digestion and metabolism. These results suggest that intestinal CD11b+ gene expression is highly influenced by regional microenvironment. Fewer differences were seen comparing CD11b$^+$ cells from CD vs UC, suggesting that this cell population is not solely responsible for the clinical differences between these diseases. The small number of differentially expressed genes in Anti-TNF refractoriness shows a clear difference between innate and adaptive immune pathways, as well as increased general cell activity in Anti-TNF responders. These genes should be further investigated to determine their precise role in refractoriness.

Figure 1. Principal component analysis of all sample genes shows separation by location (colon vs ileum) but not by disease type (CD vs UC).

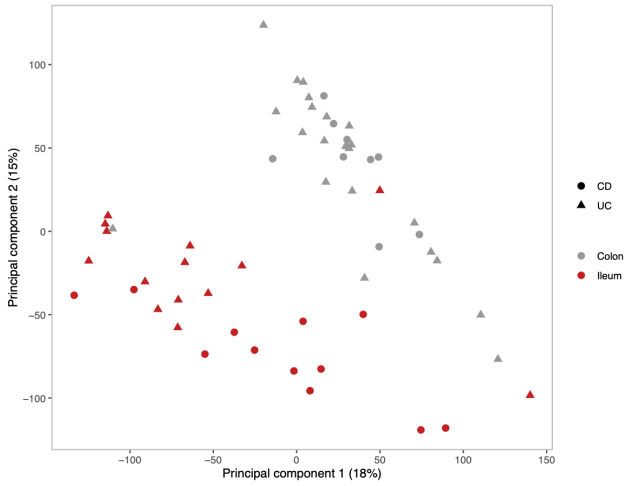
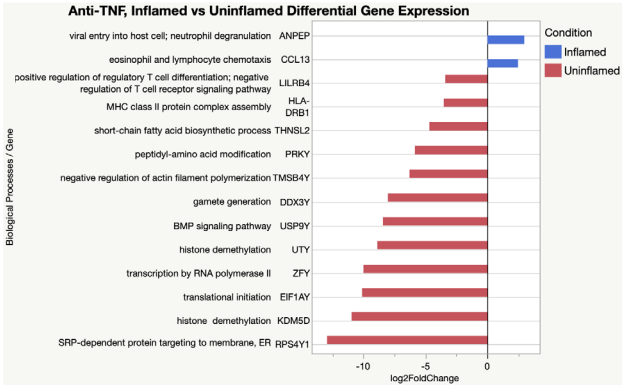


Figure 2. CD11b$^+$ cells from patients on Anti-TNF therapy express innate immune genes when inflamed, but trend towards adaptive immunity, SCFA synthesis, and increased general cellular activity when uninflamed.



UTILIZATION OF WHOLE EXOME SEQUENCING DATA TO IDENTIFY CLINICALLY RELEVANT PHARMACOGENOMIC VARIANTS IN INFLAMMATORY BOWEL DISEASE

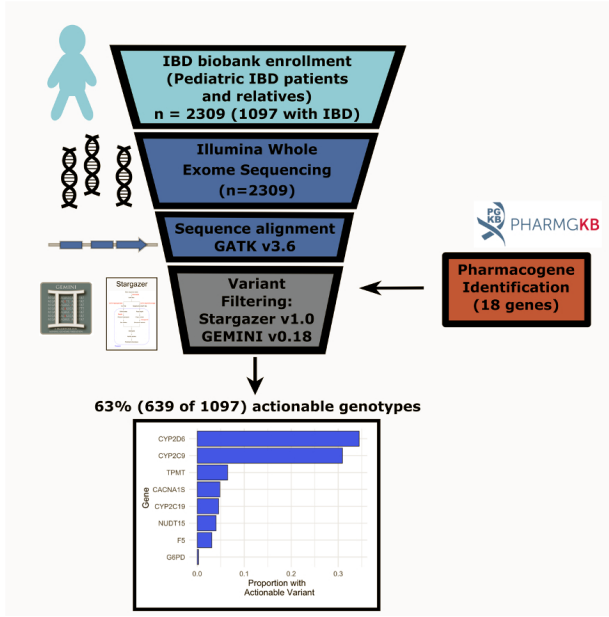
Daniel Mulder, Sam Khalouei, Neil Warner, Claudia Gonzaga-Jauregui, Peter Church, Tom Walters, Arun Ramani, Anne Griffiths, Iris Cohn, Aleixo Muise

Objectives: We hypothesized that variants within clinically relevant pharmacogenes could be identified using a whole exome sequencing (WES) dataset derived from a cohort of over 1000 IBD patients.

Methods: Pediatric patients diagnosed with IBD underwent WES. We selected 18 genes with supporting literature where specific exonic variants would influence clinical care.

Results: We identified actionable pharmacogenes variants in 63% of patients. Importantly, 5% of IBD patients were at risk for serious adverse effects from anaesthesia and 3% were at increased risk for thrombosis.

Conclusions: We identified exonic variants in the majority of our IBD patients that directly impact clinical care.



Flowchart of our pharmacogenomic analysis pipeline. After enrolment (n=2309), each patient underwent whole exome sequencing and sequence alignment. Available family members were also sequenced. Analyzed samples were limited to patients and family members with IBD (n=1097). Pharmacogenes relevant to patients with IBD were identified by literature review and evaluation of pharmGKB (total of 18 genes). Variant filtering was performed using Stargazer and GEMINI frameworks. In our cohort, there were 8 relevant pharmacogenes with variants that would alter clinical care based on current guidelines and standard of care. 63% of the patients had at least one variant that could impact care.

Utility Category	Gene	Impacted drug or complication risk	Metabolizer/Function Status	Number of Affected Individuals	Percent of Cohort	Clinical Impact	Inheritance
Direct IBD Management	TPMT	Azathioprine (immunomodulator)	Intermediate	66	6.02	Increased risk of leukopenia, neutropenia, myelosuppression	Complex multiallelic
			Poor	3	0.46	Greatly increased risk of leukopenia, neutropenia, myelosuppression	
	NUDT15	Azathioprine (immunomodulator)	Intermediate	42	3.83	Increased risk of leukopenia, neutropenia, myelosuppression	Complex multiallelic
			Poor	2	0.18	Increased risk of leukopenia, neutropenia, myelosuppression	
Adjunct IBD Therapy	CYP2C19	Citalopram, escitalopram, and sertraline (antidepressants)	Poor	50	4.56	Increased probability of side effects - arrhythmias, GI dysfunction, sexual dysfunction, headache	Complex multiallelic
	CYP2D6	Fluvoxamine and paroxetine (antidepressants)	Poor	44	4.01	Increased probability of side effects - arrhythmias, GI dysfunction, sexual dysfunction, headache	Complex multiallelic
		Venlafaxine (antidepressant)	Intermediate	325	29.63	Increased probability of side effects - arrhythmias, GI dysfunction, sexual dysfunction, headache	
			Poor	53	4.83		
		Codeine and tramadol (analgesic)	Poor	44	4.01	Treatment failure - insufficient pain control	
	CYP2C9	Ibuprofen (analgesic, antipyretic)	Intermediate	312	28.44	Increased risk of GI bleeding, hypertension, cardiac event, renal damage	Complex multiallelic
			Poor	27	2.46		
General Risk Variants	F5	Thrombosis Risk	Leiden variant	34	3.10	Thrombosis risk	Intermediate
	G6PD	Hemolysis Risk	Decreased	1	0.09	Hemolysis due to physiologic stress or drug interaction (including sulfasalazine)	X-linked intermediate
			Decreased	2	0.18		
	CACNA1S	Anesthetic Risk	Decreased	53	4.83	Hypokalemic periodic paralysis risk, malignant hyperthermia risk	Intermediate

Table 1: List of identified pharmacogenomic variants.

Innate and Mucosal Immunology and Immunity

A NOVEL LANGERIN EXPRESSING TYPE 2-CONVENTIONAL DENDRITIC CELL IS SIGNIFICANTLY DECREASED IN CROHN'S DISEASE

Chloe Doyle, Kirstie Bertram, Jake Rhodes, Anthony Cunningham, Grahame Ctercteko, Scott Byrne, Andrew Harman

Crohn's disease is a chronic relapsing auto-inflammatory condition of the gastrointestinal tract that primarily affects young individuals with an increasing incidence rate. There is still no cure for Crohn's disease and current treatment-options are limited to controlling inflammatory molecules such as TNF. Unfortunately, these biologics can produce significant side effects and not all patients respond to anti-TNF

treatment. New treatments are therefore urgently needed. Targeting the cells and molecules of the immune system still represents the most promising approach which is why we have conducted an in-depth study of the immune cells and molecules associated with a healthy intestinal immune system and compared that to what is happening in Crohn's disease.

Our group has privileged access to large human ileum explants as well as draining lymph node samples isolated from patients undergoing colorectal surgery to remove Crohn's affected tissue. We have designed and optimised enzymatic tissue digestion protocols to isolate these cells in an immature state with minimal receptor cleavage. We have also developed high-parameter flow and mass cytometry panels to comprehensively identify and characterise all known subsets of mononuclear phagocytes (MNP), innate lymphoid cells, mucosal-associated invariant T cells, natural killer cells and T and B lymphocytes.

We have discovered that the newly described Langerin$^+$ type 2-conventional dendritic cell (cDC2) is significantly decreased in Crohn's affected ileum compared to healthy ileum. Furthermore, we have shown this decrease corresponds with lower TGF-b levels, a known driver of Langerin expression. After 7 days of co-culture, sorted Langerin$^+$ cDC2 induced significantly higher levels of IL-17 and IL-22 in allogenic naïve CD4$^+$ T cells compared to other MNP subsets, including cDC2 which did not express Langerin. These differentiated T cells expressed high levels of RORt and aryl hydrocarbon receptor (AHR) – transcription factors that are associated with CD4$^+$ T helper 17 cells, implying that they may play a crucial role in intestinal barrier repair and regeneration that is absent in Crohn's disease. Together these results suggest that Langerin$^+$ cDC2 may have an anti-inflammatory role in human tissue and their reduction in Crohn's disease may contribute to the pathogenesis of this disease, highlighting a potential therapeutic target for Crohn's disease.

EVALUATING THE ROLE OF GOBLET CELL ASSOCIATED ANTIGEN PASSAGES (GAPS) IN THE DEVELOPMENT OF MUCOSAL IMMUNE TOLERANCE IN THE *CFTR* KO INTESTINE

Sarah Young, Keely McDonald, Rodney Newberry, Lane Clarke

Greater than 70,000 individuals worldwide are living with the monogenetic disease cystic fibrosis (CF). The development of chronic intestinal inflammation, with clinical signs resembling inflammatory bowel disease-like conditions, is a common yet poorly understood occurrence in CF patients. This inflammation is typically neutrophilic in human and animal models with a heightened basal pro-inflammatory cytokine release. Prior research utilizing intestinal organoids (enteroids) cultured from *Cftr* knockout mice has shown that goblet cells in the CF mouse intestine demonstrate defective clearance of mucin granules and abnormal mucus retention. Goblet cell-associated antigen passages (GAPs), located in the small intestine and colon, deliver intraluminal antigens to antigen-presenting dendritic cells in the submucosa. This mechanism serves as an important step in the development and maintenance of tolerogenic dendritic cell populations expressing receptors to luminal antigens with involvement of regulatory T cell activation and release of IL-10. We hypothesized that mucus plugging of goblet cells in the CF intestine leads to defective GAP formation and a consequent decrease in the expansion of tolerogenic dendritic cells. To test this hypothesis, *Cftr*^{tm1Unc} (*Cftr* KO) and wild type (WT) sex-matched littermate pairs (n=2) maintained on a commercially available liquid diet (Peptamen®) were anesthetized with ketamine/xylazine for a laparotomy to inject a luminal fluorescent 10kD dextran dye into the mid-jejunum. After 30 min, the mice were euthanized with CO_2, and the intestine was collected for immunofluorescent staining to evaluate GAP formation. In the WT intestine, the dextran dye was observed within goblet cells outlined by CK18 immunofluorescence, a goblet cell marker. Punctate dextran dye was observed in the submucosa, suggestive of dendritic cell uptake. In contrast, the *Cftr* KO mice demonstrated defective GAP formation, i.e., without dye penetration of goblet cells, and the lack of punctate dextran fluorescence in the submucosa. To evaluate the population of tolerogenic dendritic cells, small intestinal segments from *Cftr* KO-WT sex-matched littermate pairs (3-female and 2-male pairs) were collected for FACS sorting of submucosal CD103+ (tolerogenic) and CD103- (pro-inflammatory) dendritic cells. The WT mice had a significantly higher population of CD103+ tolerogenic dendritic cells compared to the CF mice (WT: 20.5+/-2, CF: 9.2+/-3, $P < 0.006$). A trend towards an increase in CD103- dendritic cells was seen in the CF intestine. In summary, the CF mice were found to have defective intraluminal antigen transfer through the GAP pathway and a significant decrease in tolerogenic dendritic cells in the intestine.

GPR120 MAINTAINS INTESTINAL HOMEOSTASIS THROUGH REGULATION OF CD4$^+$ T CELL IL-10 PRODUCTION

Wenjing Yang, Suxia Yao, Sara Dann, Yingzi Cong

Irregular CD4$^+$ effector T cell responses play an essential role in the intestinal inflammation, while IL-10 produced by effector T cells limits their pathogenesis to maintain the intestinal homeostasis. Dietary free fatty acids are actively involved in regulating immune responses, and mammalian G protein-coupled receptor (GPR) 120, a receptor for long-chain fatty acids, has been implicated in metabolic syndrome. However, the effect of GPR120 on intestinal homeostasis is still unknown. Here, we showed that deficiency of GPR120 resulted in more severe colitis in mice induced by dextran sodium sulfate (DSS) and *Citrobacter Rodentium*. Interestingly, CD4$^+$ T cells expressed a high level of GPR120, and mice specifically lacking GPR120 in CD4$^+$ T cells were more susceptible to DSS-induced colitis. Besides, GPR120-deficient CD45Rb^{hi} CD4$^+$ cells are more colitogenic in Rag$^{-/-}$ mice, in which IL-17 and IFNg producing CD4$^+$ T cells were increased but IL-10 production by CD4$^+$ T cells was reduced. Furthermore, CpdA, the GPR120 agonist, promoted CD4$^+$ T effector cell production of IL-10 through upregulating Blimp1 and inducing glycolysis, which were regulated by mTOR pathway. Besides, docosahexaenoic acid, a dietary long-chain fatty acid, also upregulated the IL-10 production in CD4$^+$ T cells. Additionally, GPR120 agonist-treated Th1 effector cells induced less severe colitis, whereas this protection was absent in Blimp1-deficient Th1 cells. Importantly, oral administration of CpdA protected mice against intestinal inflammation. Thus, our findings demonstrate the roles of dietary fatty acids receptor GPR120 in regulating intestinal CD4$^+$ T cell production of IL-10 and intestinal homeostasis.

NEUTROPHILS ALTER DSB REPAIR PATHWAY IN INFLAMED MUCOSA TO PROMOTE COLON CARCINOGENESIS

Triet Bui, Veronika Butin-Israeli, Stephen Hanauer, Julien Sebag, Ronen Sumagin

Due to exacerbated inflammation and recurring tissue injury patients with Inflammatory Bowel Disease (IBD) are at higher risk of developing colorectal cancer (CRC). Although, PMN infiltration of the intestinal mucosa is a hallmark of IBD, and is associated with tissue injury, the contribution of PMNs to CRC and more so to IBD associated CRC is not known. We recently showed that PMNs can acutely exacerbate tissue injury and promote genomic instability by promoting miR-23a and miR-155-dependent accumulation of double-strand breaks (DSBs) and inhibition of DSB-repair by homologous recombination (HR). We now demonstrate that in chronic gut inflammation and recurring epithelial injury, as seen in IBD, via similar miRNA-dependent mechanism, PMNs alter DNA damage responses (DDR) to promote CRC progression. In murine model of Colitis-associated CRC (Azoxymethane, AOM/Dextran sodium sulfate, DSS) and human CRC xenografts, intratumoral PMNs intriguingly, functional dualism, suppressing tumor onset, but promoting tumor cell survival and growth in progressive tumors. The observed PMN effects were mediated by persistent suppression of HR-mediated DSB repair. HR suppression by PMNs, initially resulted in elevated replication stress and increased tumor cell apoptosis, however, longer term, altered DDR transcriptional profile and facilitated the upregulation of DSB repair by non-homologous end-joining (NHEJ). NHEJ upregulation enhanced CRC progression and tumor cell survival. PMN depletion, CRSPER-mediated deletion of miR-155 responsive sequence in RAD51 (preserves HR activity) or inhibition of NHEJ by small molecule inhibitors increased tumor cell death and diminished tumor development. Collectively, our data define a novel link between PMN-mediated mucosal injury and colon carcinogenesis.

OBSTRUCTED LYMPHATIC TRANSPORT AND LEAKAGE DRIVEN BY MESENTERIC TERTIARY LYMPHOID ORGANS IS A FEATURE OF CROHN'S DISEASE MOUSE MODEL

Rafael Czepielewski, Emma Erlich, Emily Onufer, Shannon Young, Ki-Wook Kim, Peter Wang, Shashi Bala, Chyi-Song Hsieh, Bernd Zinselmeyer, Michael Davis, Gwendalyn Randolph

Pioneer reports of Crohn's disease (CD) suggested that impaired lymphatic flow might drive its pathogenesis but remains unsettled. Nodules of tertiary lymphoid organs (TLO) are found in association with collecting lymphatic vessels (CLVs) of the mesentery that normally conducts lymph outflow from the intestine. Whether TLOs affect lymph transport is unknown. In the TNF$^{\Delta ARE}$ mouse model of Crohn's-like ileitis, TLOs are found in valves regions. Using lymphatic reporters and photoconversion to study cell trafficking from the intestine, our findings indicated that TLOs halts immune cells traveling from the inflamed ileum to the lymph node, effectively trapping DCs, B, and T cells, and impacting the development of microbe tolerogenic regulatory T cells. Lymphatic transport defects were intrinsic to the CLVs because the soluble fluorescent tracer's passage through TLO was also blocked. Lymph blockage promoted retrograde lymphatic flow returning towards the gut wall due to incapable valves. Moreover, significant lymph leakage was found, specifically at the TLOs. Neutralizing anti-TNF mAb treatment into TNF$^{\Delta ARE}$ mice is ineffective in eliminating TLOs or restoring lymphatic trafficking when administered in female mice with advanced disease. In males, the therapy was able to restore forward flow to the lymph node. However, even in the presence of TNF inhibition, both sexes demonstrated TLO lymph leakage. Thus, mesenteric TLOs that form during chronic ileitis drive broadly impaired lymph transit of molecules and cells from the intestine that is only partially reversible by neutralizing the cytokine cascade underlying the disease and establish a perennial tissue alteration.