Caspase recruitment domain 9, microbiota, and tryptophan metabolism: dangerous liaisons in inflammatory bowel diseases.

CURR OPIN CLIN NUTR METAB CARE. 2017 APR

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Aim

How caspase recruitment domain 9 (CARD9), one of the numerous IBD susceptibility genes, participate to colitis susceptibility by shaping gut microbiota to produce tryptophan metabolites.

KEY POINTS

- Alteration of intestinal microbiota is observed in IBD.
- CARD9 is one of the identified IBD susceptibility genes.
- CARD9 has a role in shaping the bacterial and fungal gut microbiota and it is required for the production of AhR ligands by the microbiota.
- Impaired ability of the microbiota to catabolize tryptophan into AhR ligands increased sensitivity to colitis by altering the interleukin 22 signaling pathway.
- Impaired microbial production of AhR ligands is observed in patients with IBD and correlates with an IBD-associated SNP within *CARD9*.

1. IMPACT OF THE CASPASE RECRUITMENT DOMAIN 9 -/- MICROBIOTA IN THE SUSCEPTIBILITY TO COLITIS (colonization of germ free mice with WT microbiota and microbiota from CARD9 -/- mice and then they exposed them to DSS)

- They observed an increased susceptibility to colitis with decreased proliferation and increased apoptosis in intestinal epithelial cell of CARD9 -/- to germfree mice.

- Down-expression of IL22,Reg3g ,and Reg3b gut limited

2. TRYPTOPHAN METABOLISM IS IMPAIRED IN CASPASE RECRUITMENT DOMAIN 9 -/- to GERM-FREE MICE

- They determined the concentration of AhR ligands in the feces of our mice. Production of IAA was impaired in feces of CARD9 -/- to germ-free mice

- Their results showed that impaired tryptophan metabolism in the microbiota of the CARD9 -/- mice lead to defective AhR activation which contributes to the susceptibility of mice to colitis by reduction of interleukin 22 production.

3. IMPAIRED ARYL HYDROCARBON RECEPTOR ACTIVITY AND TRYPTOPHAN METABOLITES IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE

- They analyzed fecal samples from individuals with IBD and healthy study participants for their ability to activate AhR. Impaired AhR activity associated with decreased concentrations of IAA and tryptophan were observed in feces of patients with IBD

- These patients were also genotyped for an IBD-associated single-nucleotide polymorphism (SNP) withinnCARD9 (rs10781499)

- The results suggest a connection between IBD, CARD9, and the ability of the microbiota to produce AhR agonists in humans

Correcting impaired microbiota functions, such as ability to produce AhR ligands, is an attractive strategy in IBD.

A long noncoding RNA signature for ulcerative colitis identifies IFNG-AS1 as an enhancer of inflammation

AMERICAN JOURNAL OF PHYSIOLOGY - GASTROINTESTINAL AND LIVER PHYSIOLOGY 2016 VOL. 311 NO. 3, G446-G457

PADUA ET AL

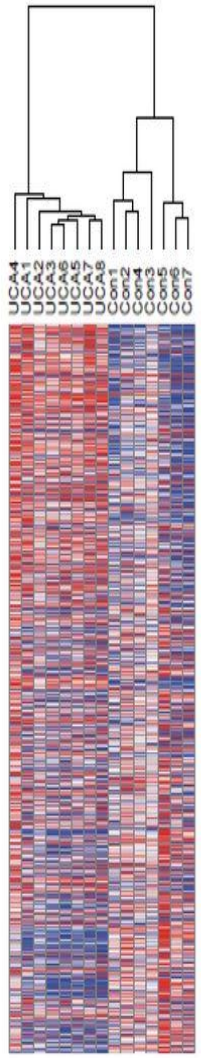
Long noncoding RNAs are defined as sequences of greater than 200 nucleotides in length. They can express introns and exons, which can be alternatively spliced and generally lack open reading frames for protein translation.

lncRNAs appear to have a diverse set of functions in chromatin remodeling, telomere activity, and subcellular structural organization.

lncRNAs have also been implicated in various steps in the posttranscriptional processing of messenger RNAs, including splicing, editing, transport, translation, and degradation.

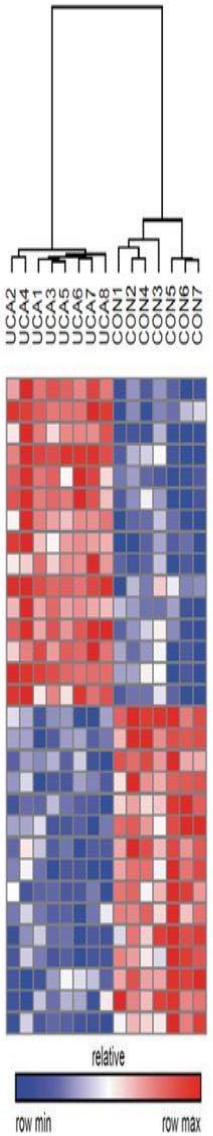
The lncRNA IFNG-AS1 was associated with the IBD susceptibility loci SNP rs7134599 and is in close proximity to the inflammatory cytokine IFNG.

A



UCA vs Con

B



C

GeneSymbol	P-value	FC (abs)	Regulation	RNAlength	chrom	strand	txStart	txEnd
AC093818.1	2.995E-08	6.5852348	up	474	chr2	-	173420153	173421135
RP11-347C18.3	3.8957E-07	6.0070404	up	709	chr8	+	95962264	95963624
AK308561	4.9638E-07	3.3843983	up	640	chr9	-	66454656	66457142
AC093818.1	8.4179E-07	6.2669089	up	1397	chr2	-	173368166	173421324
BC015977	1.50809E-06	3.9365962	up	271	chrX	-	46185713	46187080
LOC100505839	1.73017E-06	21.3462294	up	2023	chr10	+	105506536	105515167
LOC339894	3.09264E-06	6.5386288	up	2016	chr3	-	156799455	156840791
CRNDE	3.11351E-06	4.6952636	up	464	chr16	-	54952965	54957691
NOS2P3	3.38483E-06	12.5807175	up	867	chr17	+	20344339	20350085
ANKRD36BP2	3.8258E-06	5.0950911	up	391	chr2	+	89065397	89076092
RP11-536O18.1	5.1115E-06	4.0617397	up	477	chr9	+	13446524	13487510
RP11-81H14.2	5.24531E-06	5.9275247	up	417	chr12	-	68825634	68826434
BC044655	6.13422E-06	27.8429609	up	2836	chr17	+	20340136	20350557
AC133109.1	6.8757E-06	5.5129747	up	1604	chr2	-	109743782	109745386
IFNG-AS1	7.05895E-06	5.2737201	up	494	chr12	+	68383308	68628466
KCNMB2-IT1	3.0995E-07	2.4886246	down	545	chr3	+	178137021	178175097
LOC389023	3.3447E-07	22.8222991	down	744	chr2	-	115901624	115918920
BC043570	2.06129E-06	3.6120905	down	2525	chr15	+	29967193	29971367
AC012507.3	4.1393E-06	2.5166143	down	544	chr2	+	231751220	231758789
RP11-349K16.1	4.7569E-06	9.2410417	down	552	chr12	-	128508332	128511711
PMS2P3	5.88004E-06	2.0229812	down	1388	chr7	-	75137068	75157453
KRT16P3	7.90624E-06	2.818404	down	681	chr17	-	20404825	20405600
HCG21	8.36127E-06	2.4359782	down	605	chr6	-	30913755	30922639
RP11-10N16.3	1.20406E-05	2.3878804	down	2337	chr1	-	24620476	24648420
RP13-455A7.1	1.2237E-05	2.018203	down	219	chr22	+	48256419	48257811
RP11-147I3.1	1.27233E-05	2.0847325	down	469	chr11	+	74624637	74630856
RP11-195E11.3	1.32175E-05	2.931462	down	1811	chr9	-	72784558	72787804
PLCD1	1.58876E-05	3.5811373	down	3053	chr3	-	38048986	38066278
RP11-627G23.1	1.72506E-05	2.3658954	down	4387	chr11	+	134349192	134375507
HOXD-AS1	1.74911E-05	2.7729262	down	3819	chr2	-	177037923	177053686

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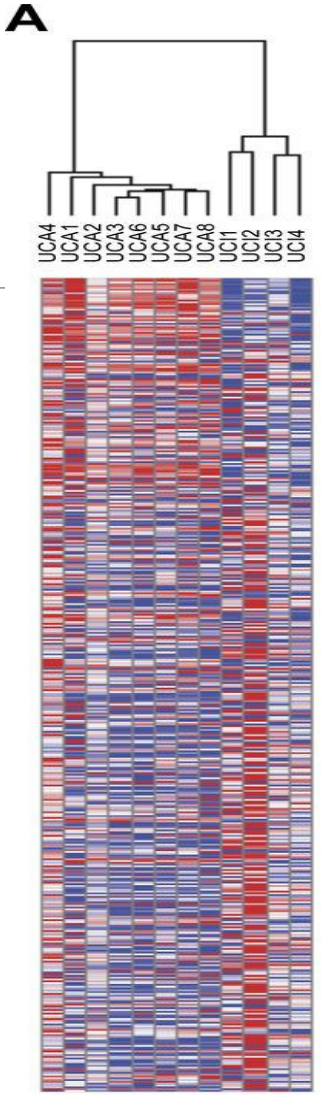

UCA vs UCI

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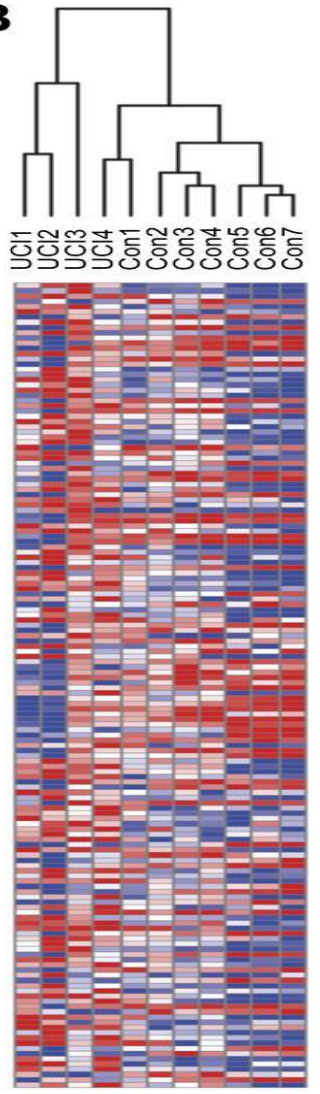
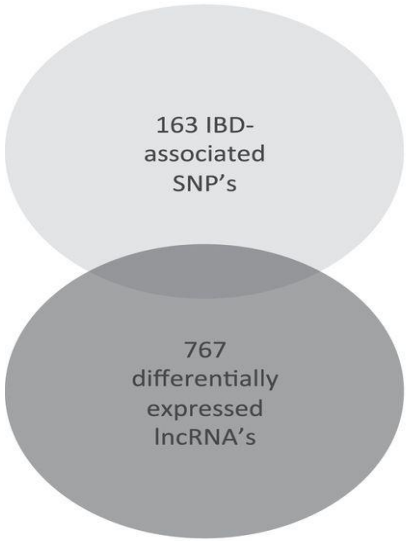
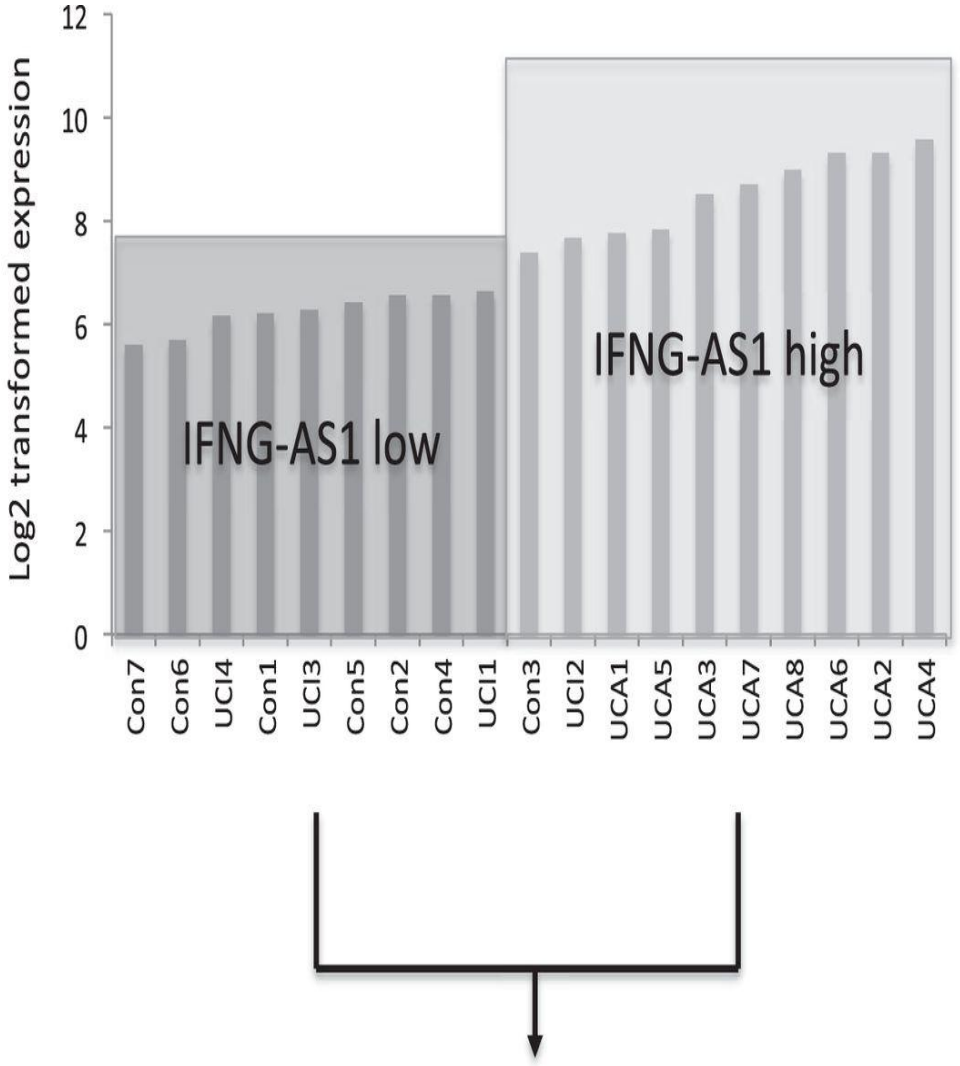


UCI vs Con

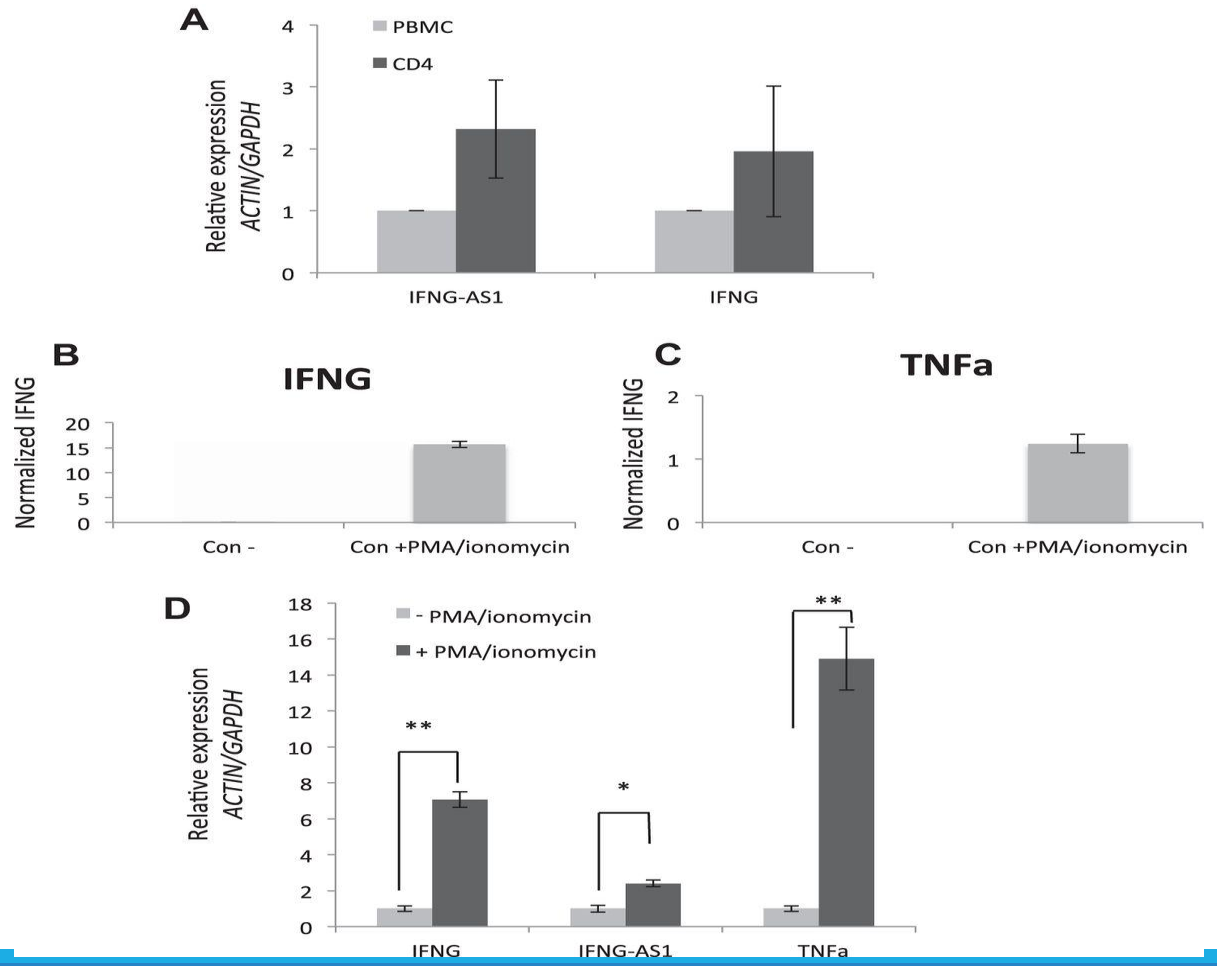


Chromosome	lncRNA	SNP	Distance
1	XLOC_001174	rs3024505	156947
	RP3-395M20.8	rs10797432	19980
	LOC115110	rs10797432	19980
	RP5-902P8.12	rs12103	62338
	XLOC_000090	rs12568930	118737
	RP11-404F10.2	rs4656958	213829
2	XLOC_002543	rs3749171	248833
6	LOC285740	rs12199775	23428
	RP11-356I2.2	rs6920220	138305
	AK124173	rs6920220	138307
	RP11-356I2.4	rs6920220	172332
9	RP11-305L7.6	rs4743820	4537
10	RP11-536K7.3	rs12722515	95778
11	RP11-21L23.2	rs2155219	212213
	RP11-655C2.3	rs10896794	73764
	FAM99B	rs907611	169573
12	IFNG-AS1	rs7134599	116767
	RP1-197B17.3	rs11168249	96995
	RP11-81H14.2	rs7134599	226592
16	RP11-401P9.1	rs2066847	178044
	RP11-615I2.1	rs1728785	16454
	XLOC_012039	rs10521318	16197
17	NOS2P1	rs2945412	134716
19	LOC80054	rs17694108	62211
20	XLOC_013845	rs6062504	238835
	RP11-410N8.3	rs4911259	227388
	RP11-112L6.4	rs913678	166298
	RP11-465L10.10	rs1569723	92807
	LOC79015	rs6017342	220063
21	AP001058.3	rs7282490	10666
22	PI4KAP2	rs2266959	95618



Upstream Regulator	Molecule Type	Predicted Activation State	p-value
TNF	cytokine	Activated	9.53E-14
IL1B	cytokine	Activated	1.59E-11
IFNG	cytokine	Activated	3.03E-17
IL1	group	Activated	1.96E-15
IL6	cytokine	Activated	1.55E-14

qPCR expression of IFNG-AS1 and IFGN among CD4 cells and total PBMC (N = 4). B and C: Jurkat T cells activated with PMA/ionomycin for 6 h and cultured media collected after 24 h show inductions of IFNG and TNF-α protein, respectively. D: Jurkat T cells activated with PMA/ionomycin for 6 h; qPCR analysis of IFNG, IFNG-AS1, and TNF-α. *P value < 0.05, **P value < 0.01; n = 3.



Conclusions

IFNG-AS1 was one of the differentially expressed lncRNAs in UC patients and found to regulate the key inflammatory cytokine, IFNG, in CD4 T cells.

The outhors identified IFNG-AS1 as a novel regulator of IFNG inflammatory responses, suggesting the potential importance of noncoding RNA mechanisms on regulation of inflammatory bowel disease-related inflammatory responses.

Mucosal Expression of Type 2 and Type 17 Immune Response Genes Distinguishes Ulcerative Colitis From Colon-Only Crohn's Disease in Treatment-Naive Pediatric Patients

GASTROENTEROLOGY, VOL. 152, ISSUE 6, P1345–1357. MAY 2017

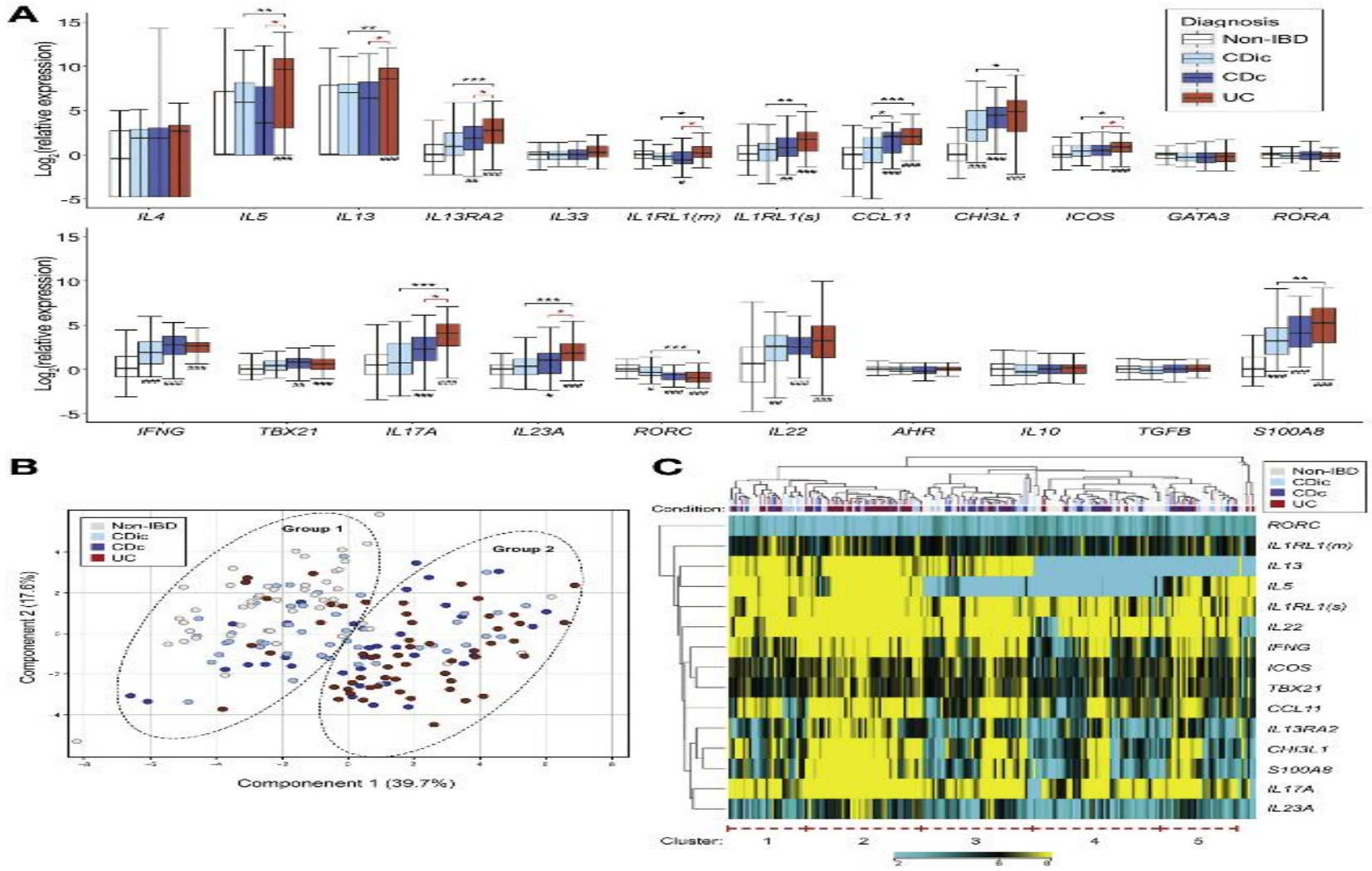
ROSEN ET AL. DIVISION OF GASTROENTEROLOGY, HEPATOLOGY AND NUTRITION, CINCINNATI CHILDREN'S HOSPITAL MEDICAL CENTER, CINCINNATI, OHIO

- There is controversy regarding the role of the type 2 immune response in the pathogenesis of ulcerative colitis (UC)
- They investigated whether genes associated with a type 2 immune response in the intestinal mucosa are up-regulated in treatment-naive pediatric patients with UC compared with patients with Crohn's disease (CD)-associated colitis or without inflammatory bowel disease (IBD), and whether expression levels are associated with clinical outcomes.

Methodology

They used a real-time reverse-transcription quantitative polymerase chain reaction array to analyze messenger RNA (mRNA) expression patterns in rectal mucosal samples from 138 treatment-naïve pediatric patients with IBD and macroscopic rectal disease, as well as those from 49 children without IBD (controls).

Results were validated in real-time reverse-transcription quantitative polymerase chain reaction analyses of rectal RNA from an independent cohort of 34 pediatric patients with IBD and macroscopic rectal disease and 17controls


A
Log2(relative expression)
Diagnosis
Non-IBD
CDic
CDc
UC
IL4
IL5
IL13
IL13RA2
IL33
IL1RL1(m)
IL1RL1(s)
CCL11
CHI3L1
ICOS
GATA3
RORA
IFNG
TBX21
IL17A
IL23A
RORC
IL22
AHR
IL10
TGFB
S100A8
B
Non-IBD
CDic
CDc
UC
Group 1
Group 2
Componenent 2 (17.6%)
Componenent 1 (39.7%)
C
Condition:
Non-IBD
CDic
CDc
UC
RORC
IL1RL1(m)
IL13
IL5
IL1RL1(s)
IL22
IFNG
ICOS
TBX21
CCL11
IL13RA2
CHI3L1
S100A8
IL17A
IL23A
Cluster:
1
2
3
4
5

Real-time RT-qPCR of rectal mucosal RNA from patients in the Cincinnati validation cohort.

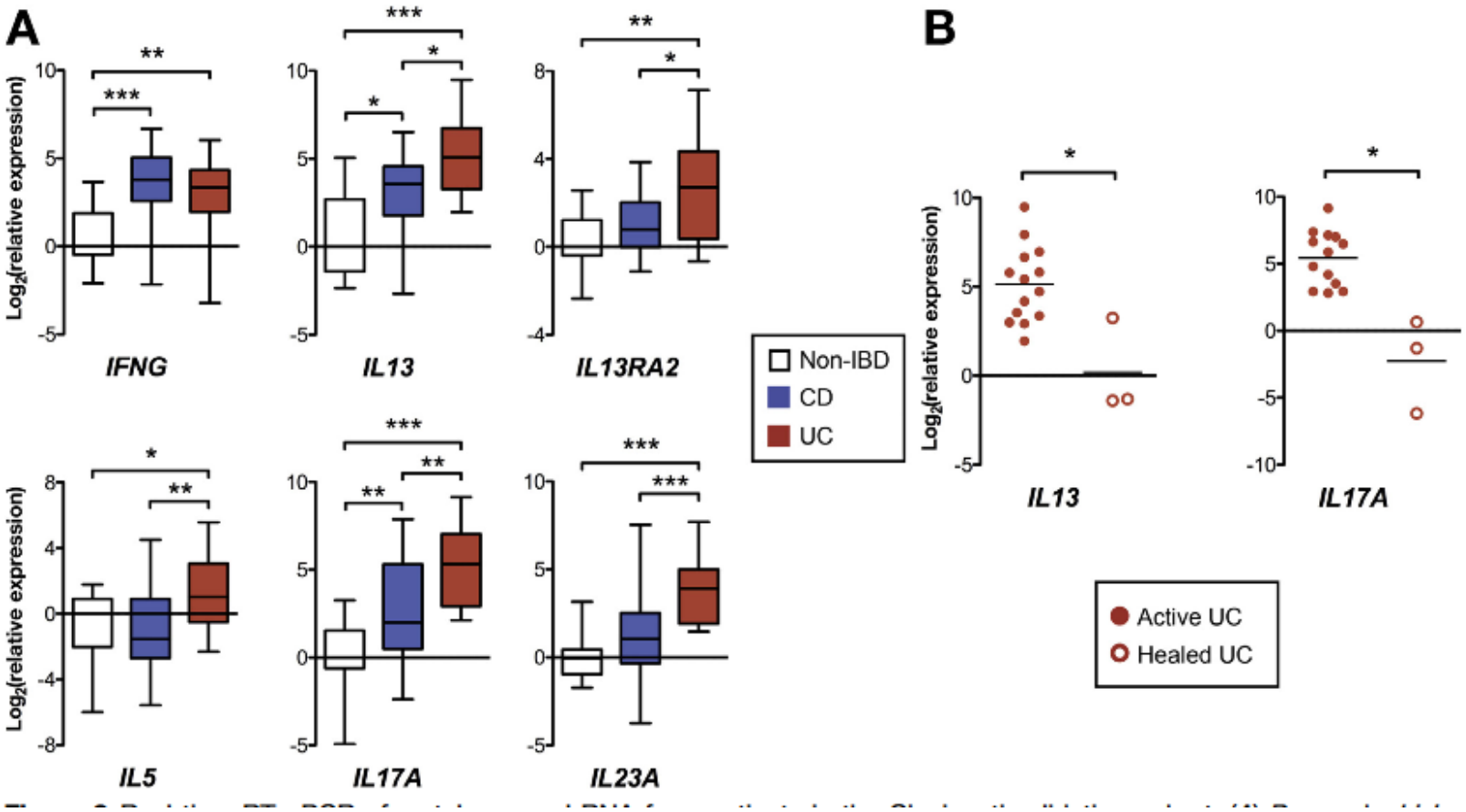


Table 3. Multivariate Logistic Regression for Discriminating UC From CDc

Gene	OR[a]	95% CI	*P* value
IL5	1.130	1.032–1.238	.009
IL17A	1.196	0.976–1.467	.085

[a]Odds of a diagnosis of UC over CDc per unit increase in Cq value for the listed gene.

The findings support a role for mucosal type 2 inflammatory responses in the early course of pediatric UC.

In treatment-naive pediatric patients, UC is distinguished from Crohn's colitis, and specifically colon-only CD by increased expression of genes associated with type 2 and type 17 immune responses.

Furthermore, an immune gene expression profile marked by increased expression of the type 2 cytokine IL13 is associated with improved clinical outcomes in pediatric UC.

Conclusions

The researchers showed that treatment-naïve pediatric patients with ulcerative colitis exhibit increased mucosal expression of genes associated with type 2 and type 17 immune responses compared to those with colon-only Crohn's disease.

Expression of type 2 and type 17 immune response genes distinguishes ulcerative colitis from colon-only Crohn's disease in treatment-naïve pediatric patients

Genome-wide association study implicates immune activation of multiple integrin genes in inflammatory bowel disease

NATURE GENETICS 49, 256–261 (2017)

DE LANGE ET AL.

They performed a genome-wide association study of 25,305 individuals and conducted a meta-analysis with published summary statistics, yielding a total sample size of 59,957 subjects.

They identified 25 new susceptibility loci, 3 of which contain integrin genes that encode proteins in pathways that have been identified as important therapeutic targets in inflammatory bowel disease.

The associated variants are correlated with expression changes in response to immune stimulus at two of these genes (ITGA4 and ITGB8) and at previously implicated loci (ITGAL and ICAM1).

In all four cases, the expression-increasing allele also increases disease risk.

They also identified likely causal missense variants in a gene implicated in primary immune deficiency, PLCG2, and a negative regulator of inflammation, SLAMF8.

Table 2 Variants fine-mapped to >50% probability of being causal in their given signal

rs ID	Chr.	Position (bp)	P_{causal}	Effect	Credible set size	Phenotype	P_{meta}	Locus type
rs34687326	1	15,979,9910	1.000	*SLAMF8* p.Gly99Ser (missense)	1	CD	1.06×10^{-8}	New
rs4845604	1	151,801,680	0.999	*RORC* (intronic)	1	IBD	7.09×10^{-14}	Known
rs1811711	2	228,670,476	0.914		2	UC	6.09×10^{-9}	New
rs56116661	3	188,401,160	0.561	*LPP* (intronic)	11	CD	5.67×10^{-10}	New
rs11548656	16	81,916,912	0.502	*PLCG2* p.His244Arg (missense)	3	IBD	5.18×10^{-11}	New
rs1143687	16	81,922,813	0.746	*PLCG2* p.Arg268Trp (missense)	5	IBD	3.83×10^{-8}	New
rs4821544	22	37,258,503	0.804	*NCF4* (intronic)	2	CD	1.76×10^{-8}	New

Chr., chromosome; CD, Crohn's disease; UC, ulcerative colitis; IBD, inflammatory bowel disease.

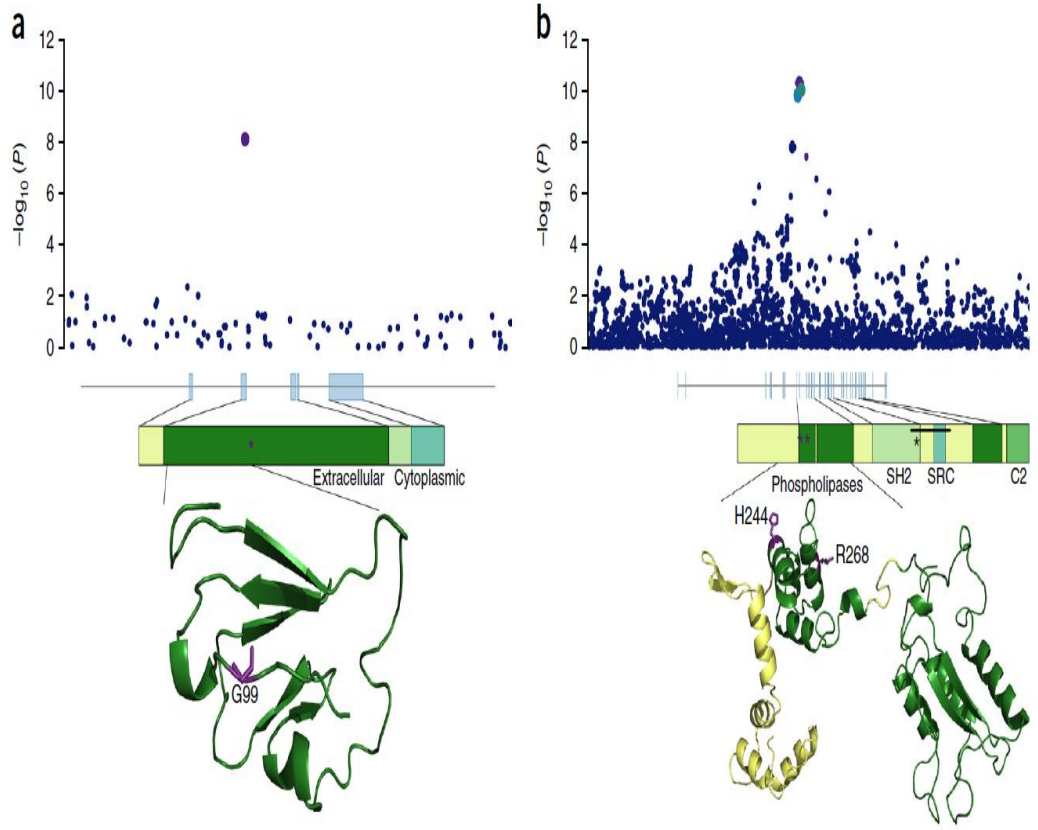


Figure 1 Likely causal missense variants. (**a**,**b**) For *SLAMF8* (**a**) and *PLCG2* (**b**), local association results are plotted with point size corresponding to LD with our lead variant and color corresponding to fine-mapping probability (purple, >50%; intermediate blue, 10–50%; navy blue, <10%). Gene body diagrams and protein domain annotations were taken from Ensembl, and partial predicted crystal structures for both proteins were obtained from the SWISS-MODEL repository.

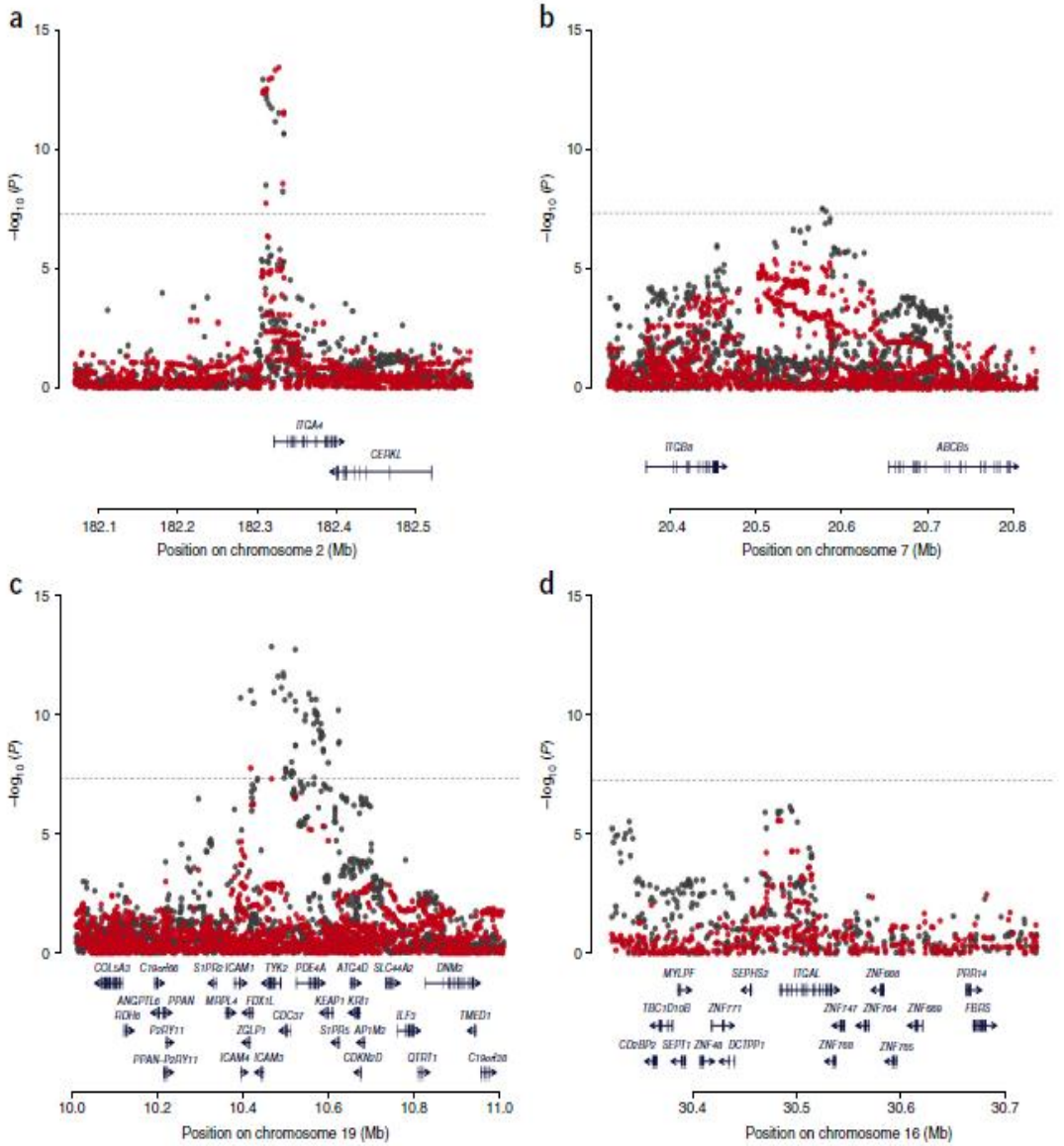


Figure 2 Colocalization of disease associations and stimulus response eQTLs in monocytes. (**a**–**d**) The local pattern of disease association for IBD at *ITGA4* (**a**), *ITGB8* (**b**) and *ICAM1* (**c**) or ulcerative colitis at *ITGAL* (**d**) is shown in gray, and the association of each variant with response to LPS stimulation is shown in red. Evidence of colocalization (probability >70%) is observed for all four loci.

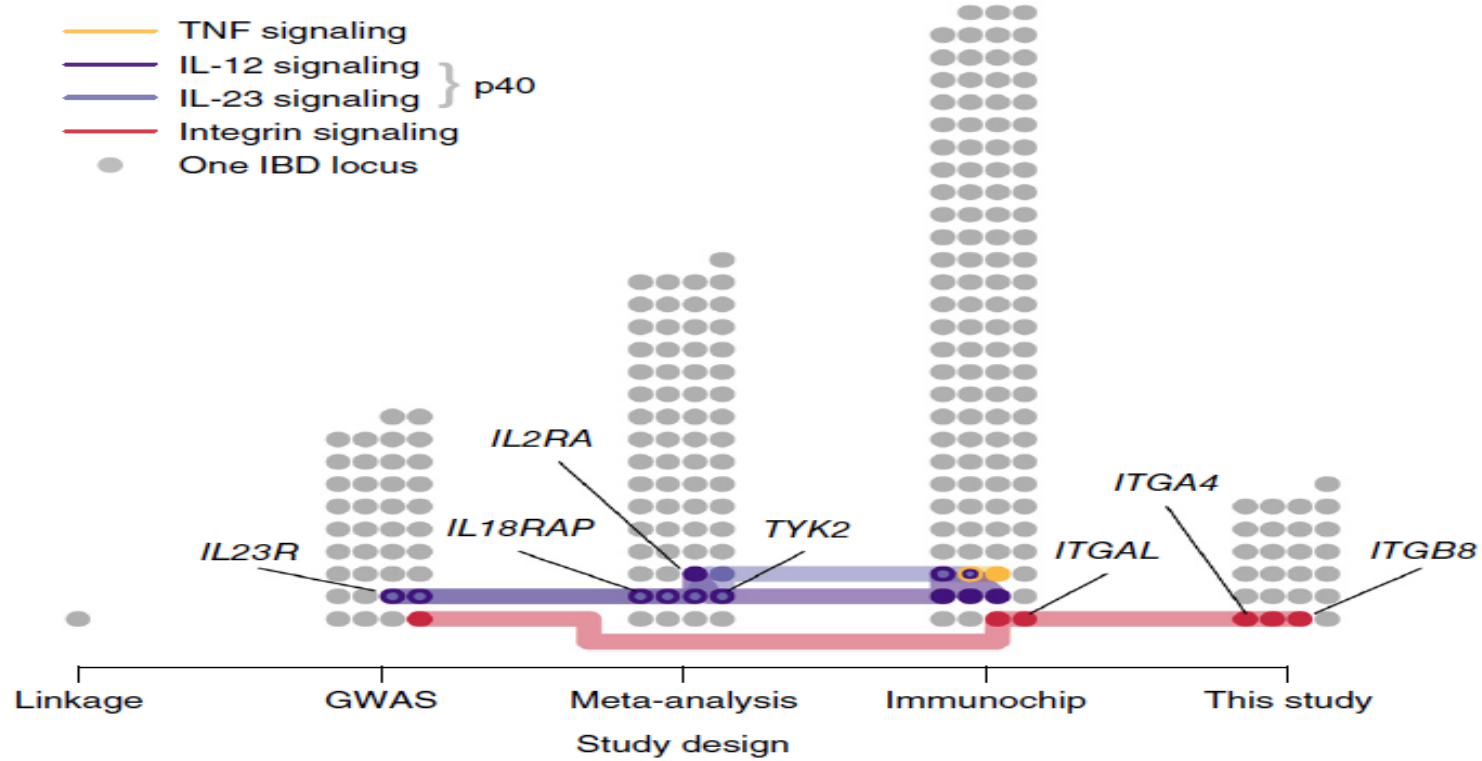


Figure 3 IBD-associated loci containing genes in immune pathways related to classes of approved therapeutics. All IBD susceptibility loci were divided into the studies where they were first identified[1]. Loci that contain a gene in one of four signaling pathways related to targets of three classes of approved IBD therapeutics (Online Methods) are highlighted, with those where the pathway gene has been confidently identified as the causal IBD gene labeled. Despite the general pattern of decrease in effect size from left to right, therapeutically relevant associations continue to be found for loci with lower effect sizes.

New associations at common variants continue to identify genes relevant to therapeutic target identification and prioritization.

The Role of the Histone Methyltransferase Enhancer of Zeste Homolog 2 (EZH2) in the Pathobiological Mechanisms Underlying Inflammatory Bowel Disease (IBD).

J BIOL CHEM. 2017 JAN 13;292(2):706-722

SARMENTO ET AL.

Regulatory T (Treg) cells expressing the transcription factor FOXP3 play a pivotal role in maintaining immunologic self-tolerance.

It has been shown previously that EZH2 is recruited to the FOXP3 promoter and its targets in Treg cells.

To further address the role for EZH2 in Treg cellular function, they have now generated mice that lack EZH2 specifically in Treg cells (EZH2Δ/ΔFOXP3+).

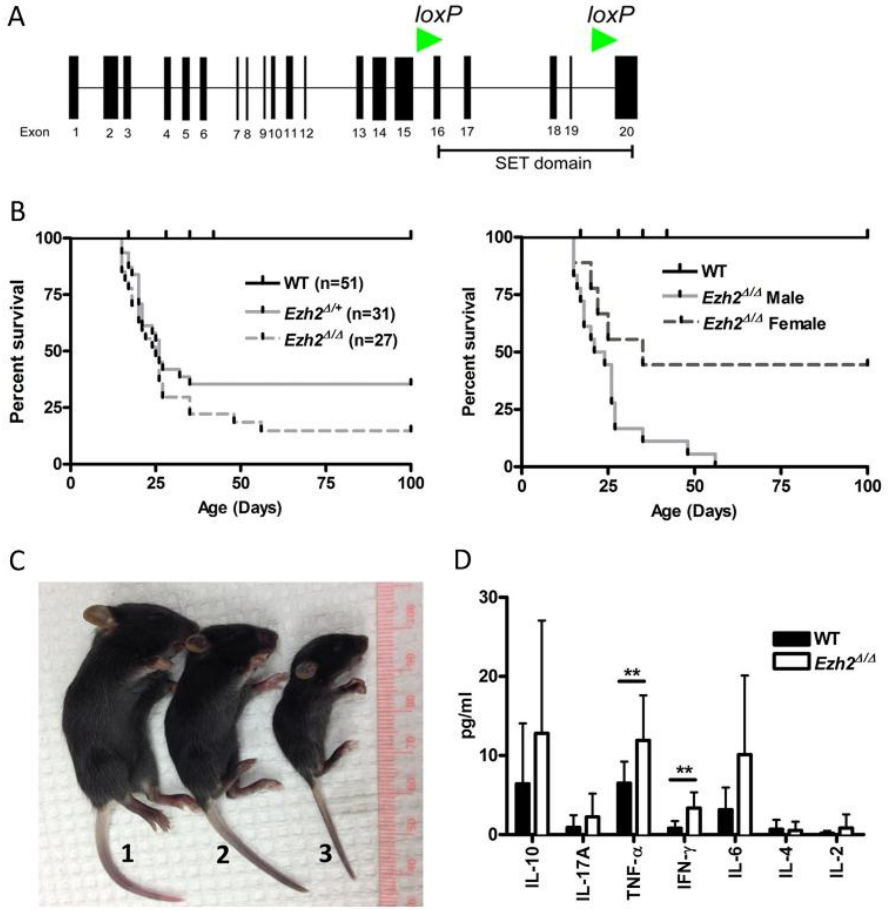


FIGURE 1. **Conditional deletion of the SET domain of EZH2 in FOXP3$^+$ Treg cells in mice (EZH2$^{\Delta/\Delta}$ FOXP3$^+$ or EZH2$^{\Delta/+}$ FOXP3$^+$) results in poor survival.** *A*, exon map of mouse EZH2 indicating the EZH2 catalytic SET domain that was conditionally deleted by flanking LoxP insertion sites (*green arrowheads*). *B*, survival analysis of EZH2$^{\Delta/\Delta}$ ($n = 27$) or EZH2$^{\Delta/+}$ ($n = 31$) mice compared with WT ($n = 51$) (*left panel*) and mutant mice distinguished by gender (*right panel*). The data are cumulative of over 10 litters, and all offspring are represented. *C*, representative images depicting the clinical appearance of experimental littermates and the size of FOXP3^{wt} EZH2$^{fl/fl}$ (WT, *1*), EZH2$^{wt/\Delta}$ FOXP3$^+$ (EZH2$^{\Delta/+}$, *2*) and EZH2$^{\Delta/\Delta}$ FOXP3$^+$ (EZH2$^{\Delta/\Delta}$, *3*) pups and lack of ear, eye, and tail inflammation 21 days after birth. D, mean (S.E.) serum cytokine concentrations as measured by multiplex cytokine analysis. Data are from 15 biological

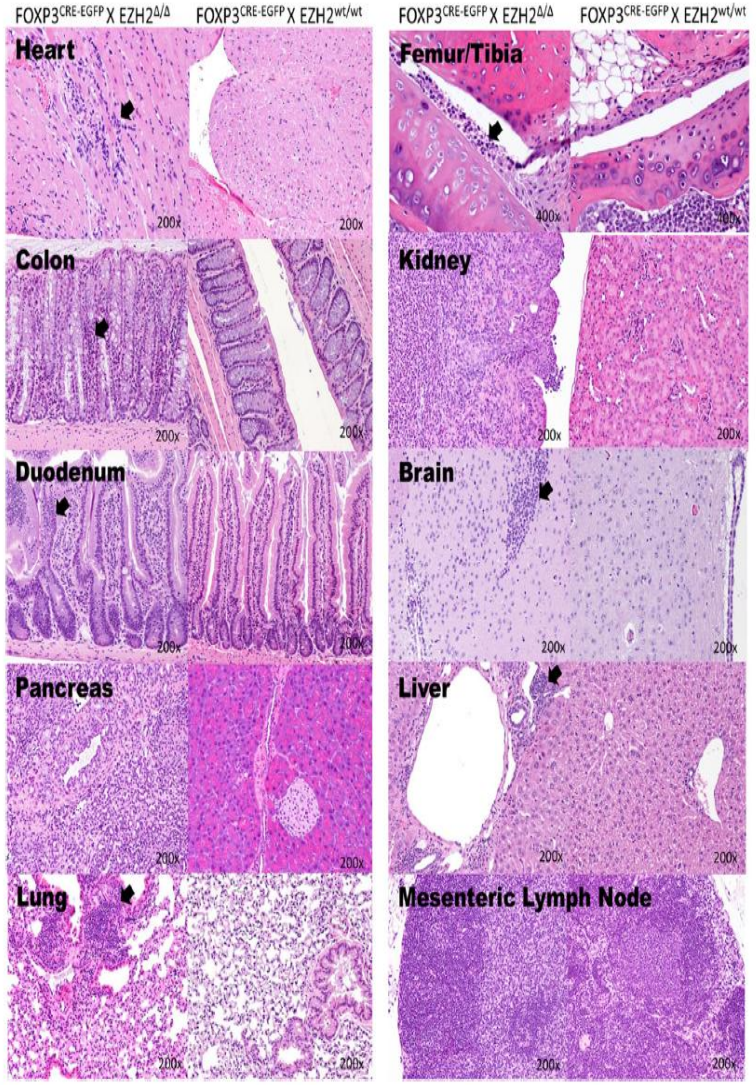
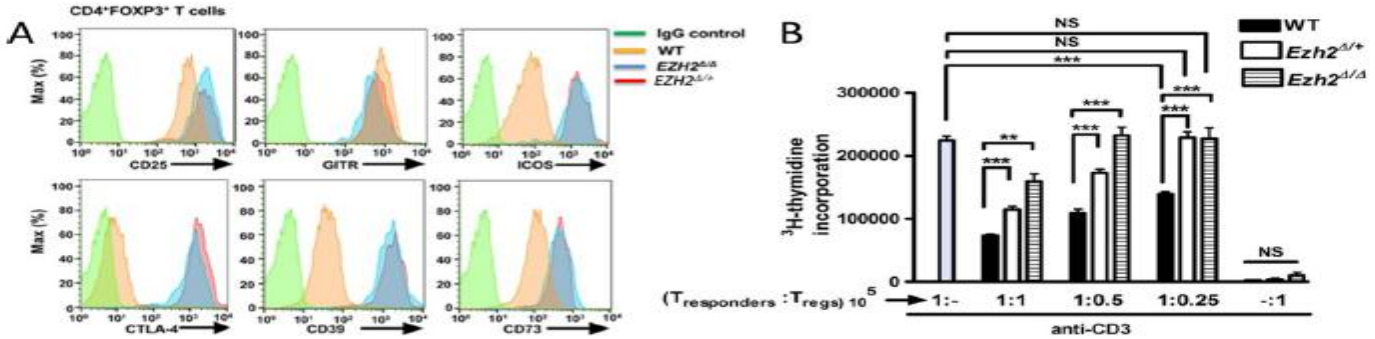


FIGURE 2. **EZH2$^{\Delta/\Delta}$ FOXP3$^+$mice develop end organ lymphoid infiltrates, either diffuse, nodular, or both.** Shown are the heart, colon, small bowel, pancreas, lung, femoral-tibial joint, kidney, brain, liver, and mesenteric lymph node. Abnormal lymphoid infiltrate is evident in both a diffuse and nodular

EZH2 as a Cofactor for FOXP3 in IBD



C

Symbol	Thelper categorization	EZH2KO Fold	EZH2het Fold
Il18rap	Th1 Epigen reg gene; cytokine	-11.37	-7.54
Igsf6	Th1 Marker	-2.37	0.00
Irf1	Th1 Marker	0.00	-2.33
Fasl	Th1 Epigen reg gene	0.00	2.02
Socs5	Th1 Marker	2.50	3.60
Tbx21	Th1 Epigen reg gene	2.82	0.00
Tnf	Th1 Marker; cytokine	4.54	4.30
Il12b	Th1 Marker; cytokine	5.05	35.73
Il18	Th1 Marker; cytokine	6.67	4.59
Tlr6	Th1 Marker	6.76	28.71
Il18r1	Th1 Epigen reg gene; cytokine	7.70	0.00
Il1r1	Th17 Epigen reg gene; cytokine	-3.27	-4.31
Il21	Th17 Epigen reg gene; cytokine	0.00	-2.17
Rorc	Th17 Epigen reg gene; TF	2.07	0.00
Il17a	Th17 Epigen reg gene; cytokine	4.24	0.00
Il17re	Th17 Epigen reg gene; cytokine	8.49	3.58
Il5	Th2 Epigen reg gene; cytokine	-5.88	-6.98
Tmed1	Th2 Marker	-2.98	-3.14
Ccl5 (Rant	Th2 Marker; cytokine	-2.59	-3.90
Ptgdr2	Th2 Marker	0.00	-3.10
Nfatc2	Th2 Marker; TF	0.00	-2.36
Asb2	Th2 Epigen reg gene	0.00	3.41
Nfatc2ip	Th2 Marker; TF	0.00	3.52
Il1rl1	Th2 Epigen reg gene; cytokine	3.21	4.94
Il4ra	Th2 Marker; cytokine	3.25	2.36
Ccr4	Th2 Marker; cytokine/receptor	3.31	2.77
Cebpb	Th2 Marker; TF	4.24	10.43
Pparg	Th2 Epigen reg gene	6.36	0.00
Ccl11	Th2 Marker; cytokine	7.98	2.36

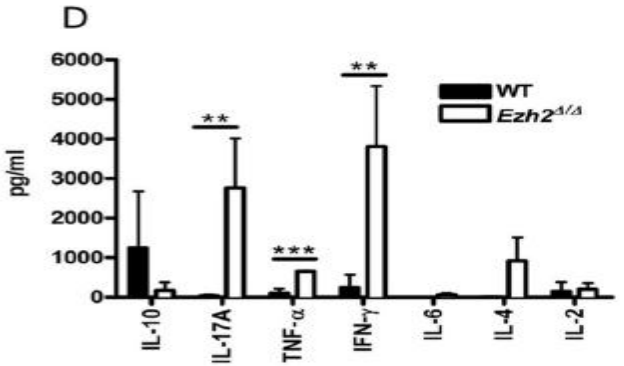


FIGURE 3. **EZH2$^{\Delta/\Delta}$FOXP3^{+} lymphocytes transform to a proinflammatory phenotype.** *A*, the expression of cell surface markers in Treg cells (CD4+FOXP3+) measured by flow cytometry. Data are representative of three independent experiments ($n = 3$ mice/experimental group). *B*, suppression assay measuring the degree of Treg-mediated suppression of CD4^{+} T responder cells (10^5) in response to anti-CD3 ± CD4^{+}CD25^{+} Treg titrations (10^5, 0.5 ×

They find that EZH2 deficiency in FOXP3+ T cells results in lethal multiorgan autoimmunity.

They demonstrate that EZH2Δ/ΔFOXP3+ T cells lack a regulatory phenotype in vitro and secrete proinflammatory cytokines. Of special interest, EZH2Δ/ΔFOXP3+ mice develop spontaneous inflammatory bowel disease.

They assessed the FOXP3 and EZH2 gene networks by RNA sequencing in isolated intestinal CD4+ T cells from patients with Crohn's disease. Gene network analysis demonstrates that these CD4+ T cells display a Th1/Th17-like phenotype with an enrichment of gene targets shared by FOXP3 and EZH2.

Combined, these results suggest that the inflammatory milieu found in Crohn's disease could lead to or result from deregulation of FOXP3/EZH2-enforced T cell gene networks contributing to the underlying intestinal inflammation.