Crohn's disease heterogeneity and new pathology-relevant research tools

1. Genetic association studies across populations
2. Sources of Crohn's heterogeneity: Predicting disease course at diagnosis & after ileal resection
3. Future research directions

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March 17, 2019

Mount Sinai

New *classes* of approved IBD therapies: early 2019

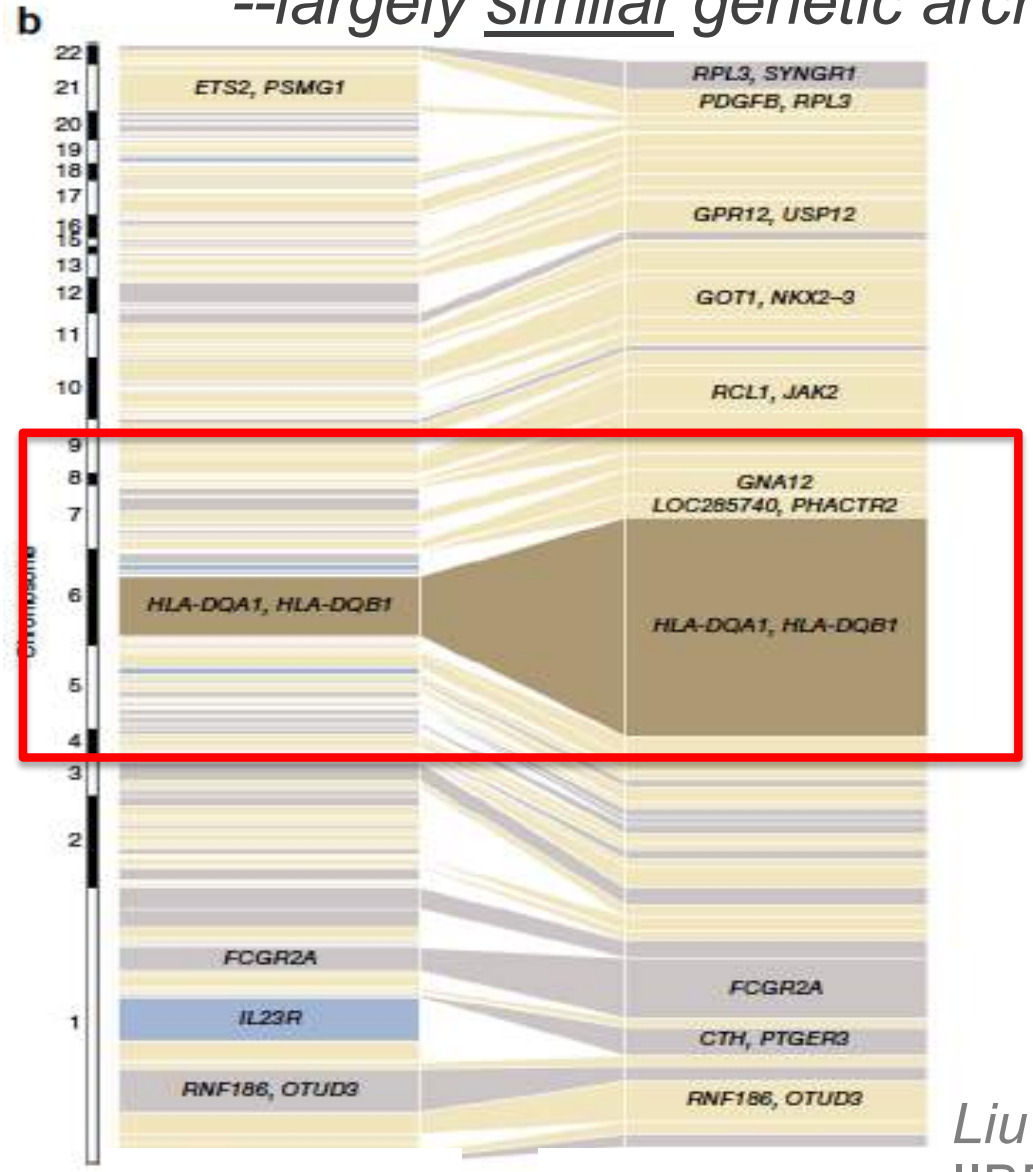
Many new agents BUT: only a minority of patients with complete one year remission/mucosal healing rates

Agent class	*Present* indication	Year of approval
Anti-TNF	Crohn's & ulcerative colitis	1998
vedolizumab (anti-integrin)	Crohn's & ulcerative colitis	2014
ustekinumab (antiIL12/23)	Crohn's	2016
tofcitinib (JAK inhibitor)	ulcerative colitis	2018

Part 1: Genetic association studies across populations

Ulcerative colitis is a more typical chronic inflammatory disease--*dominant MHC class II*

--largely <u>similar</u> genetic architecture across populations



Similar effect sizes, similar allele frequencies

Different effect sizes

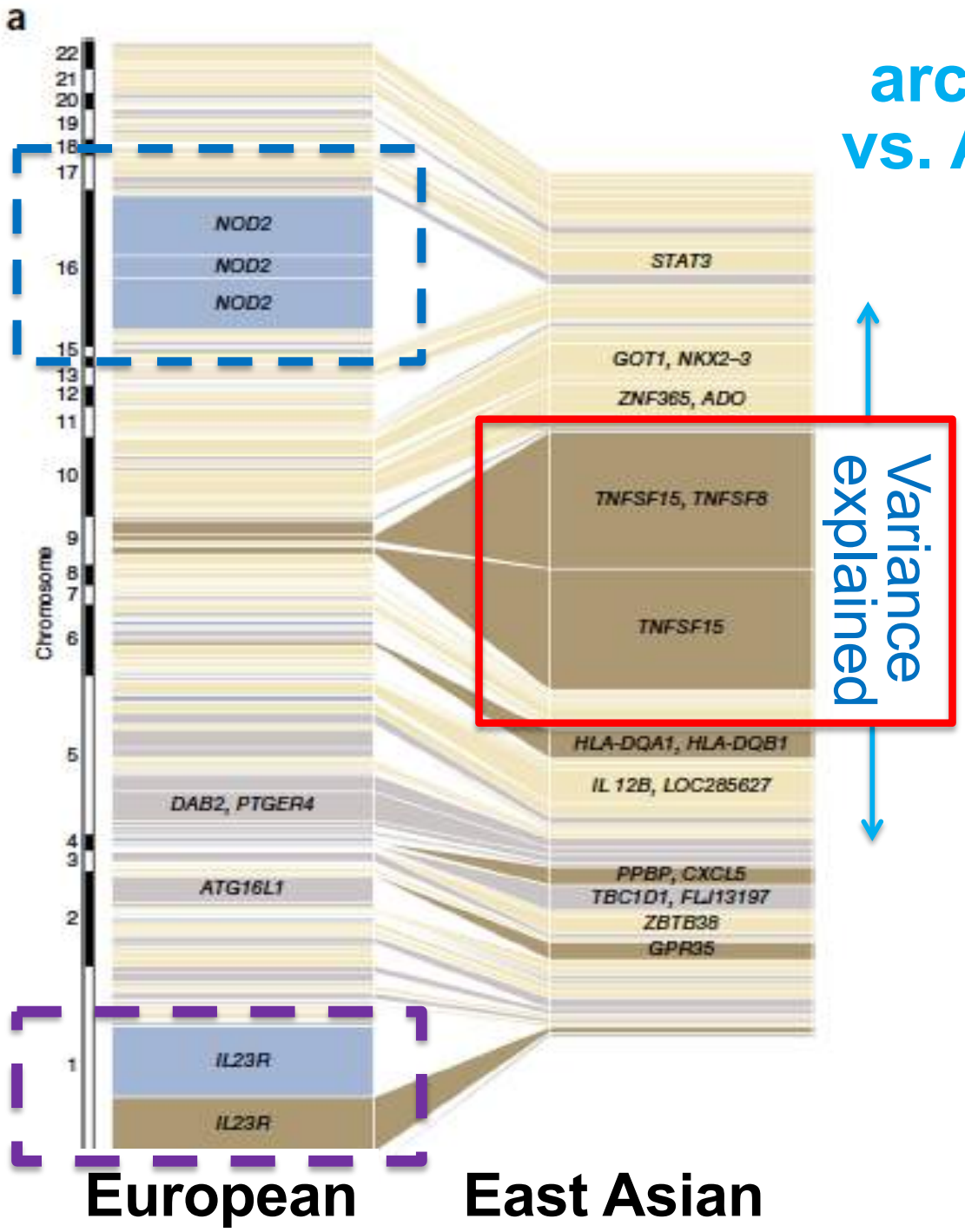
IL23R Arg381Gln: Monomorphic in Asians

Liu et al., Nature Genetics 2015; 979
IIBDGC

Population differences in major Crohn's disease genetic associations

European ancestry	Far East Asian	African-American
NOD2 (ileal stricturing)	TNFSF15	PTGER4
IL23R (CD & UC)	MHC/HLA class II	IL23R (CD & UC)
PTGER4		NOD2 admixture

Markedly distinct architectures: European vs. Asian *Crohn's disease*

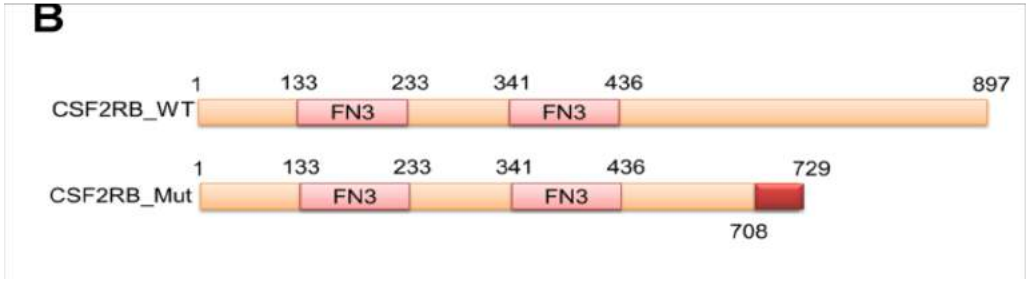


Chr9: ***TNFSF15* locus—similar alleles, much larger effects in Asians**

Monomorphic in Asians

Different effect sizes

Uncommon risk alleles: Jewish-predominant frameshift mutation in *CSF2RB (GMCSF, IL3, IL5 signaling)*



	N_{CD}	N_{ctrl}	MAF_{CD}	MAF_{ctrl}	P-value	OR
AJ Discovery cohort	1477	2614	0.032	0.020	8.52×10^{-4}	1.6
AJ Replication cohort	1515	7052	0.033	0.022	5.39×10^{-4}	1.5
Total	2992	9666	0.032	0.022	3.42×10^{-6}	1.5

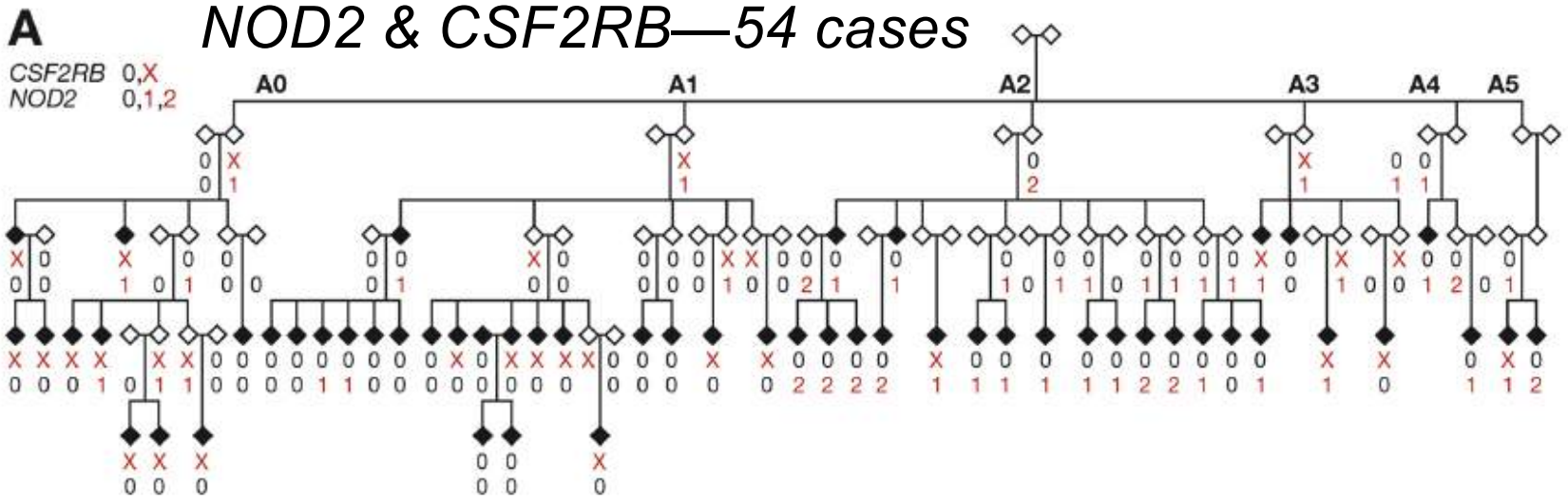
P-values: function of effect size (OR), allele frequency (3%) and sample sizes (2992 cases)

As many as one-third of Crohn's patients carry neutralizing antibodies against GM-CSF

Ling-Shiang Huang

Huang et al., Gastroenterology 2016, 710

Multiple uncommon risk alleles contribute to familial clustering

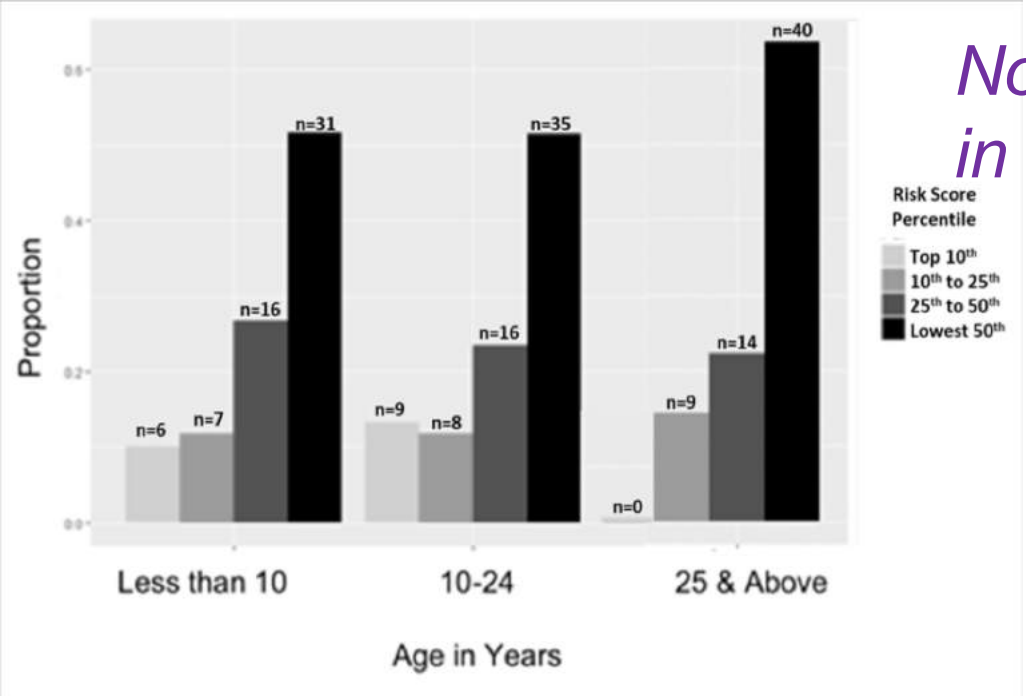


Levine et al., Gastroenterology 2016; 698

Road to Prevention: Family based studies in the highest at risk populations

Prediction (polygenic risk scores, P ~ < 10^{-4}) vs. hypothesis testing (P < 5 x 10^{-8})

Interim analyses: 77 affected, 191 unaffected



No unaffecteds with PRS in top 10% above age 25

~1/2 carry NOD2, LRRK2 or CSF2RB

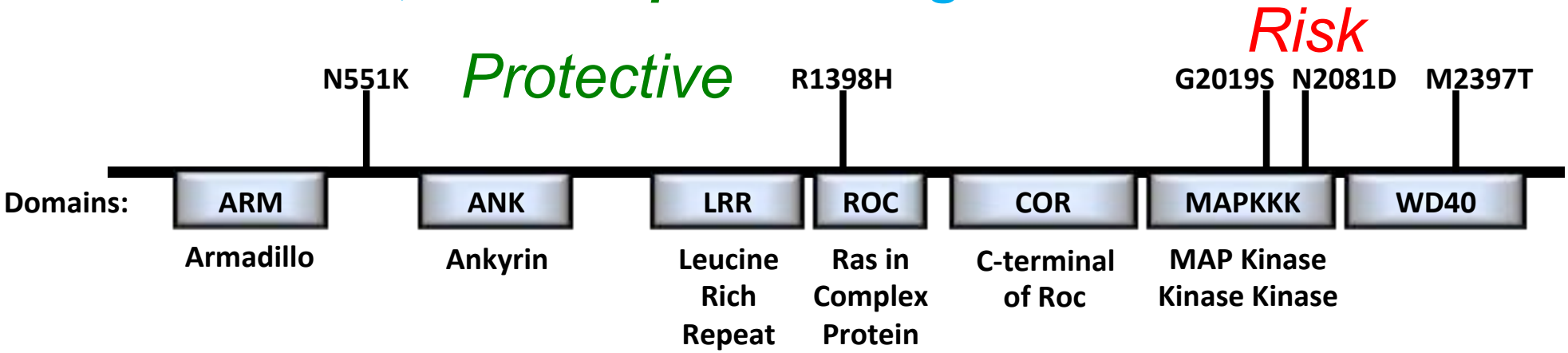
Marla Dubinsky

Elizabeth Spencer

Janssen
Shannon Telesco
Amy Hart

Kyle Gettler

Jewish predominant association: One gene (LRRK2), two diseases, *risk* and *protective* gene domains



Crohn's disease

1. Intestine
2. Disease onset: 15-30
3. Common protective allele: R1398H (ROC domain)
4. Unique, gain of function kinase: N2081D

Parkinson's disease

1. Brain
2. Late onset: 50's & above
3. Common protective allele: R1398H (ROC domain)
4. Unique, gain of function kinase: G2019S

Hui et al., Sci Trans Med, 2018

Like *NOD2*, *LRRK2* is associated with an earlier age of onset & ileal location

N2081D	Age of CD onset (SD) [N]	Ileal disease [N]
AA	26.5 (14.0) [5601]	80.5% [5311]
AG	24.6 (13.1) [483]	86.1 [453]
GG	20.8 (9.0) [12]	90.9% [11]
	P=0.002	p=0.01

NOD2 & LRRK2 effects on Paneth cells?
--Zhang et al., Nature Immunology 2015: 918

Ken Hui *Inga Peter*

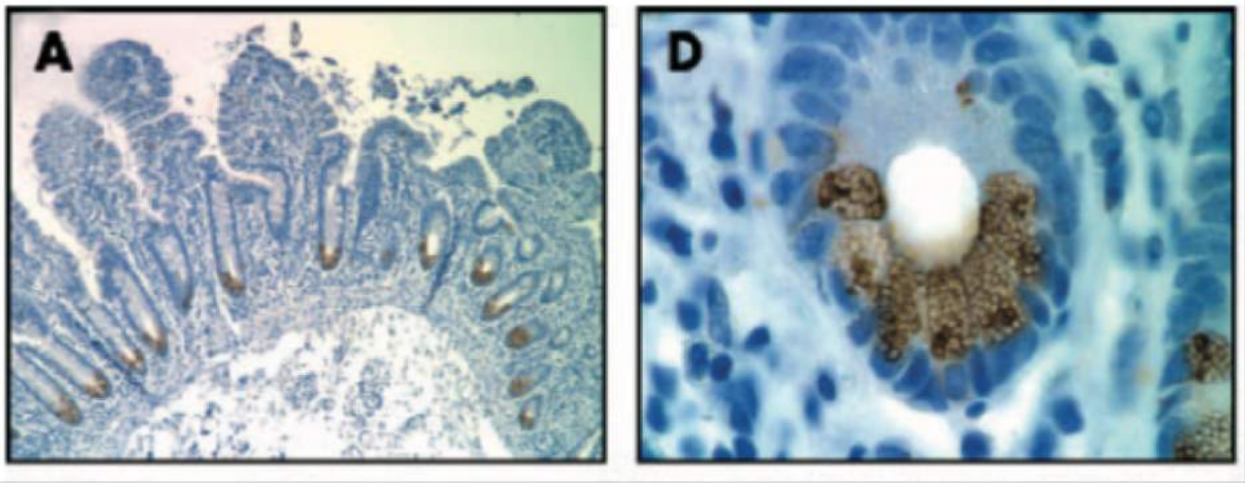
Hui et al., Sci Trans Med 2018;

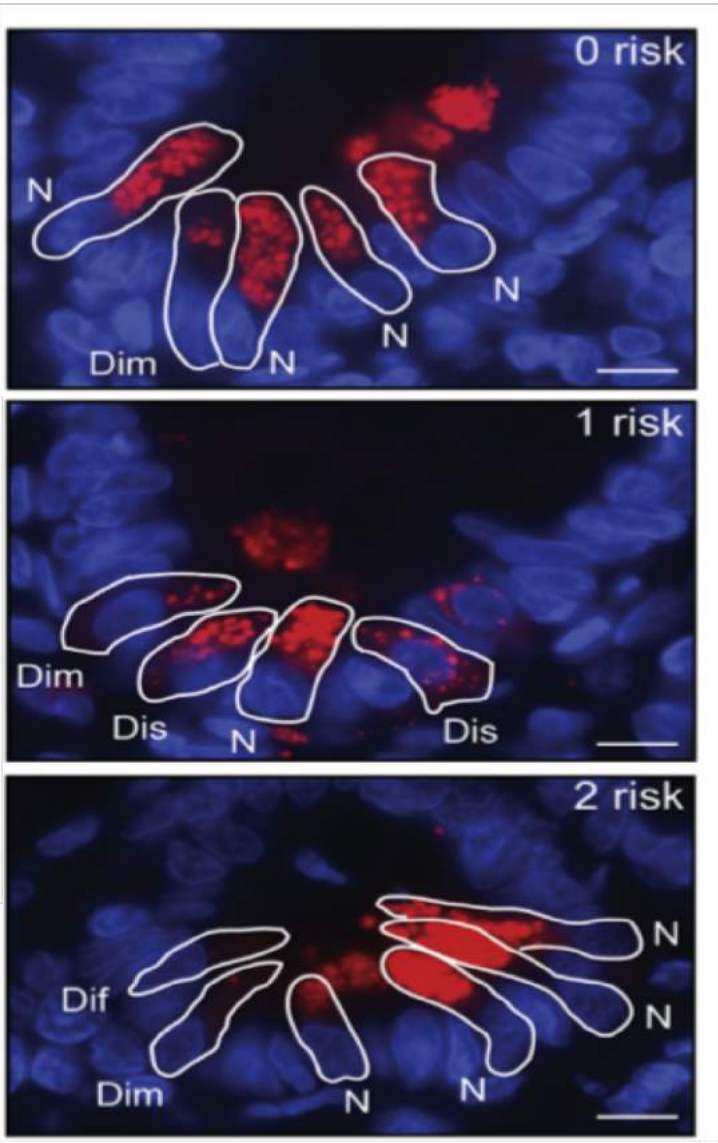
NOD2 is highly expressed in Paneth cells and NOD2 risk alleles correlate with "Panethopathies"

Expression of NOD2 in Paneth cells: a possible link to Crohn's ileitis

Y Ogura, S Lala, W Xin, E Smith, T A Dowds, F F Chen, E Zimmermann, M Tretiakova, J H Cho, J Hart, J K Greenson, S Keshav, G Nuñez

Gut 2003;**52**:1591–1597





*Panethopathies: **early** change in Crohn's??*

Van Dussen et al., Gastroenterology 2014; 200

Part 2: Sources of clinical heterogeneity in Crohn's disease

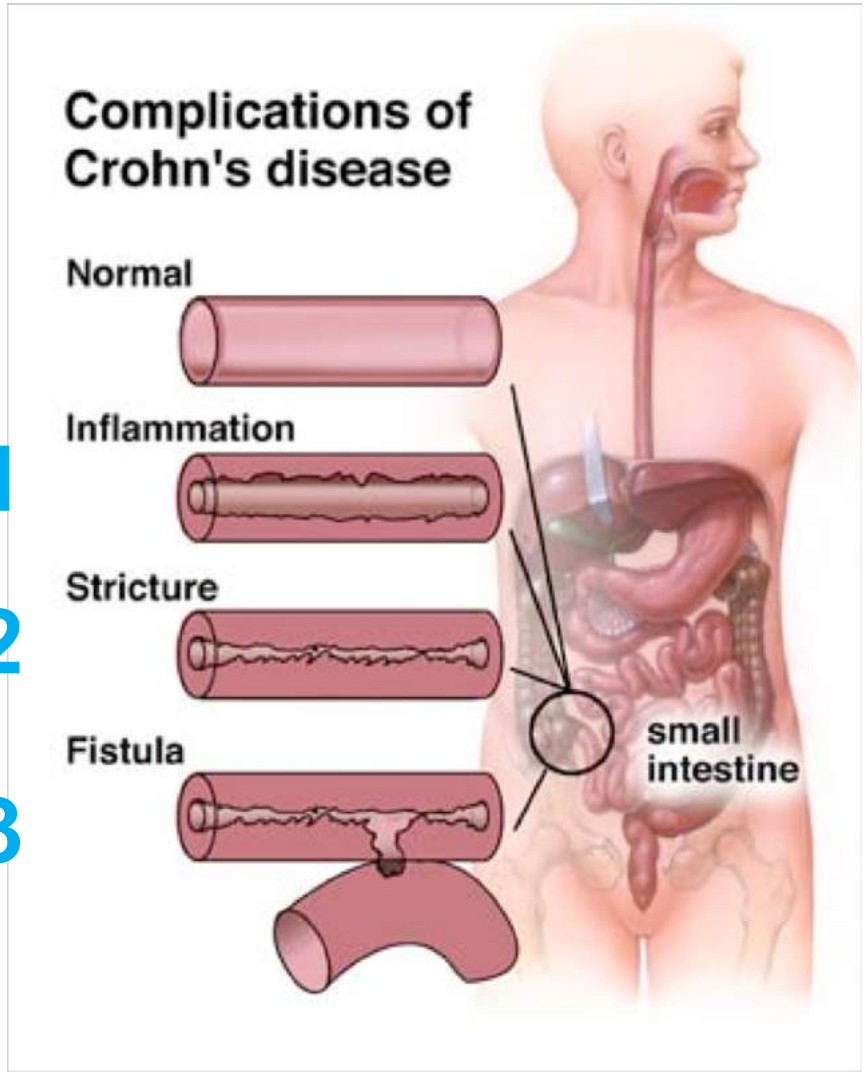
Assessments at

- *diagnosis*
- *after ileal resection*

Sources of heterogeneity: classic clinical considerations

1. *Longitudinal* disease location: ileal-only (30-40%), ileocolonic (40-50%), colonic only (10-20%) + perianal
2. *Transmural* disease location: correlated with complications; behavior classification
3. At diagnosis: *age* of onset, *number* of antibodies (ASCA, Cbir, anti-GMCSF)
4. Tobacco use
5. *Quality of clinical care,* esp. wrt early and appropriate biologic use

Categorical classification of behavior (B): stricturing Crohn's disease (B2) vs. fistulizing (B3)

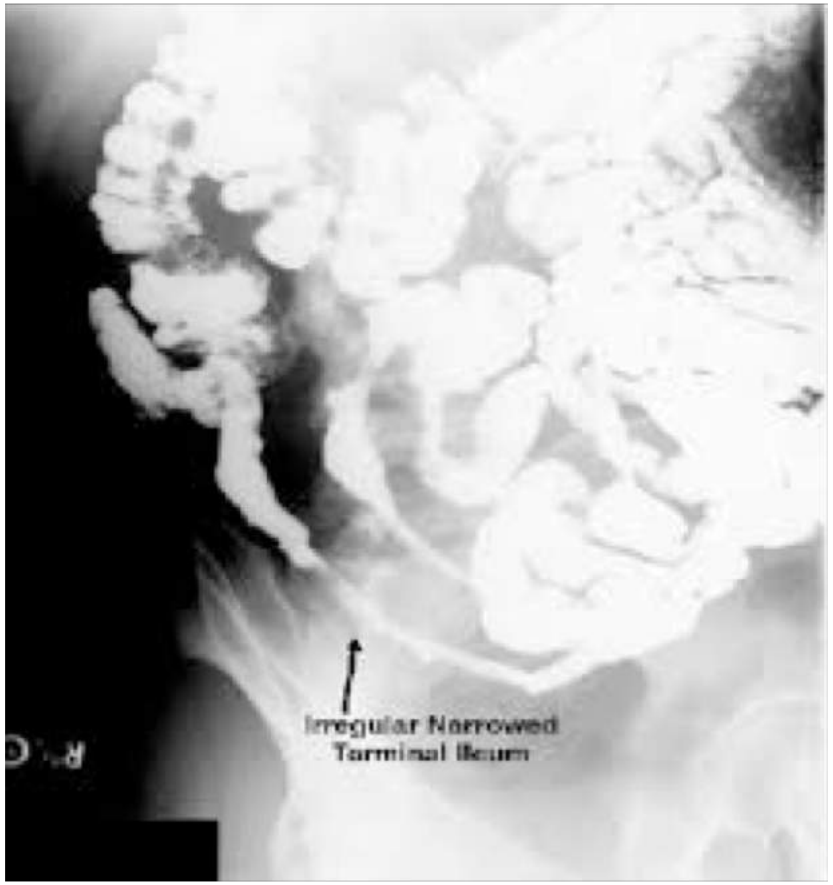


B1

B2

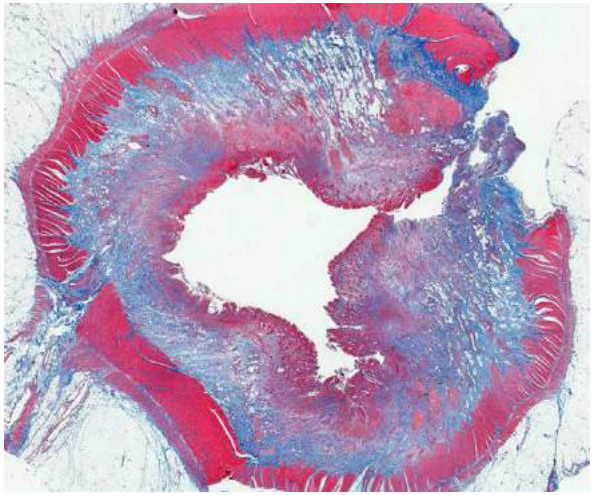
B3

B2 Crohn's disease

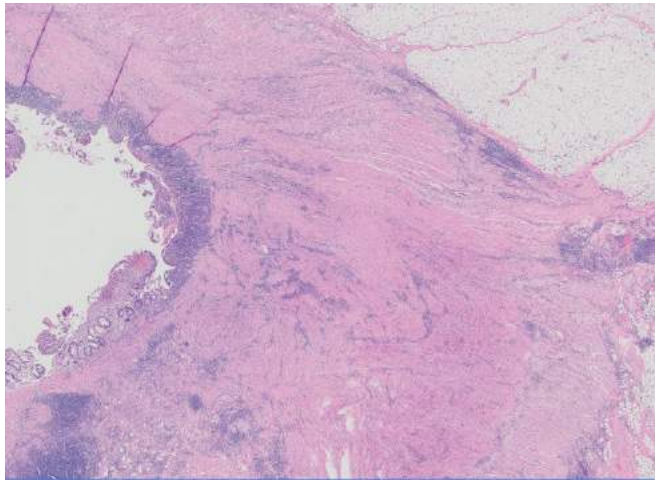


Tissue types within Crohn's strictures

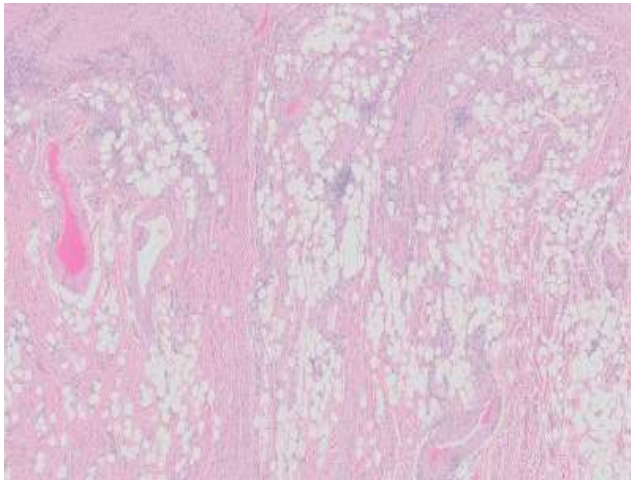
Fibrosis



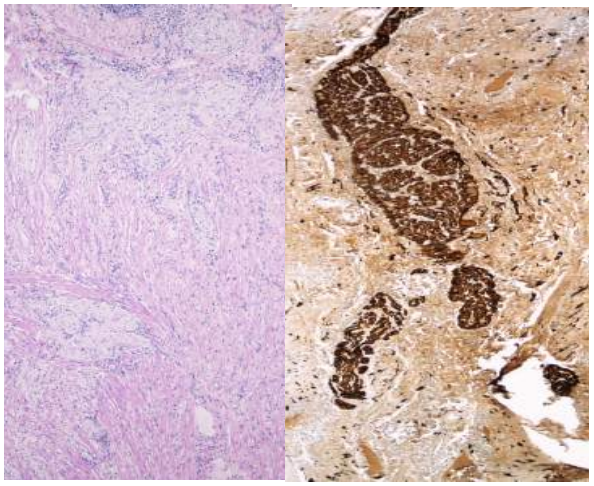
Smooth muscle



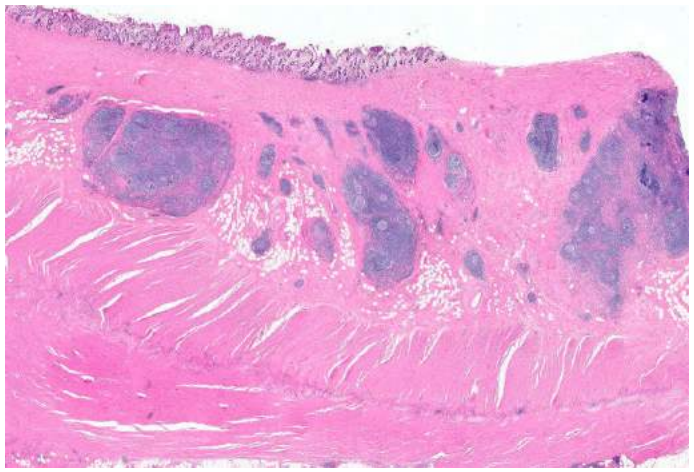
Adipose tissue



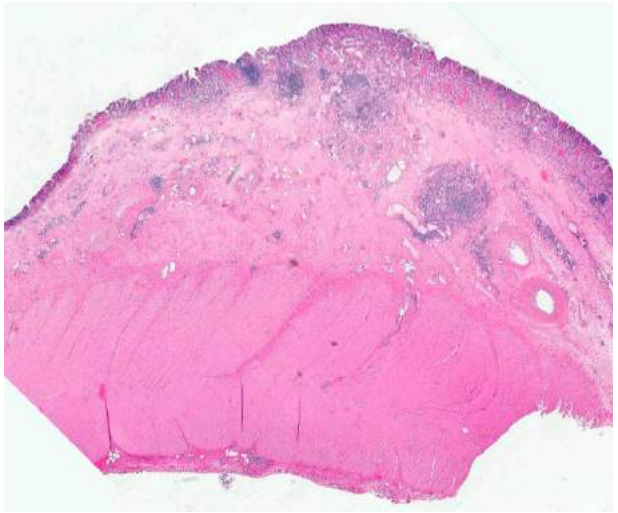
Neural tissue



Lymphoid tissue



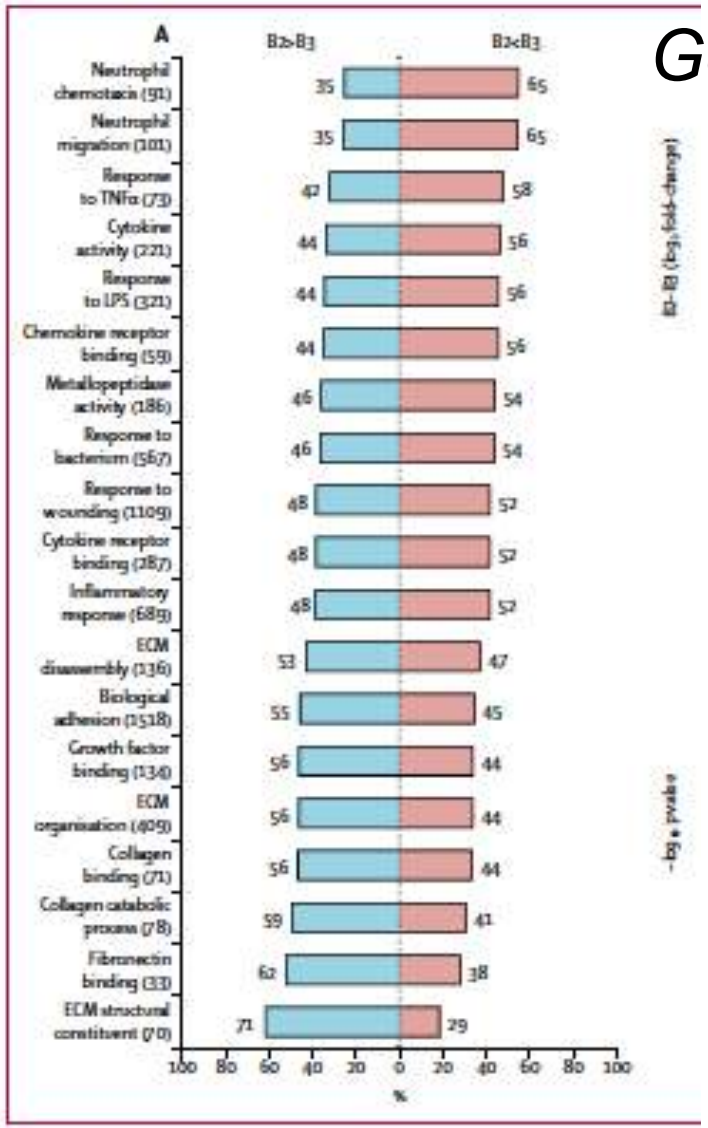
Edema



Courtesy of Noam Harpaz

Triumph of bulk RNASeq with clearly defined outcomes

Pediatric inception cohort followed for complications

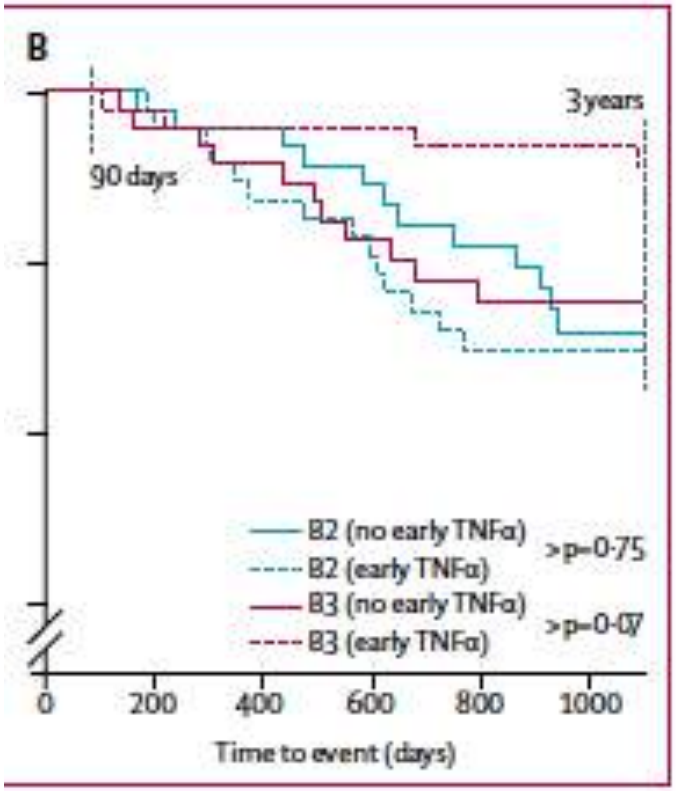


Gene expression profiles of B2 vs. B3 Crohn's disease

- B3
  - Neutrophil recruitment
  - Response to TNF
  - Response to LPS
- B2
  - Extracellular matrix components: collagen
  - Fibronectin binding
  - Growth factor binding

Kugathasan Lancet 2017; 1710

Early anti-TNF (dashed lines) <u>trends</u> toward protecting against B3, but not B2 complications



Agent	Year of approval
Anti-TNF	1998
vedolizumab (anti-integrin)	2014
ustekinumab (anti-IL12/23)	2016
tofacitinib (JAK inhbitior)	2018

RNASeq at diagnosis→ different class of first agent??

Present scenario: after anti-TNF failure→ which agent?

Ileal resection--> what next??

Kugathasan Lancet 2017; 1710

POCER study (Lancet 2015) De Cruz et al

Role for early post-op surveillance

1. N=174 patients, 17 centers, randomized (2:1) to active care (colonoscopy at 6 months + 18 months) or standard care (optimal therapy, colonoscopy at 18 months)
2. Primary outcome: endoscopic recurrence at 18 months
3. All received metronidazole x 3 months; initial therapy based on risk (smoker, prior surgery, penetrating disease)
4. 6 month colonoscopy→ 39% stepped up
5. 18 month recurrence rates: 49% in “active”; 67% in “standard” P= 0.03
6. Comment: US relevance—higher rates of pre- & post-op anti-TNF use? Higher rates of tobacco (31%) in Australia

Prevent study (Gastro 2016) Regueiro et al.

1. N=297 patients, 104 centers, *randomized* (1:1, within 45 days) to infliximab 5 mg/kg
2. Primary outcome: clinical recurrence and endoscopic recurrence at week 76
3. Patients: only 20-25% with prior anti-TNF
4. 76 week results (~18 months)
    1. Clinical recurrence (CDAI> 200; delta >70): 12.9 vs. 20.0% recurrence (P = 0.097)
    2. Endoscopic recurrence 30.6% vs. 60% (P < 0.001)
5. *Prior anti-TNF use: at risk for clinical recurrence*

NIDDK IBDGC post-op ileal resection protocol

Purpose: Defining the earliest stages of disease pathogenesis; to map altered gene expression with genetics

1. Six sites recruiting: started ileal resection protocol 2014. 4 U.S. (NYC, Pittsburgh, Los Angeles, Baltimore), 2 Canadian (Toronto, Montreal)
2. Observational
3. Bulk RNASeq biopsies taken at neoterminal ileum at first and second post-operative colonoscopy
4. Plasma samples taken at time of colonoscopies

Margaret Walshe
Mark Lazarev
Phil Schumm
Mark Silverberg

Patient summary & recurrence rates at #1 & #2 post-op colonoscopy

Patient characteristics at resection	Median (IQR) / % (n = 133)
Age (years)	32 (24–41)
Gender (female)	47%
Disease behaviour (Montreal classification) B1. Non-stricturing, non-penetrating B2. Stricturing B3. Penetrating	 1% 41% 58%
Time since diagnosis (years)	9 (3–15)
Disease location (Montreal classification) L1. Ileal L3. Ileocolonic	 44% 56%
Smoking post-operatively	12%
Anti-TNF use post-operatively	40%

Chemokines as blood biomarkers!!!

Rutgeert's score	1st colonoscopy, n=125	2nd colonoscopy, n=55
i0 n(%)	62 (50%)	19 (35%)
i1 n(%)	30 (24%)	11 (20%)
i2 n(%)	20 (16%)	15 (27%)
i3 n(%)	9 (7%)	9 (16%)
i4 n(%)	4 (3%)	1 (2%)

74% non-recurrence

55% non-recurrence

26% recurrence

45% recurrence

Part 3: Future Research Directions

a. Single cell RNASeq
b. Natural language processing

Aim: define the immune cellular architecture of the terminal ileum in active & inactive CD + blood

- 15-20 biopsies from inflamed & uninflamed tissues + peripheral blood from 11 Crohn's disease patients
- Cell isolation protocols: EDTA-treatment x 2
- scRNASeq performed using 10X Genomics Chromium
- Seq reads mapped to build 38. UMI counts matrices generated by CellRanger
- Joint cell clustering: all 22 tissue samples clustered together
  - Correction for batch-aware cell-to-cell contamination

Ephraim Kenigsberg

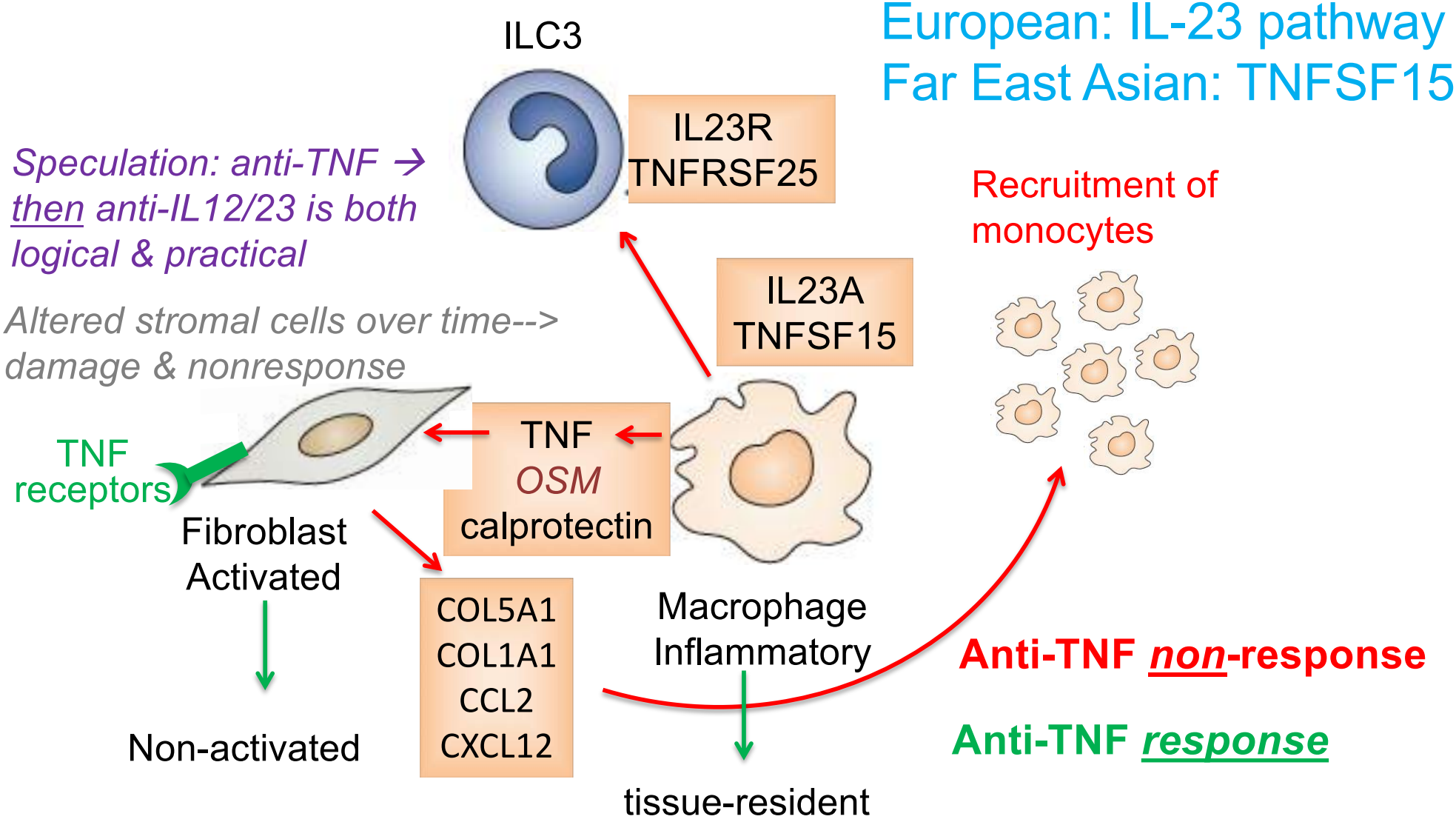
Jerome Martin

Miriam Merad

- NIDDK IBDGC
- Boehringer-Ingelheim
  - Jay Fine
  - Charles Whitehurst
- *BioRxiv 503102*

Dissecting pathophysiologic & population-based heterogeneity based on major genetic associations

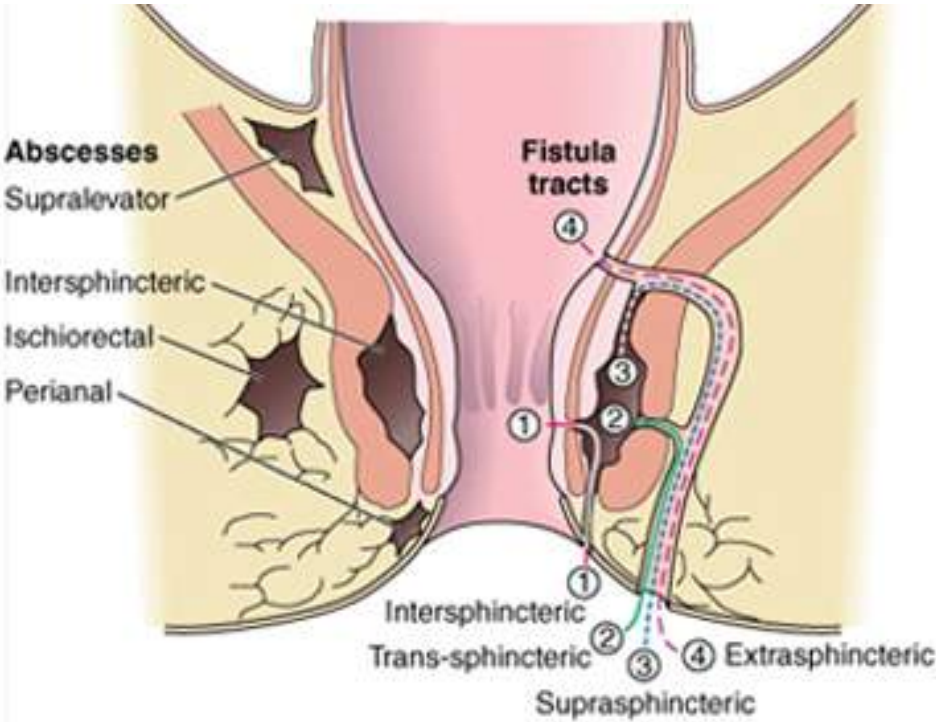
Source-target mappings in innate immunity



West et al., Nature Medicine 2017, 579

Next scRNASeq--Perianal Crohn's disease: unmet need, esp. in African populations

Challenges in relevant, live cell sampling



Harrison's Internal Medicine

Perianal Crohn's disease: unmet need, esp. in African populations. Colon-only CD

Site of intestinal lesion	*Patients (no.)*		
	Total	*With fistulae*	
		(No.)	*(%)*
Small intestine	339	40	12
Combined ileocolic	341	51	15
Large intestine			
No rectal involvement	68	28	41
With rectal involvement	71	65	92

Hellers et al., Gut 1980, 525

Plan: Comparative African vs. EA Crohn's

From texts to structured data: natural language processing

\R\9 cm long segment distal - terminal ileum wall thickening, enhancement, and hyperemia of surrounding vessels compatible with active terminal ileitis. Length of this segment is similar to slightly shorter than prior exam, although severity of disease is similar.

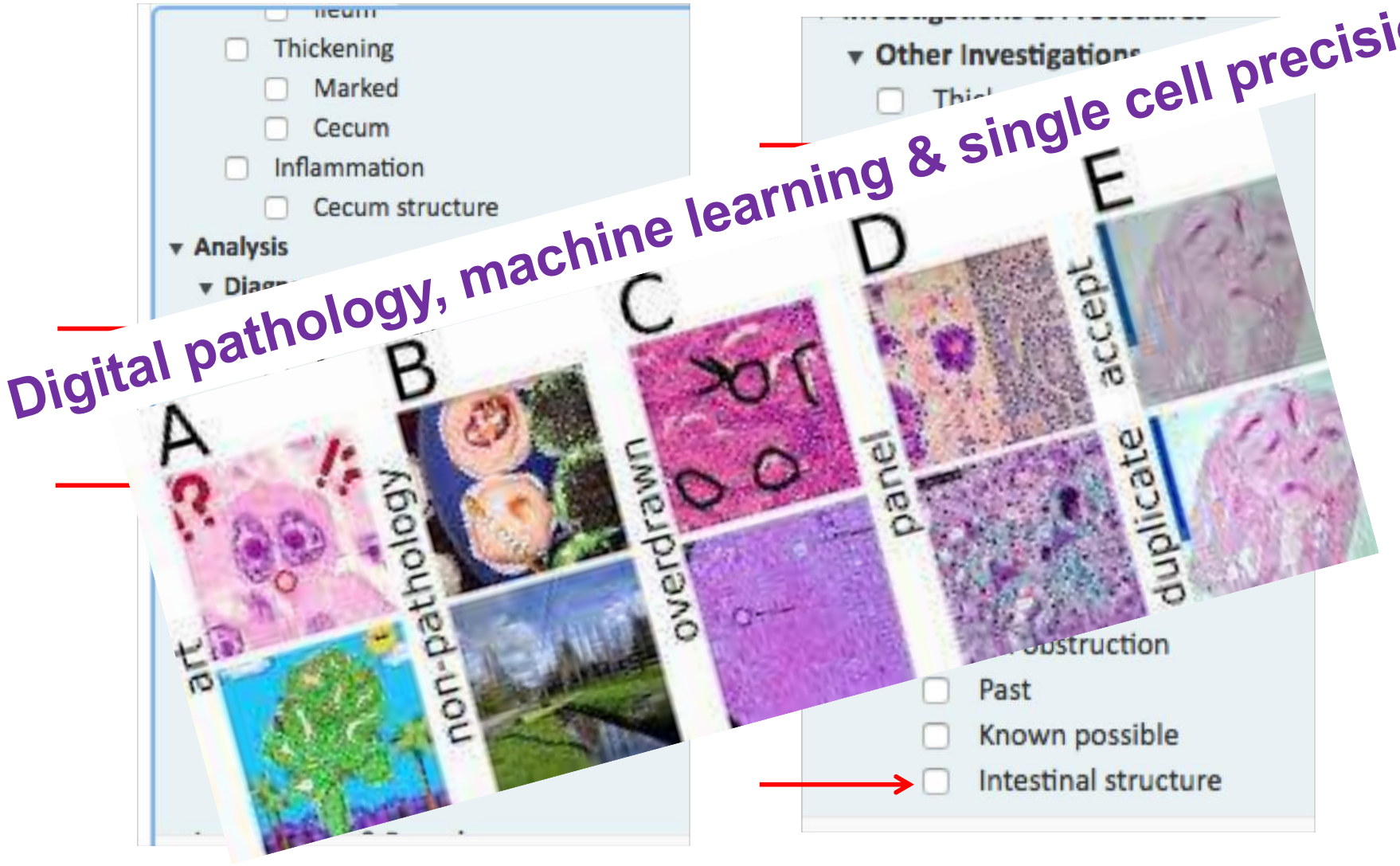
Mild dilatation of multiple ileal loops proximal to the TI disease, although improved from prior, cannot exclude component of partial obstruction secondary to TI disease / stricturing, versus ileus.

Marked cecal wall thickening compatible with inflammation / cecitis. Probable small intramural fistula at the cecum

Probable1-2 cm length fistula or tract, right lower quadrant, near the terminal ileum

Right mid abdomen drain in place, with resolution of previously noted collection, and no current drainable collection.

To structured data: scaling longitudinal data collections from radiology and pathology reports



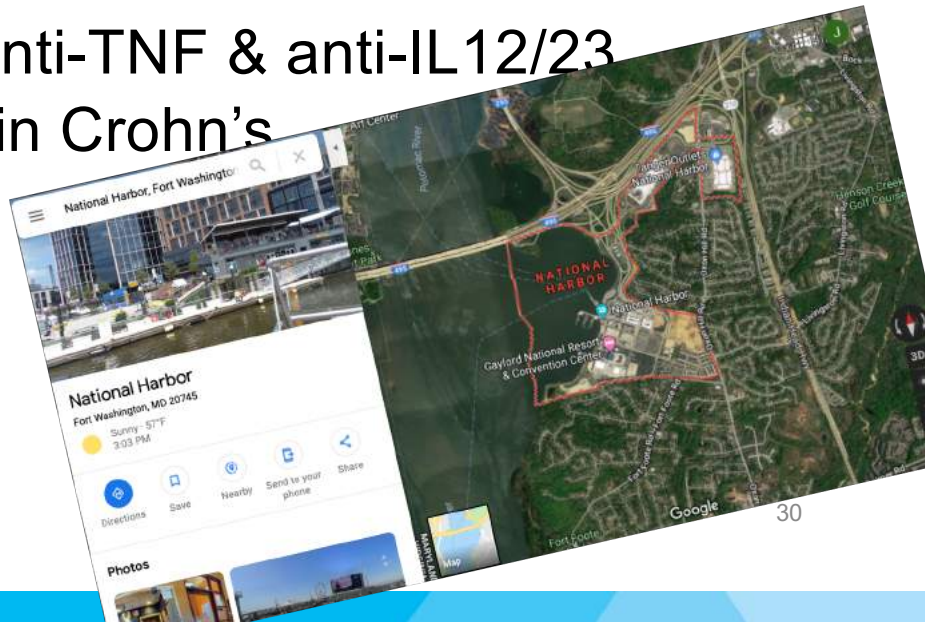
Conclusions

1. *Numerous cell types* contribute to Crohn's pathophysiology & heterogeneity: Paneth & other epithelial cells, innate & adaptive immunity, stromal cells

2. Crohn's disease heterogeneity: *classic clinical* features
   -- *Future:* genetics, RNA analysis of tissues, blood proteins (chemokines)

3. **Single cell sequencing: revolution in biology**. Can project onto bulk RNASeq cohorts in larger numbers
   -- Cellular differences between anti-TNF & anti-IL12/23
   -- anti-TNF: most effective *early* in Crohn's

4. GI & cell *atlases*: ***surgical pathology!!***



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