2

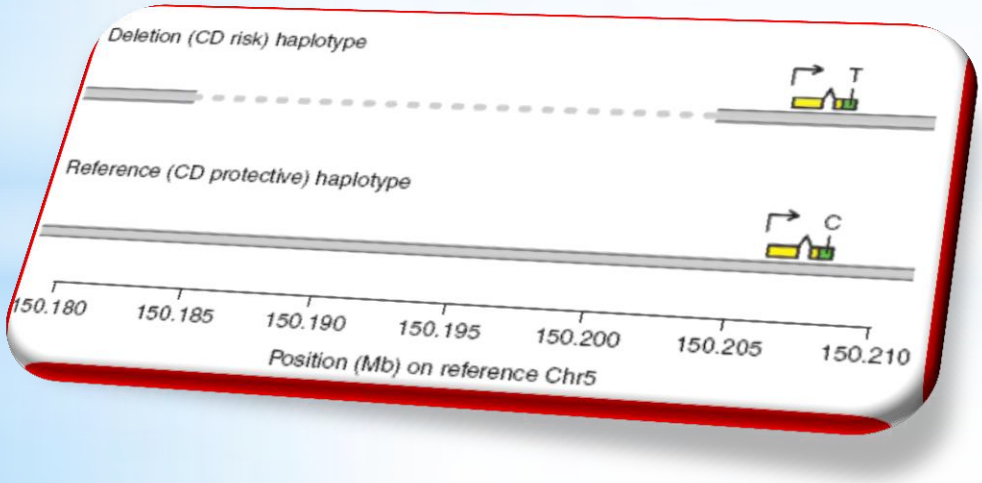
Autophagy

Dysbiosis

*ATG16L1 & IRGM

* 2 novel, replicated CD susceptibility genes clearly implicating *(?defective)* autophagy in CD pathogenesis



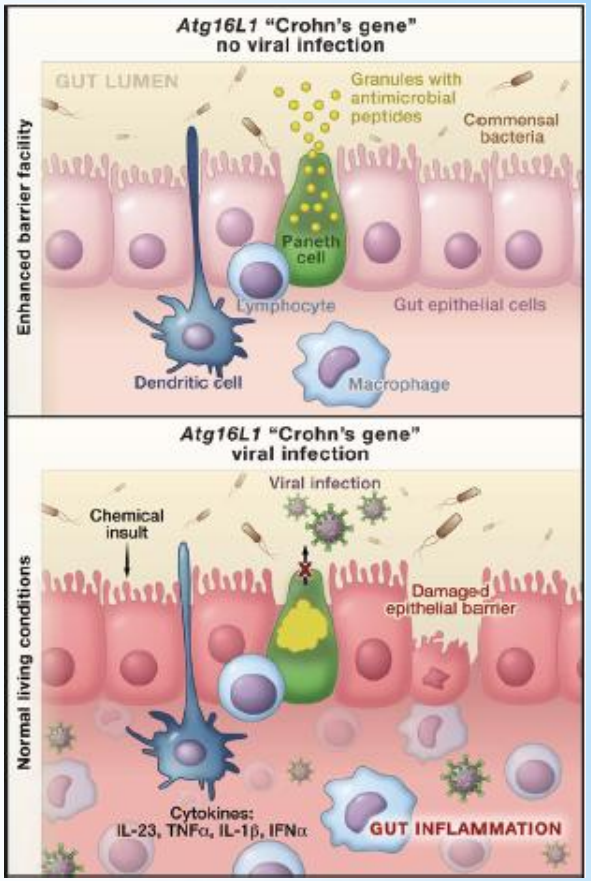


*Autophagy

*ATG16L1


LETTERS
Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1β production
A key role for autophagy and the autophagy gene Atg16l1 in mouse and human intestinal Paneth cells
nature medicine
NOD2 stimulation induces autophagy in dendritic cells influencing bacterial handling and antigen presentation
Nod1 and Nod2 direct autophagy by recruiting ATG16L1 to the plasma membrane at the site of bacterial entry

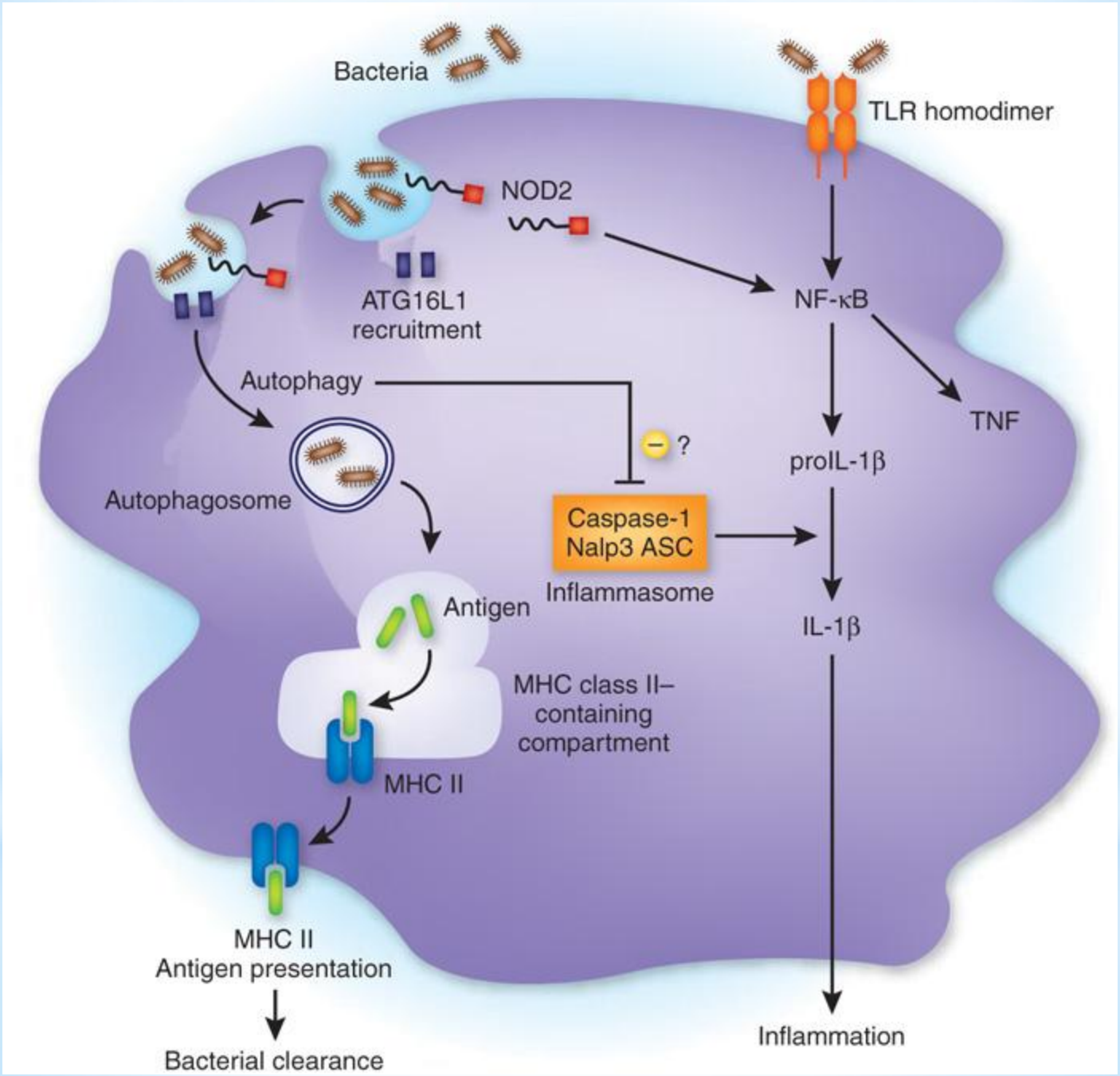
D'oh! Genes and Environment Cause Crohn's Disease



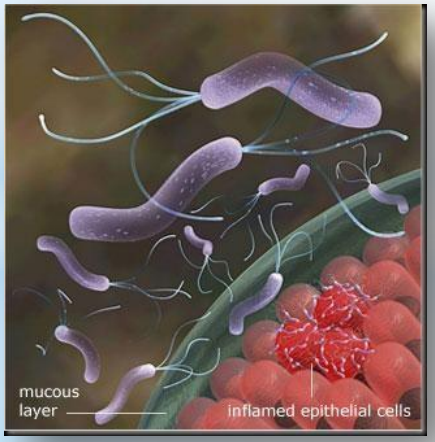
* *Atg16l1^{HM}* + MNV CR6 + DSS = hallmarks of Crohn's disease
  * Inflammation of muscularis, mesenteric fat and blood vessels
  * ↑ lymphoid aggregate
  * Subserosal fibrosis
  * Hypertrophy of muscularis propria
  * *These effects (but not standard DSS injury) are ↓↓ by blocking TNFa or IFNy or with broad-spectrum antibiotics*

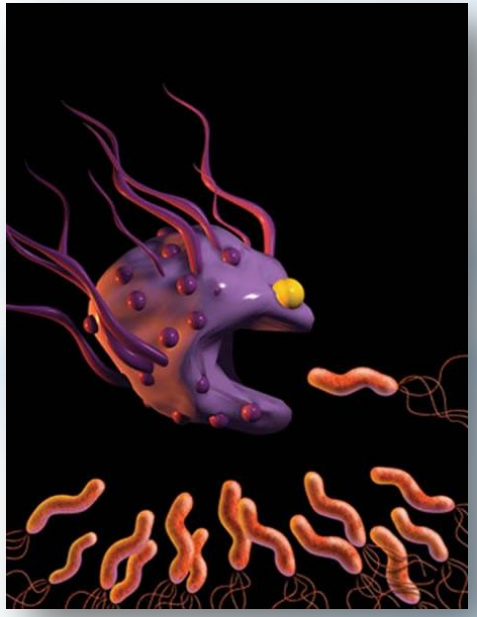
Gene defect + bacteria + virus + chemical injury ALL required to reproduce Crohn's-like phenotype

Cadwell K et al Nature 2008;456:259-63
Cadwell K et al CELL 2010;141:1135-45


Bacteria
TLR homodimer
NOD2
ATG16L1
recruitment
NF-κB
Autophagy
TNF
?
proIL-1β
Autophagosome
Caspase-1
Nalp3 ASC
Inflammasome
Antigen
IL-1β
MHC class II–
containing
compartment
MHC II
MHC II
Antigen presentation
Bacterial clearance
Inflammation

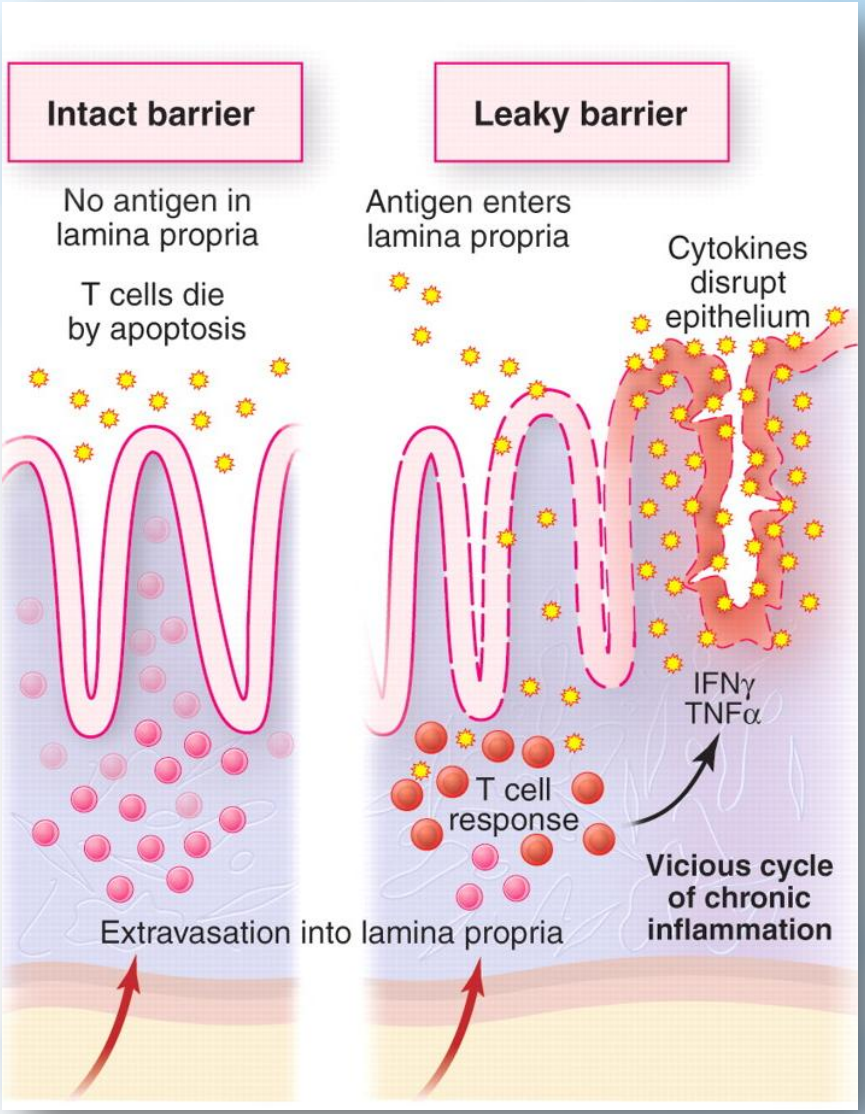
* The association of *NOD2*, *ATG16L1 and IRGM* strongly indicates that alterations in the intracellular processing of bacteria constitute a central feature of the pathogenesis of Crohn's disease.



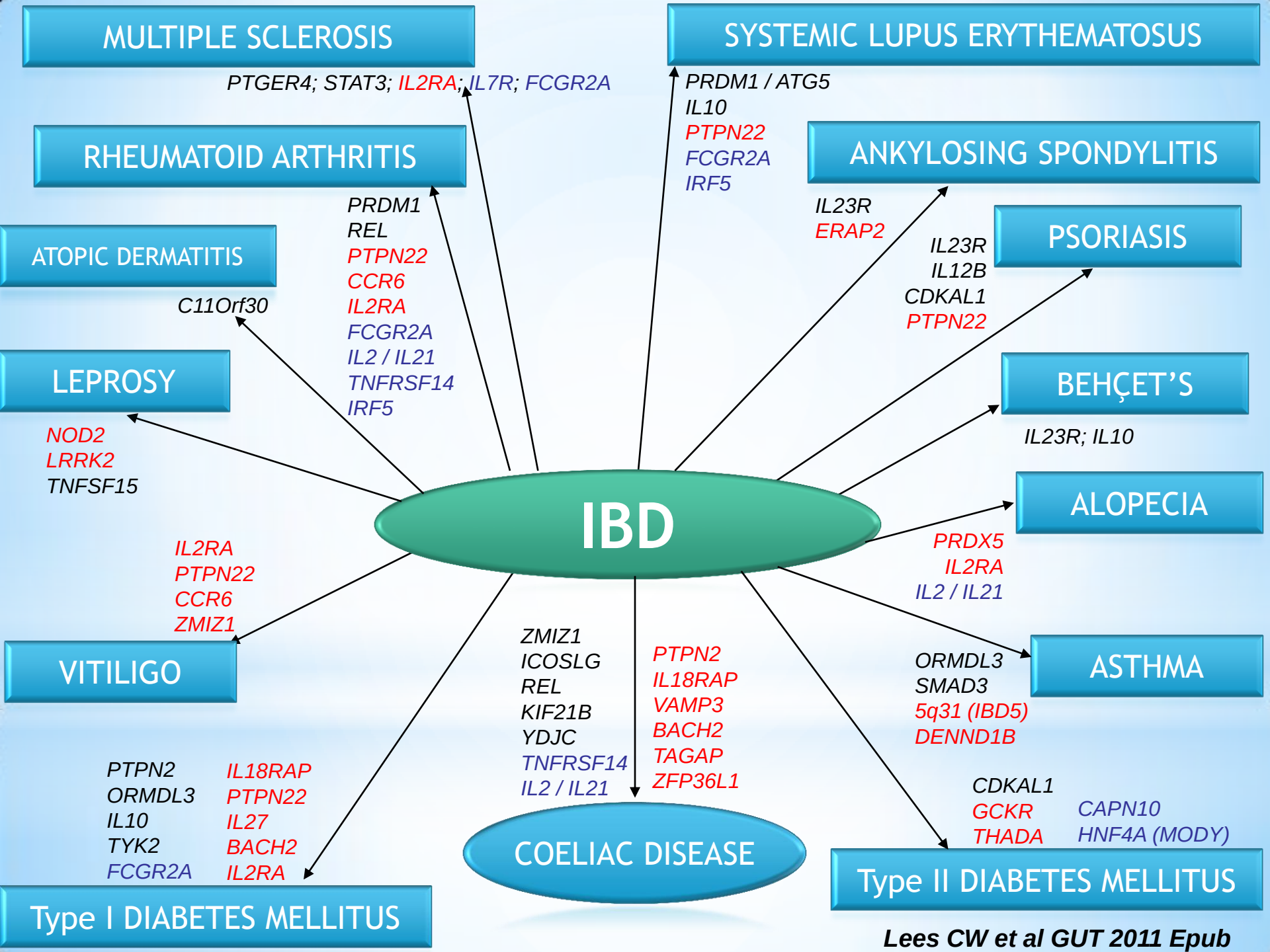


Genetics

ECM1
E-cadherin
Laminins
HNF4α
GNA12



*Defective barrier function in UC

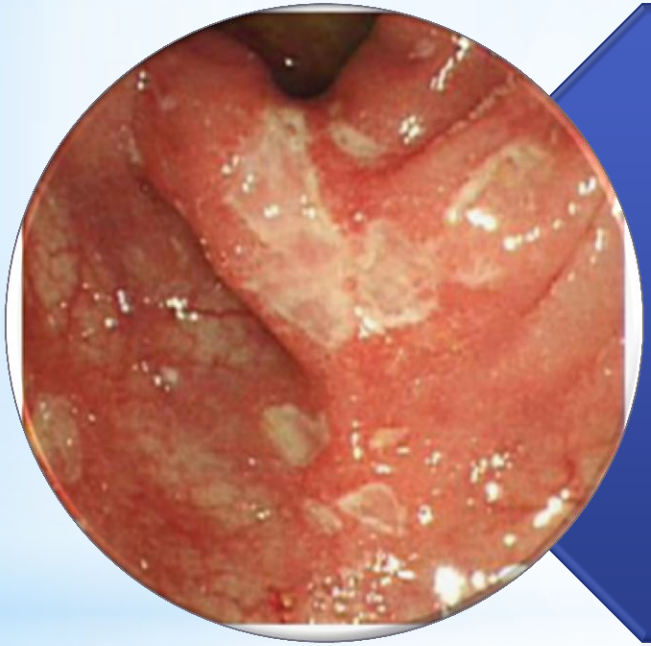


Lees CW et al GUT 2011 Epub

Genetics

* **cSNP;** Lead GWAS SNP highly correlated (r2>0.8) with common coding mutation (p.G428A / p.W142X)
* Non-secretor variant
  * 20% of background population
  * Ass. with no secretion of blood group antigens at epithelial surfaces
  * Near-complete protection from symptomatic norovirus infection
  * Partial protection from HIV and H pylori infection
  * BUT increased risk of Crohn's disease

* ***FUT2***



Clinical advance

- Therapeutics
- Prevention
- Biomarkers

* ***HMG CoA Reductase & statins***
  * SNPs within the gene ass. with small effects on cholesterol levels
  * BUT … statins which target the gene product are pretty effective & widely prescribed!

* Learning from other phenotypes …

GASTROENTEROLOGY 2008;135:1130–1141

CLINICAL-ALIMENTARY TRACT

A Randomized Trial of Ustekinumab, a Human Interleukin-12/23 Monoclonal Antibody, in Patients With Moderate-to-Severe Crohn's Disease

WILLIAM J. SANDBORN,* BRIAN G. FEAGAN,‡ RICHARD N. FEDORAK,§ ELLEN SCHERL,‖ MARK R. FLEISHER,¶ SEYMOUR KATZ,# JEWEL JOHANNS,** MARION BLANK,** and PAUL RUTGEERTS,‡‡ for the Ustekinumab Crohn's Disease Study Group

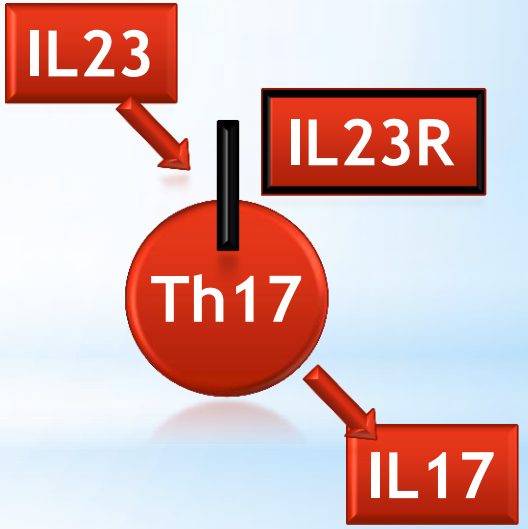
*Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, Minnesota; ‡London Health Sciences Center, London, Ontario, Canada; §Division of Gastroenterology, University of Alberta, Edmonton, Alberta, Canada; ‖Jill Roberts Inflammatory Bowel Disease Center, Weill Medical College of Cornell University, New York Presbyterian Hospital, New York, New York; ¶Borland–Groover Clinic, Jacksonville, Florida; #Long Island Clinical Research Associates, LLP, Great Neck, New York; **Clinical Biostatistics, Centocor, Inc, Malvern, Pennsylvania; and the ‡‡University Hospital Gasthuisberg, Leuven, Belgium

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

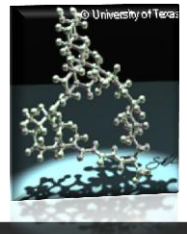
Anti–Interleukin-12 Antibody for Active Crohn's Disease

Peter J. Mannon, M.D., M.P.H., Ivan J. Fuss, M.D., Lloyd Mayer, M.D., Charles O. Elson, M.D., William J. Sandborn, M.D., Daniel Present, M.D., Ben Dolin, M.D., Nancy Goodman, R.N., B.S.N., Catherine Groden, R.N., M.S., Ronald L. Hornung, Ph.D., Martha Quezado, M.D., Markus F. Neurath, M.D., Jochen Salfeld, Ph.D., Geertruida M. Veldman, Ph.D., Ullrich Schwertschlag, M.D., Ph.D., and Warren Strober, M.D., for the Anti–IL-12 Crohn's Disease Study Group



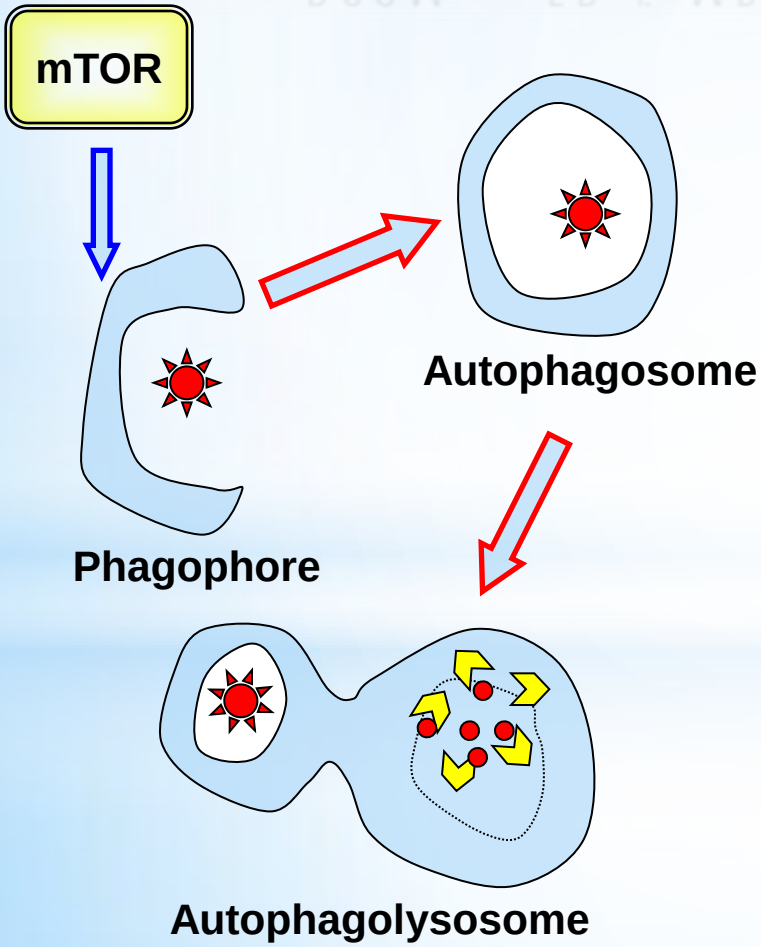
*Anti-IL23

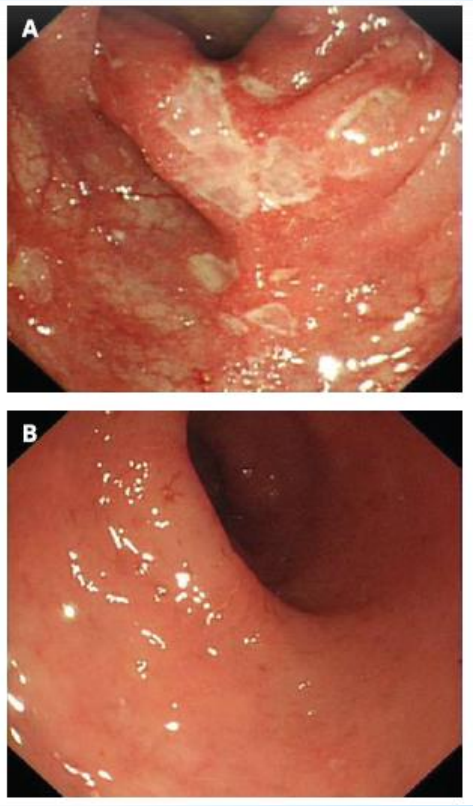
Case report



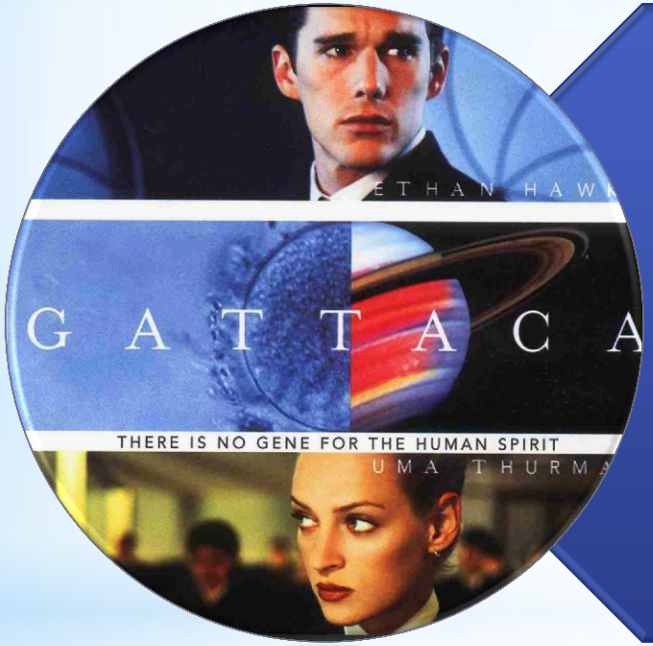
Use of sirolimus (rapamycin) to treat refractory Crohn's disease

D C O Massey, F Bredin, M Parkes



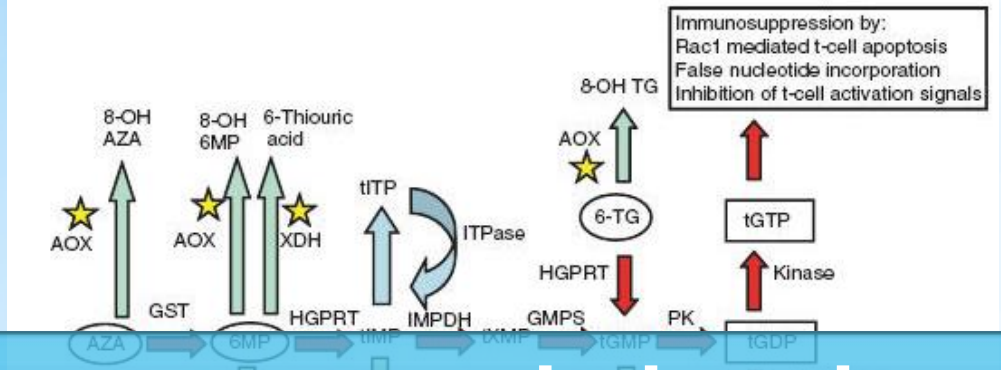


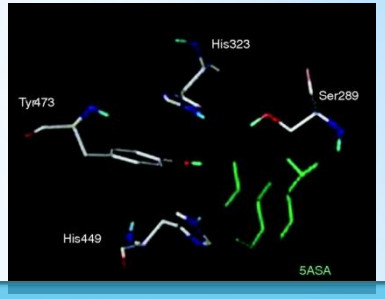
*Autophagy



Personalised medicine

- Diagnostics
- Prognostics
- Therapeutic optimisation





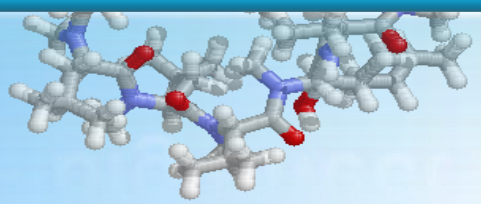
Can we use existing drugs better?

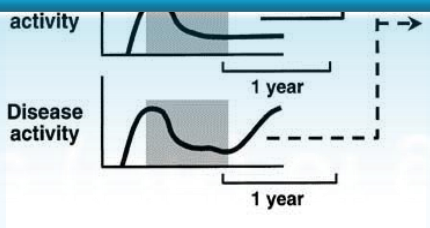
Predict response

- and avoid toxicity in non-responders

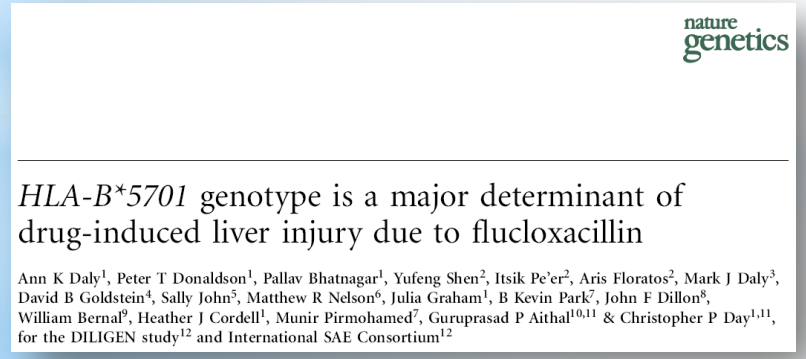
Predict risk of adverse events

- big effect size (OR) of germline mutations





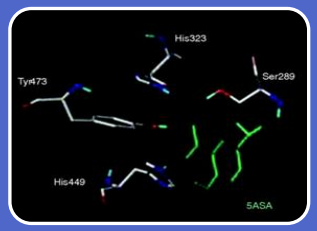
DRUG	RESPONSE	N	Genome-wide sign / replication	Implicated gene
Simvastatin	Myopathy	85/90	Yes / Yes	SLCO1B1
Flucloxacillin	Liver injury	51/282	Yes / Yes	HLA-B*5701
Iloperidone	QT prolongation	183 / 0	No / No	None found



*GWAS on ADR

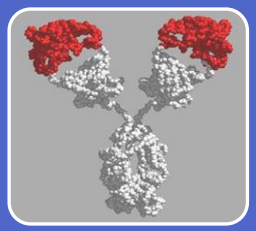
Adapted from Ann Daly Nature Reviews Genetics 2010

Genetics



5-ASA induced nephrotoxicity

- Currently recruiting
- Tariq.ahmad@doctors.org.uk

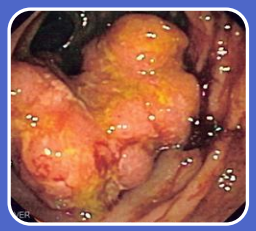


Rare anti-TNF side-effects

- Demyelination
- Lymphoma

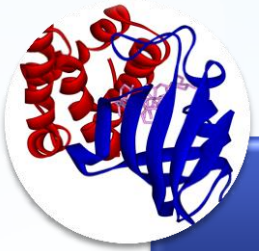
Response to anti-TNF drugs

- Acute severe UC (rescue therapy)
- Primary non-response in Crohn's



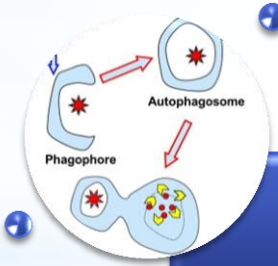
Colitis-associated colon cancer

‘Designer’ Clinical trials



Existing therapies?

sirolimus Rapamune® Tablets and Oral Solution



Biology of causal variants

Gene discovery

* Drug pipeline from gene discovery

Address issue of missing heritability

Sub-phenotypes

True biological understanding

Apply the technology to patients

*IBD Genetics ‘to do’ list

IBD Genetics

ECCO - European Crohn'... | IBDGenetics - Home page | IBD Genetics Research ib...

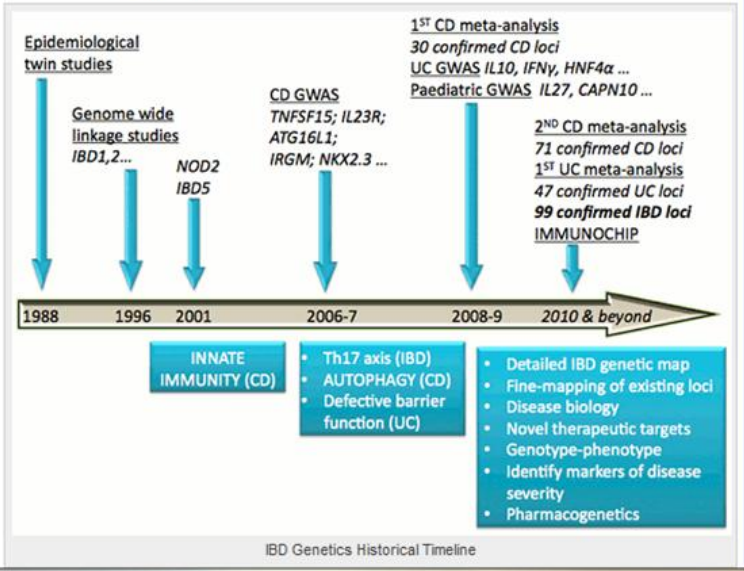
www.ibdgenetics.org

IBD Genetics

- Home page
- About IBD
- Groups Involved
- Current Projects
- Publications
- Links
- Downloads
- Contact us

International Inflammatory Bowel Disease Genetics Consortium (IIBDGC)

The International IBD Genetics Consortium is a network of researchers working on the genetics of inflammatory bowel disease (IBD). We have undertaken a number of large scale genome-wide association studies of both Crohn's disease and ulcerative colitis, which have identified dozens of genomic loci implicated in these diseases (99 at the end of 2010). We hope that this research can be translated into a more complete understanding of the biology of IBD which might lead to improved diagnoses and treatment .



IBD Genetics Historical Timeline

16:35
20/02/2011

wellcome trust

Advanced Courses and Scientific Conferences

Complex Disease Genetics for Clinicians

26-27 January 2012

Registration deadline: 7 November 2011

Full details at:
www.wellcome.ac.uk/hinxton

Wellcome Trust Genome Campus, Hinxton, Cambridge, UK