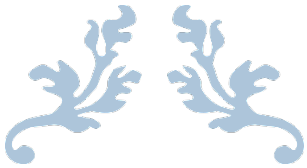

THE ROLE OF ABCB1 AND ABCB5 IN PAEDIATRIC INFLAMMATORY BOWEL DISEASE

Thesis submitted in accordance with the requirements of the University of Liverpool

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THE UNIVERSITY OF LIVERPOOL

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Abstract

ABC transporters play an important role in drug disposition. ABCB1, the most studied representative member of this transporter superfamily, can influence drug response and shape multi-drug resistance in cancer and various diseases including inflammatory bowel disease. Adult studies have shown inter-individual variability in expression between patients with inflammatory bowel disease and healthy controls, which can be modified by corticosteroid administration, a common treatment agent for inflammatory bowel disease. ABCB5 is a novel transporter highly homologous to ABCB1.

This thesis focusses on an inception pilot cohort of children and young people with newly diagnosed inflammatory bowel disease and healthy controls. The purpose of this thesis is to investigate the expression of ABCB1 and ABCB5 *in vitro* and *ex vivo*, to test the hypothesis that both are important in children with inflammatory bowel disease with respect to expression, to investigate how expression varies with genotype, and to explore the possible role of ABCB5 as corticosteroid transporter.

De novo production of stable and transient overexpressing mammalian ABCB5 clones confirmed high gene expression at the mRNA level, but no ABCB5 protein isoforms were detectable, despite repeated attempts and investigation of human malignant cell derived ABCB5 expressing clones donated by experts in the field. Radiolabeled drug uptake studies with corticosteroids commonly implemented in the treatment of inflammatory bowel disease, showed that corticosteroid efflux was not mediated by any of the ABCB5 overexpressing mammalian cell lines.

Children with newly diagnosed inflammatory bowel disease and healthy controls recruited at a regional tertiary children's hospital were genotyped for common ABCB1 and novel ABCB5 single nucleotide polymorphisms. The genotype variation observed between cases and controls was not statistically different at the point of diagnosis. The minor allele frequency for rs2032582 (G2677T in exon 21 of the *ABCB1* gene) was significantly different between cases and controls (p=0.01), but this did not withstand correction for multiple testing.

ABCB1 and *ABCB5* gene expression profiling of blood and intestinal biopsies from 16 patients with inflammatory bowel disease and 20 controls was determined. No significant variability was observed in *ABCB1* gene expression across *ABCB1* genotypes or clinical phenotypes (inflammatory bowel disease versus healthy state). *ABCB5* expression was not detectable in blood or intestinal biopsies taken from the small intestine and sigmoid colon of the study participants.

ABCB1 and ABCB5 protein expression was characterized by immunohistochemistry in intestinal biopsies from study participants. ABCB1 showed inter and intra-individual variability in expression across blood samples and intestinal biopsies from cases and controls, but this was not significantly different. There was significant correlation between gene expression and protein expression in sigmoid colon (p=0.007). Non-specific background staining for ABCB5 by immunohistochemistry was noted, which did not allow for reliable quantification of ABCB5 protein expression in the small intestine and sigmoid colon. Treatment response to

corticosteroids in a subgroup of patients with ulcerative colitis showed weak correlation with ABCB1 expression.

In conclusion, in this pilot set of experiments, no difference was identified in ABCB1 and ABCB5 expression at mRNA and protein level in peripheral blood cells, small intestine and colonic biopsies, taken from children with inflammatory bowel disease and healthy controls. The study was limited by the small sample size; further studies will be needed for confirmation of these results. Importantly, there was no ABCB5 expression at the protein level and no functional activity was identifiable with respect to corticosteroid efflux in transfected cell lines. Further work is required to appreciate the role of ABCB5 in intestinal physiology and pathology.

Acknowledgements

This academic journey commenced in 2012 and ended in 2018. I would like to express my deep gratitude to Professor Sir Munir Pirmohamed and Doctor David Dickens for their continuous support and guidance.

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This piece of work has been conducted in partnership with the wonderful Alder Hey Gastroenterology Team that I have had the pleasure and honor to be part of, as a junior doctor, clinical research fellow, sub-specialty registrar and Consultant. I would like to thank Doctors Mark Dalzell and Marcus Auth, Consultant Paediatric Gastroenterologists at Alder Hey Children's Hospital, who believed in this original piece of work and supported me at the start of this research journey. This is the first thesis undertaken in collaboration with the Alder Hey Paediatric Gastroenterology Department.

I would like to thank my dear husband Adonis and my beloved sons Manthos and Yiannis for their patience and resilience.

I dedicate this thesis to my father Yannis and my mother Effie, as a small token of my gratitude for their unconditional love, tremendous support and relentless encouragement to this day.

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Abbreviations used in this thesis

Abbreviation	Key
ABCB5 α	ABCB5 alpha
ABCB5 FL	ABCB5 full length
ABCB5 β	ABCB5 beta
ABCB5 ts	ABCB5 testis specific
ABCB5 ε	ABCB5 epsilon
5 ASA	5-aminosalicylates
anti-CBir1	anti-flagellin expressed by Clostridia phylum
Anti-GFP	Anti-green fluorescence protein
anti-OmpC	anti-outer membrane porin of Escherichia coli
anti-I2	anti- Pseudomonas fluorescence-associated sequence
AP1	Activator protein 1
AP-1	Apoprotein 1
ATG16L1	autophagy related 16 like protein 1
ATP	Adenosine triphosphate
BCRP	Breast cancer resistant protein
BSA	Bovine Serum Albumin
CD	Crohn’s Disease
cDNA	Complementary DNA
Ci	Curie
CMV	Cytomegalovirus
CRC	Colorectal cancer
CRF	Corticotropin-releasing factor
CRP	C-reactive protein
CS	Corticosteroids
CSF2RA	Colony Stimulating factor 2 receptor alpha subunit
CTLA-4	Cytotoxic T lymphocyte associated protein 4
CXR1	Chemokine receptor 1
CYP	Cytochrome P

DMEM	Dulbecco’s Modified Eagle Medium
DNA	Deoxyribonucleic Acid
dNTPs	Deoxynucleotide triphosphate solution
DPM	Disintegrations per minute
DTT	Dichlorodiphenyltrichloroethane
EDTA	Ethylenediaminetetra-acetic acid
EEN	Exclusive enteral nutrition
ELISA	Enzyme linked immunosorbent assays
Em-GFP	Emerald green fluorescent protein
ER	Endoplasmic reticulum
ESR	Erythrocyte sedimentation rate
EPCAM	Epithelial cell adhesion molecule precursor
FACS	Fluorescence activated cell sorting
FBS	Foetal Bovine Serum
FDA	Food and Drug administration
FFPE	Formalin fixed, paraffin embedded
FOXBP3	Fork head Box P3
FL	Full length
5-FL	5 fluorouracil
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase
GCP	Good Clinical Practice
GM-CSF	Granulocyte macrophage colony stimulating factor
GR	Glucocorticoid receptor
GTEx	Genotype-Tissue Expression
GWA	Genome Wide Association
HBI	Harvey-Bradshaw index
HBSS	Hanks Balanced Salt Solution
HDAC2	Histone deacetylase-2
HEK 293	Human epithelial kidney cells 293
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

HLA	Human Leucocyte Antigen region
HMEC	Human melanoma epithelial cells
HPA	Human Protein Atlas
HPLC	High performance Liquid Chromatography
HTA	Human Tissue Authority
HWE	Hardy Weinberg equilibrium
IBD	Inflammatory Bowel Disease
IBDU	Inflammatory Bowel Disease Unclassified
IgA	Immunoglobulin A
IHC	Immunohistochemistry
Ikba	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor
IL10	Interleukin 10
IL10R	Interleukin 10 receptor
IL-23R	Interleukin 23 receptor
IPEX	immune dysregulation, poly-endocrinopathy, enteropathy, X-linked syndrome
IRAS	Integrated Research Application Form
IRGM	immunity related GTPase family M
KLH	Keyhole Limpet hemocyanin
LAT1	Large neutral amino-acid transporter 1
LB	Lysogeny broth
LD	Linkage Disequilibrium
LRBA	Lipopolysaccharide responsive and beige-like anchor protein
LSC	Limbal stem cells
MAF	Minor allele frequency
MAPK	Mitogen-activated protein kinase
MCC	Merkel Cell Carcinoma
MDR1	Multidrug resistance 1
6-MMPR	6-methylmercaptopurine ribonucleotides

mRNA	Messenger RNA
MRP2	Multidrug resistance associated protein
MYO5B	Myosin-Vb
NADPH	Nicotinamide adenine dinucleotide phosphate oxidase
NBD	Nucleotide-binding domain
NFkB	Nuclear factor-kappa beta
NHS	National Health System
NR3C1	Nuclear receptor subfamily 3 group C member 1
NOD2	Nucleotide-binding oligomerization domain protein 2
NUDT15	Nydix Hydrolase 15
PBMCs	Peripheral blood mononuclear cells
P-CDAI	Paediatric - Crohn's Disease Activity Index
PDR5	Pleiotropic Drug Resistance 5
PILs	Patient information leaflets
PgP	P glycoprotein
Poly-His tag	Poly-histidine tag
P-UCAI	Paediatric - Ulcerative Colitis Disease Activity Index
PXR	Pregnane X Receptor
RIPA	Radio-immuno Precipitation Assay buffer
RT-qPCR	Real time quantitative polymerase chain reaction
SCFA	short chain fatty acids
SDS	Sodium dodecyl sulfate
siRNA	small interfering RNA
SK-MEL28	Human melanoma cell line 28
SLC	Solute load carrier
SMAD3	Small mothers against decapentaplegic 3
SNP	Single nucleotide polymorphism
TLR	Toll like receptors
TLR	Toll like receptors
TMD	Transmembrane domain

6TGN	6-thioguanine nucleotides
TNF-α	Tumor necrosis factor-alpha
TNFAIP3	Tumor necrosis factor alpha-induced protein 3
TPMT	Thiopurine methyltransferase
TTC37	Tetratricopeptide repeat protein 37
UC	Ulcerative Colitis
STAT3	Signal transducer and activation of transcription 3
VEO-IBD	Very early onset IBD
XIAP	X linked inhibitor of apoptosis
ZEB1	Zinc finger e-box binding homeobox 1

Chapter 1

General Introduction

1.1 Overview and nature of IBD

Inflammatory bowel disease (IBD) encompasses three major forms of chronic intestinal inflammation, including Crohn's disease (CD), ulcerative colitis (UC) and unclassified IBD (IBDU), previously known as indeterminate colitis. The definition of clinical phenotypes follows internationally agreed standards based on a constellation of clinical, endoscopic, radiologic and histologic features. IBD is recognized as a heterogeneous spectrum of disorders with distinct clinical courses and symptom chronicity, which may present anytime from early childhood to adulthood (Castellaneta, Afzal et al. 2004, Actis, Rosina et al. 2011, Horjus Talabur Horje, Meijer et al. 2016).

The burden of disease can be modified by treatment, but in principle IBD presents with relapsing and remitting course throughout life (Silverberg, Satsangi et al. 2005). CD and UC, while sharing several similar pathologic and clinical features, have distinct differences in prognosis and management (Guariso and Gasparetto 2017). UC is characterized by chronic continuous and circumferential mucosal inflammation extending proximally from the rectum, principally affecting the colon and terminal ileum (backwash ileitis). Sub-classification of UC is made according to the degree of disease extension to ulcerative proctitis, left sided (distal) and extensive colitis (pancolitis) (Aldhous, Drummond et al. 2007). By contrast, the stereotypical inflammation seen in CD is patchy and transmural and may affect any part of the gastrointestinal tract from the oral cavity to anus and perianal region; granuloma formation is typical of CD (Gajendran, Loganathan et al. 2017).

The Montreal classification for Crohn's disease relates to the age at diagnosis, disease location (ileal, colonic, ileo-colonic) and disease behavior, either non-stricturing/ non- penetrating, or stricturing/ penetrating (Satsangi, Silverberg et al. 2006).

IBD has been increasing in prevalence in the entire world (Hou, Abraham et al. 2011, Molodecky, Soon et al. 2012). It affects approximately 3.7 million people in North America and Europe (Molodecky, Soon et al. 2012). Over the past two decades, an increasing incidence of IBD has been observed in Asian and Middle Eastern countries, such as China, India (Ananthakrishnan 2015), South Korea (Hou, Abraham et al. 2011) and Saudi Arabia (Al-Mofarreh and Al-Mofleh 2013). The incidence and prevalence may however vary according to the geographic region, environment and ethnicity. For instance in North America the annual incidence of CD is reported to be 3.1-20.2 per 100,000 with a prevalence of 201 per 100,000 population (Gajendran, Loganathan et al. 2017). The prevalence of UC in the same region ranges from 37 to 249 cases per 100,000 people, with similar rates observed in other developed countries (Loftus 2004).

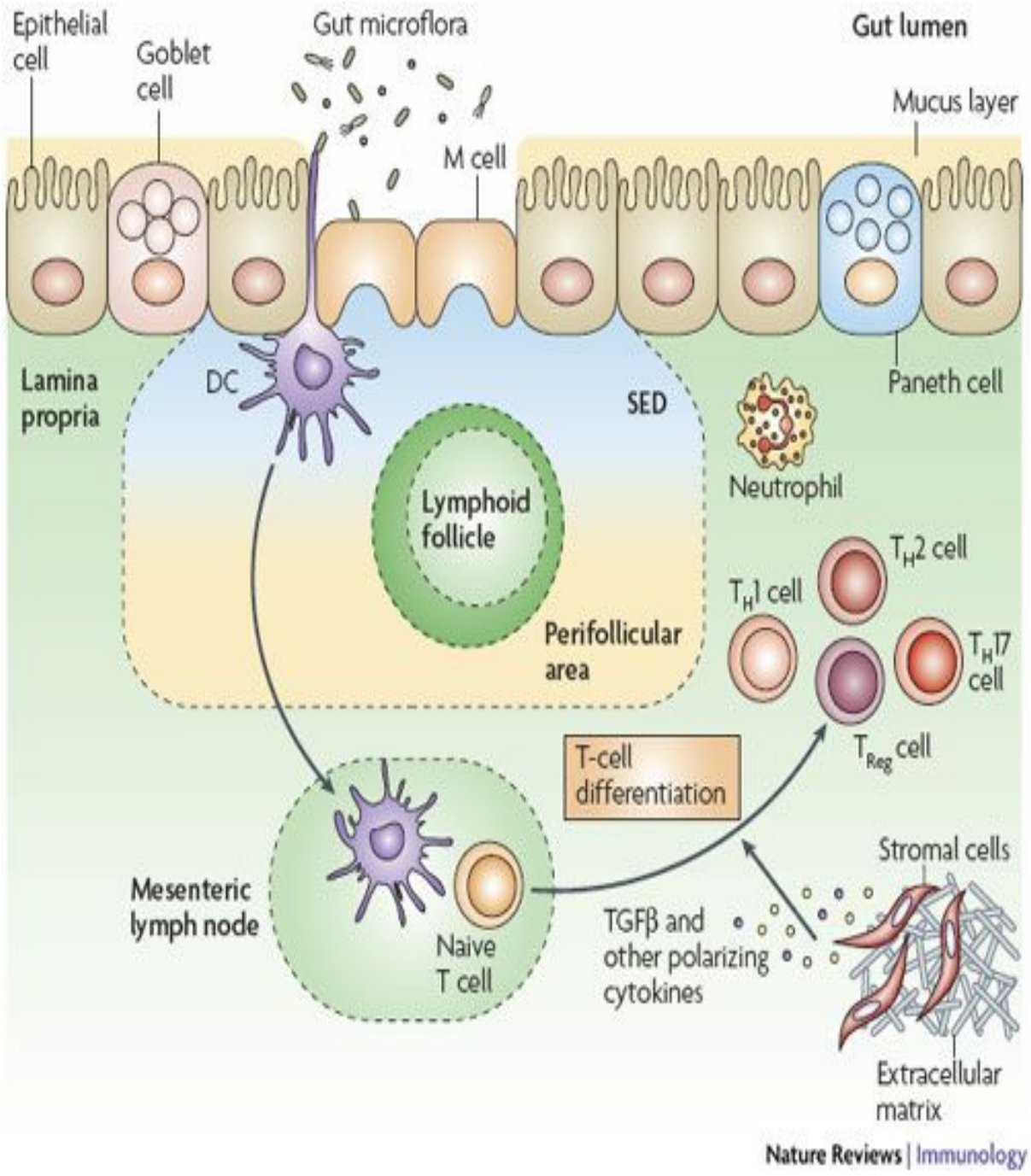
1.1.1. Aetiology of IBD

The aetiology of IBD is incompletely understood, however an increasing number of studies support the hypothesis that IBD results from a complex interplay of genetic components, environmental triggers and immune dysregulation that may exert their effect through alteration of the intestinal microbiota (Dubinsky, Mei et al. 2010, Glymenaki, Barnes et al. 2017). The pathogenesis of IBD has indeed been a major challenge for researchers over the last decades. The currently widely accepted hypothesis is that the disease is a product of interactions between host predisposing

factors and environmental triggers (Baumgart and Carding 2007, Davenport, Poles et al. 2014).

The intestinal epithelium plays a pivotal role in this interaction (Martini, Krug et al. 2017). It functions as a semi-permeable physical barrier, which protects the interior of the body from invasions of pathogens and allows selective passage of nutrients. The epithelium is believed to be a highly dynamic tissue with complex crosstalk of signals, involving an interplay with commensal gut bacteria and host immune cell responses (Saleh and Elson 2011). This interaction results in shaping the intra-luminal bacterial content and in preserving mucosal immune homeostasis within the lamina propria. Dysfunction of the intestinal epithelium and mucous layer may contribute to intestinal barrier failure and excessive translocation of commensal bacteria into the lumen (Heazlewood, Cook et al. 2008, Gunther, Martini et al. 2011, Vancamelbeke, Vanuytsel et al. 2017). Non-physiological interaction triggers immune dysregulation of the host, resulting in the onset of chronic inflammation observed in IBD and is depicted schematically in Figure 1.1 (Cho 2008, Frank, Robertson et al. 2011, Halfvarson, Brislawn et al. 2017, Rapozo, Bernardazzi et al. 2017, Nishida, Inoue et al. 2018). The highly variable natural history and spectrum of disease severity, relate to the extent and depth of intestinal inflammation, extra-intestinal manifestations and disease complications.

Figure 1.1. Interaction between intraluminal bacteria and intestinal immune system: disruption of the protective mucous layer and interaction of gut microflora with dendritic cells generates an inflammatory cascade with cytokine production at the level of the intestinal epithelium, chronic stimulation of mesenteric lymphnodes, maturation of different types of T helper cells and neutrophils and distortion of the crypt architecture. Adapted from Cho et al 2008, with permission. The genetics and immuno-pathogenesis of IBD. Nature Reviews Immunology 8, p.458-466.



1.2 Pathogenesis of IBD

1.2.1. Environmental factors

The common environmental factors which influence intestinal homeostasis include diet, lifestyle, smoking, stress, probiotic and antibiotic use (Castano-Rodriguez, Kaakoush et al. 2017, Sheehan and Shanahan 2017).

Dietary factors, mainly a Western type diet, may affect disease pathogenesis, through modulation of gut bacteria (Simpson, Campbell et al. 2014). Diet plays a pivotal role in altering the microbial content of the intestinal lumen, and therefore it is believed to have an important contribution to the individual's susceptibility to intestinal inflammation (Rapozo, Bernardazzi et al. 2017). Metabolite profiling in serum, plasma, urine and faeces implemented in animal and clinical studies can differentiate healthy subjects from individuals with IBD (Schicho, Nazyrova et al. 2010, Schicho, Shaykhutdinov et al. 2012). The increase in pathogenic bacteria that adhere to intestinal epithelial cells affects intestinal permeability, alters the diversity and composition of gut microbiota, and induces inflammatory responses by regulating pro-inflammatory gene expression in the gut (Ahmed, Roy et al. 2016, Glymenaki, Barnes et al. 2017). There is emerging evidence as to how dietary manipulations can influence the gut microbiota environment (Harusato and Chassaing 2017), thereby shaping the immune response of the host. Dietary factors can influence the presence of intestinal inflammation (Wu, Chen et al. 2011); for instance, the higher content of plant derivatives and fiber appears to be protective, in contrast to high carbohydrate and animal fat-based diet (Keshteli, van den Brand et al. 2017). There is increasing evidence that high fiber content favors

gut homeostasis and promotes intestinal health through increased microbiota diversity and production of high levels of fermentable carbohydrates (Simpson, Campbell et al. 2014). One of the reported mechanisms of the direct effect of diet is the reported abundance of *Prevotella* genus (*Bacteroidetes phylum*) in plant-based diets, shifting the production of short chain fatty acids (SCFA) in the gut lumen. Food additives alter the gut bacteria and induce histologic changes of the intestinal epithelium in animal studies, therefore can play a role in chronic intestinal inflammation; this hypothesis needs further investigation (Martino, Van Limbergen et al. 2017).

Current theories of IBD pathogenesis postulate that pathologic alterations in the intestinal microbiome trigger an aberrant mucosal immune response in genetically predisposed individuals, leading to the development of chronic intestinal inflammation (Peterson, Frank et al. 2008). These pathologic alterations in gut microbial composition seen in IBD are referred to as intestinal 'dysbiosis' (Lane, Zisman et al. 2017). The term "dysbiosis" was first devised by Metchnikoff in the early twentieth century to describe changes in intestinal bacteria (Hawrelak and Myers 2004). *Firmicutes* (Gram-positive) and *Bacteroidetes* (Gram-negative) are the predominant phyla found in the human gut, accounting for over 90% of all bacteria. The total number and composition of bacteria vary in different segments of the gastrointestinal tract, with a few species residing in the stomach and the proximal small intestine. The number of microbes increases from the jejunum, to each subsequent part of the gut, with the maximum concentration noted in faeces. The disruption of intestinal bacteria may contribute to the pathogenesis of IBD via their metabolites (Glymenaki, Barnes et al. 2017). The production of SCFA decreases in IBD-affected patients because of less *Faecalibacteria prausnitzii*, a butyrate-

producing bacterium in the gut (Thibault, Blachier et al. 2010, Geirnaert, Debruyne et al. 2012). On the other hand, the concentration of sulfide-reducing bacteria is higher in IBD-affected patients; this can result in the metabolic production of hydrogen sulfide that is damaging to the gut epithelium (Devkota, Wang et al. 2012). It however remains unclear whether the observed changes are causative for the development of histological changes described in IBD, or simply a consequence of an altered intestinal environment during the progression of the disease itself (Ley, Peterson et al. 2006).

Relative microbial diversity characterized by an increase in *Enterobacteriacae* family (*Phylum Proteobacteria)* and *Sphingomonas* genus (*Phylum Porphyrobacter,)* and a decrease in *Fusobacterium (Phylum Firmicutes)* and *Ruminococcus* genera (*Phylum Firmicutes*), has been described in the gut of patients with colitis associated colorectal cancer, differing from healthy individuals (Richard, Liguori et al. 2017).

The beneficial effect of probiotics is attributed to the restoration of goblet cells and the stimulation of the mucosal immune system to secrete protective immunoglobulins, such as secretory immunoglobulin A (IgA), protective defensins, and bacteriocidins in the intestinal tract (Nami, Abdullah et al. 2014). The phenomenon of 'dysbiosis' therefore reflects a perturbation in the symbiotic relationship between host and microbes through regulation of host signaling pathways and modulation of co-metabolism of the host and the microbiota (Geirnaert, Calatayud et al. 2017, Ong and Yim 2017, Yang and Jobin 2017).

Probiotics may promote intestinal health by modulating the microflora such as genera *Lactobacillus* (*Plylum Firmicutes*) and *Bifidobacterium (Phylum Acinetobacteria*), thereby hindering pathogen colonization, at the same time

enhancing mucosal trophic effects by stimulating intestinal epithelial cell barrier responses (Tamboli, Caucheteux et al. 2003). Probiotics express pathogen-associated molecular patterns and can therefore mimic the function of commensal bacteria by engaging and/or activating the pattern recognition receptors on epithelial mucosal surfaces, thus regulating the expression of genes involved in the host immune response (Shanahan 2010). Probiotics such as lactobacilli and bifidobacteria have a track record of safety (Sokol, Pigneur et al. 2008, Llopis, Antolin et al. 2009, Deshpande, Rao et al. 2010), however the overall safety of probiotics should be considered on a strain by strain basis and may be dependent on host susceptibility or disease (Shanahan 2012).

Antibiotic therapy is an identified environmental risk factor for development of persistent diarrhea and colitis. Severe enterocolitis associated with antibiotic treatment in critically ill patients is characterized by severe apoptosis of the gastrointestinal epithelium that may be the consequence of a microbiota deplete gastrointestinal tract following long-term antibiotic administration in the critical care environment (Wurm, Spindelboeck et al. 2017).

Stress is another risk factor implicated in the development of IBD, mediated by corticotropin releasing factor (CRF) signaling (Brzozowski, Mazur-Bialy et al. 2016). In response to stress, the released CRF produced in multiple brain regions stimulates the production of adrenocorticotropic hormone from the pituitary gland, subsequently transported to the adrenal cortex to induce the synthesis and secretion of cortisol; CRF signaling can act in the stomach, small intestine, and lymphocytes. Importantly, the activation of CRF has been reported to be associated with inflammation of the colonic mucosa and increased intestinal permeability in patients

with CD or UC, through release of inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), known to play a pivotal role in the intestinal inflammation cascade (Kawahito, Sano et al. 1995, Brzozowski, Mazur-Bialy et al. 2016).

Smoking can increase susceptibility and adversely affect CD progression with recurrence after surgery, poor response to medical treatment and rapid progression to more aggressive disease; the exact mechanism is largely unexplored (Parkes, Whelan et al. 2014). It is plausible that smoking exerts its effect through modulation of the host microbiota. For instance, studies have used fluorescent in situ hybridization to show that smokers had increased proportion of *Bacteroides Prevotella*, associated with tendency for CD4 T cell mediated colitis in animal models (Hoentjen, Tonkonogy et al. 2007). Lower *Faecalibacterium prausnitzii* is associated with higher risk of endoscopic recurrence after surgery in CD (Sokol, Pigneur et al. 2008). Nicotine directly inhibits the innate immune system in animal and cell culture models (Wang, Yu et al. 2003). It enhances small intestinal but not colonic inflammation in interleukin 10 (IL-10) knock out mice (Eliakim, Karmeli et al. 2001). Epigenetic changes of the genome, such as post translational methylation, histone modification or micro-RNA influence could otherwise explain how smoking exerts its complex effect in CD pathogenesis (Archanioti, Gazouli et al. 2011, Scarpa and Stylianou 2012). Heterogeneity between studies with regards to definition of the smoking status may have influenced conflicting study outcomes as reported by Mahid et al (Mahid, Minor et al. 2007). In UC smoking is largely reported to have a protective effect (Mahid, Minor et al. 2006). Nicotine and corticosteroids (CS) can enhance colonic mucous layer in patients with UC (Finnie, Campbell et al. 1996); nicotine and its metabolites may reduce intestinal permeability to pathogens, however the conflicting evidence from in vivo and in vitro studies have not shed light

to the actual mechanism of the reported protective effect in UC (Benoni and Prytz 1998, McGilligan, Wallace et al. 2007).

1.2.2. Genetic susceptibility

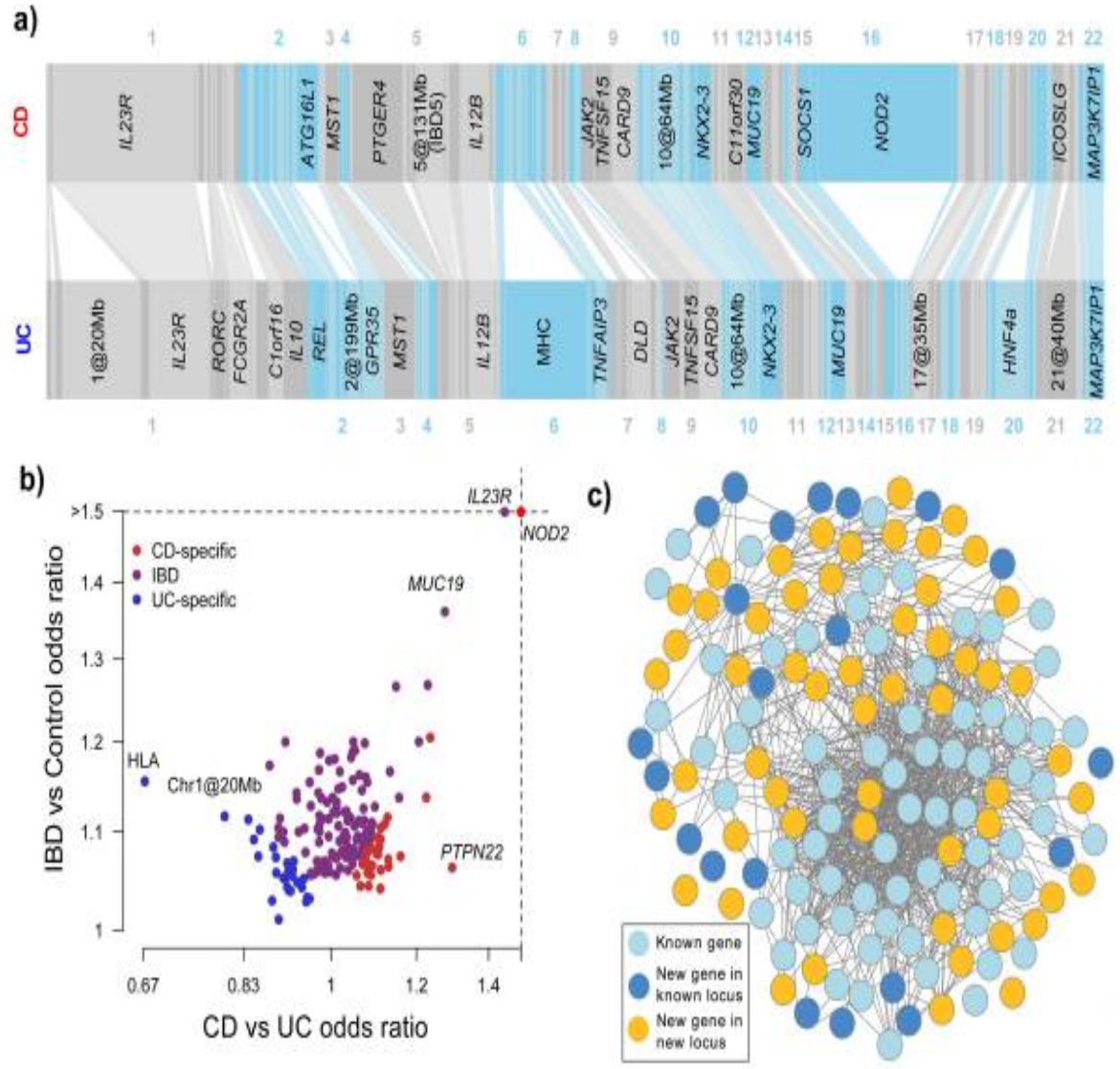
Mapping of the interaction between genetic and environmental factors may inform molecular classification of disease in the future (Khor, Gardet et al. 2011, Lane, Zisman et al. 2017). Our knowledge about susceptibility loci per se has been gradually accumulating through genome wide association studies; these are however not adequate to shed light on disease pathogenesis (Tennessen, O'Connor et al. 2011). The molecular mechanism by which, genetic variability predisposes individuals to disease, is poorly characterized, impeding the development of therapeutic interventions (Ardlie, DeLuca et al. 2015). There are gaps in our knowledge of the complexity of gene expression regulation and furthermore their influence on disease presentation and prognosis (Ahmad, Armuzzi et al. 2002). Epigenetic alterations, such as DNA methylation, may influence disease progression (Harris, Shah et al. 2016). The main genetic locus *CARD15/NOD2* has emerged as the most significant from the largest genotype-phenotype analysis to date (Economou, Trikalinos et al. 2004). Based on epidemiological, genetic and immunological data, IBD is a heterogeneous disorder with multifactorial etiology, where genetic make-up and environment interact to manifest the disease (Morgan, Tickle et al. 2012). Recently a trans-ethnic analysis identified an additional 38 new IBD loci with an overlap of directionality of odds ratios (ORs) between European and Asian ancestry cohorts (Liu, van Sommeren et al. 2015). Disease distribution in part influences disease progression and prognosis (Roussomoustakaki, Satsangi et al. 1997, Ho, Soranzo et al. 2006); Genome wide association (GWA) and other studies

have demonstrated how genetics can be used for phenotype stratification (Haritunians, Taylor et al. 2010, Plevy, Silverberg et al. 2013).

GWA studies have provided an overview of relative contribution of various genomic loci to a number of human diseases including IBD, through genotyping of a large number of single nucleotide polymorphisms (SNPs) across the human genome (International HapMap, Frazer et al. 2007). Over the last decade, various studies have reported that multiple genomic loci increase genetic susceptibility to IBD (Senhaji, Nadifi et al. 2017). To date more than 163 IBD susceptibility genetic loci have been found in cohorts of European descent, as shown in Figure 1.2 (Jostins, Ripke et al. 2012), with emerging data of how genetic variation can determine disease distribution (Cleynen, Boucher et al. 2015). Of these loci, 110 confer risk to both IBD subtypes, whereas 30 and 23 loci are unique to CD and UC respectively. The 163 loci explain 13.6% of CD and 7.5% of UC total disease variance respectively (Jostins, Ripke et al. 2012). A large number of genes found to increase susceptibility to IBD play a role in the innate and adaptive immune systems (Franke, McGovern et al. 2010). Genes implicated in the immune dysregulation observed in IBD are involved in intracellular identification and processing of intraluminal, commensal and pathogenic bacterial components. Susceptibility genes encode proteins such as the nucleotide-binding oligomerization domain protein 2 (*NOD2)* with mutations affecting nuclear factor-kappa beta *(NFkB)* production, autophagy related 16 like protein 1 (*ATG16L1*), immunity related GTPase family M (*IRGM),* functional Toll like receptors *(TLR)* (Torok, Bellon et al. 2017), signal transducer and activation of transcription 3 *(STAT3)* protein and interleukin 23 receptor *(IL-23R)* (Duerr, Taylor et al. 2006).

Liu et al have conducted the first trans-ethnic association study in IBD with use of genome wide association Immunochip data from 86,640 Europeans and 9,846 subjects of Asian descent, which revealed 38 new IBD susceptibility loci; the majority of IBD loci show similar direction and magnitude of effect between European and non- European populations. Nevertheless genetic heterogeneity due to difference in allele frequencies and effect sizes have been observed in many of the risk conferring IBD loci established in GWAS to date such as *NOD2, ATG16L1, IL23R* (Liu, Wu et al. 2015). The evidence of shared IBD susceptibility loci from Liu et al as well as large transethnic studies for other complex diseases show that combining genotyping data from various cohorts may lead to the identification of new disease associated susceptibility loci (Okada, Wu et al. 2014, Replication, Meta-analysis et al. 2014).

Figure 1.2. The IBD Genome taken from Jostins et al with permission (2012). a) Variance explained by the 163 IBD genetic loci. The width of the bar is proportional to the variance explained by that locus in CD and UC. b) The independent signals plotted by IBD odds ratio and phenotype effect. c) Network of genes connected to CD and UC, including known and newly associated genes.



1.3 Childhood onset IBD

Validated algorithms classify childhood onset IBD into small bowel CD, colonic CD, IBDU, atypical UC, and UC (Birimberg-Schwartz, Zucker et al. 2017). The Paris modification of the Montreal classification by Satsangi et al was the outcome of an evidence-based consensus process by a group of IBD experts, in a coordinated effort to address the weaknesses of the Montreal classification, at the same time taking into account the complexity and evolving nature of patient phenotypes in childhood IBD. For instance, upper gastrointestinal tract involvement has been reported in up to 25% of children diagnosed with UC (Sullivan, Wei et al. 2017). The reasons therefore behind the Paris modification were a) to facilitate research by creating uniform standards, b) to address the need to include age and disease behavior, not only the extent in UC classification and c) to include the parameter of growth in both childhood onset UC and CD (Levine, Griffiths et al. 2011).

Furthermore, emerging evidence supports the need for a 'top-down' approach in the implementation of treatment modalities, especially in children, because of the observed aggressive childhood onset IBD phenotype, in CD and UC (Duricova, Burisch et al. 2014, Walters, Kim et al. 2014, Fumery, Duricova et al. 2016). For example, population based, paediatric cohort studies show up to twice the risk of developing steroid dependency within the first year from diagnosis in children, when compared to adults (Duricova, Pedersen et al. 2011).

Paediatric IBD is associated with greater disease burden and morbidity because it affects children at a critical period for growth and development and is characterized by extensive and severe disease, which if untreated, results in increased risk of intestinal strictures necessitating surgical treatment (Schoepfer, Santos et al. 2019), growth failure, nutritional deficiencies, delayed puberty, metabolic bone disease and

increased risk of extraintestinal manifestations such as hepatic (Mahfouz, Martin et al. 2019), eye and skin disease (Annese 2019) and psychiatric morbidity (Butwicka, Olen et al. 2019). Complicated diseases course is associated with choice of treatment agents, drug efficacy and sustained treatment response or lack of it.

IBD incidence and prevalence is increasing especially in children (Henderson and Wilson 2012). Further research is required in the ontogeny and pathogenesis of IBD in this age group (Benchimol, Guttmann et al. 2009). The only prospective national survey of IBD in children aged <16 years in the UK was carried out during 1998-1999 and involved 739 children aged below 16 years. This survey showed that the incidence was 5.2 per 100,000 individuals per year (60% CD, 28% UC and 12% IBDU); disease was slightly more common in boys, with a marginally higher rate of UC in Asian children than in other ethnic groups. The mean age at diagnosis was 11.9 years. For CD there were approximately equal proportions of ileitis, colitis and ileocolitis, and for UC almost 90% of children had pancolitis (Sawczenko, Sandhu et al. 2001). Other national cohort studies have been included in a recent systematic review, which has confirmed the rising global trend of paediatric IBD in both developed and developing countries (Benchimol, Guttmann et al. 2009). The paediatric phenotype involves extensive disease with an overall higher burden of morbidity and mortality compared to adults (Olen, Askling et al. 2017). As far as gut microbiota profiling in children with IBD is concerned, de Meij et al reported a lack of abundance and diversity in comparison to gut microbiota of healthy controls, rather than a prevalence of pathogenic bacteria (de Meij, de Groot et al. 2018).

Very early-onset IBD (VEO-IBD) presenting below 6 years of age is predominantly a monogenic condition; genetic testing such as whole genome/exome sequencing or

next generation DNA sequencing may establish the molecular diagnosis to some extent, allowing for patient-specific early intervention and the opportunity to screen family members for carrier detection and genetic counselling. IBD-like monogenic disorders are related to primary immunodeficiency, for example defects of T and B lymphocyte selection and activation, disorders affecting regulatory T cell activity and interleukin 10 (*IL10)* and *IL10 receptor A/B (IL10R) A/B)* signaling (Moran, Walters et al. 2013). Other monogenic conditions shown by VEO-IBD cohort studies, include dysfunction of nicotinamide adenine dinucleotide phosphate (*NADPH*) oxidase complex, X linked inhibitor of apoptosis (*XIAP)*, lipopolysacharide responsive beige-like anchor protein (*LRBA)*, cytotoxic T lymphocyte associated protein 4 (*CTLA-4)*, *STAT3* and chronic granulomatous disease (neutrophil oxidation burst dysfunction) (Shouval, Kowalik et al. 2018). Mutations in the epithelial cell adhesion molecule (*EPCAM)*, myosin Vb (*MYO5B)* and Fork head Box P3 gene (*FOXBP3)*, 'immune dysregulation, poly-endocrinopathy, enteropathy, X-linked' (*IPEX* syndrome) (Charbit-Henrion, Parlato et al. 2018) and tricho-hepato-enteric syndrome caused by mutations in Tetratricopeptide Repeat Domain 37 (*TTC37)* have been implicated in VEO-IBD (Fabre, Breton et al. 2014). Tumor necrosis factor alpha-induced protein 3 (*TNFAIP3*) mutations cause infantile onset IBD (Zheng, Huang et al. 2018). Monogenic IBD is also caused by genes related to intestinal epithelial defects, such as intestinal epithelial cell adhesion and generation of reactive oxygen species (Leung and Muise 2018). Targeted gene panel sequencing emerged as a useful tool for screening of genes of interest identified in GWA studies (Kammermeier, Drury et al. 2014, Uhlig, Schwerd et al. 2014, Petersen, August et al. 2017).

Until fairly recently, there has been a paucity of studies about genes predisposing to childhood onset IBD. Denson et al reported the results of whole exome sequencing

in 543 children with IBD. Low/normal neutrophil intrinsic granulocyte macrophage colony stimulating factor (GM-CSF) signaling was found to be associated with colony stimulating factor 2 receptor A subunit (CSF2RA) missense mutations, where neutrophils from IBD patients exhibited alterations in gene expression regulating cytokine production, wound healing, cell survival and proliferation (Denson, Jurickova et al. 2018). Further study by same group performed whole exome sequencing of deoxyribonucleic acid (DNA) from children with IBD and healthy controls to identify mutations in genes encoding nicotinamide adenine dinucleotide phosphate oxidase NADPH and neutrophil gene expression associated with reactive oxygen species production. Patients with specific mutations in NADPH oxidases had more aggressive disease course in CD (Denson, Jurickova et al. 2018).

The human leucocyte antigen region (HLA) in chromosome 6 has been attributed to have a strong association in childhood onset UC susceptibility (Venkateswaran, Prince et al. 2018). Chandradevan *et al* analyzed serological markers and gene expression profiles from ileum and rectum of children with IBDU were similar to the patients diagnosed with UC from the same prospective multi-center inception control (Chandradevan, Hofmekler et al. 2018).

Recent paediatric GWA by Cutler et al in 1008 paediatric onset IBD patients and 1633 controls confirmed overlap with common adult IBD susceptibility loci such as NOD2 and IL23R (Cutler, Zwick et al. 2015). The contribution of rare variants in paediatric onset IBD has been explored comparing exome sequencing in 368 children with IBD to publicly available and matched 625 controls of European ancestry. Pathway enrichment of the genes annotated to the 200 most common genetic loci (Jostins, Ripke et al. 2012, Liu, van Sommeren et al. 2015) were tested

by logistic regression; genome wide significance level was reached for NOD2. When enrichment of rare variants with MAF<0.05 in IBD associated loci was tested, the only significant variants were those in CD associated loci such as NOD2 and CARD9. A possible susceptibility relationship of rare variants in genes that are implicated in neutrophil function may exist in the case group (Shaw, Cutler et al. 2019).

1.4 IBD Therapeutics

1.4.1. Disease burden, complications, personalized treatment

Research into novel personalized therapies based on the understanding of disease pathogenesis, patient susceptibility and interindividual variability to treatment is ongoing (Cascorbi 2017, Cross 2017, Timmer, Stark et al. 2017). Optimal clinical outcome, less adverse reactions related to treatment and better resource utilization are at the core of personalized medicine. For instance, individual carriers of risk allele HLA DRB1 03:01, have been shown to be at three-fold risk of 5ASA related nephrotoxicity (Heap, So et al. 2016). Genetic variants of TPMT and NUDT15 are associated with higher risk of myelotoxicity; allele frequency differs in various populations and the presence of mutant variants can inform initiation of thiopurine treatment as well as safe dosing regime in cases of dose dependent side effects. The Clinical Pharmacogenetics Implementation Consortium has published guidance on thiopurine dosing recommendations based on TPMT and NUDT15 genotypes (Relling, Schwab et al. 2019). Thiopurine induced pancreatitis has been related to HLA DQA1- HLA DRB1 homozygous haplotypes, however this association has not

been yet included in routine pre-treatment pharmacogenetic panels (Heap, Weedon et al. 2014).

The need for personalized therapy especially in children is also indicated by recent studies in childhood onset CD which have shown that non-sustained response to treatment may be more important in predicting disease relapse and overall complications than actual disease severity and extent at diagnosis (Ziv-Baran, Hussey et al. 2018). The problems of achieving best drug efficacy and predictability of drug toxicity can be addressed with the use of relevant genetic markers; however currently this largely remains an unmet need, due to the lack of patient stratification based on a constellation of clinical indices, genetic and molecular signatures. Research that involves large international consortia of rigorously characterized patients, is necessary to identify, validate and implement complex personalized treatment algorithms with emphasis on avoidance of significant adverse reactions as well as optimized and sustained treatment outcomes. Differences in ethnicity, high power required for identification of rare allele variants, observed variability in phenotype and disease progression in adult and childhood onset IBD, pose further challenges with regards to identification of biomarkers with translational value. Clinical validity of biomarkers will be achieved by replication of findings in independent cohorts and by addressing cost issues (Voskuil, Bangma et al. 2019).

Clinical activity indices have been standardized and implemented as proxy markers of clinical disease activity, such as the (Paediatric)-Crohn's Disease Activity Index (P)-(CDAI), the (Paediatric)- Ulcerative Colitis Disease Activity Index (P)-(UCAI) and the Harvey-Bradshaw index (HBI) (Zittan, Kabakchiev et al. 2016). 'Treat to target' and 'top- down' with targeted clinical, endoscopic remission and mucosal healing,

versus ‘step up’ treatment are the modern treatment paradigms and endpoints of induction and maintenance treatment (Lee, Briars et al. 2016, Berg, Colombel et al. 2019). Endoscopic scoring systems such as the Mayo endoscopic score have in addition been implemented so as to more accurately describe disease status (Samaan, Mosli et al. 2014). Alternative potentially useful biomarkers to objectively describe disease progress and mark relapses include acute-phase proteins, faecal markers, serum antibodies and novel genetic determinants. C-reactive protein (CRP) is the most studied and is an overall good marker for measuring disease activity in acute relapses of CD (Fabisiak, Fabisiak et al. 2017). The faecal markers (calprotectin and lactoferrin) may be helpful in triaging patients with suspected IBD for colonoscopy from those with functional disorders and to predict disease relapse and response to induction of remission treatment; however their overall predictive value can be poor (Toyonaga, Kobayashi et al. 2017). Serologic markers, such as anti-Saccharomyces cerevisiae antibody, perinuclear anti-neutrophil cytoplasmic antibody, antiglycan antibodies, anti-flagellin expressed by Clostridial phylum (anti-CBir1), anti-outer membrane porin of Escherichia coli (anti-OmpC), anti-Pseudomonas fluorescence-associated sequence, have been used to enable differentiation between UC and CD mainly for screening purposes, however their diagnostic and prognostic values are equivocal (Olbjorn, Cvancarova Smastuen et al. 2017, Smids, Horjus Talabur Horje et al. 2017). Recent evidence from multicentre prospective paediatric studies have shown increased risk of penetrating CD phenotype in children of African American descent, anti-ASCA IgA, C-Bir 1 (Kugathasan, Denson et al. 2017). As far as paediatric UC is concerned, high PUCAI score at diagnosis and 3 months post diagnosis, as well as a low rates of CS free remission one year post diagnosis, are considered poor prognostic factors

(Schechter, Griffiths et al. 2015). Early poor response to treatment and severe disease extent at diagnosis as predictors of colectomy in paediatric UC further support the need for early initiation of patient tailored treatment. Use of biologics provides a treatment paradigm showing exactly how loss of clinical response to anti-TNF a can negatively influence disease progression in IBD (Allez, Karmiris et al. 2010).

Clinically meaningful pharmacogenetic associations are therefore increasingly necessary and are gradually implemented in pre-treatment and therapeutic drug monitoring algorithms, in order to guide personalized medicine and tailor pharmacotherapy to individual patient profiles, so as to minimise adverse side effects and optimise treatment outcomes (Voskuil, Bangma et al. 2019). The treat to target approach with treatment adjustments guided by the use of modern biomarkers, needs however further prospective evaluation and validation, compared to repeated endoscopic examinations to ensure mucosal healing (Colombel, Panaccione et al. 2018).

In conclusion, IBD patients cannot be treated with 'one size fits all' approach', because poor treatment response, loss of response, adverse drug reactions and therapeutic failure are observed, resulting in increasing morbidity and health care costs (Molodecky, Soon et al. 2012).

1.4.2. The role of CS in IBD

Current therapeutic options for induction of remission consist of exclusive enteral nutrition for CD (Connors, Basseri et al. 2017), anti-inflammatory medicines such as CS and immunosuppressive biological agents, and experimental methods such as faecal microbiota transplantation for both CD and UC (Narula, Kassam et al. 2017).

Individuals may fail to respond, lose response over time to these therapies or face adverse effects. The introduction of exclusive enteral nutrition (EEN), such as polymeric or elemental feeds for 6-8 weeks, results in remission rates of up to 80% in paediatric CD (Connors, Basseri et al. 2017). Furthermore, EEN may contribute to avoidance of treatment escalation (Sigall Boneh, Sarbagili-Shabat et al. 2017).

The first line pharmacologic agent implemented in the majority of patients for induction of clinical remission in CD, UC and unclassified IBD is CS (Ruemmele and Turner 2014). Inadequate efficacy of induction with other medicines, except 5-aminosalicylates (5ASA) for mild UC, has limited the choice of pharmacotherapy induction agents (8). CS can be administered either as oral prednisolone (from 6 up to 12 weeks), budesonide, or parenteral methylprednisolone, hydrocortisone (Munkholm, Langholz et al. 1994). The use of the terms 'responsive', 'dependent' and 'refractory to CS treatment', as described by Munkholm and Truelove are as follows (Truelove and Jewell 1974, Munkholm, Langholz et al. 1994).

Steroid responsive: clinical improvement after treatment with high-dose oral CS (40-60 mg prednisone or equivalent) within 30 days, or clinical improvement after treatment with high-dose intravenous CS within 7-10 days

Steroid refractory: patients who fail to respond to CS within this timeframe are CS refractory

Another term also used is ***steroid dependency***, however, this definition has varied with each study, leading to lack of uniformity when analyzing therapeutic outcomes (Farrell and Kelleher 2003, Bianchi Porro, Cassinotti et al. 2007). Nevertheless, the more commonly used definition of steroid dependency, developed by Munkholm and Truelove (Munkholm, Langholz et al. 1994) is about patients who initially respond to

CS but then relapse during weaning or shortly after drug discontinuation of CS, requiring CS re-introduction to maintain symptoms control. These definitions allow us to define subgroups of patients based on observed clinical criteria (Tung, Loftus et al. 2006). They have the advantage of incorporating time from first CS use, allowing for stratification of patients' steroid responsiveness at predefined time points following CS introduction (Ho, Chiam et al. 2006, Newby, Croft et al. 2008).

Medical evidence shows that initial induction of remission with CS is largely successful in UC and IBDU; however, subsequent relapses of disease are characterized by increasing steroid resistance or steroid dependence. Both of these outcomes, although by definition completely different, translate into similar consequences for patients, which is either a need for alternative pharmacotherapies to achieve induction of remission, or consideration of surgical management, with associated risks and comorbidities (Asl Baakhtari, McCombie et al. 2017). Up to one half of children with CD, and most if not all (about 90%) with UC, will need treatment with CS, with remission rates close to 85% and 80% respectively (Newby, Croft et al. 2008). However one year later, around 60% of CD patients and 40% of UC patients are steroid dependent or require surgery (Ho, Chiam et al. 2006). Continued CS treatment is therefore considered a predictor of relatively poor outcome after one year (Selinger, Parkes et al. 2017). A different cohort study examined outcomes associated with steroid use in 358 adult IBD patients (185 with ulcerative colitis and 173 with Crohn's disease). Among UC patients, 84% demonstrated complete or partial response by 30 days, and at one year after introduction of CS, 49% had prolonged response within one year from diagnosis, 22% were steroid dependent and 29% required surgery. Among CD patients, 84% demonstrated complete or partial response by 30 days, and at 1 year, 32% had prolonged response, 28% were

steroid dependent, and 38% required surgery (Faubion, Loftus et al. 2001). According to a retrospective review, adult lifetime risk of major intra-abdominal surgery in cases of failed CS treatment response may be up to 50% in patients with CD and 20% in patients with UC (Farrell and Kelleher 2003).

De Iudicibus *et al* recently published original research in potential pharmacogenomic biomarkers observing the micro-RNA profiles in children with IBD treated with CS. Next generation sequencing was performed in leucocytes from children at diagnosis and four weeks after the introduction of CS; 18 micro-RNAs were significantly differentially expressed within four weeks; their expression was validated by real time quantitative polymerase chain reaction (RT q PCR). Three of these micro-RNAs contained glucocorticoid responsive elements in their gene promoters and could putatively be directly regulated by the glucocorticoid receptor (GR); others could recognize 3' untranslated region of GR gene (De Iudicibus, Lucafo et al. 2018).

1.4.3. Modern treatment paradigms: therapeutic drug monitoring and step up therapy

Thiopurines such as azathioprine and 6-mercaptopurine can be administered as first or second line agents in maintenance treatment. They undergo extensive hepatic and intestinal metabolism by a number of enzymes including hypoxanthine phosphoryltransferase, thiopurine methyltransferase (TPMT), xanthine oxidase and inosine monophosphate hydrogenase. Various active metabolites such as 6-thioguanine nucleotides (6TGN) and 6-methylmercaptopurine ribonucleotides (6MMPR) mainly mediate the pharmacological effect of thiopurines (Lennard 1992). Patient response to treatment exhibits inter-individual variability thought to result from variation in drug metabolism (Liang, Geske et al. 2013, Konidari,

Anagnostopoulos et al. 2014, Lennard, Cartwright et al. 2015). TPMT genotype and activity can partially explain the variability in patient response. Hematological toxicity, commonly manifesting as leukopenia is associated with the homozygous recessive TMPT genotype and intermediate or low TPMT activity. Optimal 6TGN levels are a surrogate marker of response to thiopurine treatment, while low metabolite concentrations are indicative of lack of compliance (Relling, Gardner et al. 2011). Additional monitoring of 6MMPR levels can be useful for identification of liver toxicity (Konidari, Anagnostopoulos et al. 2014). TPMT genotyping is therefore undertaken routinely prior to thiopurine introduction in patients with IBD (Liu, Xu et al. 2015). NUDT15 mutations resulting in genetic variants of exons have been associated with thiopurine-induced leucopenia in Asian patients (Kojima, Hirotsu et al. 2018). Sutiman et al has also reported higher risk of thiopurine induced myelotoxicity in Asian patients with IBD (Sutiman, Chen et al. 2018).

Anti-TNF α agents are a class of biologic drugs (infliximab, adalimumab, golimumab, certolizumab pegol) commonly used as ‘step up’, second, or third line maintenance treatment in patients with loss of response or suboptimal response to 5ASA and thiopurines. Furthermore, they are used in patients who have experienced side effects, in cases of non-compliance, steroid dependent patients or others with severe disease or flare-ups requiring rescue treatment as well as in perianal CD (Papamichael and Cheifetz 2016, Icht, Yanai et al. 2017). In an adult European Crohn's disease registry, 58% of patients had received treatment with anti-TNF-α agent such as infliximab, and 42% had received non-biologic therapy. Over the 13-month follow-up period, treatment emergent serious infections were seen significantly more often in patients receiving steroids (P = 0.009), but were not significantly increased in patients receiving infliximab (P = 0.30) (D'Haens, Colombel

et al. 2008). There is emerging evidence about benefits of early infliximab and adalimumab administration ('top-down approach') with regards to disease remission, avoidance of later progression to stricturing/penetrating disease in CD, improved quality of life and reduction of hospital admission (Roda, Jharap et al. 2016, Kerur, Machan et al. 2018). Primary loss of response to anti-TNF a occurs in up to a third of patients, with up to half of the patients losing response over time (Scaldaferri, D'Ambrosio et al. 2017). A recognized mechanism for loss of response is immunogenicity due to the formation of antibodies. These antibodies hinder the binding of anti-TNFα to its receptor or promote the drug clearance through the reticuloendothelial system (Rojas, Taylor et al. 2005). Dose increases, shortening of intervals between infusions, and switch within or out of drug class are currently implemented practices, combined with re-evaluation of clinical symptomatology, endoscopic, histologic and radiologic indices (Perezgonzalez and Rojas 1976). There are a few studies exploring the significance of anti-TNFα therapeutic drug monitoring, for example the impact of drug trough levels and antibody titers on clinical outcomes (Tighe, Hall et al. 2017). There is evidence of HLA DQA1*05 haplotype associated with two fold increase of anti-TNF a immunogenicity risk (Sazonovs and Barrett 2018). There is no evidence-based consensus to date for change of clinical practice based solely on interpretation of anti-TNFα therapeutic drug monitoring, however it is used to guide ongoing therapy (Billiet, Cleynen et al. 2015, Dreesen, Van Stappen et al. 2017). The association of drug levels and antibodies with clinical outcomes requires further investigation, however recent systematic review and meta-analysis overall support the finding that regular therapeutic drug monitoring contributes to avoidance of higher disease relapse rate (Ricciuto, Dhaliwal et al. 2018).

Methotrexate can be used for maintenance of steroid free remission in IBD, mainly CD, especially in cases of non-response, lack of compliance or in patients experiencing side effects from use of thiopurines (Colman and Rubin 2015, Haisma, Lijftogt et al. 2015). More recent meta-analysis showed that use of methotrexate as monotherapy can achieve clinical remission in about half of the patients with CD within 3-6 months, however less than half of patients would remain in clinical remission at 12 months post methotrexate introduction (Colman, Lawton et al. 2018). Use of 'step up', combination therapy including immunomodulators (such as methotrexate or thiopurines) and anti-TNFα agent, improves trough anti-TNFα drug levels and suppresses anti-TNFα antibody formation, however long term efficacy and safety profile of combination therapy has not been fully explored (Dulai, Siegel et al. 2014).

Use of new biologic agents such as vedolizumab, a monoclonal antibody to α-4 β-7 integrin has shown preliminary encouraging data for escalation of treatment in complex patients with refractory or steroid dependent disease (Samaan, Pavlidis et al. 2017). The mechanism of action is by influencing disease effector T-cell homing and trafficking in the gut (Feagan, Rutgeerts et al. 2013, Sandborn, Feagan et al. 2013, Zundler, Becker et al. 2017).

1.4.4. Therapeutic challenges

Therapeutic approaches involve potent inhibition of inflammatory pathways and immune suppression. As medication are not universally effective (Colombel, Reinisch et al. 2015) and can be associated with significant toxicity, it is important to elucidate how the expression of drug efflux transporters and common genetic polymorphisms may shape response to treatment (Russell, Wilson et al. 2011).

Frequent use of CS in IBD and CS resistance/dependence offer an opportunity for translation of clinical pharmacology research into clinical practice as CS are ABCB1 substrates. The availability of robust clinical end-points to define response to CS treatment is necessary prior to undertaking larger prospective cohort studies or randomized controlled trials (Faubion, Loftus et al. 2001).

Clinical and basic science research studies aim to explore cellular and molecular mechanisms that can have significant impact on clinical management. Targeted approaches in drug development would empower clinicians to tailor pharmacotherapy according to individual genetic, molecular or clinical biomarker profiles. Loss of response or severe refractory disease remain the main challenges clinicians and patients face during the disease course. Disease complications or failing medical management increases the lifetime risk of necessary surgical intervention in the large majority of patients with IBD. Safety profile concerns such as organ toxicity, accumulated cancer risk and drug interactions influence clinician and patient choices. Future prospective cohort studies and randomized controlled trials are necessary to clarify disease and patient related factors that play a role in treatment response and disease progression. At the same time, basic science experiments at cellular and molecular level can promote translational research and facilitate application of research from bench to bedside.

1.5 Pharmacology of ABC transporters

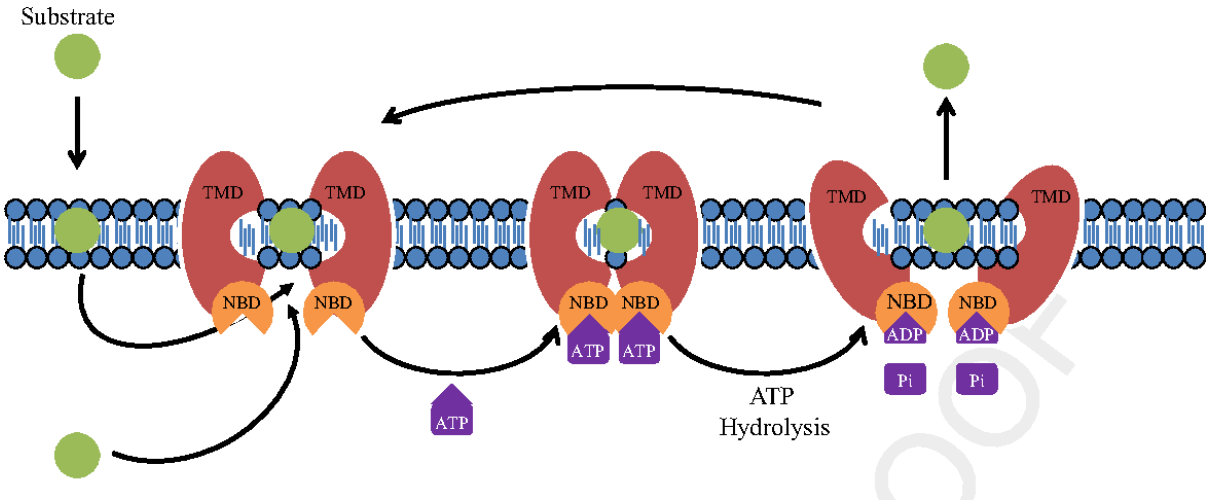
Members of adenosine triphosphate (ATP) binding cassette transporter subgroup, briefly called ABC transporters, comprise a superfamily of 48 members classified to seven subfamilies according to sequence homology (ABCA-ABCG). ABC

transporters are important efflux pumps, localized across intra- and extracellular membranes, and are categorized in seven distinct subfamilies. They use ATP as high energy molecule allowing them to transport substrates against the concentration gradient. Substrates include natural substances, various endogenous and exogenous molecules into and out of cells, such as amino-acids, peptides, metabolites, fatty acids, metals, phospholipids, vitamins, androgens, estrogens and xenobiotics for example environmental carcinogens and drugs (Abu-Qare, Elmasry et al. 2003, Leslie, Deeley et al. 2005), with the main objective of ensuring cells function optimally, and protecting cells from toxic substances. Function may differ in different cell types and tissues. They have a large impact on the pharmacokinetics of numerous drugs, including modulation of effectiveness of drug therapy. The ABC superfamily is well conserved through evolution with observed protein homology between bacteria and eukaryotic species; proteins are expressed in plasma membrane and intracellular organelles (Moitra and Dean 2011). The ATPase dependent efflux transporters exist in tissues involved in absorption, detoxification and elimination (Figure 1.3).

The ABC transporters are considered major determinants of drug disposition, safety and efficacy (Aye, Singh et al. 2009). Efflux transporters, through playing a role in trafficking of various organic and non-organic molecules, influence regulation of cell membrane function and protect tissues from deleterious substances. For example, aberration in the balance of the intestinal epithelium interface results in activation of the inflammatory cascade at cellular level (Andersen, Svenningsen et al. 2015). Inflammatory triggers such as cytokines can further affect protein function (Deuring, de Haar et al. 2012). Dietary factors, such as fat and fiber, and commensal bacterial by-products act as ABC substrates, or influence their expression by activating

nuclear transcription factors, thereby playing a key regulatory role in the intestinal inflammatory cascade as shown in Figure 2.1 (Jowett, Seal et al. 2004, Herfarth, Martin et al. 2014, Sarrabayrouse, Bossard et al. 2014). Impaired protein folding causes endoplasmic reticulum stress and alters transporter protein function in the intestinal epithelial cells, thereby lowering the protection of the epithelium against xenobiotics in inflamed tissue (Deuring, Peppelenbosch et al. 2011).

Figure 1.3. Function of ABC transporters. ABC transporters are energy dependent transporters and exhibit a conformational change upon substrate binding; ATP hydrolysis which drives the transport process of the substrate. Adapted from 'Mammalian drug efflux transporters of the ATP binding cassette (ABC) family in multidrug resistance: a review of the past decade. Chen et al. Cancer letters. January 1, 2016, 370 (1): 153-164 (reproduced with permission). TMD: transmembrane domain, NBD: nucleotide binding domain, ATP: adenosine triphosphate, ADP: adenosine diphosphate, Pi: phosphate



The widely studied transporter proteins are encoded by the multi-drug resistance gene *ABCB1* (also called multi-drug resistance 1, *MDR1*), the multi-drug resistance associated proteins 1 and 2 (*MRP1* or *ABCC1* and *MRP2* or *ABCC2*), and the breast cancer resistance protein (*BCRP* or *ABCG2*). They are located at the apical side of the endothelial and epithelial membranes in the intestine, liver, kidney, testis, blood brain barrier, placenta and peripheral blood mononuclear cells (PBMCs). These transporter proteins play a pivotal role in endogenous substrate trafficking as well as xenobiotic efflux. They transfer substrates against the concentration gradient by using energy (Taipalensuu, Tornblom et al. 2001), thereby play a key role in promoting drug resistance. There is an overlap of endogenous substrates between proteins such as ABCB1, ABCC1 and ABCG2, for example peptides, phospholipids,

vitamins, flavonoids, phytoestrogens and xenobiotics (Panwala, Jones et al. 1998, Johnson and Fleet 2013).

1.5.1. ABCB1 and relevance to IBD susceptibility and response to CS

Studies have investigated how genetic variation in different genes can be implicated in disease susceptibility. It has proven challenging to pinpoint causal or functional variants that play a pivotal role in disease pathogenesis or treatment (Doecke, Simms et al. 2013). *ABCB1* SNPs have been implicated in multidrug resistance in diseases such as epilepsy (Leschziner, Jorgensen et al. 2006), UC (Ho, Gaya et al. 2005) (Hirano, Onda et al. 2004) and colorectal cancer (Jin and Song 2017). *ABCB1* SNPs have been associated with IBD, but their role in disease susceptibility remains unclear. The association of the three common *ABCB1* polymorphisms in IBD patients has been investigated; these are C1236T (rs1128503), G2677T/A (rs2032582) and C3435T (rs1045642). Mijac et al (2018) showed that the T allele of above *ABCB1* SNPs contributed to UC susceptibility in an adult Serbian cohort; however no significant association was noted for the specific allele or genotypes in CD patients from the same cohort (Mijac, Vukovic-Petrovic et al. 2018).

When compared to combined 1236 CC/CT genotypes, the 1236 TT genotype in the ABCB1 gene was associated with an increased risk of UC (OR 3.7, CI: 1.3-10.7, p=0.03). A recent meta-analysis has shown increased risk for developing UC, conferred by the ABCB1 SNP rs1045642 C>T (Zhao, Wang et al. 2015). The G allele of ABCB1 rs2032582 was less frequent among CD patients than in controls (OR 0.668, 95% CI 0.484-0.921, P=0.014). G allele carriers were also less likely to develop non-stricturing and non-penetrating disease (OR 0.661, 95% CI 0.462-0.946, P=0.023) and ileocolonic disease (OR 0.669, 95% CI 0.472-0.948,

P=0.024) (Liu, Zhou et al. 2015). ABCB1 polymorphisms could therefore play a role in susceptibility to IBD (Senhaji, Kassogue et al. 2015).

ABCB1 plays a pivotal role in intestinal inflammation and colorectal cancer (Panwala, Jones et al. 1998, Davenport, Poles et al. 2014). Annese et al and Andersen et al have described lower ABCB1 mRNA expression in inflamed colon when compared with healthy colon (Annese, Valvano et al. 2006, Andersen, Svenningsen et al. 2015). Efflux transporters have been described to be reduced in inflamed intestine of patients with UC when compared to non UC patients (Ho, Soranzo et al. 2006). Disease activity can possibly modify expression of ABCB1 in the intestinal mucosa, with significantly lower expression in more severe UC (Ufer, Hasler et al. 2009). Chiba et al, on the other hand, investigated cis-regulated allele specific methylation in IBD susceptibility genes and the association between rs36221701 genotype and small mothers against decapentaplegic homolog 3 (*SMAD3) gene* expression. This study provided supporting evidence of epigenetic changes, such as allele-specific DNA methylation, which can mediate genetic effects on disease susceptibility (Chiba, Kakuta et al. 2018). SMAD3 specific inhibitors may reverse ABCB1 mediated multidrug resistance in cancer cell lines (Wu, Murakami et al. 2018).

Substrate overlap and cellular co-localization suggest a concerted function of the ABC transporters that forms a significant barrier for the intestinal absorption of xenobiotics. ABCB1 protein is localized in increasing frequency from the proximal to the distal part of the intestine and is expressed in the liver, kidneys, Sertoli cells, blood brain barrier and PBMCs. ABCB1 has been shown in animal and clinical studies to play a role in drug disposition (Misaka, Muller et al. 2013).

CS include glucocorticoids and mineralocorticoids; they are steroid hormones released by the adrenal glands in a circadian manner, regulated by hypothalamic-pituitary-adrenal (HPA) axis. They regulate diverse cellular functions with profound immunomodulatory actions and exert their action upon binding to the glucocorticoid receptor (GR). GR binding sites vary between tissues and various protein isoform expression, can be modulated by inflammatory processes (Kumar and Thompson 2005). GR protein is the product of nuclear receptor superfamily 3 group C member 1 (*NR3C1)* translation; polymorphisms can alter the amino-acid sequence and lead to impairment of GR function as a transcriptional activator or repressor, resulting in altered GC sensitivity. Non-genomic action of CS is mediated via activation of various kinases (Ramamoorthy and Cidlowski 2016).

Endogenously produced glucocorticoids are also key players in regulation of intestinal inflammation (de Souza, Sales-Campos et al. 2016). Interferon γ, interleukin 6 and other cytokines are upregulated in adrenalectomised mice with colitis, thereby perpetuating the inflammation, normally counteracted by endogenous adrenal CS (de Souza, Sales-Campos et al. 2016). Extra-adrenal production of CS originating from the intestinal epithelial cell layer exert an immune-regulatory role in intestinal homeostasis; this has been described in animal and human studies where direct correlation between cortisol production and peroxisome-proliferator-activated receptor γ (PPARγ) levels was noted, signifying impaired cortisol production by the colonic epithelium of patients with UC (Bouguen, Dubuquoy et al. 2015, Bouguen, Langlois et al. 2015).

Systemically active glucocorticoids such as dexamethasone, prednisone (and its active drug prednisolone), methylprednisolone, budesonide, beclomethasone

propionate are ABCB1 substrates. Transport studies performed in Caco-2 monolayers showed variable drug efflux with the use of high performance liquid chromatography (HPLC) protocols (Crowe and Tan 2012). Patients who receive CS demonstrate variable rates of treatment response and adverse drug reactions, which to some extent depend on proteins involved in the disposition of CS such as ABCB1 (Steinhart, Ewe et al. 2003, Gabryel, Skrzypczak-Zielinska et al. 2016). For instance, T helper 17 ABCB1 expressing cells are shown to be refractory to CS use in IBD (Ramesh, Kozhaya et al. 2014). CS dependent ABCB1 inhibition has been reported in overexpressing mammalian cell lines (Pedersen, Khan et al. 2017). Budesonide pharmacokinetics may be influenced by ABCB1 and CYP3A5 expression in sigmoid biopsies (Ufer, Dilger et al. 2008). ABCB1 SNP rs2032583 may confer protection from CS dependency in children with CD (Krupoves, Mack et al. 2011). Prednisolone and dexamethasone can induce ABCB1 via interaction with lymphocytes (Manceau, Giraud et al. 2012). Mechanisms of multi-drug resistance include activation of detoxifying pathways such as cytochrome P 450 (CYP 450), increased expression of transporters and disruption in apoptotic signaling pathways that do not allow the destruction of cancer cells (Moitra 2015). ABC expression in stem cells of various tissues, in healthy state but mainly in cancer, can be regulated through targeting signaling pathways or by using transporter modulating agents such as inhibitors (Moitra 2015). The manipulation of their expression may be useful in cancer treatment and other diseases, where transporter overexpression plays a role. Low levels of efflux transporters expression may contribute to altered transport of bacterial toxins, drugs, carcinogens (Englund, Jacobson et al. 2007).

Inter-individual and intra-individual variability in transporter protein expression influences drug pharmacokinetics. Inhibition of these intestinal efflux pumps lead to

increased plasma levels whereas induction can reduce absorption and cause decreased plasma concentrations (Misaka, Muller et al. 2013). There is ongoing controversy as to how genetic polymorphisms influence susceptibility to various diseases and response to treatment (Yue, Xiong et al. 2015). Genetic polymorphisms in the genes encoding these transporters may have a further effect on bioavailability and drug-drug interactions, as for example happens with calcineurin inhibitors used for immunosuppression following solid organ transplantation, and in resistant cases of IBD (Lasa and Olivera 2017). Tacrolimus response to treatment has been associated with the presence of ABCB1 variants. Drugs such as tacrolimus require close therapeutic drug monitoring and their levels are unpredictable and not fully explained from current scientific knowledge (Knops, Levtchenko et al. 2013, Ramesh, Kozhaya et al. 2014).

Research in IBD suggests that the phenomenon of CS resistance occurs via T lymphocytes. Three described key molecular mechanisms are: a) decreased cytoplasmic concentration due to increased ABCB1 efflux of drug, b) impaired signaling due to dysfunction at receptor level (Garcia-Carrasco, Mendoza-Pinto et al. 2017) and c) activation of pro-inflammatory mediators resulting in inhibition of glucocorticoid receptor transcriptional activity (Vandevyver, Dejager et al. 2013). CS such as hydrocortisone, prednisolone, methylprednisolone, budesonide are ABCB1 substrates. Orally administered CS absorbed by enterocytes are transported in the gut lumen by ABCB1 (Dilger, Schwab et al. 2004).

A recent retrospective cohort study conducted in two tertiary paediatric gastroenterology units in Canada showed that *ABCB1* gene was associated with CS dependence in CD. Thirteen tagging single nucleotide polymorphisms and a synonymous variation (C3435T) in ABCB1 gene were genotyped. The tag-SNP

rs2032583 was statistically significantly associated with CS dependency. The rare C allele (odds ratio OR+0.56, 95% confidence interval CI: 0.34-0.95, p=0.029) and TC heterozygous phenotype (OR+0.52, 95% CI 0.28-0.95, p=0.035) were noted more in children from Canada with symptoms who did not relapse following tapering or at the end of CS treatment (Krupoves, Mack et al. 2011). In CD, CS response can therefore vary depending on *ABCB1* gene polymorphisms. Yang et al have reported that the CC genotypes of rs1128503 (1236T>C) and rs1045642 (3435T>C) in the *ABCB1* gene were more frequent in Chinese patients with CD who had demonstrated CS dependency (OR 6,583, 95% CI1.76-24.62, P=0.019 and OR 3.873, 95% CI 1.57-9.506, p=0.009, respectively) (Yang, Chen et al. 2015). ABCB1 expression is upregulated in PBMCs from patients with IBD, treated with CS (Farrell, Murphy et al. 2000, Hirano, Onda et al. 2004). SNPs including 1236C>T, 2677G>T, A and 3435C>T polymorphisms are associated with significantly higher remission rates in subjects with CS refractory UC (Herrlinger, Koc et al. 2011).

Table 1.1 ABCB1 allele frequencies significantly associated with treatment response to CS in Canadian children with CD and Chinese adults with UC

	CD	UC
ABCB1 SNP	↑CS dependency	↑CS responsiveness
rs2032583	C allele	G allele
rs1128503	C allele	C allele
rs1045642	C allele	C allele

SNPs in general seem to play a role in CS resistance, for example in various other diseases such as idiopathic nephrotic syndrome (Han, Xu et al. 2017), glucocorticoid

induced avascular necrosis of the femur (Li, Zhao et al. 2014) and hepatocellular carcinoma (Wang, Liu et al. 2015). As with many inflammatory conditions (Farrell and Kelleher 2003, Chikanza and Kozaci 2004, Barnes and Adcock 2009), failure to respond (CS resistance) and wean CS (CS dependence) are significant clinical problems (Barnes and Adcock 2009). Despite the identification of several molecular mechanisms of glucocorticoid resistance, including activation of mitogen-activated protein kinase (MAPK) pathways by certain cytokines, excessive activation of the transcription factor activator protein 1 (AP1), reduced histone deacetylase-2 (HDAC2) expression, raised macrophage migration inhibitory factor, and increased ABCB1-mediated drug efflux, inherent factors that influence glucocorticoid response remain poorly defined and ill-understood.

SNP determined differential expression, as well as mutations and aneuploidy, are few of the recognized mechanisms of differential mRNA tissue levels of transporters with subsequent inter-individual and intra-individual variability in observed patient multi- drug resistance. *ABCB1* SNPs have been implicated in multidrug resistance in diseases such as epilepsy (Leschziner, Jorgensen et al. 2006), UC (Ho, Gaya et al. 2005) (Hirano, Onda et al. 2004) and colorectal cancer (Jin and Song 2017). Low ABCB1 mRNA expression occurs early in colorectal carcinogenesis (Andersen, Vogel et al. 2013). Low levels of ABCG2 have been reported in affected intestines from patients with mild to moderate colorectal dysplasia and high levels of ABCC2 (Andersen, Svenningsen et al. 2015). Resistance mediated by ABCB1 may therefore play a pivotal role in chemotherapy failure (Wu, Yang et al. 2014). Expression of ABCB1 mRNA was higher in mutated tissue samples, i.e. in patients with gastric cancer where higher frequencies of A/G and G/G- for ABCB1 rs28381943- and G/G genotype -for ABCB1 rs2032586- were observed, in

comparison with the genome of healthy controls. For C3435T, genotype differences were not significant. Observed genotype differences may be tumor specific (Mansoori, Golalipour et al. 2015). Mrowicka et al (2017) noted the dysregulated antioxidant capacity in patients suffering from IBD in a Polish population; the *SOD1* A/C, *GSHPX1 C/T* and *T/T* genotypes could be associated with a reduced risk of IBD in this population (Mrowicka, Mrowicki et al. 2017). Mutant SOD1 astrocytes can drive ABCB1 upregulation in blood brain barrier endothelial cells via NFkB nuclear translocation (Qosa, Lichter et al. 2016). Arisawa and colleagues (2017) concluded that genetic polymorphisms in the *MAPK* gene were significantly associated with susceptibility to UC development. In particular, rs4268033 was closely associated with an increased risk for the development of UC (Arisawa, Nakamura et al. 2017). Inhibition of MAPK pathway has been found to partly depress ABCB1 activity in leukemic and solid tumour cells; when treated with hypomethylation agents, drug sensitivity is restored (Wang, Wang et al. 2017). Activation of MAPK pathway upregulated ABCB1 expression in leukemic cell lines in a different study by Tomiyasu et al (Tomiyasu, Watanabe et al. 2013).

1.5.2. ABCB5 structure and relevance to ABCB1

ABCB5 is a close homologue of ABCB1 and is the third and the most recently identified member of the human MDR family, shown to bring resistance in human cancer cells. The ABCB5 gene is predicted to be highly polymorphic and can produce at least 6 ABCB5 mRNA transcripts (Figure 1.5). Phylogenetic and functional motif analyses have shown that ABCB1 and ABCB5 are closely related (Tusnady, Sarkadi et al. 2006, Fukuda, Aguilar-Bryan et al. 2011). In-silico analysis revealed that large number of non-synonymous coding SNPs map to important

functional regions of the protein and therefore, if present in human populations, may play a role in associated diseases. (Moitra and Dean 2011). Four isoforms; ABCB5 alpha (ABCB5α), beta (ABCB5β), ABCB5.ts (testis specific or full length) and ABCB5 epsilon (ABCB5e) have been described (Frank and Frank 2009). The two most frequently isolated ABCB5 mRNA transcripts from melanoma complementary DNA (cDNA) libraries are ABCB5α and ABCB5β. ABCB5α and ABCB5 epsilon (ε) encode proteins of 131 and 134 amino acids respectively and have no transporter role (Table 1.1) (Chen, Szakacs et al. 2005). The ABCB5β protein is 812 amino acids long (89,831 Dalton) and has a very unusual structure, in that it lacks the first transmembrane domain (TMD1), and is missing a large section of the first nucleotide-binding domain (NBD1). ABCB5β would therefore have an unusual structure for an ABC half-transporter or full transporter (Chen, Szakacs et al. 2005). Frank et al predicted that ABCB5 has five transmembrane helices flanked by intracellular and extracellular domains (Frank, Kho et al. 2006). Tusnady et al predicted that there are six transmembrane helices (Tusnady, Sarkadi et al. 2006). The sequence homology between ABCB5 and ABCB1 would suggest a similar number and arrangement of transmembrane helices. Only one transcript, ABCB5.ts (testis specific) encodes a full length transporter containing 12 transmembrane helices and two nucleotide-binding domains, is expressed in the prostate and testis and represents the longest transcript named ABCB5 FL (Frank, Kho et al. 2006, Moitra, Scally et al. 2011). Splice variants of ABCB5 are expressed in other organs such as liver, spleen, pancreas, ovary and small intestine as detected by polymerase chain reaction (Kawanobe, Kogure et al. 2012).

Figure 1.4. ABCB5 predictive topology; a) ABCB5 full transporter with 12 transmembrane helices and 2 nucleotide binding domains b) ABCB5 beta with six transmembrane helices and two nucleotide-binding domains. Taken from Moitra et al. Evolution of ABC transporters by gene duplication and their role in human disease. Biol Chem, 2011, 392, 29-37 (reproduced with permission)

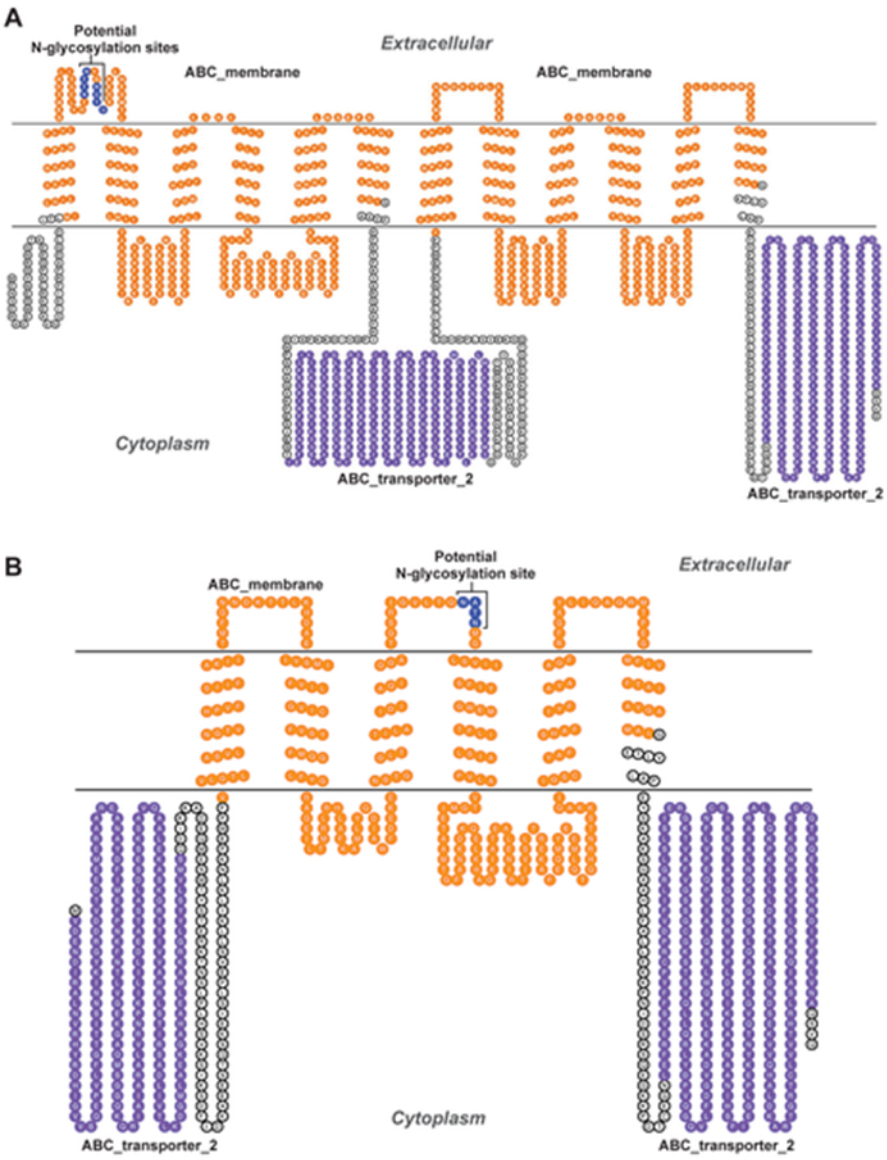


Table 1.2. ABCB5 Ensembl; ABCB5 ENSG00000004846 Description; ATP-binding cassette, sub-family B member 5 [Source: HGNC Symbol; Account: 46]. Location Chromosome 7: 20,687,045-20,795,247 forward strand. This gene has six transcripts.

Name	Transcript ID	Length (base pairs)	Length (amino-acids)	Biotype	Expression
ABCB5-001 -ABCB5 β -ABCB5"half transporter"	NM_178559 19 exons total Alternative transcription start site at exon 10 of full length 108Kb	2906	812 (446-1257 amino-acids of full length)	Protein coding	melanoma cells and melanocytes (Chen et al 2005)
ABCB5-002	NM_001163993 20Kb	1483	126 (446-569 amino-acids of full length plus two additional amino-acids)	Protein coding	
ABCB5-003 ABCB5 α	NM_001163942	2247	131 (446-569 amino-acids of full length plus 7 additional amino-acids)	Protein coding	melanoma cells and melanocytes (Chen et al 2005)
ABCB5-004 -ABCB5 full length -ABCB5.ts	NM_001163941 28 exons 141Kb	5811	1257	Protein coding	"testis specific"
ABCB5-007		2204	314	Nonsense mediated decay	
ABCB5-006		673	No protein	Processed transcript	

1.5.3. Role of ABCB5

ABCB5 FL is a putative transporter and shares approximately 70% similarity with ABCB1 (Moitra and Dean 2011). ABCB5 FL has been hypothesized to have a transport function with potential clinical implications in multidrug resistance; however very little is known about ABCB5 substrates and physiological role (Moitra, Scally et al. 2011). ABCB5 FL mRNA is physiologically expressed in prostate, testis (Gen Bank Accession Number AY353947) (Frank and Frank 2009), in retinal epithelial cells (Chen, Szakacs et al. 2005), as well as in human melanoma cells, where it promotes drug resistance by acting as a doxorubicin efflux pump (Frank, Kho et al. 2006). ABCB5 represents the only known cellular molecular marker regulating the membrane potential in normal melanocytes expressing the stem cell marker CD133, thereby influencing progenitor cell fusion, growth and differentiation of human epidermal melanocytes (Frank, Pendse et al. 2003). This process is regulated via alteration of membrane potential.

Chen et al have also reported that ABCB5 FL may encode a full-length functional transporter, expressed in human melanoma lines (Chen, Szakacs et al. 2005). ABCB5 FL as a very close homologue of the multidrug transporter ABCB1 (approximately 70% similarity), was initially named ABCB5β, first described by Chen et al to have pigment-cell specific expression (Chen, Szakacs et al. 2005). High-throughput sequencing performed on both mRNA and total RNA, depleted of ribosomal RNA, supported this finding, but no protein isoform was detectable. Characterizing as well as understanding ABCB5 role and regulation could potentially reveal novel targets for treatment of intractable melanoma and possibly other diseases, where ABCB5 plays a role.

ABCB5 protein expression was shown using immunohistochemistry in paraffin embedded tissue in the placental cytotrophoblast layer; perinuclear and membranous/cytoplasmic staining was observed in villous trophoblasts in first trimester placentas, with progressive loss of expression in term placentas; lack of m RNA expression with in situ hybridization was observed in the multinucleated syncytio-trophoblast (Volpicelli, Lezcano et al. 2014).

To determine the physiological role of ABCB5, Gillet et al have subsequently created animal model (personal correspondence). ABCB5 knock out mice developed by Gillet et al did not express any of the ABCB5 splice variants. The mice were viable and fertile, but grew at a slower rate in comparison to the wild-type mice. Phenotyping revealed altered bioenergetics (decreased lactate and increased oxygen consumption). Decreased liver weight and increased heart weight were observed in the knockout males. These results suggest a possible role of ABCB5 in intermediary metabolism and were presented at the 8TH Annual North American ABC Genetic Workshop, in Maryland Bethesda, USA, in September 2011 by Professor JP Gillet in abstract form, titled 'Functional studies on ABCB5: a pleiotropic phenotype in knock-out mice'.

1.5.4. ABCB5 and multidrug resistance

Melanoma cells expressing ABCB5 show increased survival when exposed to cytotoxic drugs (Chartrain, Riond et al. 2012). Frank et al have shown that ABCB5β, with 73% homology and 54% amino-acid identity to ABCB1, acts as rhodamine-123 and doxorubicin efflux transporter and is selective to human malignant tumors, including lung cancer and melanoma (Frank, Pendse et al. 2003, Frank, Kho et al. 2006). In silico analysis has shown that a large number of coding (non-synonymous)

SNPs map to important functional regions of ABCB5 protein and this could play a role in associated diseases (Moitra, Scally et al. 2011). For instance *ABCB5* SNP rs2301641 (c.343A>G) has recently been associated with a lack of clinical response to thiopurine treatment (Smith 2010). ABCB5 genetic variation in expression is reportedly involved in altering the brain concentration of haloperidol, thereby influencing induced central nervous system toxicity as part of the blood brain barrier, when tested in knock out mice (Zheng, Zhang et al. 2015); the same research group showed that ABCCB5 is expressed in brain capillaries. A GWAS in a haloperidol treated human cohort suggested that ABCB5 variants showed interindividual differences in susceptibility to haloperidol brain toxicity, by altering the brain concentration of haloperidol as part of the blood brain barrier.

Transfected ABCB5 human epithelial kidney cells 293 (HEK 293) clones generated by Kawanobe et al showed higher resistance to doxorubicin, paclitaxel and doxetacel compared to parental HEK 293. The observed difference in efflux of chemotherapeutic agents was attributed to higher ABCB5 expression in ABCB5 transfected cells, however no direct drug uptake studies were performed; instead, cell growth inhibition was implemented to gauge multidrug resistance. The transfected ABCB5 clones treated with small interfering RNA (siRNA) exhibited lower resistance to docetaxel. ABCB5 FL was hence reported to confer resistance to taxanes and anthracyclines (Kawanobe, Kogure et al. 2012). Huang et al assessed drug transporter relationship at genomic level and showed how three efflux transporters (ABCB1, ABCC3, and ABCB5) showed significant negative correlations with multiple drugs, suggestive of a mechanism of drug resistance. ABCB1 gene expression correlated negatively with potencies of 19 known ABCB1 substrates. Use of siRNA reduced ABCB1 mRNA levels concomitantly increased sensitivity to

ABCB1 substrates. Similarly, siRNA mediated silencing of ABCB5 in human melanoma SK-MEL 28 cell line increased sensitivity to several drugs, including camptothecin and 5 fluorouracil (5-FU) in melanoma cells (Huang, Anderle et al. 2004).

ABCB5 β and ABCB5 *FL* cDNA were cloned and expressed at the pleiotropic drug resistance 5 (PDR5) genomic locus of the *Saccharomyces cerevisiae* model, downstream of a promoter under the control of a mutant transcription regulator; deletion of seven endogenous ABC transporters was performed in the same clones. The β isoform did not confer resistance to known ABCB1 substrates, such as rhodamine 123, daunorubicin and FK506, in the ABCB5 transfected yeast model, and was presumed to be truncation of a canonical full-size ABC transporter protein. No functional dimerization of ABCB5β was noted in this experiment. The ABCB5 FL model showed limited resistance to rhodamine 123. The resistance effect conferred by expression of the protein efflux pump was determined by simple yeast growth inhibition experiments. The ABCB5 FL protein detection was lower when compared to ABCB1. Immunoblot for detection of protein expression measured in relation to that of the parental yeast strain was performed in total protein cell extracts with the use of a polyclonal, commercially available antibody recognizing both FL and β isoforms. An additional, synthetic ABCB5 FL cDNA was codon harmonized for expression in yeast, cloned and tested in order to avoid codon bias and ensure effective heterologous expression of human proteins in yeast. This clone conferred resistance to rhodamine 123, daunorubicin and FK506. ABCB1 antibody used in the same experiments also reacted with homologous sections of ABCB5 protein isoforms.

ABCB5 FL conferred resistance to substrates of human ABC transporters but showed lower than ABCB1 expression; this may have occurred because of antibody cross-reactivity with other transporters such as ABCB1, due to the similarity of epitopes. Keniya et al recommended that further optimization of expression models and use of more specific, less cross-reactive antibodies is needed (Keniya, Holmes et al. 2014).

1.5.5. ABCB5 and malignancy

Oncogenic function of ABCB5 in breast cancer is through enhanced tumor cell migration and invasion; as reported by Yao et al, the effect is zinc finger e-box binding homeobox 1 (ZEB1) mediated, acting as a downstream factor for ABCB5. Knocking down ZEB1 had a similar effect on tumorigenicity, while ABCB5 silencing in vitro decreased ZEB1 promoter activity (Yao, Yao et al. 2017).

High expression of the NH2 terminal truncated isoform of tumor suppressor gene p73 called ΔNp73 (Ishimoto, Kawahara et al. 2002) has been correlated with increased ABCB5 expression in breast cancer and melanoma cell lines. Its reduction in expression leads to increased intracellular concentration of doxorubicin, whereas its knock down suppresses ABCB1 and ABCB5 expression, and results in concomitant decrease in tumor cell proliferation. The effect was further confirmed in melanoma cell lines (Sakil, Stantic et al. 2017).

Govindan et al discovered three lung cancer samples with non-synonymous point mutations (LUC11:G347R, LUC12: M521L, LUC9: P580S and A687S) in the ABCB5 gene (Govindan, Ding et al. 2012), suggesting a possible role in resistance.

ABCB5 causes 5-FU resistance in colorectal cancer cells (Barretina, Stransky et al. 2011). ABCB5- mediated chemo resistance to 5-FU in primary colon cancer was influenced by the c-MYC gene. MYC oncogene deregulates cell growth and death, and its presence in cancer cells is associated with poor prognosis and rapid clone proliferation (Oster, Ho et al. 2002). Using a human colon cancer xenograft murine model, Kugimyia et al, demonstrated that c-MYC promotes ABCB5 expression; the use of a chromatin immunoprecipitation assay showed how c-MYC could bind to ABCB5 promoter region (Kugimiya, Nishimoto et al. 2015).

Wilson et al have recently investigated the intrinsic molecular function of ABCB5 involvement in melanoma tumorigenicity; the ABCB5 effect occurs through functional regulation of the IL8 receptor chemokine receptor type 1 (CXCR1). A broadly important mechanism potentially applicable to variable human malignancies such as liver and breast cancer that share the IL8 signaling pathway in their cancer stem cells has been elucidated (Ginestier, Liu et al. 2010, Tang, Ma et al. 2012). Following first line chemotherapy with carboplatin and etoposide in patients with Merkel cell carcinoma, resistance to treatment increased in the presence of ABCB5 mRNA in the tumor cells (Kleffel, Lee et al. 2016).

High ABCB5 expression is associated with tumor progression and recurrence in oral squamous cell carcinoma patients, as demonstrated by immunohistochemistry techniques and RT q PCR in cancer cell lines and human samples (Grimm, Krimmel et al. 2012). ABCB5 gene expression has also been higher in a small case series of patients with hematological malignancies who were treatment refractory (Yang, Li et al. 2012).

1.5.3. ABCB5 and potential relevance to IBD

ABCB5 is therefore a novel drug transporter with a putative key role in health and disease, as far as response or resistance to drug treatment are concerned. The research hypothesis pursued in this thesis derives from the similarity of ABCB5 with ABCB1, while anecdotal data suggests that ABCB5 expression variability may influence observed clinical response to CS in adult IBD patients (Gwo Tzer Ho et al, Clinician Scientist at the Edinburgh Medical Research Council Centre for Inflammation research, personal communication). The hypothesis of CS being putative ABCB5 substrates, as a biologically plausible mechanism, has been formed.

1.6 Aims of thesis

This research focuses on paediatric IBD, a life-long incurable disease with severe impact on the lives of children and families, requiring multiple clinic visits, hospital admissions, surgery and cancer screening. The disease mechanisms and the individual differences in response to drugs warrant further study, which will inform the application of new technologies to unpick how patients respond to drugs and to allow for ‘personalized treatment’.

Hypotheses tested in this thesis include that ABCB5 is a CS transporter and that ABCB5 genotype and expression differ between healthy children and children with IBD at diagnosis; ABCB5 may be impacting on observed CS treatment response in a similar fashion to ABCB1, by ‘effluxing’ the drug out into the intestinal lumen.

For in vitro (bench) testing, direct transfection of eukaryotic cells with clones of synthetic ABCB5 cDNA sequence will be undertaken, followed by drug transport

assays, to clarify whether CS are ABCB5 substrates. Transporters can move various drugs out of the cells and cause drug resistance. It is therefore important to check in vitro if commonly used IBD drugs, such as CS, are substrates of this transporter. The hypothesis that CS are ABCB5 substrates stems from the observed homology with ABCB1. Expression of ABCB5 and ABCB1 in blood, small intestinal and colonic mucosa will be investigated by implementation of molecular and immuno-histochemical (IHC) techniques. Genotyping of included study participants with emphasis on six ABCB1/ABCB5 genetic polymorphisms, reported to be associated with conferring resistance to CS, will be undertaken. Translation of this research into something clinically meaningful for the patients would be possible, if genotype variation and/or interindividual transporter expression indeed influenced treatment outcomes.

This thesis also combines ‘bench’ work, to ‘bedside’, through the undertaking of an original clinical pilot study in a tertiary children’s hospital, involving the study of ABCB1 and ABCB5 transporter expression in recruited subjects and the exploration of how their expression relates to genotype in health and disease. The clinical part involves recruitment of an inception cohort of children newly diagnosed with IBD and healthy controls in a tertiary children’s hospital, following ethical and local approval processes, and according to research ethics and Good Clinical Practice (GCP) Standards.

The rationale of this thesis adheres to the principles of translational research. Cellular studies and molecular techniques can improve our insight in drug efficacy and the clinical management of complex disease, by exploring the function and inter-individual expression of drug efflux transporters at cellular and tissue level in health

and disease. Observed clinical outcomes regarding treatment response and drug efficacy could be the product of complex interaction between genotype, gene or protein expression of drug transporters in a given individual.

In conclusion, the aims of this thesis are the investigation of function and expression of the new drug transporter ABCB5 in human cells, as well as the exploration of ABCB1 and ABCB5 genotype and expression in healthy subjects and treatment naïve children with newly diagnosed IBD.

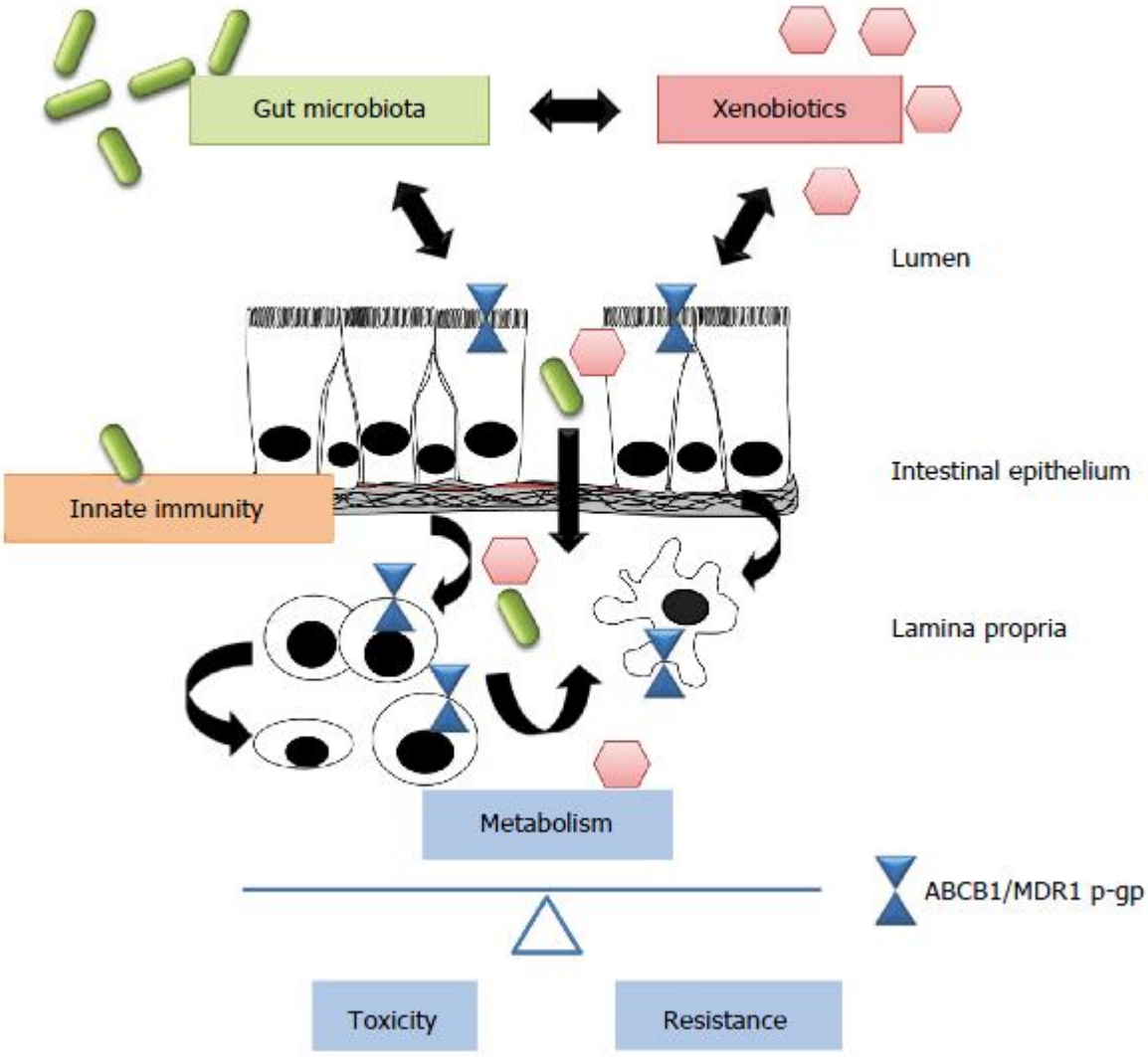
Chapter 2

The expression and function of the novel ABC efflux transporter ABCB5

2.1 Introduction

There are conflicting clinical studies about the role of SNPs in influencing oral bioavailability of ABCB1 substrates; drugs that induce or inhibit intestinal ABCB1 expression play a role in drug interactions, thereby affecting drug efficacy and safety. Evidence from clinical studies has shown low expression of ABCB1 and ABCG2 mRNA in the colon and rectum in active UC compared with healthy individuals (Englund, Jacobson et al. 2007). Langmann et al have also observed lower levels of expression of ABCB1 and ABCC2 in the inflamed colon (Langmann, Moehle et al. 2004). Deuring et al reported reduced levels of ABCG2 transporter expression in inflamed versus healthy colon (Deuring, de Haar et al. 2012). Further evaluation of the roles of ABC transporters in disease susceptibility and CS treatment response is required (Farrell, Murphy et al. 2000, Huebner, Browning et al. 2009). Cellular in vitro work focusing on the function of ABC transporters in relation to their substrates could inform animal and human studies, allowing for investigation of their role in vivo (Figure 2.1).

Figure 2.1. Xenobiotics, microbiota and host immunity interact in a multi-dimensional network in the gut. Taken from Cario et al with permission. Role of ABCB1/PGP in drug resistance or toxicity depending on expression at the intestinal epithelium in inflammatory bowel disease: more questions than answers. World Journal Gastroenterol. March 7, 2017 23 (9): 1513-1520.



Various types of systemically active CS are implemented in the treatment of IBD, including dexamethasone, prednisolone, hydrocortisone, methylprednisolone, beclomethasone propionate and budesonide (Friend 1998, Pan, Ju et al. 2001, Ruemmele and Turner 2014, Ruemmele, Veres et al. 2014, Triadafilopoulos 2014, Abdalla and Herfarth 2016, Rizzello, Mazza et al. 2018). These inhibit the expression and action of multiple inflammatory genes encoding cytokines. At the same time they increase the transcription of genes encoding anti-inflammatory proteins (Beato and Klug 2000). They all exert their action via the glucocorticoid receptor (GR); ligand binding induces conformational change and migration of the GR from cytoplasm to nucleus, where it interacts with GR responsive elements of gene promoters. The activated form inactivates key inflammatory transcription factors such as apoprotein-1 (AP-1) and NFkβ (Adcock, Nasuhara et al. 1999). The latter activates genes encoding for cytokines, cytokine receptors and adhesion molecules such as TNF a. CS mainly exert their effect through repression of NFkβ, by stabilizing the cytosolic inhibitor Ikba, against activation-induced degradation. For example, oral dexamethasone and prednisolone reduce the detectable levels of NFkβ in nuclear extracts from sigmoid biopsies of patients with CD (Schreiber, Nikolaus et al. 1998). The negative modulation of anti-inflammatory cytokines is the primary action of CS. Sequestration of NFkβ in the cytoplasm is a different possible mechanism of GC action (Auphan, DiDonato et al. 1995).

GC effects may be exerted through intestinal microbiota and their roles in regulating mucin expression, visceral sensitivity, intestinal barrier function and gut motility (Lutgendorff, Akkermans et al. 2008). Gut microbiome such as *Bifidobacteria* may inhibit lipopolysaccharide induced NFkβ activation, thereby exerting an anti-inflammatory effect (Riedel, Foata et al. 2006). GCs as part of their anti-

inflammatory action decrease mucin synthesis, with the gut microbes being an important mediator in this process. CS related changes in gut microbiome have been important in targeted pharmacological approaches to restore intestinal homeostasis in animal studies of Muc2 knockout, germ free mice (Huang, Inoue et al. 2015). Other CS such as dexamethasone increase PD1 expression via the GC receptor; PD-1 is a cell surface receptor that triggers cytokine inhibitory pathways, therefore negatively regulates T cell activation. The mechanisms of patient response to CS remain however unclear (Barnes and Adcock 2009). The effect of CS on intestinal inflammation itself may be influenced by the function of efflux ABC transporters, but this phenomenon needs further exploration in vivo and in vitro.

The expression and function of ABCB5 is not well known. The high degree of homogeneity with ABCB1 (Moitra, Scally et al. 2011) and the anecdotal pilot data from clinical adult studies about the potential impact on clinical outcome after CS administration in IBD, have shaped the rationale behind this original work. The aim of this chapter was to investigate the localization of ABCB5 and to investigate whether CS (dexamethasone, prednisolone, methylprednisolone, budesonide) are putative ABCB5 substrates. Transient, mock and stable transfected ABCB5 FL and β isoform overexpressing cell lines were produced. An additional ABCB5 stable overexpressing cell line for further testing was donated as a gift from Professor Jean-Pierre Gillett, Department of Biomedical Sciences University of Namur, Belgium.

2.2 Materials and Methods

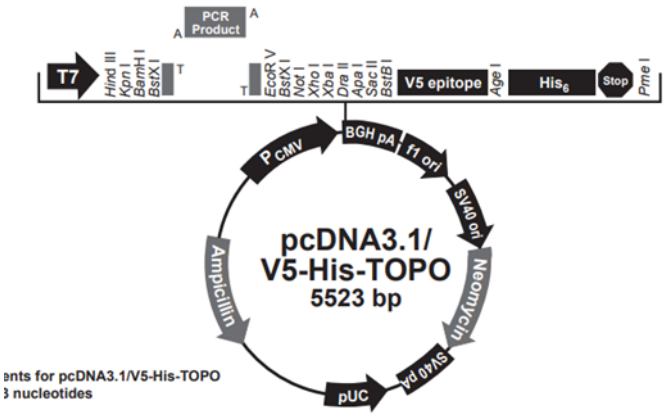
2.2.1. Drugs and chemicals

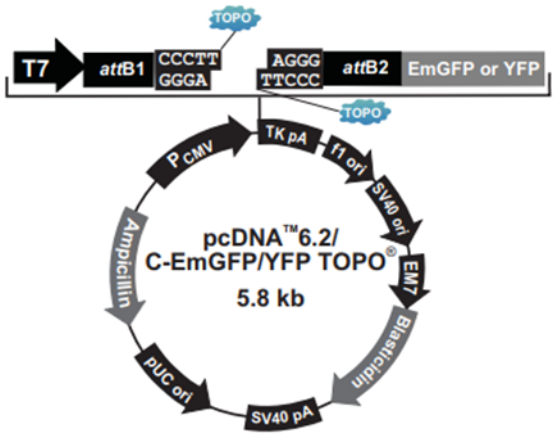
Radiolabeled ^{3}[H] CS were acquired including the following. ^{3}[H] budesonide with specific activity of 39 Curie/mmol (Ci/mmol) was purchased from Quotient Bioresearch (Manchester UK). ^{3}[H] Prednisolone with specific activity of 20 Ci/mmol was obtained from American Radiolabeled Chemicals (St Louis, USA) and ^{3}[H] methylprednisolone with specific activity of 5 Ci/mmol was purchased from Movarek Biochemical (Los Angeles, USA). ^{3}[H] dexamethasone with specific activity of 82.8 Ci/mmol was obtained from American Radiolabeled Chemicals. All other drugs and chemicals were purchased from Sigma (Poole, Dorset, UK) unless stated otherwise.

2.2.2. Plasmids

Commercially synthesized cDNA encoding for ABCB5 full length (NM 001163941) and ABCB5 β (NM178559) were purchased from Gene Art, Gene Synthesis (part of Thermo-Fisher group). 'Empty' plasmids or t-tailed versions, TOPO-R vector of pcDNA3.1 V5-6xHis and pc DNA ™ 6.2/C emerald-green fluorescent protein (em-GFP), were purchased from the Thermo-Fisher group. Figure 2.2 below shows the vector maps for utilized plasmids.

Figure 2.2. Vector maps of plasmids utilized in this project.





PCR projects of either ABCB5FL or ABCB5β sub-cloning into the respective plasmid is described below. DNA extraction and purification of plasmid DNA involved growth of bacterial culture, lysis of bacteria and preparation of plasmid DNA yield; in increasing order of magnitude these are called mini-maxi-mega preparations (preps). Mini preps were used to analyze bacterial clones. Lysogeny broth (LB) with ampicillin stock was mixed to a final concentration of 100 µg/ml for ampicillin.

Scrapings of bacteria were inoculated in tubes or conical flasks and were shaken at 250 RPM overnight at 37^{o}C. Tubes were centrifuged at 3000g for 15 minutes, and mini-preps (4-5 ml) or maxi-preps (200 ml) were prepared. Alkaline lysis based plasmid DNA purification kits were utilized following manufacturer's instructions. To obtain glycerol bacteria stocks, overnight bacterial cultures were centrifuged at 2000 g for 5 minutes. Bacterial pellets were suspended in LB broth containing 20% of glycerol. These glycerol stocks were stored at -80^{o}C. Nano-drop spectrophotometry (ND-100 TF Scientific, MA, USA) was used for DNA quality control and nucleic acids with A260/A280 between 2-2.2 were used for downstream applications.

TOPO sub-cloning into pc DNA 3.1 v5 6xHis plasmid and pc DNA ™ 6.2/C-Em-GFP was completed. The open reading frames of ABCB5 full or ABCB5 beta were sub-cloned into the t-tailed plasmids, utilizing PCR amplification of insert and was verified by sequencing. The primers used were as follows:

Primers for ABCB5 full length

ABCB5 full forward primer for 5'-GCCACCATGGAAAATTCAGAAAGAGC-3'

ABCB5 reverse primer 5'- CTGCACTGACTGTGCATTCACTAACT-3'

Primers for ABCB5 β

ABCB5 β forward primer 5'-GCCACCATGGTGGATGAGAATGACAT-3'

ABCB5 reverse primer: same primer as for full length

2.2.3. HEK 293 cells

The human embryonic kidney cell line (HEK 293, ATCC Middlesex UK) is a cell line established from primary embryonic human kidney cells, transformed with sheared human adenovirus type 5 DNA. The adenovirus genes allow production of high level of recombinant protein. HEK are vessels for effective transfection of functional genetic material to eukaryotic cells; this cell line is appropriate for functional, radiolabeled drug transport assays, to determine the impact of transporter inhibition on the exposure of drugs, through calculation of kinetic constants in monolayer cultures, with a fraction-excreted approach (Nettleship, Watson et al. 2015). HEK 293 cells were therefore cultured, and passaged after every two to three days. ABCB5 cell lines were developed through transfection of plasmid DNA containing ABCB5 gene in mammalian cells in two separate experiments; stable and transiently transfected ABCB5 FL and β overexpressing cell lines were produced, as per standard practice described below. A wild type, mock transfected, control cell line was developed by using an 'empty', non-protein expressing plasmid.

2.2.4. ABCB5 protocol of transient transfection

The protocol implemented to achieve transfection of HEK 293 cells with use of Lipofectamine 2000 under aseptic conditions is described in detail below. Lipofectamine is a plasmid DNA specific cationic lipid transfection reagent that provides maximum expression and viability with minimum cytotoxicity. HEK 293 (ATCC, Middlesex UK) cells were cultured in six well plates adherently in monolayers, seeded at a density of 1 million cells per well. After 24 hours growth in antibiotic free medium, the cells were transfected by adding ABCB5 full/β-

lipofectamine 2000 complexes; control wells were mock transfected using lipofectamine 2000 with an empty plasmid.

On day one, plates were prepared, coated with poly-lysine using 1:20 dilution with sterile de-ionized water. One million cells per well were plated in a six well plate and 2ml of media was added per well, i.e. Dulbecco's Modified Eagle Medium (DMEM) with fetal bovine serum (FBS). On day two, transfection commenced with the dilute plasmid DNA in minimum essential medium (Opti-MEM). 2.5 micrograms (μg) of plasmid DNA was added to 250 micro-liters (μl) of Opti-MEM. 7.5μl lipofectamine was diluted in 250μl Opti-MEM, dilution was scaled up by number of wells, plus 10%. 250 μl of diluted lipofectamine was added to each plasmid DNA containing tube. After 20 minutes' incubation at room temperature for complexes to form, 500μl of DNA/lipid complex mixture was added to the appropriate well. On day 3, selection by passaging cells onto T25 flask (up to 10 ml of medium) occurred with use of complete DMEM to a final G418 concentration of 800μg/ml. Single cell clone selection took up to two weeks. Cells were sub-cultured at log phase and before reaching confluence (every 2-3 days), to facilitate cell division, as G418 normally inhibits protein synthesis. Trypsin induced dissociation was implemented as per standard protocol of passaging adherent cells. Cell morphology was inspected under the microscope each time the cells were handled to detect any contamination and to ensure healthy status. Two weeks after transfection, single cell ABCB5 transfected clones were generated with a serial dilution method using 96 plates with 100 μl per well. The same experiment was repeated after integration of reporter proteins, such as green fluorescent proteins (GFP) tagging the gene of interest within the transfected cells. This facilitated the exploration of dynamics of protein movement and expression over time, by creating fusion with protein of interest.

2.2.5. Cell culture and production of ‘stable’ overexpressing ABCB5 cell line

The same transfection protocol was used with plasmid pc DNA3.1 after adaptation for production of stably transfected ABCB5 cell lines overexpressing, FL, β and mock transfected (empty or wild type) plasmid cell lines. Pc DNA 3.1 plasmids were 5.4 kb vectors derived from pc DNA3, commonly used for high efficiency of stable and transient transcription in mammalian cells. The vector also contained a cytomegalovirus (CMV) promoter, multiple cloning sites in forward and reverse orientation to facilitate cloning, neomycin resistant gene and SV40 early promoter, which allows high-level transfection and episomal replication in cells (figure 2.2). A control plasmid in the cell line of choice was implemented as positive control for transfection and expression. Genes of interest were cloned in pcDNA 3.1 plasmids, inserts were ligated into the appropriate vector and were transformed into suitable *Escherichia coli* host. Transformed cells were selected by restriction digestion on LC plates containing ampicillin 50-100 µg/ml and analyzed were for the presence of inserts. The inserts contained a Kozak translation initiation sequence with ATG initiation and stop codon. Transformed plasmids with correct restriction pattern were sequenced, to confirm efficient cloning. A positive control vector and ‘mock-transfection’ negative control was used for evaluation of results. Following isolation, plasmid DNA was used for transfection of mammalian HEK 293 cells. Two weeks after transfection, single cell derived ABCB5 transfected clones were generated with the serial dilution method using 96 plates with 100 µl per well.

2.2.6. SK-MEL28 cell lines

'Wild type' SK-MEL 28 melanoma cell line and a stable overexpressing ABCB5 FL FLAG fusion protein cell line was provided as a gift by Jean-Pierre Gillet, Department of Biomedical Sciences, University of Namur, Belgium. Cells were cultured in DMEM high glucose supplemented with 10% FBS at 37 ^{o}C in 5% CO_2. Poly-lysine coated six well plates were seeded with 1 million cells from each cell line, and were incubated for 24 hours. RIPA and 300 μl triple lysates were prepared from a single well for each cell line. RNA extracted from both versions of the cell-lines was reverse-transcribed; the c-DNA was subjected to RT-q PCR. The same assay probes as for HEK293 cell transfection were used (assay ID Hs00698751_m1, Life Technologies UK), as well as an additional probe that binds to all ABCB5 isoforms (assay ID Hs02889060_m1, Life technologies UK). The experiments were repeated on three separate occasions.

2.2.7. RNA extraction and real time quantitative polymerase chain reaction (RT-q PCR)

Cells were lysed using TRI-reagent and RNA was extracted from cells by acid guanidinium thiocyanate-phenol-chloroform extraction. mRNA was reverse transcribed as per standard practice described below, with the resulting c-DNA amplified by RT-q PCR. The experiments were replicated on three separate occasions. The two ABCB5 assay probes were purchased from Life Technologies, UK; these were Hs 02889060_m1, which detected ABCB5 FL and β isoforms, and Hs 03676541-m1, which detected only β isoform of ABCB5. Cells lysed with Tri reagent on 12 well plates were transferred to the micro-centrifuge tube; 0.2 ml of chloroform was added per 1 ml of Tri reagent and was incubated at room

temperature for 10 minutes, subsequently centrifuged at 12000 g for 15 minutes at 4^{o}C. The upper aqueous phase was transferred to a different tube and 0.5 ml of isopropyl alcohol was added per 1 ml of Tri reagent, was mixed and incubated for 10 minutes at room temperature, subsequently centrifuged for 10 min at 4^{o}C. Supernatant was discarded and 1 ml of 75% alcohol was added per ml of Tri reagent and was centrifuged at 7500 g for 5 minutes at 4^{o}C. The supernatant was removed and the pellet was air-dried. RNA was dissolved in deionized water and was mixed by repeat pipetting at 55-60^{o}C for 10-15 minutes. The extracted RNA yield was measured and quality controlled by the ratio of absorbance at 260 and 280 nm, using Nano-drop spectrophotometry technology (ND-100 TF Scientific, MA, USA). The nucleic acid purity A260/A280 ratio measured between 2 and 2.2 for RNA. Only nucleic acids that fulfilled this premise were used in downstream applications. These were reverse transcribed with use of random hexamers for priming.

The protocol used for reverse transcription was as follows: 4.5 µl of Master Mix was made up from adding 50 ng/µl random hexamers to 2.5 µl water and 10 µM d NTPs and was heated for 5 minutes at 65^{o}C and subsequently chilled to 4^{o}C. Samples were pulse spinned at maximum speed; 4.5 µL of enzyme mix was added to the samples which were inserted in the thermal-cycler program for five minutes at 25^{o}C, 50 minutes at 42^{o}C, 15 minutes at 70^{o}C, then chilled at 4^{o}C. cDNA was subjected to RT q PCR using a probe that detected both ABCB5 β and FL isoforms (assay ID Hs00698751_m1, Life Technologies, UK) on the Taqman platform. GAPDH VIC probe was the endogenous control expressed in all cells. RT q PCR was performed, by adding 2µl of single stranded cDNA to18 µl of mixture containing 10 µl of 2xMaster Mix, 6µl of molecular grade water and Taqman ABCB5 expression assays,

i.e. 1 µl VIC dye probe and 1 µl of FAM dye probe for multiplexing. Samples ran for 50 cycles in the 'Applied Biosystems' machine.

mRNA expression was analyzed with the delta-delta Ct ($\Delta\Delta$CT) method, using the relative fold abundances of each isoform in relation to that of mock-transfected cells (control cell line or 'empty plasmid'). $\Delta\Delta$CT logarithmic analysis (Pfaffl 2001) is used as a method of relative quantification of gene expression; the change of expression of the target gene is quantified in relation to a control or untreated sample. The control is the sample used to normalize the PCRs for the amounts of RNA added to the reverse transcription reaction. The 2^{-DDCt} equation gives the fold change in gene expression relative to the control.

2.2.8. ABCB5 protein expression

Immunoblotting was conducted in stable and transient ABCB5 FL and β isoforms, 'empty plasmid', ABCB5 SK-MEL28 and wild type SK-MEL28 cell lines, to detect ABCB5 protein isoforms. Lysis was performed with 300 µl of RIPA lysis buffer in each well, consisting of 25mM of Tris-HCl, 150mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS); cells were scraped off, pipetted up and down in Eppendorf tubes and placed on ice for 10 minutes. As per established Western Blot protocol, RIPA buffer prepared cell lysates were ran on a 10% SDS-PAGE gel at 200V and transferred to a PVDF membrane using the iBlot 2-gel transfer device form Life Technologies. The membrane was blocked in 1% milk for 1 hour at room temperature, probed with a mouse anti-FLAG antibody (1:1000) overnight in the cold room followed by incubation with secondary anti-mouse horseradish peroxidase conjugated antibody for 1 hour at room temperature.

Detection was by chemo-luminescence on GE's Image Quant LAS 4000 or by film. The experiment was repeated to compare protein expression via Western Blot between LAT4 HEK293 overexpressing cell line tagged with Em-GFP reporter protein specific antibody and ABCB5 FL Em-GFP tagged cell line. LAT4 HEK 293 cell line was donated as a gift from Doctor David Dickens, University of Liverpool, and had been produced with use of above transfection protocol and replacement of 12ABEZNC ABCB5 FL custom synthesized plasmid with T7 promoter before ABCB5 insert. The transiently transfected HEK293 ABCB5 FL tagged with Em-GFP was subsequently compared to the LAT4 Em-GFP cell line implemented as control to verify anti-GFP antibody specificity.

2.2.9. Radio-ligand accumulation assay

Functional drug uptake assays were conducted on two separate occasions in triplicates in stable HEK 293 overexpressing ABCB5 FL, β, mock transfected 'empty plasmid' cell lines, stable ABCB1 overexpressing and wild type MDCK cell lines, using tracer tritium labelled ^{3}(H) CS, including dexamethasone, prednisolone, methylprednisolone. Radiolabeled budesonide was tested according to the same protocol, separately, in triplicates on two different occasions, according to the same protocol, using wild type SK-MEL28 and SK-MEL28 ABCB5 stable overexpressing cell lines donated by Professor Jean Paul Gillet.

The protocol implemented for drug uptake studies is described below. The amount of radiation in the dissolved cells was estimated by measuring liquid scintillation in average disintegrations per minute (DPM) per well, and then used to calculate the amount of radiolabeled drug taken up in pico-moles (pmol) per million cells (average DPM/pmol/million cells). Disintegrations per minute (DPM) is a measure of

radioactivity; it is the number of atoms in the given quantity of radioactive material that decay in one minute using a scintillation counter as radiation detector.

On day one, cells were layered on polylysine coated plates (1:20 from stock diluted with sterile DI water); 1 ml/well on a six well plate was added and washed after 5 minutes with 1 ml Hanks Balanced Salt Solution (HBSS) from Sigma. 2 million cells per well were plated on a six well plate with 2ml media per well (1 million cells per ml). On the day of the assay, cell count was performed on a well from each cell line so that the total number of cells (per well) was determined. The transport buffer was made by mixing 500 ml HBSS with 25 mM HEPES (2.98g) to pH of 7.4. On the day of the assay, the complete transport buffer was made from the above solution with added bovine serum albumin (BSA) at a concentration of 50 mg/ml (50x stock solution). The complete transporter buffer was added to equal volumes of 1 mCi/ml radiolabeled drug and 1mmol/L cold drug, to achieve a final drug concentration of 5 µmoles/L. The drug cocktail and added HBSS aliquot were incubated for 10 minutes to 37^{o}C. Cells were washed in HBSS using the aspirator (pre-warmed to 37^{o}C). Subsequently HBSS was aspirated and replaced with1ml drug cocktail, and was incubated at 37^{o}C for 30 minutes (each plate was staggered by 5 minutes). Reaction ended after the drug cocktail was aspirated off and was washed three times with ice cold HBSS. HBSS was aspirated and 500 µl of 10% SDS was added; the plate was incubated at 37^{o}C for 30 minutes to dissolve cells. Dissolved cells were transferred to a scintillation tube, and 4 ml of scintillation fluid was added with dispenser. 100µl of drug cocktail was used for determination of the extracellular DPM. Samples were transferred to the scintillation counter and F2 run was activated with protocol tag for ^{3}H isotopes (tag 4). Rhodamine 123, a known ABCB5 substrate (Frank, Pendse et al. 2003), was used as positive control, and verapamil was used

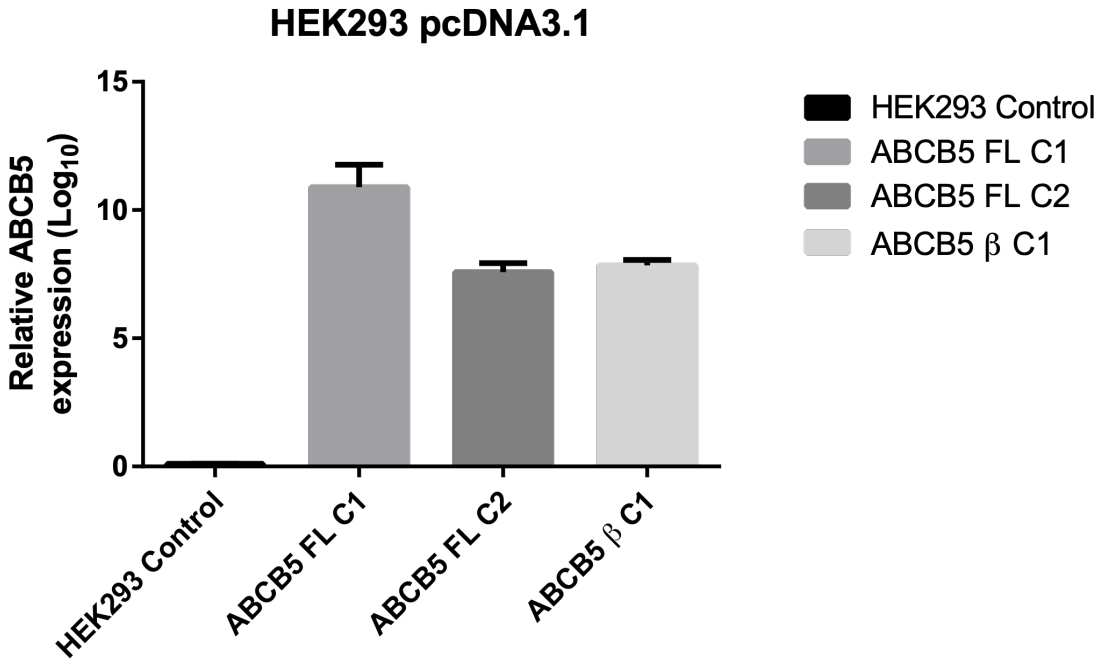
to block endogenous ABCB1 transporters (Mukkavilli, Jadhav et al. 2017), which are also known to affect the cellular efflux of rhodamine 123 (Zijlmans, Visser et al. 1995). Verapamil is not known to block ABCB5.

2.3 Results

2.3.1. Gene and protein expression in transient or stably overexpressing ABCB5 cells

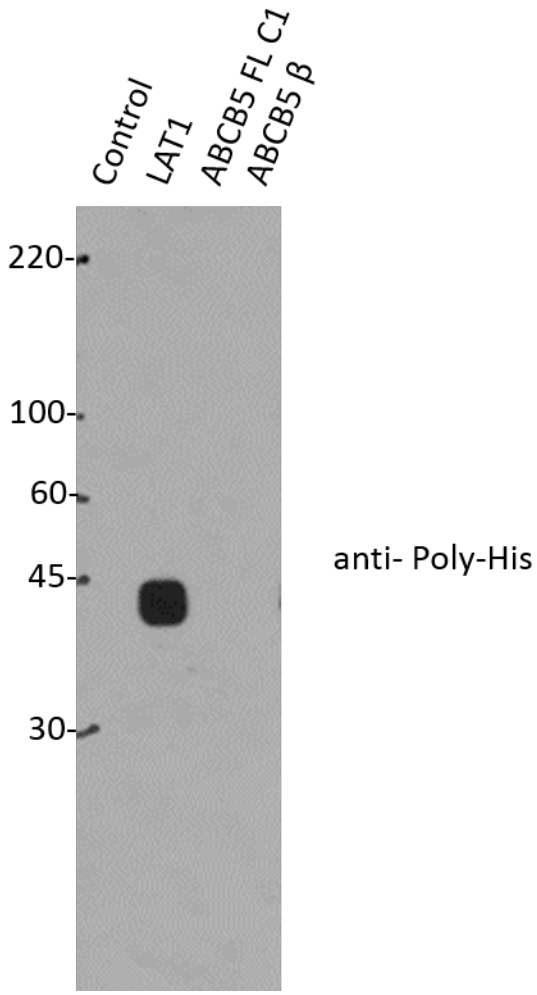
A comprehensive set of experiments were performed with overexpressing tagged-ABCB5 cell lines, to investigate both their potential for protein expression, and as a candidate efflux transporter of CS. The successful production of ABCB5 FL stable overexpressing cell clones, ABCB5 β and control (empty plasmid) cell clones derived from single cell culture, was confirmed with high mRNA expression via RT q PCR for the first two as shown in figure 2.3 below. The ABCB5 mRNA expression levels of the ABCB5 FL and β isoform cells were multiple orders of magnitude higher than the control cells (‘empty plasmid’), indicating successful assimilation of plasmid DNAs and overexpression of the investigated gene.

Figure 2.3. Relative ABCB5 mRNA expression in HEK 293 (control) cell lines and stably expressing ABCB5 full length and ABCB5 ßcell lines determined by RT q PCR (n=3, where n number of experiments).



Immunoblotting was used to detect and determine protein expression of the fusion proteins ABCB5 FL and ABCB5β tagged to poly-His in the HEK293 cell lines. The positive control used for this experiment was the LAT1 transporter tagged to a poly-His tag (Dickens, Webb et al. 2013). The immunoblot from the poly-His antibody tagged cell lines showed no bands for ABCB5 FL and ABCB5 β fusion protein lanes; however, for the HEK293 LAT1 positive control cell line, a strong band was observed at the predicted size for this poly His tagged transporter (figure 2.4).

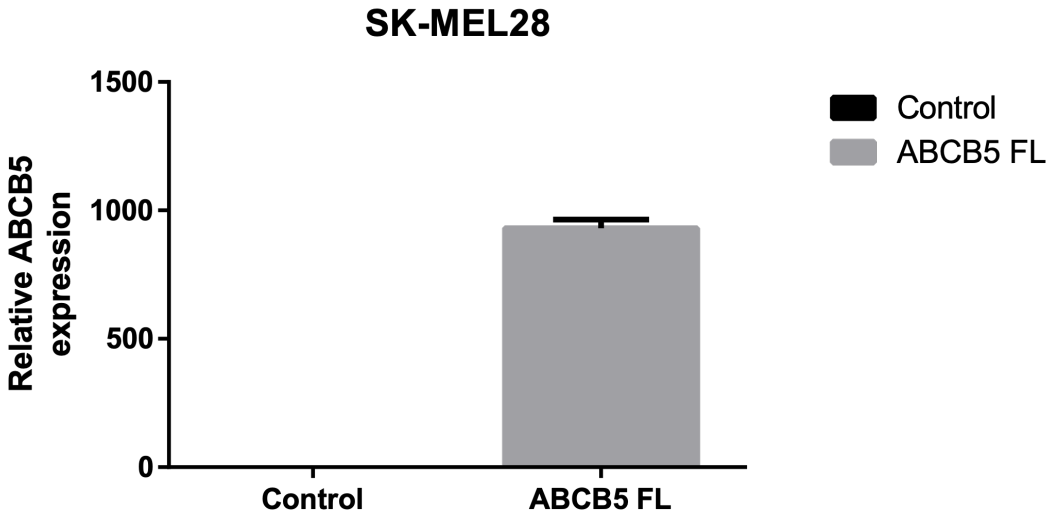
Figure 2.4. Immunoblot shows no detection of ABCB5 FL and protein (tagged with Poly-His) in transfected HEK 293 cell lines. The use of the anti-Poly-His antibody successfully detected Poly-His tagged LAT1 (SLC7A5) HEK 293 stably overexpressing LAT1 tagged to 6xHis cell line.



2.3.2. ABCB5 overexpression in a melanoma cell line

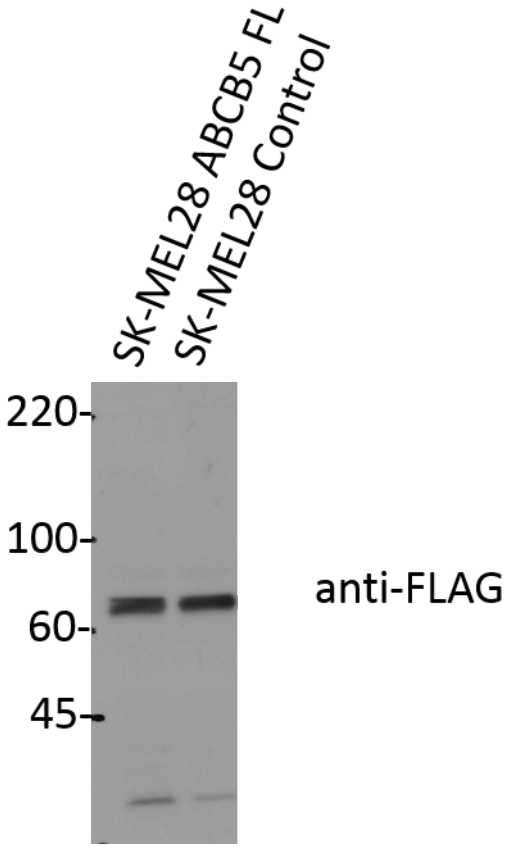
Due to the negative results with the stable cell line for ABCB5 protein expression, a different stably overexpressing ABCB5 FL cell line where ABCB5 was tagged to FLAG (DYKDDDK) was used, donated as a gift from the research group of Professor Jean-Paul Gillet. The RT q PCR result of the target transcript in ABCB5 SK-MEL28 cells was compared with that of the wild-type SK-MEL28. ABCB5 mRNA expression was enriched in the overexpressing cell line (figure 2.5).

Figure 2.5. Relative gene expression of m RNA in stable ABCB5 full length overexpressing SK-MEL28 cell line versus wild type SK-MEL28 cell line (shown as control on the graph below, n=3 where n number of experiments).



Immunoblotting was used to detect and determine correlation between the apparent presence of mRNA and the expression of the fusion protein ABCB5-FLAG in both overexpressing ABCB5 SK-MEL28 and control SK-MEL28 cell lines. The results, shown in figure 2.6, showed no difference in the detectable signal using the anti-FLAG monoclonal antibody in ABCB5 SK-MEL28 and control SK-MEL28 cell lines except for the non-specific signal shown below, which did not correspond, in molecular weight, to any of the known ABCB5 isoforms, and was also observed in the control cell line.

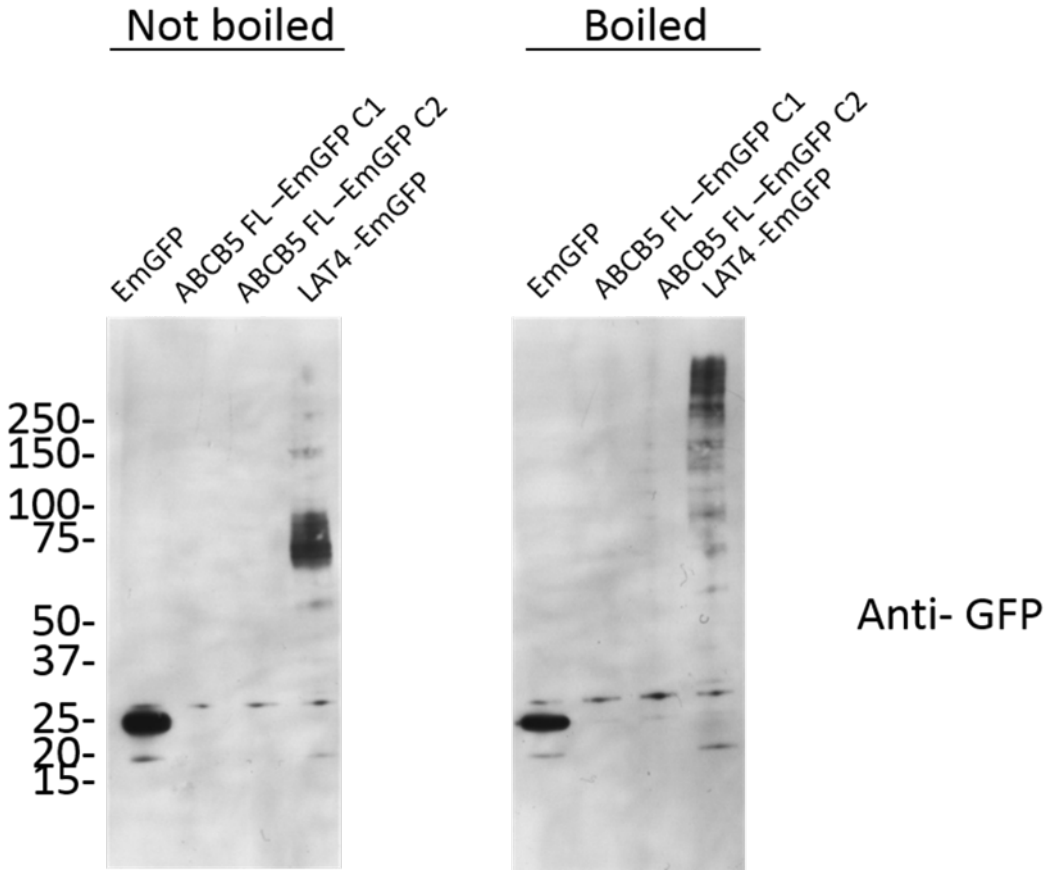
Figure 2.6. Western Blotting shows no relative difference in ABCB5 protein expression between ABCB5 overexpressing SK-MEL28 and control SK-MEL28 cell lines as described in section 2.3.2 above.



2.3.3. ABCB5 tagged to Em-GFP

The tags utilized for ABCB5 so far were 6xHis and FLAG tags which were relatively small, so could in theory and under the denaturing conditions of immunoblot be obscured so that the tags are inaccessible to the respective antibodies, and thus give a negative immunoblot. Therefore ABCB5 FL opening reading frame was sub-cloned into an Em-GFP plasmid (pcDNA6.2 Em-GFP) so that ABCB5 FL was tagged to a large protein tag (30 kilo-dalton), highly likely to be assessable with an antibody for immunoblotting staining applications. As a positive control for this approach and utilizing the same tagging protein strategy, a transporter tagged to Em-GFP (LAT4) was utilized. However, no specific signal was observed on the Western Blot from the transiently transfected ABCB5 FL Em-GFP cells. This compared to the positive control sample that showed a strong signal in boiled and non-boiled conditions (figure 2.7).

Figure 2.7. Western Blotting shows no anti-GFP immunofluorescence signal above background in HEK 293 cells transiently transfected with ABCB5 full length and tagged with Em-GFP, compared with LAT4 tagged with Em-GFP.

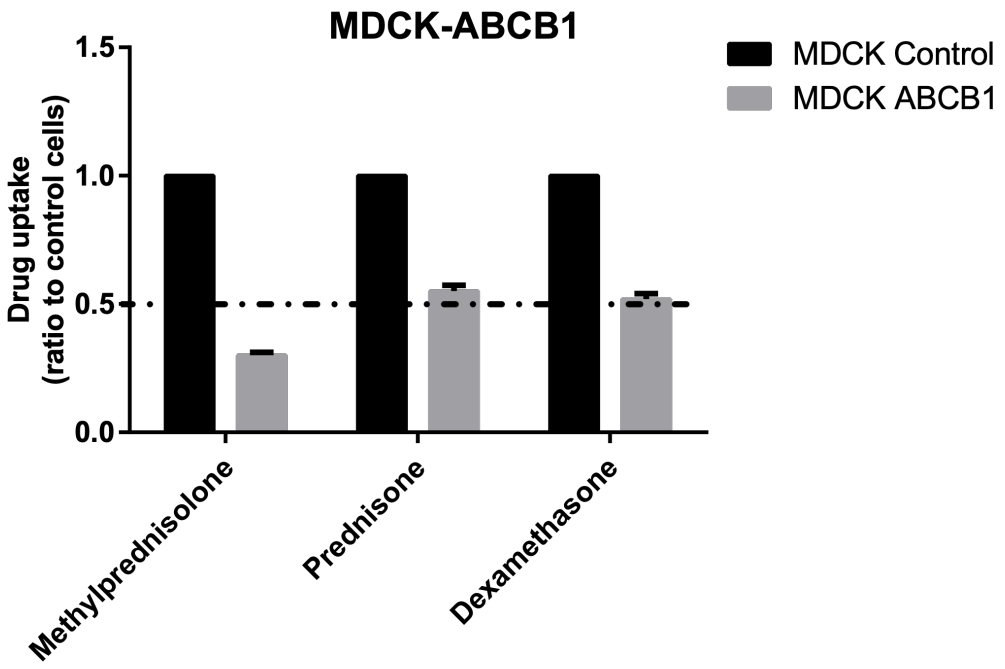


2.3.4. Radiolabeled drug uptake studies for ABCB1 and ABCB5 cell lines

A rule of two is applicable to *in vitro* drug transporter studies for both uptake and efflux transporters when comparing the uptake of a potential substrate in an overexpressing cell line compared to the control cells. For uptake transporters this refers to a two-fold increase in the drug uptake of a substrate when compared to the control cells, and for efflux transporters this can refer to a two-fold decrease in the drug uptake in the overexpressing cells when compared to the control cells (Brouwer, Keppler et al. 2013).

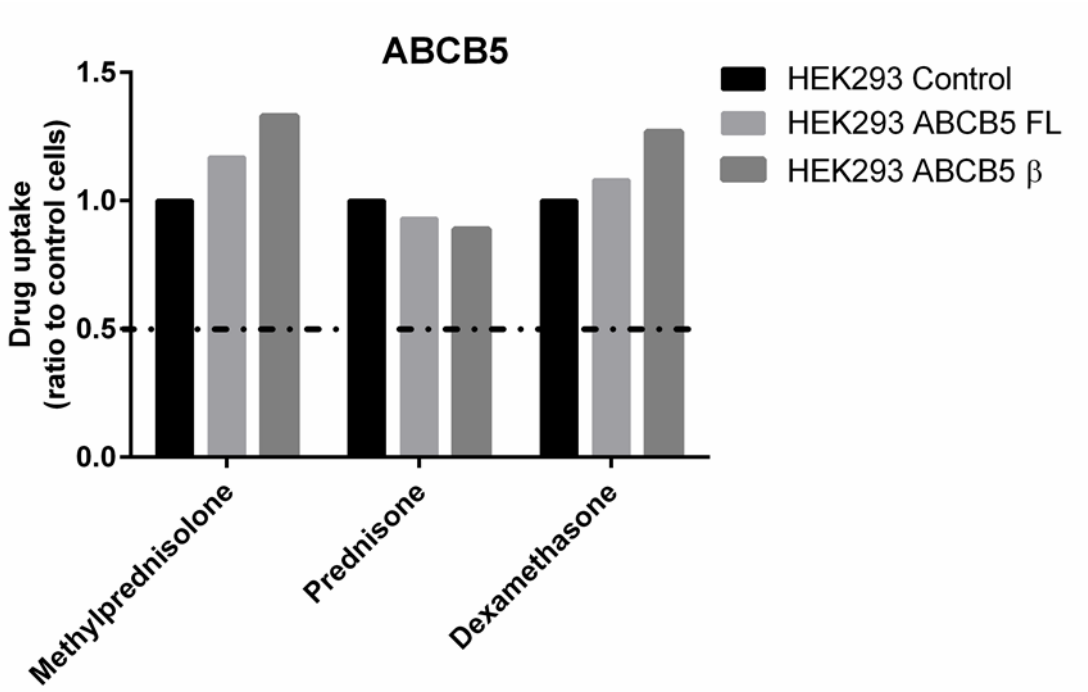
As a positive control for the functional uptake studies, a stably transfected ABCB1 cell line and a matched control cell line were utilized. CS are well-characterized substrates of ABCB1. For the three CS used, a nearly two-fold reduction in the uptake was observed in the ABCB1 overexpressing cells when compared to the matched control cell line (figure 2.8). This provides a functional positive control for this experimental approach.

Figure 2.8. Relative ratio of radiolabeled corticosteroids uptake in MDCK control and MDCK ABCB1 cell lines performed in triplicates in one independent experiment. Dotted line annotates the 2-fold reduction in uptake threshold compared to the MDCK control cells.



The uptake of CS in the HEK293 cells expressing ABCB5 FL and β shown below did not show a decreased uptake to the two-fold cut-off unlike the ABCB1 overexpression cells (figure 2.8). Drug uptake studies therefore did not show a difference in efflux of radiolabeled CS when ABCB5 is overexpressed (figure 2.9).

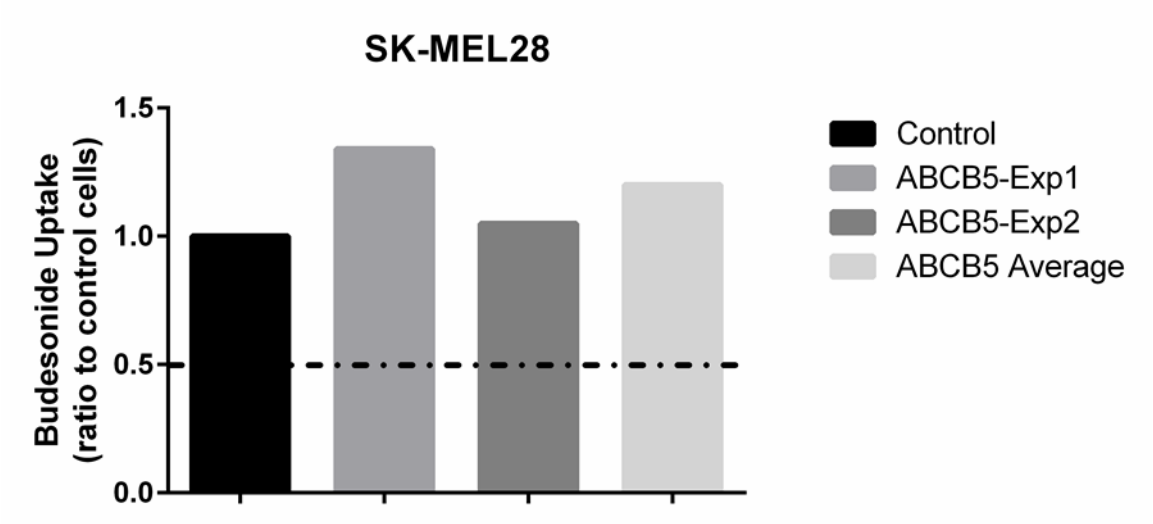
Figure 2.9. Average relative ratio of radiolabeled uptake in HEK293 control and HEK293 ABCB5 FL and β cell lines performed in triplicates in three independent experiments. Dotted line annotates the 2-fold reduction in uptake threshold compared to the HEK293 control cells.



The ratio of budesonide that accumulated in the control (wild type) SK-MEL28 versus the SK-MEL28 ABCB5-FLAG overexpressing cell line (WT: ABCB5-FLAG) was calculated. Twenty per cent increase in budesonide was detected in the ABCB5-FLAG cells; this increase is noted in the intracellular compartment of the cell as the drug. This was not reproducible in an independent repeat experiment, where the ratio of drug concentration between the cell types was unchanged. The uptake of budesonide from the SK-MEL28 cells expressing ABCB5 FL did not show a decreased uptake to the two-fold cut-off unlike the ABCB1 overexpressing cells. Drug uptake studies therefore did not show difference in the efflux of radiolabeled

budesonide between SK-MEL28 ABCB5 FL and control (wild type) SK-MEL28 cell lines (figure 2.10).

Figure 2.10. Relative ratio of radiolabeled budesonide uptake from ABCB5 FL SK-MEL 28 and control (wild type) SK-MEL28 cell lines performed in triplicates in two independent experiments. Average ratio of budesonide uptake by SK-MEL 28 ABCB5 FL cell lines compared to the control SK-MEL28 cells is shown. Dotted line annotates the two-fold reduction in uptake threshold compared to the SK-MEL28 control cells.



2.4 Discussion

The findings from ABC study show that although we were able to transfect a cell with ABCB5 and detect mRNA transcripts (Figures 2.3 and 2.4), this was not translated into detectable protein isoform. There was no protein transporter function evident in the expressed cell lines using CS as substrates.

ABCB5 is normally expressed in limbal stem cells (LSC) where it plays a role in corneal development and repair (Ksander, Kolovou et al. 2014), and in skin progenitor cells where it functions as a regulator of cellular differentiation (Frank,

Pendse et al. 2003). It is also expressed in human mammary epithelial cells (Braker 2014), testis, adipose tissue, skin melanoma, haematological malignancies (Yang, Li et al. 2012), breast, ocular surface squamous neoplasia, oral squamous cell carcinoma, Merkel cell carcinoma (neuroendocrine skin cancer), leukaemia, liver and colorectal cancer (Cheung, Cheung et al. 2011, Wilson, Schatton et al. 2011, Grimm, Krimmel et al. 2012, Farawela, Khorshied et al. 2014, Jongkhajornpong, Nakamura et al. 2016, Kleffel, Lee et al. 2016, Sakil, Stantic et al. 2017).

It is interesting to note that despite the fact that ABCB5 overexpressing cell lines with proven high mRNA expression have been developed by various scientific groups, identification of the respective protein isoforms within the cell with conventional proteomics has been problematic (Frank, Margaryan et al. 2005, Chartrain, Riond et al. 2012, Sakil, Stantic et al. 2017). In the author's view, there has been insufficient evidence of direct ABCB5 protein and function. The protein coding potential has been largely extrapolated from mRNA or cDNA expression, but not from direct protein expression experiments as direct predictor of ABCB5 function (Kim, Pinto et al. 2014). It is plausible that other transporters have been responsible for the observed drug efflux capacity or drug resistance phenotype, wrongly ascribed to ABCB5. ABCB5 gene expression could potentially be induced or downregulated upon treatment with drugs other than CS, but not necessarily be directly involved in their efflux; therefore observed differences in mRNA expression, as for example described with doxorubicin by Frank et al (Frank, Margaryan et al. 2005), cannot be interpreted as direct ABCB5 transporter mediated drug efflux. ABCB5 may play an adjunct but distinct role in drug resistance through mediation of cell fusion by altering cell membrane potential in breast cancer cell lines, rather than via reduction of drug accumulation (Yang, Ha et al. 2010).

There is a commonly preserved ratio between mRNA and protein expression in cells and tissues, tightly controlled by regulating mRNA, non-coding RNAs and transcription factors (Wilhelm, Schlegl et al. 2014). It is plausible that ABCB5 gene encodes a non-coding mRNA. In such case, its biological significance would be unclear and any reported ABCB5 transcripts would be of uncertain function (Kopp and Mendell 2018). Long and short non-coding RNAs regulate gene expression by regulating chromatin modification, affecting transcriptional factors by binding in DNA regions or acting as scaffolds for protein assembly into functional complexes. Thereby they influence mRNA stability and translation (Deveson, Hardwick et al. 2017)

The non-conventional ABCB5 structure and the lack of detectable protein isoforms could be secondary to a defective transporter not fit for purpose. Undetected ABCB5 protein expression in HEK 293 cells can be cell/tissue or disease specific. Cell lines retain protein expression characteristics of their respective primary tissue, and therefore it is plausible that HEK293 was not the optimal cell line to test ABCB5 expression. Antibody based methods of protein detection have their limitations due to non-specific binding; antibodies used can bind to different protein isoforms, for example beta, full length or alternative splice variant, or to other homologous transporters in proximity.

A further plausible explanation is that the ABCB5 protein is expressed transiently and rapidly degrades. About one third of human genes produce multiple protein isoforms with distinct functional properties and differential tissue expression, whereas the remaining genes correspond to single protein product or non-coding RNAs (Lieberman 2018). Functionally uncharacterized proteins may be the products of

pseudogenes that may have lost their function during course of human evolution or demonstrate expression in tissue/disease specific pattern only (Kim, Pinto et al. 2014).

ABCB5 unlike ABCB1 may not be expressed in the plasma membrane, but in the internal membranes of cell organelles, such as endoplasmic reticulum (ER), (ABCB2 and ABCB3) (Abele and Tampe 2011), mitochondria (ABCB7, ABCB8) (Schaedler, Faust et al. 2015) or lysosomes (ABCB6, ABCB9, ABCB10) (Fujimoto, Kamakura et al. 2011, Kiss, Brozik et al. 2012, Kiss, Kucsma et al. 2015). In that case, the observed drug resistance could be due to sequestration of the substrate within the cell. Protein expression would have however been detectable in whole cell lysates used in this project.

Other members of the ABC superfamily, for instance ABCE1, ABCF1, ABCBF2, ABCF3, are not functional transporters but are thought to have an intermediary role in protein translation(Alexander 2017-2018). Alternative cellular processes, for instance post translational modifications such as the unfolded protein response whereby protein folding impairment occurs inside the ER leading to ER stress, can affect expression of genes encoding respective proteins, or protein function itself, and host protection against xenobiotics (Schroder and Kaufman 2005). This results in intraepithelial cell malfunction with dysregulated immune response in organs including the gut (Faitova, Krekac et al. 2006).

ABCB1, a transporter protein related to ABCB5, plays a pivotal role in the colitis-adenoma-carcinoma sequence leading to colorectal cancer. The presumed molecular mechanism in pathogen free ABCB1 knock out mice is via defective transport of inflammatory mediators; deletion of ABCB1 leads to fundamental

changes in gut microbiota-host interactions. Molecules produced through this interaction, and influenced by diet, may regulate ABCB1 transcription, thereby affecting expression and function (Zhang, Xu et al. 2014). Trafficking of vesicles across cell membranes, altered phagocytosis and impaired function of tight junctions may also play a role (Andersen, Svenningsen et al. 2015). CS are ABCB1 substrates; the hypothesis tested in this chapter is that CS are also substrates of the highly homologous ABCB5.

CS are part of induction of remission treatment in IBD (Ruemmele and Turner 2014). ABCB1 expression can identify subpopulations of T helper 17 pro-inflammatory cells resistant to CS treatment, a reason for perpetuation of inflammation (Ramesh, Kozhaya et al. 2014). Furthermore, drug sensitivity data has enabled the identification of proteins predicting resistance or sensitivity, through analysis of mRNA and protein expression profiles (Barretina, Caponigro et al. 2012). Protein expression correlates with drug sensitivity but this process is signposted by relevant regulatory mRNAs (Wilhelm, Schlegl et al. 2014).

Variability in ABCB5 expression as a mechanism of impaired clinical response to CS is therefore an attractive hypothesis to test, as it would potentially be translatable to clinical practice. Clinically this requires demonstration that ABCB5 transports CS. ABCB5 in that case could potentially become an important prognostic marker and a target for pharmacologic manipulation in drug resistance studies. The effect of common polymorphisms on the efficacy of CS treatment in IBD could also be potentially significant.

Experiments conducted in this study however did not confirm ABCB5 protein expression within the cell, despite use of overexpressing ABCB5 cell lines in human

melanoma and HEK293 cell lines. Furthermore, negative transport of CS was demonstrated which presumably reflects the lack of detectable protein rather than direct proof that ABCB5 does not transport CS.

The negative results of the above experiments may be due to many reasons, including levels of expression beyond the sensitivity limits for detection, poor choice of cell line used to express ABCB5, limitations related to antibody specificity to detect ABCB5 and the possibility of production of non-coding transcripts. Moreover, it is possible that ABCB5 expression has a tissue and/or disease specific pattern not captured in this study. Gene expression of drug metabolizing enzymes and transporters is regulated by nuclear receptors and transcription factors and is modulated through signal transduction, post-translational modifications, cell membrane trafficking and subcellular organization pathways (Correia and Liao 2007, Klaassen and Aleksunes 2010). Gene expression can also be modified by DNA methylation proteins acting on the promoter regions of transporter genes and histone modification (Zhong and Leeder 2013). Post-transcriptional gene regulation mediated through micro-RNA could alternatively explain the lack of ABCB5 protein detection; for instance, it is possible that mRNA is degraded or that translation is repressed (Yu, Tian et al. 2016).

Braker et al showed exogenous expression of ABCB5 mRNA, but the recombinant protein was poorly expressed, and localized to the ER of human melanoma epithelial cells (HMEC). Point mutations introduced in the ATP catalytic domain to improve expression levels also failed, suggesting that protein function was not deleterious to the cell. Several commercial antibodies used in this study did not identify ABCB5, except for one that recognized both ABCB5 FL and β, which was subsequently used

to evaluate protein expression levels in HMEC with siRNA knock down of ABCB5. ABCB5 knock down of HMEC resulted in reduced p16INK4a expression, but no native ABCB5 β protein was detected in HMECs. This finding led the author to conclude that ABCB5 may be acting as a long non-coding RNA, regulating p16INK4a expression, a protein that traps the cell at the restriction point of the cell cycle (Braker 2014).

On the other hand Ksander et al published flow cytometry experiments with implementation of various commercially available polyclonal antibodies that showed positive ABCB5 protein expression in mouse and human limbus (Ksander, Lewis et al. 2015). ABCB5 had anti-apoptotic function similar to that of homologous ABCB1, which promoted cell survival in gastric cancer cells (Rocco, Compare et al. 2012). Specificity of the binding pattern was checked by RNA in situ hybridization. Other protein detecting methods used by the same group were western blotting, immunohistochemistry and confocal microscopy, which confirmed ABCB5 expression in mouse ocular tissue with the use of monoclonal antibodies and isotype-matched controls. Ksander et al developed ABCB5 knock out mice which had impaired corneal development and wound healing, therefore concluded that ABCB5 was an important marker for LSC isolation and clinical transplantation (Ksander, Kolovou et al. 2014). Kleffel et al demonstrated immunohistochemically-determined positive ABCB5 expression in skin biopsy samples from three patients with Merkel cell carcinoma (MCC), which varied pre and post first line chemotherapy (Kleffel, Lee et al. 2014). Flow cytometry experiment was also performed with use of monoclonal anti-ABCB5 antibody which induced compensatory increases in mRNA expression compared to treatment with isotype control; the specificity of this antibody used in flow cytometry was tested by comparing inhibition of rhodamin123 efflux with

antibody mediated blockade of ABCB5 in MCC cells (Kleffel, Lee et al. 2016). De Waard et al has also shown small but defined stem cell ABCB5 expression in immune deficient mice inoculated with orthotopic xenografts of human conjunctival melanoma cells. Subpopulations of ABCB5 expressing cells were positive in all three conjunctival melanoma cell lines, primary tumors and metastases in animal models. ABCB5 as stem cell marker was associated with tumor progression (de Waard, Kolovou et al. 2015). ABCB5 protein expression was demonstrated with indirect single color flow cytometry experiments with the use of IgG1 κ anti-ABCB5 3C2-1D12 monoclonal antibody developed by essentially the same research group (Frank, Pendse et al. 2003), and commercially available rabbit anti-ABCB5 pAb (Novus) antibody with MOPC-31C mouse monoclonal antibody used as isotype control (Sigma Aldrich, St Louis, Mo, USA). According to the authors, both tested ABCB5 antibodies had similar specificity and stained same number of cells (about 0.05% of total cell number). ABCB5 mediated rhodamine123 efflux was reported in flow cytometry experiments by other group (Adamiak, Walkiewicz-Jedrzejczak et al. 2013). Borchers et al used intracellular cytokine staining for interferon-γ and TNFα in PBMCs from melanoma patients and checked ABCB5 reactivity in T cells in a small heterogeneous cohort. Positive ABCB5-reactive CD8 cells were found in just over half of the patients (Borchers, Mabetalo et al. 2018). Xiao et al have very recently published about variability in ABCB5 expression using RT q PCR and Western blotting in three different melanoma cell lines, with lower levels observed in SK-MEL 28 in comparison to other melanoma cell lines; some but not all of these were BRAF-inhibitor (vemurafenib) resistant. In the SK-MEL2 cell line with wild type BRAF ABCB5, expression was lower than in SK-MEL2 with BRAF mutation. Authors concluded that ABCB5 related resistance may play a role but not always in BRAF-

inhibitor resistant melanoma, and may be associated with extracellular signal regulated kinase pathway reactivation (RAF-ERK). ABCB5 knockdown did not re-sensitize BRAF-inhibitor resistant melanoma cell lines, unless RAF-ERK were inhibited at the same time. Other melanoma chemotherapeutic agents, such as temolozomide, were also discussed in the same paper with ABCB5 presumed to play a role towards drug response (Xiao, Egger et al. 2018). The authors concluded that ABCB5 expression varied in different melanoma cell lines when exposed to different chemotherapeutic agents. Vasquez-Moctezuma et al observed variability in ABCB5 expression in various ABCB5 melanoma cell lines (Vasquez-Moctezuma, Meraz-Rios et al. 2010).

The Human Protein Atlas (HPA) is an online database launched in 2005 aiming to map all human proteins in cells, tissues and organs including antibody-based imaging, mass spectrometry, confocal microscopy, transcriptomics and systems biology (Thul, Akesson et al. 2017). The HPA website, accessed online on 16/09/2018, confirmed that ABCB5 enriched mRNA expression was observed in testis, epididymis and retina, adipose tissues, breast, colon; these findings derived from various transcriptomic data repositories, including Fantom5 (database of long noncoding RNAs and mi-RNAs) (de Rie, Abugessaisa et al. 2017) and Genotype-Tissue Expression (GTEx) projects. Normal tissue annotation at protein level was still pending confirmation. According to the HPA website, protein expression evidence is available for only part of the human chromosome 7 where ABCB5 resides (ranging from 56% to 86.7%). According to the UniProt Knowledge Database (The UniProt 2017), a freely accessible resource of protein sequence and functional information, ABCB5 is present in stem cells in a number of malignancies and acts as regulator in cell differentiation; it mediates efflux of rhodamine123 dye

and doxorubicin from cells. It plays a role in melanoma drug resistance; at protein level, ABCB5 is expressed in CD-133 progenitor cells among epidermal melanocytes. There are four described isoforms in humans (ABCB5 FL, α, β, ε) as mentioned at the beginning of this chapter.

Further work is required in this area to explore the possibility of ABCB5 expression in intracellular organelles or tissue specific and disease specific expression in vivo. Of course, it will be important to understand whether ABCB5 functions as a non- coding RNA and its relevance to physiology, pathology and pharmacology. Direct protein sequencing technologies and deep proteomic profiling methodologies can complement genomic annotations. Examples of such methodologies are mass spectrometry, use of multiple proteases, direct capture of N-termini as validated translational start sites for identification of novel signal peptide cleavage and post-translational modifications of peptides, mapped to open reading frames located upstream, or in alternate reading frames within coding regions of annotated genes (Kim, Pinto et al. 2014). The human proteome project will be largely dependent on new generation mass spectrometry proteomics technology. Human proteome project is a coordinated effort to ascertain the human genes translated to proteins, by investigating human tissues, body fluids and cell lines for organ specific proteins and RNAs, in order to assess the function of the final gene products. The use of combined proteomic and genomic sequencing methods will consolidate basic and translational research and will accelerate a better understanding of gene-protein pathway networks in health and disease, as well as individual gene/drug transporter function including novel ABCB5.

In conclusion, the work presented in this chapter shows that ABCB5 can be expressed in mammalian cells but only mRNA expression was observed, without detectable protein or function, at least as tested by using CS. There are many possible reasons for this. Disease specific effect (paediatric IBD) will be tested in following chapters.

Chapter 3

The ABC study methodology: from inception to clinical recruitment

3.1 Introduction

Inflammatory bowel diseases are chronic gastrointestinal inflammatory disorders that affect millions of people worldwide, usually due to unknown etiology. The pathogenesis of IBD has been attributed to genetic factors as well as microbial, immunological and environmental factors (Rapozo, Bernardazzi et al. 2017). The integration of these complex factors in a comprehensive diagnostic and treatment algorithm follows the principles of personalized medicine with associated patient benefits with regards to enhanced treatment efficacy, avoidance of serious adverse drug reactions and predictability of disease prognosis.

The ABC study is a pilot, observational, non-interventional, case control prospective study, which took place in a tertiary children's hospital in partnership between the University of Liverpool and Alder Hey Children's Hospital NHS Trust. This study tests the hypothesis that there is genotypic variation and variability in gene and protein expression of ABCB1 and ABCB5 in health and disease.

The aim of this chapter is to describe the methodology of the ABC study from inception until the end of recruitment. The study protocol, ethical and regulatory approval process, eligibility and exclusion criteria, the methodology of case ascertainment and control selection, the clinical outcome and sample size definition, the number of participants at every stage and the process of inclusion of final participants will be explained.

3.2 Study methods

3.2.1. Study approvals and permissions

The study went through the designated process of ethical approval body. The National Research Ethics Service Committee North West–Greater Manchester East (reference number 12/NW/0207), an independent external ethics and peer review committee, approved the study at a meeting held on 26/4/2012. The committee meeting took place following online submission of application (project ID 101025, IRAS) on 2/3/2012, in accordance with the Declaration of Helsinki principles. Relevant permission was granted from the Research and Development Department at Alder Hey NHS Trust and joint sponsorship between the NHS Trust and the University of Liverpool was obtained. A Material Transfer Agreement was arranged and authorization was granted for tissue storage at the Human Tissue Authority (HTA) approved tissue bank (license number 12020) at the Wolfson Centre for Personalized Medicine, University of Liverpool. University and NHS indemnity was in place and study was fully compliant with the UK Department of Health Research Governance Framework (Appendices 1-3, ABC study protocol, case report files, ethical approval).

3.2.2. Study design and recruitment

The NIHR portfolio adopted the ABC study, which was registered in the publicly accessible database of the United Kingdom Clinical Research Network (UKCRN). Participating researchers were included in the ABC study delegation log after successful completion of Good Clinical Practice (GCP) training as per Declaration of Helsinki principles. Researchers accessed necessary information from medical records to facilitate completion of approved case record files. Access was gained to

NHS premises and medical records through substantive or honorary clinical contracts with Alder Hey Children's hospital on a strict need-to-know and confidential basis.

Taking into consideration the annual incidence of newly diagnosed IBD patients in the geographical area covered by Alder Hey Children's Hospital, as shown in annual records held in the department for over 5 years, i.e. around 70 new patients per year, a recruitment target of 60 patients in 12 months was proposed, with a plan to review feasibility, following the recruitment of the first 30 patients. ABC study was subsequently adopted by NIHR portfolio as commercial, non- interventional, industry funded and investigator led study; the initial recruitment target was agreed on a pilot basis so as to ensure a realistic recruitment target based on available time, resources and patient throughput, however ethical approval had been granted for recruitment up to 85 patients. ABC study started on a pilot basis, in order to draw preliminary descriptive data about putative role of ABC transporters in IBD susceptibility in the first instance, in parallel to the bench data, where the biologically plausible functional role of ABCB5 as CS transporter had been investigated. Correlation of gene and protein expression and clinical outcomes following CS administration was undertaken. The samples from UC, CD and IBDU patients were grouped under the umbrella term 'IBD' for the purpose of the analysis only, reflecting the key aim to describe variation of ABCB1/ABCB5 in health and disease (IBD).

Sample size calculation was done; the aim of the study was that ABCB1/ABCB5 genetic polymorphisms would be genotyped and correlated to the expression of the respective transporters at both mRNA and protein levels. Based on pilot data on ABCB5 gene expression in the large intestine of healthy controls from the NCBI geoprofiles database (http://www.ncbi.nlm.nih.gov/geoprofiles/49799877), mean

expression in both unaffected tissue and in wild-type patients was assumed to be 4.05 (SD:0.44). Based on this assumption and the patient annual throughput, a number of 85 study participants would give us 78% power to detect a difference of 5% between diseased and unaffected tissue and 99% power to detect a difference of 10%.

We would also have 35% power to detect a difference of 10% in mean expression between wild-types and carriers of the minor allele for SNPs with minor allele frequency (MAF) of 10% and 78% power to detect the same increase for SNPs with MAF of 30%. To detect a difference of 20%, we have 97% power where MAF is 10% and 99% power for SNPs with MAF of 30%.

Study participants were recruited in a consecutive, prospective manner under the guidance of the direct care consultant. Patients between 2 and 16 years of age who had been listed for diagnostic upper and lower gastrointestinal endoscopy at Alder Hey Children's Hospital NHS Foundation Trust were approached.

Patients diagnosed with pathology other than IBD, such as coeliac disease, enterobius vermicularis infection, eosinophilic gastro-intestinal disease, polyposis, malignancies, or patients/parents who did not consent/assent, were excluded from the study. Genotype data that will not pass standard quality control criteria, would also be excluded retrospectively.

In detail, cases were prospectively screened from planned endoscopy lists in a chronological order; written comprehensive information in the form of age appropriate patient information leaflets (PILs, Appendix 4-6) was posted out to families and patients a week prior to the planned date of the endoscopy. The same patients were then approached on the day of the endoscopy by the researchers who

further explained the study aims, answered questions and asked for permission to recruit. Once the process of informed consent according to GCP principles was completed, researchers obtained written informed consent from parents of eligible participants < 16 years of age, and assent on a case by-case basis, judging the participants' ability to understand the information presented. Demographic and clinical information obtained from patients included clinical symptomatology, indication for endoscopy, growth parameters, comorbidities, previous medical and, family history, medication and allergies. This information in addition to investigational reports (radiological, histological and laboratory information) were used to confirm or exclude a diagnosis of IBD. Recruited patients underwent upper and lower gastrointestinal endoscopy under general anaesthetic; additional mucosal endoscopic biopsies were taken according to the study protocol (Appendix 1) from different segments of the gastrointestinal tract, including, duodenum, terminal ileum and sigmoid. The inflammation status of a sample was based on the histology of a paired sample taken within 2 centimeters of samples at the same time of the endoscopy. Children with macroscopically and histologically normal mucosa with a diagnosis of IBD ruled out, served as the non-disease control group.

Biological samples collected during gastrointestinal endoscopy were assigned a study identification number for partial anonymization, and were transferred in designated containers for storage at the tissue bank of the Wolfson Centre for Personalized Medicine, University of Liverpool, (linked anonymized format, license held by the University of Liverpool, licensing number 12020). Coded and anonymized data files were safely stored in password protected university computers. Manual files were locked in designated offices at the University of

Liverpool. Chief Investigator (Professor Munir Pirmohamed) was the named Data and Sample custodian.

The study commenced recruitment on 31/05/2012 and initially recruited until 03/09/2012. The first substantial amendment was submitted to the ethics committee on 25/03/2013 (Appendix 7), and was approved on 13/04/2013 (Appendix 8). The substantial amendment temporarily paused the study recruitment to allow for prioritization of laboratory work on samples collected, including cell culture work and sample processing to ascertain satisfactory sample quality and quantity. The study recruitment resumed on 08/05/2013 following a second substantial amendment, ratified on 25/03/2013 by the same ethics committee mentioned previously. The second study amendment enabled additional collection of duodenum and terminal ileum biopsies, and extended the recruitment period of the ABC study until 30/09/2015. The study recruitment period therefore ended on 30/09/2015 after recruitment of 61 consecutive patients.

3.2.3. Clinical phenotypes and CS treatment outcome data

The diagnosis of IBD was established from the nature and duration of clinical symptoms, in addition to laboratory parameters, imaging, endoscopic and histological data from biopsies obtained during diagnostic gastrointestinal endoscopy (Ruemmele, Veres et al. 2014). Clinical phenotypes at diagnosis were categorized, according to the Montreal classification (Paris modification) for childhood onset CD and UC respectively as shown in Tables 3.1 and 3.2 below (Levine, Griffiths et al. 2011).

Table 3.1. Montreal Classification (Paris modification) for patients diagnosed with Crohn's disease: L1 ileal disease, L2 colonic disease, L3 ileo-colonic, L4 upper gastrointestinal disease, B1 non-stricturing, non- penetrating disease, B2 stricturing, B3 penetrating, p perianal disease modifier. CD severity increases with extended disease location, penetrating, stricturing or perianal disease.

Crohn's disease (CD)	**Clinical phenotype as per Montreal classification**
Age at diagnosis	A1 below 16 years A2 17-40 years A3 >40 years
Disease location	L1 ileal L2 colonic L3 ileo-colonic L4 isolated upper gastrointestinal disease
Disease behavior	B1 non-stricturing, non-penetrating B2 stricturing B3 penetrating P perianal disease modifier

Table 3.2. Montreal classification (Paris modification) of severity (S) and extent (E) of ulcerative colitis

Disease severity (S) or extent (E)	Clinical phenotype	Clinical symptoms
S0	Clinical remission	Asymptomatic
S1	Mild UC	Passage of four or fewer stools/day (with or without blood), absence of any systemic illness and normal inflammatory markers (erythrocyte sedimentation rate, ESR)
S2	Moderate UC	Passage of more than four stools per day but with minimal signs of systemic toxicity
S3	Severe UC	Passage of at least six bloody stools daily, pulse rate of at least 90 beats per minute, temperature of at least 37.5°C, haemoglobin of less than 10.5 g/100 ml and ESR at least 30 mm/h
E1	Ulcerative proctitis	Involvement limited to the rectum (that is, proximal extent of inflammation is distal to the recto-sigmoid junction)
E2	Left sided UC (distal UC)	Involvement limited to a proportion of the colon and rectum distal to the splenic flexure
E3	Extensive UC (pancolitis)	Involvement extends proximal to the splenic flexure
E4	Right sided	Limited to caecum, ascending colon and proximal to splenic flexure

According to current practice (Turner, Ruemmele et al. 2018), the majority of children with UC should have CS at diagnosis for induction of remission. Patients with UC in this study therefore received the recommended dose of steroids 1-1.5 mg/kg for 3-5 days post diagnosis; the dose was tapered by 5 mg every week and stopped (Ruemmele, Veres et al. 2014). The clinical response to steroids at 12 months was ascertained as per Tung et al (Tung, Loftus et al. 2006) and was performed according to physician global assessment in a prospective fashion. Clinical outcomes were also categorized according to a validated PUCAI at 3, 6 and 12 months after diagnosis (Turner, Otley et al. 2007, Hyams, Davis et al. 2017, Gasparetto, Wong-Spracklen et al. 2018). The PUCAI score was calculated by adding up individual scores for parameters, such as abdominal pain severity score, rectal bleeding frequency, type of stool consistency, average number of stools in 24 hours, presence of nocturnal stooling and physical activity category level calculated over 24-hour period; high PUCAI score indicated clinically more severe course.

According to recent guidelines (Ruemmele, Veres et al. 2014), CD patients who do not respond or do not comply with 6-8 weeks of exclusive enteral nutrition (EEN), require steroids for induction of remission.

3.3 Results

Sixty-one patients were recruited to the ABC study; of these ten patients were diagnosed with UC, nine patients were diagnosed with Crohn's and one patient was diagnosed with IBDU. Forty-one study participants were healthy subjects; 17 patients were excluded as per study protocol due to a diagnosis of enterobius vermicularis infestation (n=5), coeliac disease (n=1), orofacial granulomatous

disease (n=1), and inadequate sampling during procedure due to poor bowel preparation and limited colonoscopy (n=10). Forty-four patients (n=44) remained for further analysis. DNA samples of insufficient quality or quantity and participants with incomplete genotype data were excluded from the analysis (n=2) were healthy, (n=1) had UC, (n=1) had CD. Non-Caucasian individuals (n=4) were excluded. The non-Caucasian study participants were healthy controls (n=2) or patients with unclassified IBD (n=1) or UC (n=1). A total number of thirty-six patients; 16 IBD cases-8 with UC and 8 with CD -and 20 healthy controls were eventually included in downstream analysis. The flow chart below shows the various stages of the recruitment process and excluded participants:

n=61 ⟹ 10 UC, 9 CD, 1 IBDU, 41 controls

↘ n=17 (excluded as per study protocol) ⟶ n= 44 participants

Further exclusions ⟹ n=4 (non-Caucasian) and n=4 (poor genotype data) ⟹ n= 36

Demographics of final ABC study participants are included in Table 3.3 below. Median values have been calculated to better reflect the raw data, which is slightly skewed across tested parameters.

Table 3.3. Demographics of ABC study participants included in the final analysis

Study population	IBD	Healthy controls
Total number N=36	N=16 (8 UC, 8 CD)	N=20
Sex (percentage)	75% male, 25% female	55% male, 45% female
Median age in years (range)	9.8 (5.9-14.6)	10.4 (2-16.6)
Median weight in kilograms (range)	26.9 (20.4-70)	46.6 (11.2-91)

Median height in centimeters (range)	138.1 (121.2-158.8)	147.1 (95-178.9)
Family history of IBD (percentage)	N=5 (31.25%)	N=3 (15%)

Clinical phenotypes of the study patients diagnosed with CD and UC respectively, classified as per Montreal classification (Paris modification) (Levine, Griffiths et al. 2011), are shown below in Tables 3.4 & 3.5. The observed response to CS treatment of UC patients measured by PUCAI scores at 3, 6, 12 months post diagnosis is graphically shown in Table 3.6.

Table 3.4. Clinical phenotype of study patients diagnosed with Crohn's as per Montreal classification: A=age, A1=below 16 years of age, B=behavior, B1=non-stricturing, non-penetrating, B2=stricturing disease, B3=penetrating disease, G=growth, G0=growth not impaired, G1=impaired growth, L=location, L1=ileal distribution, L2=colonic distribution, L3=ileocolonic distribution, L4=upper gastrointestinal tract involvement, p=perianal disease phenotype.

Crohn's disease n=9	Age	Disease behavior	Disease location	Growth
Clinical phenotype asper Montreal classification	A1 (<16 y)	B1 n=6 B2 n=1 B3 p n=2	L2/L4 n=1 L3 n=2 L3/L4 n=5 L1/L4 n=1	G0 n=5 G1 n=4

Table 3.5. Clinical phenotypes for ABC patients diagnosed with ulcerative colitis (UC) shown as per Montreal classification (Paris modification) with disease extent (E) and severity (S). S1=mild UC, S2=moderate UC, S3=severe UC. Disease extent (E): E1=proctitis, E2=left sided UC, E3=pancolitis, E4=right sided UC.

Ulcerative colitis (n=10)	Disease extent	Disease severity
Clinical phenotype	E2 n=2 E3 n=8 E4 n=0	S1 n=1 S2 n=7 S3 n=2

The observed response to CS treatment of UC patients measured by PUCAI scores at 3, 6, 12 months post diagnosis is graphically shown in Table 3.6.

Table 3.6. Clinical outcomes according to PUCAI score and response to CS at 3,6,12 months post diagnosis as per Tung et al (Tung, Loftus et al. 2006). PUCAI<10=quiescent UC, PUCAI 10-34= mild UC activity, PUCAI= 35-64 moderate UC activity, PUCAI> 65=severe UC activity.

UC Study number	PUCAI 3 months	PUCAI 6 months	PUCAI 12 months	Response to steroids (as per Tung et al)
1	<10	<10	<10	Steroid responsive

6	<10	<10	40	Steroid responsive
14	<10	<10	<10	Steroid responsive
15	40	20	<10	Steroid dependent
17	<10	<10	45	Steroid responsive
26	<10	<10	<10	Steroid responsive
34	<10	<10	<10	Steroid responsive
36	20	40	40	Steroid dependent
45	<10	<10	<10	Steroid responsive
55	25	60	30	Steroid dependent

As far as CD patients are concerned, the majority of ABC study patients with CD, except two patients, successfully responded to EEN administration for induction of remission and showed sustained clinical remission at 12 months post diagnosis without the need for CS administration. One patient had perianal fistulizing disease and required early anti-TNFα (infliximab) administration and surgical input at diagnosis. The second patient improved clinically in the first two weeks of EEN, but due to lack of compliance, eventually required a course of CS to achieve clinical remission.

3.4 Discussion

3.4.1. Interpretation of key results and limitations

Children with UC exhibited a phenotype of pancolitis, with 30% steroid dependence and 70% steroid responsiveness at 3 months post diagnosis and CS introduction; no patients from the ABC cohort were steroid resistant. PUCAI at 3 months was predictive of clinical outcome at 12 months in 2 out of 3 cases. CD patients mostly had an ileocolonic distribution of disease with upper gastrointestinal involvement; the

disease phenotype at presentation is known to be relatively more aggressive in childhood onset IBD as described in Chapter 1.

There was a male preponderance in IBD patients. Positive family history of IBD, was noted almost twice more commonly in cases than controls.

The large majority of recruited patients were Caucasian; ethnicity could not be matched and this should have addressed during the consideration of study design, however selection bias had to be avoided. Furthermore, in terms of ethics, all patients were given the chance to be considered for recruitment, irrespective of ethnicity. As the study was carried out in a country with large majority of Caucasian inhabitants, researchers had anticipated lower throughput of non-Caucasian patients, however the exact final numbers could not be predicted prospectively. Normally, subgroups of different ethnicities would have been included in the analysis and/or adjustment for genetic markers of ethnicity not linked to the disease in question could have been taken into consideration. Transethnic studies in large numbers, collaboration between various research groups internationally or development of tissue banks could have addressed this limitation of the ABC pilot study.

The power calculation was done based on ABCB1/ABCB5 SNP MAFs from public data repositories, therefore had some predictive value with regards to advance planning for number of participants; the final number of study participants was actually smaller, mainly due to time and resource constraints and due to the significant extension of necessary bench work.

3.4.2. Future work

Future work would include funding application to charitable bodies to ensure adequate funding and early consultation with experts with proven track record in genomics of childhood onset IBD; this research field has been expanding dramatically since inception of the ABC study and collaboration with other paediatric IBD centres and/or use of existing genetic data repositories in order to increase the power of the study would be facilitated. The study protocol would give emphasis to recruitment across different ethnicities and power calculation would address the aim of assessing treatment response for various commonly used therapies in IBD, such as biologics, over different timepoints post diagnosis. The integration of useful parameters related to therapeutic drug monitoring other than clinical activity indices, for instance anti-TNF a levels and antibodies would be implemented. Susceptibility genes associated with more aggressive clinical outcomes requiring therapy intensification or top down approach, or even early surgery would also be ascertained from public data depositories and concurrent GWAS or transethnic studies. Epigenetic parameters affecting disease pathogenesis, diet, stress, administration of concurrent medication and other potentially confounding factors that may affect disease course would also need to be considered. The primary and secondary outcomes for example time to mucosal healing and time to surgery would shape the study protocol and subsequent statistical analysis. The future work would focus on key ABC transporters known to have proven pharmacogenomic influence in vivo and in vitro rather than novel ones where relatively limited data on disease susceptibility or treatment outcomes has been published.

Chapter 4

ABCB1 and ABCB5 genotypes in children newly diagnosed with Inflammatory bowel disease and healthy controls

4.1 Introduction

IBD onset during childhood can present with a more extensive disease phenotype than in adults. Furthermore, a diagnosis of IBD can be detrimental for growth and psychosocial development with higher surgery rates and poor outcomes (Hansen, Jakobsen et al. 2015). The clinical response to CS in healthy and treatment naïve children with newly diagnosed IBD has been the topic of paediatric retrospective clinical studies

(Gasparetto, Wong-Spracklen et al. 2018). Small retrospective cohorts have shown that up to10 per cent of children with UC who receive CS for induction of remission will be resistant, with the remainder showing a fairly equal split, between steroid responsiveness and steroid dependence (Newby, Croft et al. 2008), similar to observations from adult UC populations (Ho, Soranzo et al. 2006). Medical evidence shows that induction of clinical remission after the first CS regime is largely successful in UC; subsequent disease relapses however signify higher CS resistance or CS dependence (Tung, Loftus et al. 2006, Newby, Croft et al. 2008). Both of these outcomes although by definition completely different, translate to similar endpoints for patients, which is escalation to alternative treatment or consideration for surgical management.

The *ABCB1* SNPs C3435T (rs1045642) in exon 26 and the missense mutation G2677T (rs2032582) in exon 21 have received a lot of attention with regards to their effect on intestinal expression of ABCB1 (Ho, Soranzo et al. 2006). Correlation between IBD susceptibility and ABCB1 expression has been shown in vivo by Langmann et al (Langmann, Moehle et al. 2004), and in vitro by Kerb et al (Kerb, Hoffmeyer et al. 2001). Soranzo et al noted a discrepancy between genetic polymorphisms and expression of ABCB1 within and across different populations, due to a presumed lack of true causality and LD between causative and non–causative genetic polymorphisms in close proximity within the genome (Soranzo, Cavalleri et al. 2004). Ho and colleagues (2006) however concluded that, accounting for haplotype variations, the contribution of germ line variations is most significant in extensive colitis (Ho, Soranzo et al. 2006). LD with a causal variant may be a plausible explanation of how a specific genetic polymorphism can play a significant role in clinical outcome (Ho, Moodie et al. 2003). Genetic association

studies are often imperfect proxies for causality alleles and can substantially underestimate the true attributable risk; the observed discrepancies amongst published studies may be due to the differences in design, heterogeneity between populations, differences in statistical methodology, variability in outcomes and result interpretation. Intricate interplay between patient, disease and treatment related factors affect the observed outcomes.

The genetic overlap between adults and children needs to be explored, therefore there is a need for more paediatric studies with focus on loci that are associated with disease susceptibility or treatment outcome and disease prognosis.

A research collaboration between the University of Liverpool, Alder Hey Children's Hospital and Institute of Genetics and Molecular Medicine, University of Edinburgh was established. Researchers from the Western General Hospital and the University of Edinburgh had studied the outcome of CS therapy in a 10-year IBD adult inception cohort. A stable inherent phenotype was shown, where about 50% of individuals had complete response to CS, whilst the remainder had a partial or no response as defined per Mayo criteria (Ho, Chiam et al. 2006). 768 tagging SNPs in 173 candidate genes related to drug transport, metabolism, effect of CS and disease susceptibility, membrane transport proteins, xenobiotic metabolism and respective transcriptional regulators, as well as glucocorticoid responsive genes, mined from PharmaGKB website, or previously identified from GWAS meta-analysis (Barrett, Hansoul et al. 2008), were targeted; patient samples from the Scottish IBD adult cohort were genotyped (Illumina Golden Gate technology). Genotype calls that deviated from HWE were excluded. 5 tagging SNPs (of 10 highest ranked associations) within the ABCB5 gene were significantly associated with CS

response, with the most significant associations detected for ABCBC5 rs10266329 (C-allelic frequencies 0.475 vs 0.292 in incomplete versus complete responders, OR 1.62, p=5,46 $x10^{-5}$ in both UC and CD. Furthermore C-allele was significantly higher when compared to healthy individuals (OR 1.35, P=0.001). A-allele frequency of rs10488577 was significantly higher in partial and non-CS responders, in comparison to healthy controls (OR 2.12, p=6,6 $x10^{-5}$). ABCB5 rs12669866 was the third SNP with higher MAF in the group of partial or non-VS responders (OR 1.31, P=3.5 $X10^{-4}$). Further anecdotal evidence from the above adult GWA study (Ho et al, personal communication), showed that *ABCB5* SNPs may play a role in increase susceptibility to UC (OR=1.16, P< 5.9 $x10^{-7}$), however this association did not reach GWAS significance levels. Extended in silico analysis of whole blood microarray dataset annotated to represent 33,296 genes demonstrated higher, however non-statistically significant ABCB5 expression in CS non-responders, compared to controls (p=0.004, delta (Δ) Mean 0.91 ± 0.27) and to CS responders (p=0.08, delta (Δ) Mean 0.44 ± 0.25), (Ho et al unpublished data). Intestinal ABCB5 expression did not differ between responders and non-responders, in contrast to the highly homologous ABCB1 inter-individual variation in expression previously demonstrated by the same research group (Ho, Gaya et al. 2005). The above three intronic tagging SNPs within the ABCB5 gene were significantly associated with CS response and treatment outcome within one year from diagnosis (Ho et al, personal communication). Genotypic variation of the three intron SNPs upstream *ABCB5* gene (rs10488577, rs12669866, rs10266329) corresponded to a size of the effect (OR=1.1, p<0.0006), with clear differentiation between CS responders when compared with the combined group of partial and non-responders. The aim of the ABC study presented in this chapter is to characterize ABCB1/ABCB5 genotypes for

common ABCB1 and selected ABCB5 SNPs as per Ho et al (personal communication) in an inception cohort of children with and without IBD.

4.2 Methods

4.2.1. Genotyping

Genomic DNA was extracted from whole blood ethylenediaminetetraacetic acid (EDTA) samples, using the Chemagic Magnetic Separation Module I according to the manufacturer's protocol. The quality and quantity of extracted genomic DNA was subsequently assessed using UV/VIS spectrophotometry on the NanoDrop 8000 (ThermoFisher Scientific). Three exonic SNPs in ABCB1 and three intronic SNPs in ABCB5 (detailed in Table 3.3) were selected and genotyped using predesigned TaqMan genotyping assays (rs1045642, rs2032582, rs1128503, rs10488577, rs12669866, rs10266329 (ThermoFischer Scientific). TaqMan assays were synthesized, optimized and quality control tested (functionally pretested on 20 human DNA samples to ensure assay amplification and sample clustering) by ThermoFisher Scientific. Each PCR reaction was carried out in a 5µl mixture, consisting of 20 ng genomic DNA, 20x genotyping assay (0.25 µl), 2xTaqMan universal PCR mastermix (2.5 µl) and DNAse free water (2.25 µl). Each sample was genotyped in triplicate on a 384-well plate, with two 'no template' controls included per assay tested.

Table 4.1. Allele frequencies of ABCB1 and ABCB5 SNPs in Caucasian /European (North Western) population. Allele frequencies and base pair position of SNPs are based on Ensembl GRCh37 release 93.

SNP Accession number	Chromosome 7 Base pair position	Gene position	Allele frequency (A1, A2)
rs1045642	87509329	ABCB1 Exon, synonymous	G=0.56 A=0.44

rs2032582	87531302	ABCB1 Exon, missense	C=0.56, A=0.43, T=0.01
rs1128503	87550285	ABCB1 Exon, synonymous	G=0.57, A=0.43
rs10488577	20707452	ABCB5 Intron	C=0.91, A=0.1
rs12669866	20742851	ABCB5 Intron	C=0.57, T=0.43
rs10266329	20752178	ABCB5 Intron	T=0.66, C=0.34

4.2.2. Blood sample and SNP (genotyping) quality control

Genotypes were categorized as 'missing' if samples had failed quality control procedures. Patients with incomplete or inaccurate data, and DNA samples of insufficient quality or quantity, were omitted from the analysis. Excluded patients as per study protocol were also excluded from downstream analysis regardless of quality of genotyping data.

All SNPs were quality checked. One of the ABCB1 SNPS was tri-allelic (rs2032582), and therefore two Taqman genotyping assays with different VIC and FAM reporter dyes linked to the 5' end of each probe for the two minor alleles A and T were tested separately for all included participants. Hardy Weinberg equilibrium (HWE), minor allele frequency (MAF) and genotyping call rate were calculated using the HaploView software v4.2 (Barrett, Fry et al. 2005) (www.broad.mit.edu/mpg/haploview/) for cases and controls. A p value less than 0.001 indicated deviation from HWE. A MAF threshold <5 % was applied. Relaxation of the genotype call rate from 90% to 80%

permitted inclusion of SNP data that had previously passed quality control check, thus avoiding introduction of selection bias if strict 90% genotype call rate had been implemented. The 90% genotype call rate has been the standard methodological approach in candidate gene analyses. However, this pilot study focuses on investigation of selected ABCB5 SNPs based on anecdotal data from GWA adult study by Ho et al (personal correspondence).

4.2.3. Haplotype and statistical analysis

Linkage disequilibrium (LD) and statistical analysis were performed using HaploView (Barrett, Fry et al. 2005). Haplotype blocks were assigned using the internally developed Solid Spine of LD method, in which the two end markers are in strong LD with intervening markers; intervening markers are not necessarily in LD with each other (Barrett, Fry et al. 2005). Differences in genotype frequencies between IBD cases and healthy controls for each SNP were calculated via the Pearson's chi squared test in HaploView (Barrett, Fry et al. 2005).

4.3 Results

4.3.1. Genotyping

Genomic DNA was extracted from whole blood ethylenediaminetetraacetic acid (EDTA) samples, using the Chemagic Magnetic Separation Module I according to the manufacturer's protocol. The quality and quantity of extracted genomic DNA was subsequently assessed using UV/VIS spectrophotometry on the NanoDrop 8000 (ThermoFisher Scientific). Three exonic SNPs in ABCB1 and three intronic SNPs in ABCB5 (detailed in Table 4.2 below) were selected and genotyped using

predesigned TaqMan genotyping assays (rs1045642, rs2032582, rs1128503, rs10488577, rs12669866, rs10266329 (ThermoFischer Scientific). TaqMan assays were synthesized, optimized and quality control tested (functionally pretested on 20 human DNA samples to ensure assay amplification and sample clustering) by ThermoFisher Scientific. Each PCR reaction was carried out in a 5µl mixture, consisting of 20 ng genomic DNA, 20x genotyping assay (0.25 µl), 2xTaqMan universal PCR mastermix (2.5 µl) and DNAse free water (2.25 µl). Each sample was genotyped in triplicate on a 384-well plate, with two 'no template' controls included per assay tested.

Table 4.2. Allele frequencies of ABCB1 and ABCB5 SNPs in Caucasian /European (North Western) population. Allele frequencies and base pair position of SNPs are based on Ensembl GRCh37 release 93.

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rs1128503	87550285	ABCB1 Exon, synonymous	G=0.57, A=0.43
rs10488577	20707452	ABCB5 Intron	C=0.91, A=0.1
rs12669866	20742851	ABCB5 Intron	C=0.57, T=0.43

rs10266329	20752178	ABCB5 Intron	T=0.66, C=0.34

4.3.2. Univariate analysis of SNP association between IBD cases and healthy controls

After excluding samples of poor quality (n=5) and non-Caucasian individuals (n=4), a total number of thirty-six patients remained for downstream analysis, of 16 were IBD cases and 20 were healthy controls. Genotyping analysis for the triallelic ABCB1 rs2032582 SNP showed that the lower frequency T allele was not identified in any of the study participants' samples. According to Ensembl GRCh37 release 93, the reported MAF of the T allele for above SNP in North-Western European population is 1% and our small sample size is underpowered to detect such low frequency. Of the remaining six SNPs investigated, all were found to be in HWE with $p>0.001$ and all MAFs were >5% as shown in Table 4.3 below. The observed MAFs for IBD cases and healthy controls in our ABC study population were comparable with the MAFs from 1000 Genomes reference population of Caucasian/European of North and Western European ancestry, as noted in Ensembl GRCh37 release 93.

Table 4.3. Haploview analysis showing Hardy Weinberg equilibrium, genotype call rate and minor allele frequencies of ABCB1 and ABCB5 single nucleotide polymorphisms for IBD patients and healthy controls.

	Healthy controls			IBD		
SNP (rs)	HWE-p value	% genotype call rate	MAF	HWE-p value	% genotyping call rate	MAF
rs10488577	0.05	97.2	0.21	0.09	100	0.18
rs12669866	0.54	80.6	0.44	1.0	81.2	0.34
rs10266329	0.79	94.4	0.29	0.29	87.5	0.32
rs1045642	0.79	97.2	0.41	1.0	100	0.34
rs2032582	0.28	100	0.47	0.92	100	0.31
rs1128503	1.0	100	0.48	1.0	100	0.46

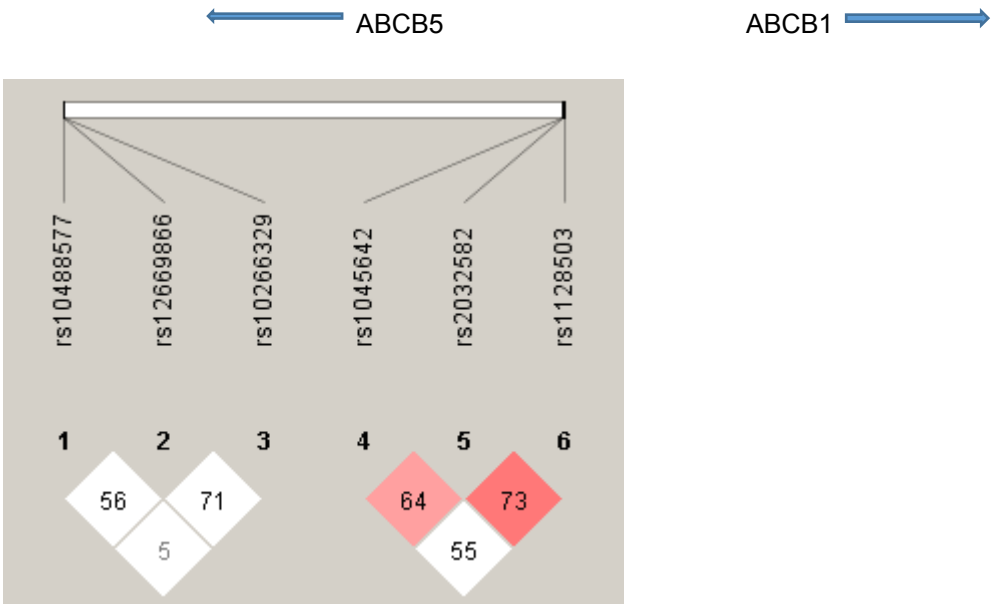
Table 4.4 summarizes the association results of the six SNPs investigated and the genotype and allele frequencies of ABCB1/ABCB5 SNPs in the ABC study population Interestingly, rs2032582 showed a significantly higher A allele frequency (p=0.01). This p value reflects higher frequency of variant allele in cases compared to controls. To account for multiple testing, the Bonferroni correction was performed; following this, the p value for rs2032582 did not remain significant. No genotype related frequency distribution therefore was observed between IBD cases and controls.

Table 4.4. Allele and genotype frequencies of ABCB1 and ABCB5 single nucleotide polymorphisms in IBD patients and healthy controls from the ABC study population.

SNP Accession number	Gene position	Gene	Chromosome 7 base-pair position		Alleles	Allele Frequency IBD patients A1 A2	Allele Frequency Healthy controls A1 A2	p value	Genotype Frequency IBD patients A1/A1 A1/A2 A2/A2	Genotype Frequency Healthy Controls A1/A1 A1/A2 A2/A2	p value
rs1045642	Exon	ABCB1	87509329		A/G	0.66 0.34	0.59 0.41	0.27	0.44 0.44 0.12	0.21 0.63 0.16	0.35
rs2032582	Exon	ABCB1	87531302		A/C	0.69 0.31	0.53 0.47	0.01*	0.5 0.375 0.125	0.4 0.4 0.2	0.08
rs1128503	Exon	ABCB1	87550285		A/G	0.54 0.46	0.52 0.48	0.49	0.19 0.56 0.25	0.3 0.5 0.2	0.73
rs10488577	Intron	ABCB5	20707452		C/A	0.82 0.18	0.79 0.21	0.61	0.75 0.125 0.125	0.64 0.22 0.11	0.59
rs12669866	Intron	ABCB5	20742851		C/T	0.66 0.34	0.56 0.44	0.15	0.38 0.54 0.08	0.31 0.375 0.31	0.16
rs10266329	Intron	ABCB5	20752178		T/C	0.32 0.68	0.71 0.29	0.67	0.43 0.57 -	0.55 0.35 0.1	0.16

ABCB1 and ABCB5 are located approximately 67 Mb away from each other on chromosome 7. Linkage disequilibrium (LD) analysis of the six SNPs across the two genes showed no distinct haplotype blocks, therefore haplotype analysis was not undertaken. LD is the non-random of association between alleles from different loci on the same chromosome. As such, the above SNPs are independent of each other within the population and the occurrence of one does not affect the occurrence of the other.

Figure 4.1. Linkage disequilibrium (LD) display of ABCB5 and ABCB1 SNPs for the ABC study population generated using Haploview 4.2. Colored squares show relatively higher probability of SNPs inherited together than in the case of white squares. Numerical values are measures of LD (D'=LD coefficient) expressed in percentages; LOD score is a statistical estimate of whether SNPs are likely to be located near each other in a chromosome and are therefore likely to be inherited. The strength of LD is shown in increasing shades of blue to red, as depicted by the colored bars.



	D'<100%	D'=100%
LOD<2	White	blue
LOD≥2	shades of pink/red	bright red

4.4 Discussion

4.4.1. Role of ABCB1 and ABCB5: interpretation of key results

In this study, samples and SNP data were quality checked with the use of standardized, established techniques. This study provides original data comparing genotypic variation of *ABCB1* and *ABCB5* in children with newly diagnosed IBD and healthy controls. This study looked was a pilot study, but its design as a prospective study, avoided bias that would have arisen from use of data from retrospective cohorts.

The calculated ABC study population MAFs for the *ABCB1* SNPs in IBD patients (cases) and healthy controls were comparable with the MAFs of the reference population of Caucasian/European of North and Western European ancestry, as noted in Ensembl GRCh37 release 93 (July 2018). There was no statistical difference between patients and healthy controls for rs1045642; the heterozygotes were the majority in the general population and likewise in the ABC study group, however in IBD patients an equal split was observed between heterozygotes and homozygotes for the ancestral allele. As far as rs1128503 is concerned, there were more heterozygotes noted than homozygotes for either ancestral or minor allele. There was no statistical difference between ABC study patients and controls.

The *ABCB1* rs2032582 SNP minor allele was significantly more common in IBD patients than in healthy controls in the ABC study population, but this association did not withstand correction for multiple testing. This is possibly due to the small sample size; difficulties in acquiring tissue samples in children pose limitations to genotype-phenotype association studies, particularly when dealing with associations that have low effect size in complex disease, where studies are not powered to detect effect of

this size. Genetic polymorphisms with low MAF require even larger sample sizes to detect variants conferring high ORs (Wong, Yang et al. 2003). MAFs of selected SNPs were equal or above 1% in the North Western European population, as per Ensembl GCHr37 release 93 (July 2018).

ABCB5 SNP rs10488577 showed comparable allele frequencies in general population and ABC study population, with no statistical difference between IBD patients and controls. As far as *ABCB5* SNP rs12669866 is concerned, the majority were heterozygotes for tested alleles in the general and study population, without any statistical difference between cases and controls. There were more heterozygote IBD patients than healthy controls in ABC study population for *ABCB5* SNP rs10266329; the ancestral gene for *ABCB5* SNP rs10266329 was observed in higher frequencies in the North Western European population, as per Ensembl GRCh37 release 93.

The function of *ABCB5* genotype variation in children is unknown. The *ABCB5* SNPs, highlighted from adult GWAS by Ho et al, have been selected for downstream analysis in the ABC study population. All selected ABCB5 SNPs have MAFs above or equal to 1% in the North Western European population, as per Ensembl GCHr37 release 93.

ABCB5 SNPs may have a functional effect on the encoded protein, by either interfering with the NBD structure (rs61732039, rs34603556, rs2301641), or by potential substrate binding (rs6461515) and communication between ATP sites (rs58795451) (Moitra, Scally et al. 2011). According to the Bayesian approach (Kemper, Bowman et al. 2018), all SNPs within a gene can be candidates for clinical phenotype variation; at the same time one polymorphism can affect multiple traits. A

genomic region conserved through evolution is more likely to affect variation in trait expression. Variants with higher MAFs have a higher chance of a link or association with clinical phenotypes. Candidate gene analysis involving exon or deep whole genome sequencing of the genomic region where the causal variants may reside could be informative. Exon SNPs can affect translation directly. Intron and promoter region SNPs such as the tested ABCBC5 SNPs can also indirectly influence gene expression. Genetic association studies are often imperfect proxies for causality alleles and can substantially underestimate the true attributable risk; the observed discrepancies amongst published studies may be due to the differences in design, heterogeneity between populations, differences in statistical methodology, variability in outcomes and result interpretation. Intricate interplay between patient, disease and treatment related factors affect the observed outcomes.

4.4.2. Study limitations

This study is a pilot study therefore limited by its small sample size, which translates to limited power. In addition, the large majority of study patients were Caucasian, and therefore results may not be directly applicable to other populations. Genotype data was available for over 80% of the population (patients and controls) for three of the six SNPs; 90% availability of SNP genotype data is the standard threshold used in candidate gene studies to proceed with inclusion of the relevant SNP in the analysis. Another limitation of this clinical pilot study is that it lacks power to investigate how genetic polymorphisms are associated or may influence clinical outcomes such as patient response to various treatment strategies post diagnosis (for example steroid responsiveness or steroid dependence). Such a small number does not allow generalizable conclusions to be drawn, and multiple testing could

cause a type 2 error, therefore no statistical attempt was made to correlate genotypes with CS response.

4.4.3. Implications for future work

Investigation of candidate genes implicated or linked to disease pathogenesis and treatment response can lead to development of novel biomarkers of treatment efficacy, so that treatment strategies could be individualized (Slager, Schaid et al. 2003). The striking overlap of susceptible genomic loci across the spectrum of chronic, immune mediated diseases, shows shared immune regulatory pathways; other chronic diseases paradigms could therefore be relevant. Working towards an integrated clinical, serological, molecular and genetic classification of IBD will require united and coordinated efforts through networking and collaboration (de Souza and Fiocchi 2018), with focus on susceptibility and treatment influencing genes in various populations (Liefferinckx and Franchimont 2018). Environmental factors, epigenetics and metagenomics will also have a role to play in future studies.

Collaboration on a large scale will help us overcome difficulties such as limited power, in order to achieve a multi-omics profiling approach and highlight specific data signatures indicative of disease status and behaviour with the potentially clinical relevance in the future; collaboration will allow us to build our knowledge of how genetic and environmental factors shape disease prognosis and clinical outcomes over time; correlating genome wide molecular signatures with treatment outcome measures remains a major challenge. Multicenter international networking will therefore be necessary; biobanks such as UK Biobank is a paradigm of a large adult data repository, continuously updated and expanded, where academia and industry can share forces in accumulating life style information, clinical and genomic data for

common diseases, with a view to understand disease pathogenesis, disease course and any shared associations in various populations over time. Such a paradigm could be replicated in paediatrics (www.ukbiobank.uk.ac. accessed 28/07/2019).

Research should ultimately target the future development of pharmacogenomic based dosing algorithms, both for maintenance and initiation of treatment in various populations. Exploring variability in drug transporter expression and function can allow appreciation of variability in response to treatment (Peng, Zhang et al. 2016). An additional key factor that will require consideration, as it would significantly influence the implementation of pharmacogenetics based dosing, is treatment cost (Cascorbi 2018).

Chapter 5

Gene expression in blood and intestinal epithelium of children newly diagnosed with inflammatory bowel disease and healthy controls

5.1 Introduction

Gene expression analysis can provide an assessment of the number of genes that change in disease providing information on genetic susceptibility and pathogenesis (Russo, Lombardelli et al. 2017). Song et al used DNA microarray data to elucidate genetic susceptibility to UC (Song, Li et al. 2018). Gene array studies from mucosal biopsies in patients with UC have identified predictive panels for genes associated with non-response to infliximab (Arijs, Li et al. 2009). Noble et al investigated gene expression in the intestinal epithelium of adult patients with CD and controls, reporting downregulation of the organic solute carriers SLC38A4 and SLC26A2, and upregulation of CD susceptibility genes identified from GWA studies, such as IL-23A, JAK2 and STAT3 (Noble, Abbas et al. 2010)

CS dependence is a predictor of relatively poor clinical outcome (D'Haens, Colombel et al. 2008, D'Haens, Reinisch et al. 2017). Response to CS can to some degree, reflect disease behavior/progression in the short, medium and long term and highlight the need for timely escalation of treatment (Ho, Chiam et al. 2006). The pathophysiology and molecular basis of CS resistance in IBD is unclear (Bousvaros, Sylvester et al. 2006).

ABC transporters have been implicated in CS resistance, but work has mostly focused on ABCB1, which can be upregulated in PBMCs of patients with UC or CD who received CS (Farrell, Murphy et al. 2000, Ho, Nimmo et al. 2005). Inter-individual variability in expression has an effect on the overall bioavailability of CS and thus drug efficacy. ATP binding cassette family transporters such as ABCB1 and ABCG2 may have impact on pharmacokinetics of numerous drugs and could modulate the effectiveness of drug treatment (Farrell, Murphy et al. 2000). More

recently, this topic has been the center of attention by the United States Food and Drug Administration (FDA) due to potential drug-drug interactions, that would need to be taken into account for the development of investigational new drugs (Giacomini and Huang 2013).

5.2 Study aim

The aim of the study presented in this chapter was to investigate variability in gene expression of ABCB1 and ABCB5 in the blood and the gastrointestinal tract of children newly diagnosed with IBD and healthy controls, with the implementation of standard gene expression methodology. Correlation of ABCB1 gene expression with clinical phenotypes of ABC study participants (IBD cases versus controls) was also performed, allowing for assessment of impact of SNP genotypes on relative ABCB1 gene expression. A subgroup analysis was conducted to assess the association between ABCB1 gene expression in blood and sigmoid colon and clinical response within 12 months for the 8 UC patients who received CS at diagnosis.

Ho et al (Ho, Soranzo et al. 2006) had previously shown ABCB1 gene expression variability in an adult IBD cohort. Other adult studies have compared healthy subjects to patients with IBD (Englund, Jacobson et al. 2007) and have shown inter-individual variability in ABCB1 gene expression (Taipalensuu, Tornblom et al. 2001) with downregulation of expression in diseased areas of the gut and upregulation following exposure to CS treatment (Farrell, Murphy et al. 2000, Hirano, Onda et al. 2004).

5.3 Methods

5.3.1. Human samples collection and storage (blood and intestinal biopsies)

Blood and sigmoid biopsies were collected for research purposes during diagnostic upper and lower gastrointestinal endoscopy from the first 28 consecutive patients recruited to the study, following informed consent/assent process. After the second study amendment additional samples were collected from duodenum and ileum of the remaining 33 recruited subjects, to allow for determination of ABCB1 and ABCB5 gene expression across different parts of the gastrointestinal tract. Routine diagnostic biopsies from different parts of the gastrointestinal tract were taken for histology as per usual practice.

Whole blood was stored in PAX gene (patented RNA stabilization technology, Preanalytix, catalogue number 762164) and EDTA tubes, designed for pure RNA and DNA isolation respectively. PAX gene tubes were transferred to -80^{o}C freezer within two hours, where they were stored until further processing, according to manufacturer's recommendations. EDTA tubes were stored at 4^{o}C. Gut biopsies were stored in tubes containing formalin as part of routine endoscopic procedure and were partially anonymized and transferred to Histopathology laboratory in Alder Hey for safe storage and routine analysis. Additional intestinal biopsy samples were stored in RNA later (RNA stabilization reagent solution), in order to avoid unwanted changes in gene expression profile. Samples were initially stored at 4^{o}C overnight and subsequently transferred to -20^{o}C freezer at the University of Liverpool for safe storage until further processing.

5.3.2. RNA extraction from gut biopsies

Tissue samples were thawed to room temperature. RNA was extracted according to the micro-RNA Easy Mini Handbook by Qiagen protocol 07/2012 (pages 23-24), titled 'Purification of Total RNA from animal tissues', using the well-established technology for RNA purification, combining the selective binding properties of a silica-based membrane with the speed of micro-spin technology. Biological samples were transferred to Precellys 2 mL soft tissue homogenization ceramic bead tubes and were homogenized with the use of the Minicelys device. Homogenized material was lysed in the presence of Qiazol Lysis Reagent, a highly denaturing guanidine-thiocyanate-containing buffer. Tubes with homogenized samples were shaken vigorously for 15 sec after 140µl chloroform was added; then they were placed on benchtop to stand for 2-3 minutes in room temperature and were subsequently centrifuged at 12000 g for 15 minutes at 4ºC. The aqueous phase of each sample was transferred to a new reaction tube and 350 µl of 70% ethanol volume was added and mixed thoroughly by vortexing. The samples were then transferred to RNA Easy Mini Spin columns placed 2 ml collection tubes, which were gently centrifuged for 30 seconds at 8000 g at room temperature. The flow through was pipetted into 2 ml reaction tubes.

5.3.3. RNA extraction from blood samples

Blood samples stored in PAX gene tubes were processed according to Blood RNA Kit Qiagen Handbook, version 2, 2015. Samples were centrifuged, and nucleic acids were pelleted. The supernatant was discarded and cell pellets were then washed and suspended in suspension buffer in micro-centrifuge tubes as per the manufacturer's protocol for purification of intracellular RNA from animal cells.

Proteinase K and binding buffer were added. After incubation, samples were transferred in 2 ml collection tubes. 700 µl of each sample was transferred to RNeasy spin column placed in a 2 ml collection tube and was centrifuged at 8000 g for 15 seconds; flow through was discarded. RW1 buffer was added (700 µl) and centrifuged as above, flow through was discarded. Buffer RPE 500µl was added to the RNeasy spin column, which was centrifuged for 15 seconds at 8000 g. The on-column DNAase digestion step was performed twice to eliminate genomic DNA contamination. RNA easy spin column was placed in a new 1.5 ml collection tube; 50µl RNase-free water was added directly to the spin column, which was then centrifuged for one minute at 8000 g to elute the RNA; this step was repeated twice in order to increase final RNA concentration. After washing, samples were eluted and RNA quantity and quality were checked with Nanodrop spectrophotometry. Samples were denatured by heating to 65ºC for use in downstream analysis.

5.3.4. Reverse transcription and gene expression assay

The extracted RNA yield was measured and quality controlled by the ratio of absorbance at 260 and 280 nanometers, using Nanodrop spectrophotometry technology (ND-100 TF Scientific, MA, USA). The nucleic acid purity test of A260/ A280 ratio measured between 2 and 2.2 for RNA; nucleic acids that passed quality control were used for downstream applications. Reverse transcription of extracted RNA was performed using the Invitrogen Superscript II reverse transcriptase kit according to the manufacturer's protocol. 500 nanograms of RNA was mixed with water, random hexamers and deoxynucleotide triphosphate solution (dNTPs) in the amounts stated in table 4.1 below and the mix was incubated for 5 minutes at 65ºC. The reaction mix was chilled to 4ºC before adding 5xfirst-strand buffer and DDT;

following incubation at room temperature for 2 minutes, superscript II enzyme was added to the reacting mix. Using a thermal cycler (Thermofischer UK), the reaction was heated to the following temperatures sequentially; 25ºC for 10 minutes, 42ºC for 50 minutes and 72ºC for 15 minutes.

Table 5.1. Reagents and volume used for reverse transcription of purified RNA to complementary DNA (cDNA)

Per reaction	
Component	**Volume (µl)**
Nuclease free Water	5.5
Random hexamers	0.5
dNTPs	1
µl per reaction	7
(after 5 min @65ºC)	
5Xfirst-strand buffer	4
0.1 M DTT	2
Total volume (µl)	20

5.3.5. Real-time Quantitative Reverse Transcription polymerase chain reaction (RT-q PCR)

The synthesized cDNA samples were diluted to a concentration of 80 ng/µL. *ABCB1* (Hs00184500_m1, Applied Bio-systems UK) and *ABCB5* (Hs00698751_m1, Hs03676541_m1, Applied Bio-systems UK) Taqman gene expression assays were used to determine respective gene expression. *GAPDH* (Hs03929097_g1, Applied Bio-systems UK) and *β actin* (Hs99999903_m1, Applied Bio-systems UK) were used as endogenous controls, i.e. housekeeping genes assumed to be ubiquitously expressed in all tested samples. Three technical repeats per each sample were performed; reference samples (calibrators) and template controls (negative controls) were used as per standard practice along with overexpressing ABCB1 MDCK-II and ABCB5 HEK 293 cell lines used as positive controls for respective gene of interest.

The normalized ΔCt was calculated as $Ct_{gene} - Ct_{control,}$ where the control Ct was the geometric mean of the Cts of β-actin and GAPDH for that given sample (Kozera and Rapacz 2013). A 384 well plate was prepared following mixing of reagents as shown in Table 4.2 below, which was placed in Thermal cycler (Applied Biosystems, Thermofischer UK) for RT q PCR analysis. The same protocol was used for human RNA, extracted from blood (leucocytes), duodenum, ileum and sigmoid colon.

Table 5.2. Reagents used in RT q PCR for determination of ABCB1/ABCB5 expression in blood and gut biopsies in cases and controls. βactin and GAPDH were endogenous controls

Reagent	X1	ABCB1	β-actin	GAPDH
FAM dye probe	1μl	12.1	12.1	12.1
VIC dye probe (for multiplexing)	1μl			
Molecular grade water	6μl	396	396	396
2x master mix Taqman gene expression assay	10μl	660	660	660
Single strand DNA 80ng/μl	2μl			

5.3.6. Association between ABCB1 gene expression, clinical phenotype and genotypic variation

Statistical methods for analysis of non-parametric data (Mann Whitney U) were used to determine relative *ABCB1* and *ABCB5* gene expression in blood, duodenum, ileum and sigmoid stratified by clinical phenotype (IBD patients and healthy controls). The same analysis was conducted for the subgroup of 8 UC patients who had received CS at diagnosis and had been subsequently classified as steroid

responsive or steroid dependent at 3 months post diagnosis. The analyses were conducted with the use of Graph Pad Prism software, version 6.01. The means of the sum of the ranks were compared between IBD patients and controls after median ΔΔCT (relative gene expression) values were calculated for blood, duodenum, terminal ileum and sigmoid biopsies from IBD patients and healthy controls. The larger sum of ranks, U values and two tailed exact p values at 0.05 significance level, were calculated for each of the four tissue types. Median and standard deviations for relative ABCB1 gene expression were calculated for IBD cases and controls for all ABCB1 SNP genotypes in SPSS version 22. The Kruskal Wallis test was undertaken to investigate whether relative ABCB1 gene expression in blood, duodenum, terminal ileum and sigmoid colon was different depending on ABCB1 genotype in IBD patients and healthy controls. Spearman's correlation coefficient was used to correlate clinical response of UC patients to CS- as defined per Tung et al within 12 months from diagnosis- and ABCB1 gene expression in blood and sigmoid colon (ΔΔCT).

5.4 Results

5.4.1. ABCB5 gene expression

No differences were observed between ΔΔCt values of human samples (including blood, duodenum, ileum, sigmoid colon) and template control. ABCB5 gene

expression was therefore not detectable in blood, duodenum, ileum, and sigmoid colon, when compared with overexpressing ABCB5 HEK293 FL and β cell lines used as positive controls (produced de novo as described in Chapter 2). ABCB5 gene expression was not detectable across all clinical phenotypes and SNP genotypes from all tissue samples of study participants. ABCB5 gene expression and treatment response to CS could therefore not be ascertained.

5.4.2. ABCB1 gene expression

ABCB1 expression variability was noted in blood samples and endoscopic biopsies from intestinal epithelium, including small intestine (duodenum, terminal ileum) and colon (sigmoid colon). No statistically significant difference between IBD patients and healthy controls was observed for ABCB1 gene expression in blood, duodenum, terminal ileum and sigmoid colon. Figure 4.1 depicts relative ABCB1 expression shown as fold change in expression (2^ΔΔCT) in the blood, duodenum, terminal ileum and sigmoid biopsies of IBD cases and healthy controls.

Figure 5.1. ABCB1 gene expression (shown as relative fold change in y axis) in various tissues, including blood, duodenum, ileum and sigmoid colon biopsies from ABC study participants.

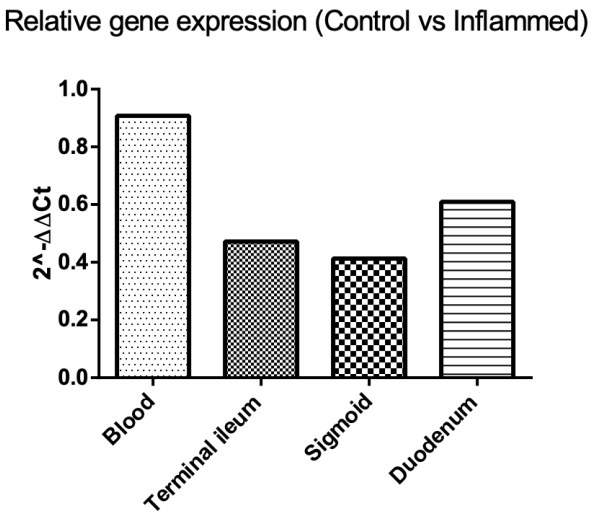
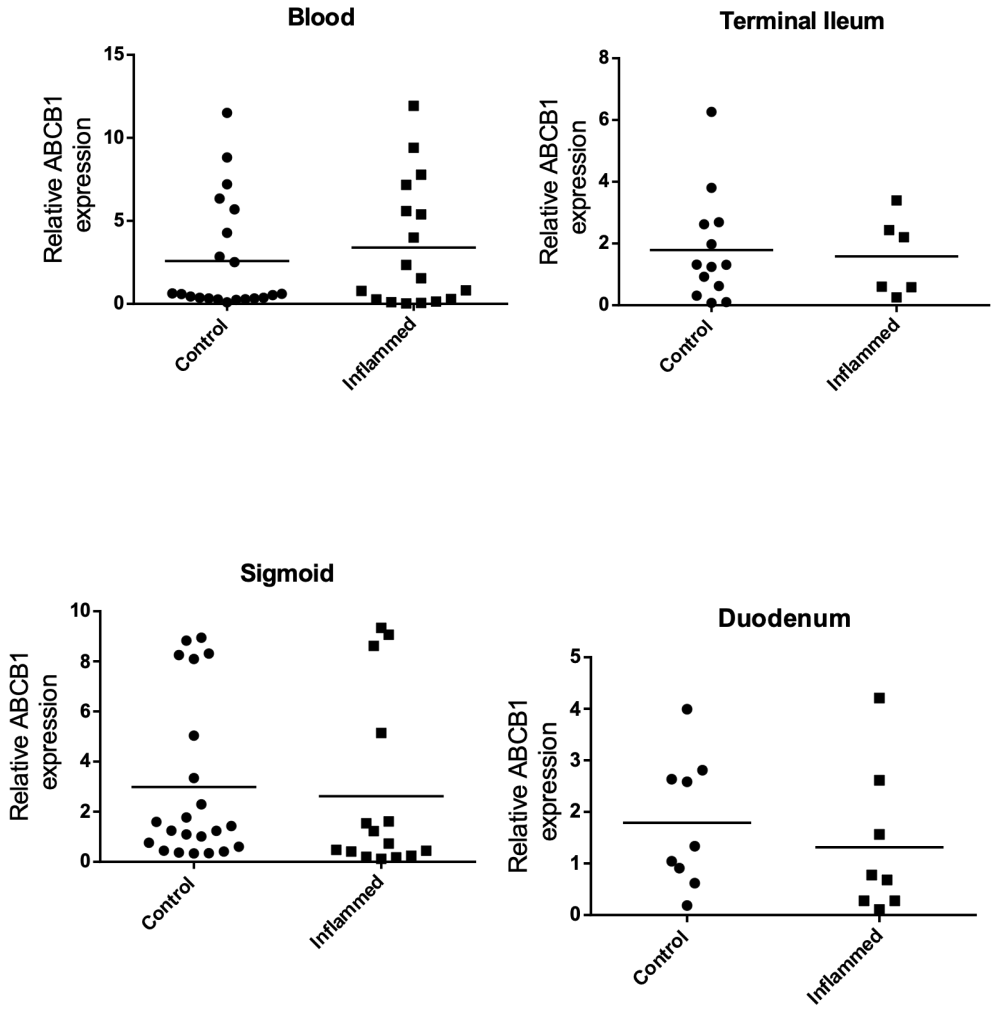


Figure 5.2 schematically shows in more detail the relative ABCB1 gene expression per tissue type of ABC study participants grouped by their clinical phenotype (IBD patients and healthy controls). The Mann Whitney test was performed which compares the means of the two ranks (IBD patients and heathy controls) and reflects the difference between the two rank totals. No statistical difference (two-tailed p value) was seen between patients and controls in any of the tissue samples tested (Turner, Levine et al. 2012).

Figure 5.2. Relative ABCB1 expression in blood, terminal ileum, duodenum and sigmoid colon of IBD cases (inflamed) and healthy controls. Horizontal lines show median values (two-tailed p values) for each tissue type.



The effects of *ABCB1* variants on ABCB1 gene expression in blood and intestinal biopsies (duodenum, terminal ileum, sigmoid colon) from IBD patients and healthy controls are summarized in Table 5.3.; no significant associations were observed.

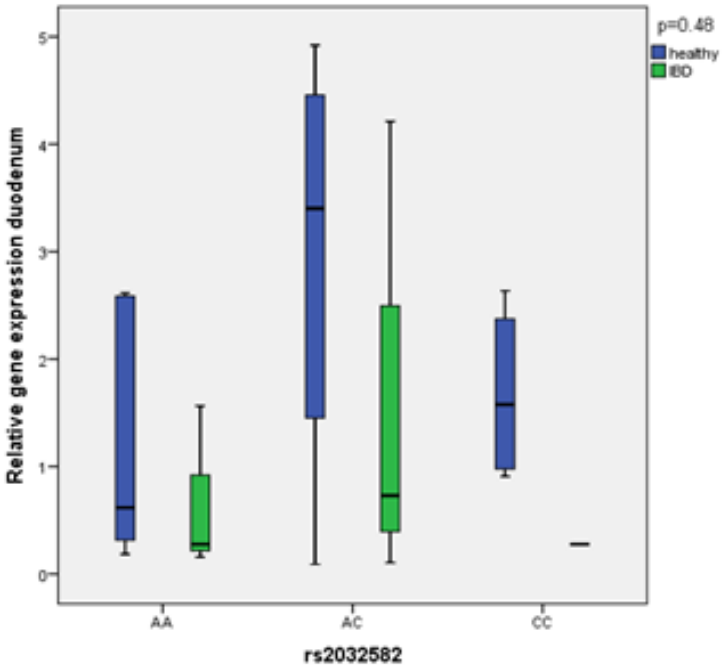
Table 5.3. Effects of ABCB1 SNPs on *ABCB1* expression levels in duodenum (D), terminal ileum (TI), sigmoid colon (sig) and blood.

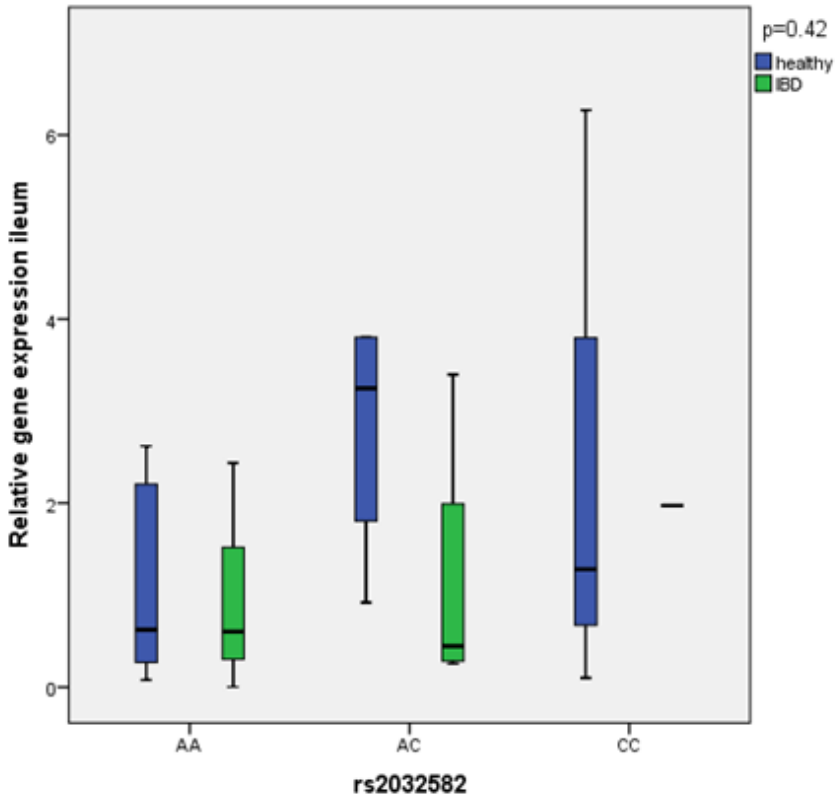
Relative *ABCB1* expression		*ABCB1* SNP							
Tissue	Median ± SD	rs2032582		P	rs1128503		P	rs1045642		P
		AA	AC		AA	AG		AA	AG	
		CC			GG			GG		
D	IBD cases (n=8)	0.46±1.06 1.04±0.95	1.79±2	P=0.48	0.35±0.37 0.87±1.17	0.31±0.35	P=0.71	2.08±1.5 0.9 ±0.4	0.72±1.72	P=0.74
	controls (n=15)	2.58±0.59 1.51±1.08	3.4±1.96		1.51±1.08 2.1±0.72	2.81±1.79		2.63±0.94 0.59±0.44	2.34±1.83	
TI	IBD cases (n=8)	0.61±1.11 1.31±2.38	1.8±1.62	P=0.42	0.25±0.69 0.34±1.22	1.56±1.43	P=0.31	0.59±1.12 1.31±0.95	2.52±1.95	P=0.13
	controls (n=15)	2.52±1.13 2.75±0.91	1.31±1.27		0.62±2.59 2.52±1.25	2.8±1.27		1.76±1.15 0.18±0.11	2.69±1.92	
Sig	IBD cases (n=16)	0.75±3.54 1.33±3.39	0.70±3.20	P=0.65	1.33±4.08 1.04±0.58	0.56±3.75	P=0.62	0.44±2.96 1.24±3.66	0.37±0.21	P=0.6
	controls (n=20)	5.47±4.5 8.09±3.89	3.59±1.59		8.09±4 1.53±0.3	7.28±3.83		4.73±3.79 5.31±5.19	6.32±3.64	
Blood	IBD cases (n=16)	2.34±2.31 1.89±2.53	3.71±6.61	P=0.98	0.81±1.9 2.34±2.67	4.6±5.44	P=0.68	0.80±2.93 1.89±2.53	5.38±5.54	P=0.44
	controls (n=20)	0.48±0.99 0.97±4.48	0.66±2.71		1.34±4.77 2.29±2.77	0.53±2.03		0.81±1.02 0.53± 6.33	0.65±2.60	

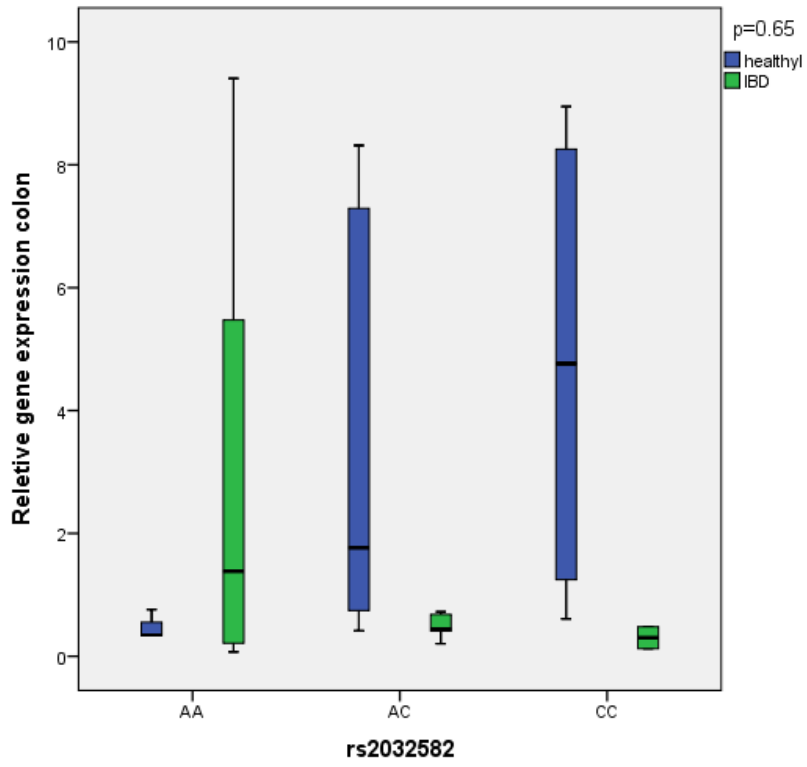
Figure 5.3 below shows relative ABCB1 gene expression in duodenum, terminal ileum, sigmoid colon and blood stratified by *ABCB1* SNP genotype and clinical phenotype (IBD versus healthy).

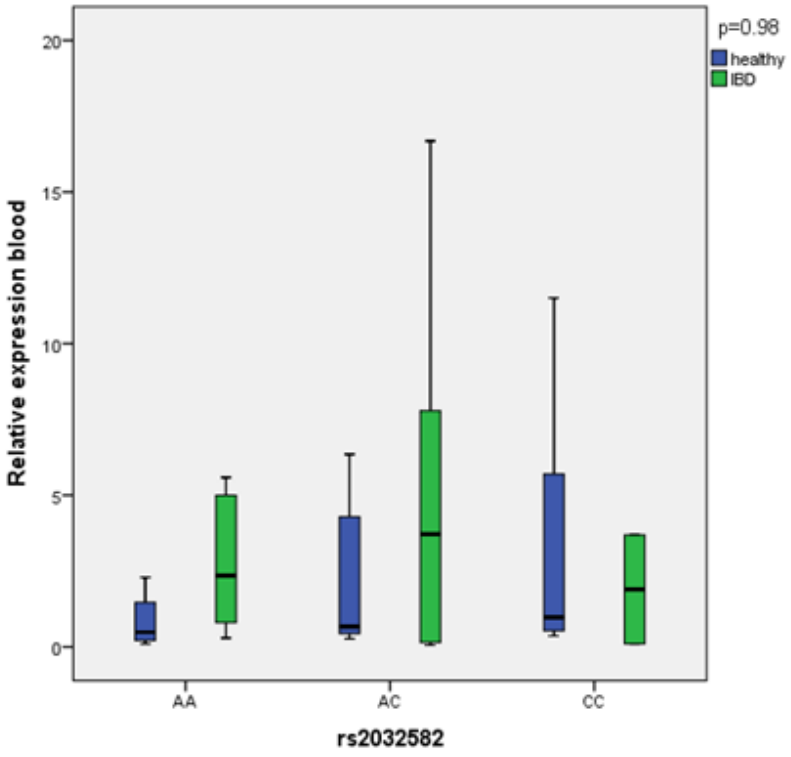
Figure 5.3. Clustered box plots show relative levels of ABCB1 gene expression in relation to ABCB1 SNP genotypes in the duodenum, terminal ileum, sigmoid colon and blood of IBD patients (green) and healthy controls (blue). SNPs examined: (a)-(d) rs2032582, (e)-(h) rs1128503, (i)-(l) rs1045642. Boxes represent 25th-75th percentiles of ABCB1 relative gene expression levels, whiskers represent 5th-95th percentiles, solid lines represent median ABCB1 relative gene expression levels in individuals with respective genotype and open dots represent outliers. P values are shown.

(a)-(d)

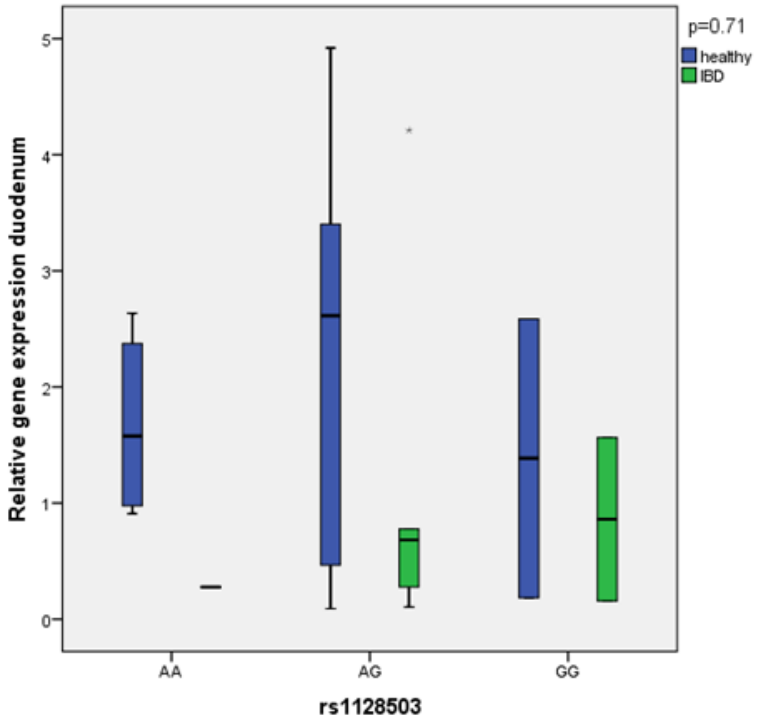


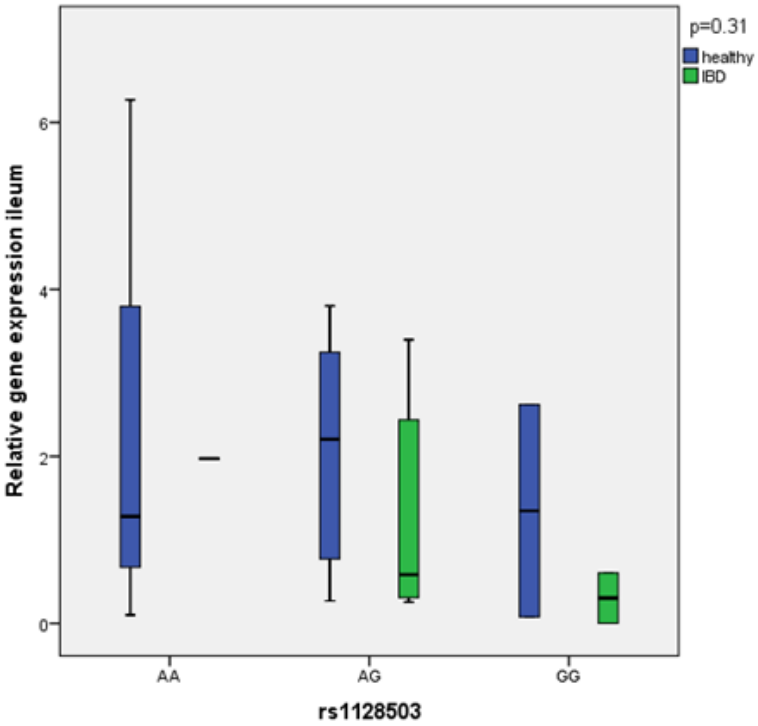

p=0.42
healthy
IBD
Relative gene expression ileum
0
2
4
6
AA
AC
CC
rs2032582

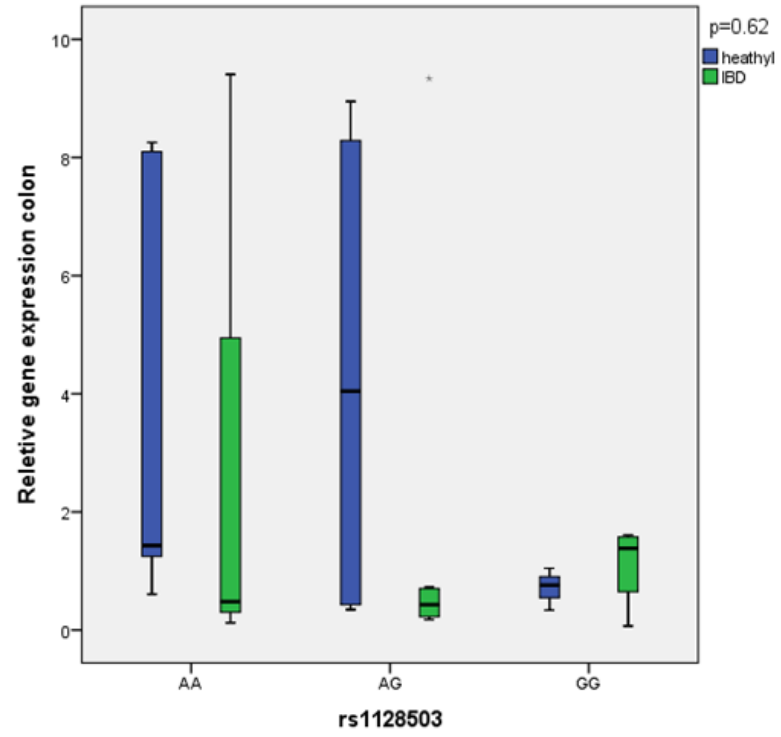

p=0.65
healthyl
IBD
Reletive gene expression colon
0
2
4
6
8
10
AA
AC
CC
rs2032582

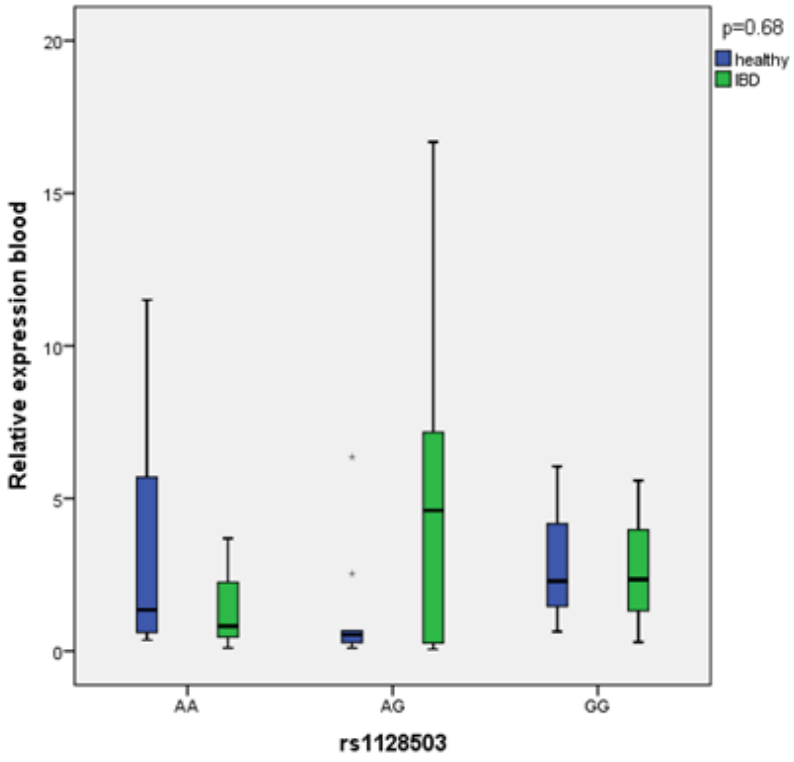

p=0.98
healthy
IBD
Relative expression blood
20
15
10
5
0
AA
AC
CC
rs2032582

(e)-(h)

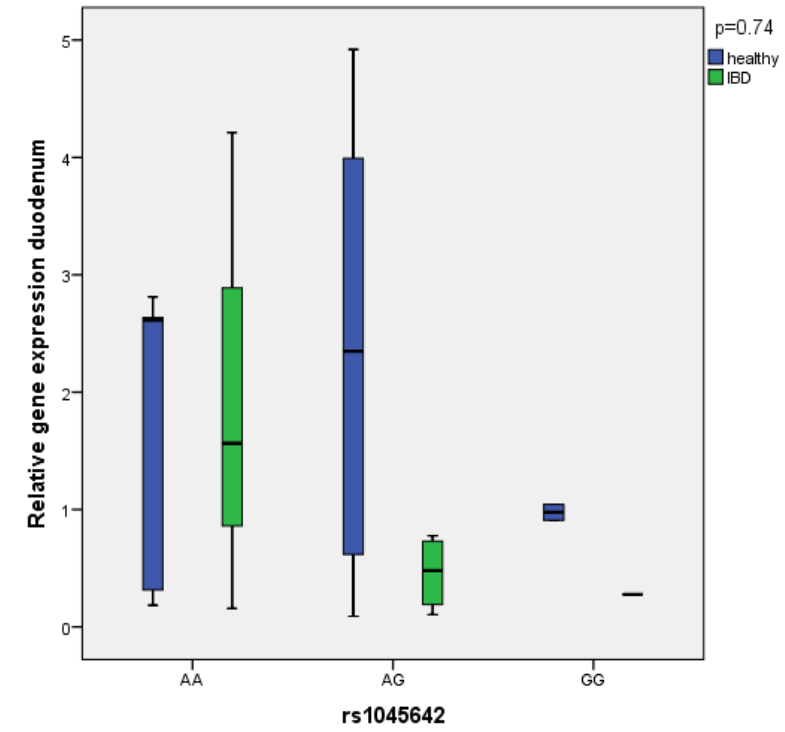

p=0.71
healthy
IBD
Relative gene expression duodenum
0
1
2
3
4
5
AA
AG
GG
rs1128503

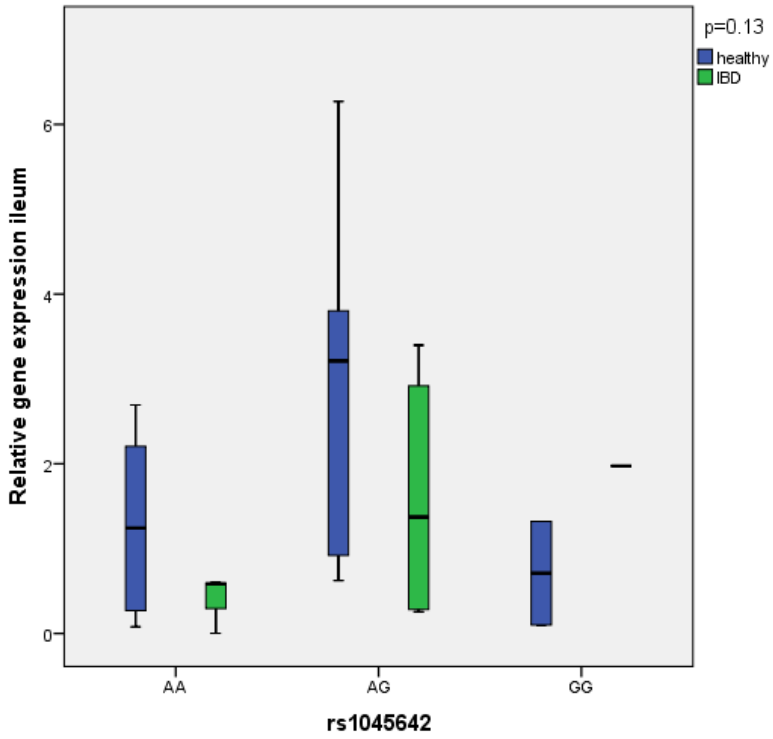

p=0.31
healthy
IBD
Relative gene expression ileum
0
2
4
6
AA
AG
GG
rs1128503

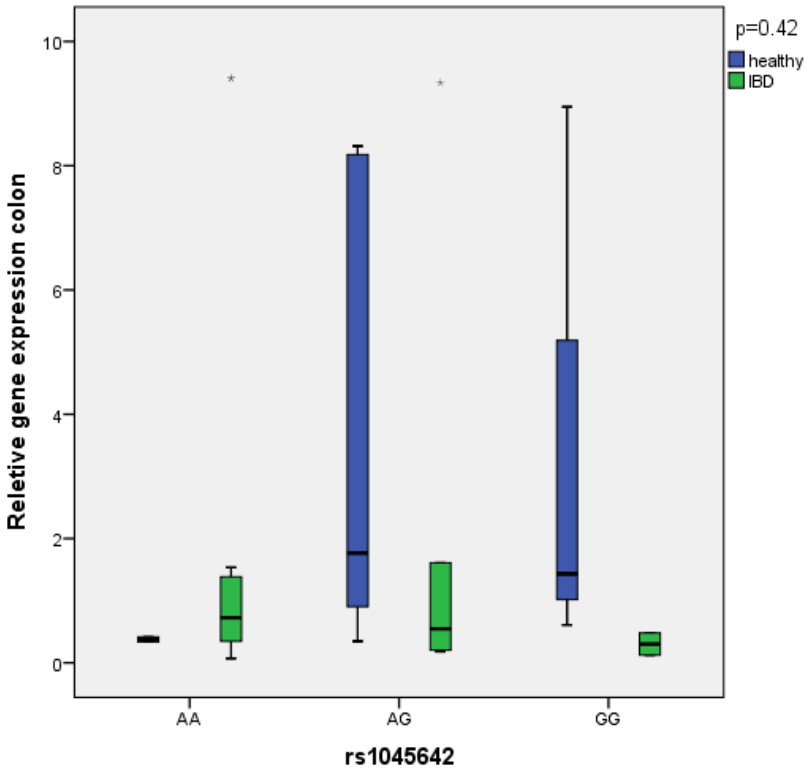

p=0.62
heathyl
IBD
Reletive gene expression colon
10
8
6
4
2
0
AA
AG
GG
rs1128503

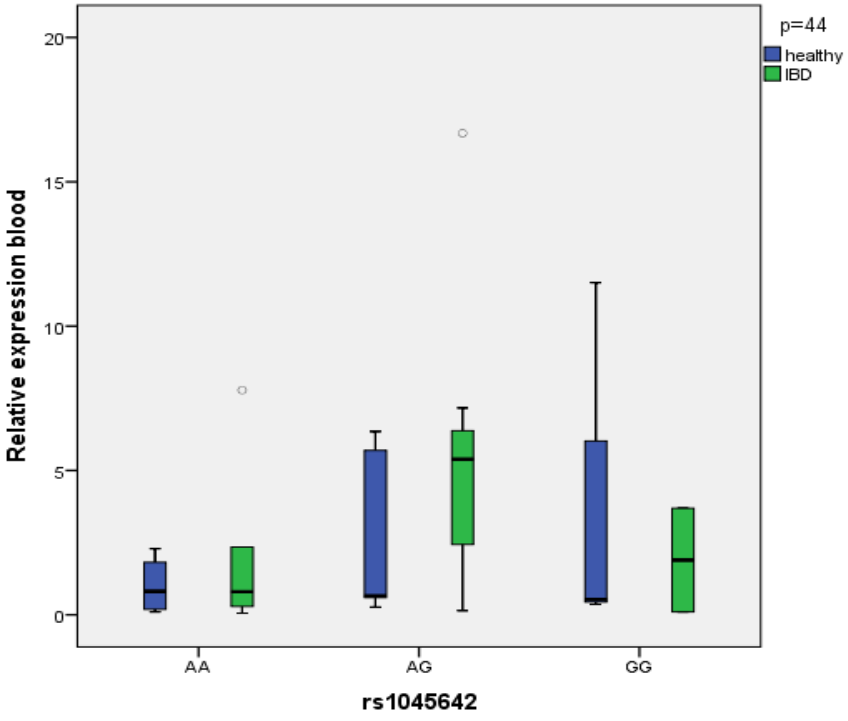

p=0.68
healthy
IBD
Relative expression blood
20
15
10
5
0
AA
AG
GG
rs1128503

(i)- (l)


p=0.74
healthy
IBD
Relative gene expression duodenum
0
1
2
3
4
5
AA
AG
GG
rs1045642


p=0.13
healthy
IBD
Relative gene expression ileum
0
2
4
6
AA
AG
GG
rs1045642


p=0.42
healthy
IBD
Reletive gene expression colon
10
8
6
4
2
0
AA
AG
GG
rs1045642


p=44
healthy
IBD
Relative expression blood
20
15
10
5
0
AA
AG
GG
rs1045642

Correlation of ABCB1 gene expression in sigmoid colon and clinical response to CS as defined per Tung et al for UC patients within 12 months from diagnosis was weak (Spearman's correlation coefficient r=0.25, p=0.54). As far as ABCB1 gene expression in blood was concerned, the correlation with clinical response to CS was also weak and non-statistically significant (Spearman's r=0.378, p=0.35).

5.5 Discussion

5.5.1 Key results, interpretation and limitations

The data presented in this chapter examined ABCB1 gene expression across various tissues of healthy children and children with newly diagnosed IBD. Although variability in ABCB1 expression was detectable in the tissue samples analyzed in this study, there was no significant differences in expression between children with IBD and healthy controls across the various tissues examined, including small intestine (duodenum, ileum) and colon (sigmoid colon). The small sample size and the lack of power may have been the explanation for the fact that the differences observed in ABCB1 gene expression between patients and controls were not statistically significant. Notably, there was weak correlation between clinical response to CS (as defined by Tung et al) and ABCB1 gene expression in sigmoid; this was an estimate of the strength and direction of the association between ABCB1 relative gene expression 2^ΔΔCt and CS response. ABCB1 SNP genotypic variations showed no effect on gene expression in the ABC study participants.

An alternative explanation about the observed pattern of ABCB1 expression in children may be that the pathogenesis of IBD varies according to the age of presentation. For instance, a clear distinction of disease phenotype and

pathogenesis, has been described with prevalence of monogenic disorders in patients diagnosed with VEO-IBD prior to six years of age as previously discussed in Chapter 1 (Uhlig, Schwerd et al. 2014). The IBD disease phenotype at presentation can overall be more severe across the paediatric age range in comparison to adult cohorts, as shown in clinical studies described in Chapter 1. It is therefore plausible that transcriptomic profiling, including drug transporter expression in blood and intestinal epithelium, may also differ in children. Possible heterogeneity of transcriptomic profiling across different age groups is further hampered by small patient numbers and various confounding factors, not allowing for clear conclusions (Ouahed, Gordon et al. 2018). A recently published transcriptomic study implemented various gene expression methodologies, such as Affymetrix Human Primeview gene microarray, RNA sequencing and RT q PCR, in colonic biopsies from 13 patients with different age and UC phenotype (children and adults). Forty genes were investigated, implicated in innate and adaptive immunity, intestinal barrier homeostasis, autophagy, inflammation, apoptosis and genes previously reported to increase IBD susceptibility. As far as drug transporter representation is concerned, the drug transporters ABCG2, SLC26A2, SLC38A4 and SLC3A1 belonged to the top 20 genes noted to be downregulated in affected (inflamed) versus unaffected (control) tissues in the combined data set. Age nevertheless did not significantly affect the analysis or the findings of the study (Ouahed, Gordon et al. 2018).

The three common ABCB1 SNPs investigated in the ABC study have also been investigated in adult IBD studies, for example in a recent paper form Mijac et al, where ABCB1 common genotypic and haplotype variations were investigated in 206 adult IBD patients and 255 healthy controls; this group concluded that the T allele of

the three common ABCB1 SNPs predisposed Serbian adult patients to UC (Mijac, Vukovic-Petrovic et al. 2018). Ho et al, as previously mentioned, showed that genotypic variation in rs1045642 and rs2032582 ABCB1 SNPs correlated with disease susceptibility and extent in UC, however effect of genotypes on gene expression was not included in the study analysis (Ho, Nimmo et al. 2005). Hoffmeyer et al showed positive correlation between increased ABCB1 protein expression in small intestine and rs1045642 genotype (Hoffmeyer, Burk et al. 2000). Juyal et al also showed that rs1045642 and rs2032582 genetic polymorphisms in Asian populations may play a role in UC phenotype with earlier presentation (<29 years) and prevalence of left sided UC (Juyal, Midha et al. 2009). Other European studies did not however report clear association between ABCB1 genetic polymorphisms and susceptibility to IBD (Croucher, Mascheretti et al. 2003, Fischer, Lakatos et al. 2007). Two recent meta-analyses focused on ABCB1 SNPs and IBD susceptibility, rather than the potential influence of the genotype on gene expression (Wang, Guo et al. 2014, Zhao, Wang et al. 2015). Interestingly, circadian modulation of ABCB1 gene expression has been observed in animal studies (Iwasaki, Koyanagi et al. 2015) and humans with active IBD (Palmieri, Mazzoccoli et al. 2015).

ABCB5 gene expression was not detectable in the small intestine, colon and blood from cases and controls when compared with stable ABCB5 overexpressing cell lines (FL and β) described in detail in Chapter 2 (positive controls). Lack of ABCB5 gene expression in blood and intestinal epithelium is the biologically plausible reason behind this finding. An alternative explanation for the lack of ABCB5 gene expression could be that the gene expression assays used were not sensitive and specific enough to detect ABCB5 expression or that ABCB5 expression in the specific tissue samples tested was extremely low and could not be accurately

detected or replicated with standard assays and methodology. The use of quantitative RT q PCR is however the gold standard for detection of gene expression and more sensitive in comparison to other methods, for instance c-DNA microarrays (Scherf, Ross et al. 2000). Alternative methodologies such as digital PCR may have also helped (Lin, Su et al. 2017).

ABC study showed that ABCB5 expression is either absent or extremely low in healthy or inflamed intestinal epithelium; this negative finding however does not deviate from previous published literature, where ABCB5 expression was not detectable in peripheral blood leucocytes of healthy individuals (Farawela, Khorshied et al. 2014). Yang et al showed ABCB5 gene expression in white blood cells from a limited number of diseased human subjects with leukaemia, implementing the use of RT q PCR in treatment refractory disease; the same group found positive correlation of ABCB5 gene expression with ABCB1 expression in this small group of cancer patients (Yang, Li et al. 2012). Taipalensuu et al reported that ABCB5 mRNA was not expressed in jejunal biopsies from 12 healthy human subjects, or in human intestinal epithelial Caco-2 cell monolayers, in contrast to other ABC transporters such as ABCB1 (Taipalensuu, Tornblom et al. 2001). Other studies have indicated ABCB5 expression in cancerous tissue. For instance, Xu et al showed that ABCB5 expression was enriched in peripheral blood specimens from patients with colorectal cancer (CRC) in comparison to non-CRC patients, and that mRNA expression correlated with tumor progression (Xu, Shu et al. 2017). Xu et al concluded that ABCB5 expression could be a regulatory factor in cellular differentiation and malignancy progression. ABCB5 gene expression has been shown in human colon cancer tissues by Kugimiya et al, where c-myc knockdown decreased ABCB5 gene

expression, resulting in less drug resistance to 5-fluouracil (Kugimiya, Nishimoto et al. 2015).

There is an observed paucity of published literature despite previous extensive work on ABCB5 transporter in cell lines, animal models and human samples (personal communication with renowned clinician and basic scientists, experts in IBD and drug transporter biology such as Ho et al and Gillet et al research groups), which possibly makes ABCB5 'elusive' to study both at the bench and at the bedside.

The pilot study presented in this thesis adds to this unchartered territory of ABC transporter expression in children with IBD and healthy controls, by investigating how ABCB1/ABCB5 expression relates to genotype and clinical phenotype and how ABCB1 expression relates to clinical outcomes post CS administration.

Clinical outcome post CS treatment and possible effect on ABCB1/ABCB5 gene expression has not been determined in this pilot study due to lack of further sampling post treatment; correlation between gene expression and clinical outcome post CS administration has nevertheless been described for ABCB1 in the small number of UC patients who received CS at diagnosis. In children, upper and lower GI endoscopy is performed under general anaesthetic and repeat endoscopy is indicated if induction of remission fails, if disease is refractory to treatment, or prior to surgery and escalation or de-escalation of medical management (Ruemmele and Turner 2014, Ruemmele, Veres et al. 2014). Therefore, from an ethical point of view, and bearing in mind this was a pilot study, it would have not been inappropriate to seek and obtain ethical approval, parental consent or patient assent to collect samples post CS at diagnosis. This would have been outside current best practice.

Instead, a correlation was described between gene expression at diagnosis and clinical outcome post CS administration.

5.5.2. Implications for future work

Exploration of the ABCB5 role outside of IBD may be more fruitful in terms of biological significance. There is a lack of published literature on transcriptomic and proteomic studies in IBD patients, particularly in children, certainly in relation to ABC transporters. Exploration of gene expression would need further investigation in healthy controls and IBD patients from various age groups and ethnicities, with variable disease behavior, severity and extent.

Tailoring of patient's management based on gene and protein expression adheres to the principles of personalized and translational medicine. This approach to disease management requires ex vivo work, combined with large-scale studies, where molecular and clinical parameters can be studied in large and more representative IBD patient cohorts, with a view to inform complete management algorithms combining genomic and transcriptomic profiles, clinical phenotypes and outcomes post treatment. This 'personalized medicine' approach has the potential to guide therapeutics in the future but will require well-designed and adequately powered studies. Patient tailored medicine benefits from collaborative efforts between academia, industry and the health sector, for instance the use of genomic data repositories and biobanks, as national or international health resources where clinical, demographic information and clinical phenotypes over time would be linked to electronic health records and genome sequencing for a wide range of serious, chronic, life threatening illnesses (www.ukbiobank.ac.uk, accessed 28/07/2019).

Chapter 6

Immunohistochemistry of intestinal ABCB1/ABCB5 expression

6.1 Introduction

Protein expression is important in biological interactions, as the genetic code end product as it ultimately exerts the direct biologic effect in vivo. Protein expression to an extent correlates to gene expression and so the latter has been used as a surrogate marker, especially in diagnosis and classification of cancer (Lopes, Rigon et al. 2018). In the case of unclear correlation between gene and protein expression, both should be ideally investigated so as to predict protein function and interaction (Greenbaum, Colangelo et al. 2003).

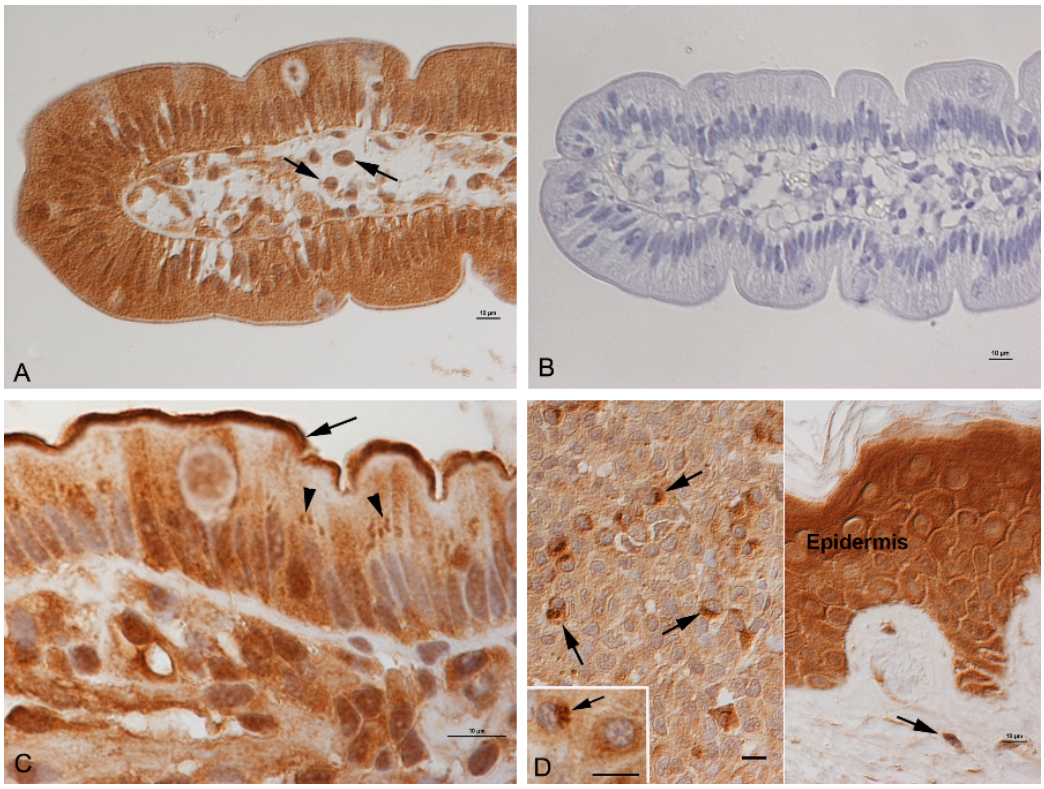
ABC transporter expression has been investigated with the use of immunohistochemistry (IHC) in formalin fixed paraffin embedded (FFPE) tissue. IHC facilitates the detection of biological processes in the context of intact tissues; for example, it allows quantification of ABC transporter expression in the human placenta and has been used for validation of other protein detection methods (Cederbye, Palshof et al. 2016, do Imperio, Bloise et al. 2018). IHC is comparable to universally accepted quantitative protein expression modalities such as Western Blotting or enzyme linked immunosorbent assays (ELISA), as it follows the same immunological principles in histological sections of tissues in paraffin blocks (Taylor and Levenson 2006).

ABCB1 is a transmembrane protein with a strong apical staining pattern in normal small intestine, colon and colorectal cancer tissue (Stein, Fleuter et al. 2012). ABCB1 is expressed in the plasma membrane of most human tissues, including kidney, liver, breast and blood brain barrier. Inter-individual variability in protein expression with immunoblotting has been described for example in human liver lysates (Abanda, Riches et al. 2017). ABCB1 is expressed to varying degrees in

human umbilical cords obtained from term gestation neonates as shown with the use of IHC by Riches et al (Riches, Walia et al. 2016).

There are few published studies looking at ABCB1 expression in healthy and inflamed human intestinal epithelium (Fakhoury, Litalien et al. 2005, Gutmann, Hruz et al. 2005). The focus of IHC research as far as the gastrointestinal tract is concerned has been in biopsies from patients with colorectal cancer (Trumpi, Emmink et al. 2015, Cederbye, Palshof et al. 2016). There is a paucity of validated scoring systems and consensus in ABCB1 expression scoring and semi-quantification of protein expression. A paradigm of ABCB5 expression staining has been described by IHC in the human enterocytes, by Dr Anya Kipar Professor of Veterinary Pathology at the University of Liverpool (unpublished data). This is depicted in figure 6.1 below, where cytoplasmic staining is observed in enterocytes and lymphocytes of the lamina propria. Intense focal perinuclear ABCB5 and apical staining in enterocytes with use of ABCB5 polyclonal antibody is shown. Intestinal negative and cutaneous melanoma positive controls were implemented.

Figure 6.1. In situ demonstration of ABCB5 protein expression by immunohistochemistry. A, C. Small intestine, villus. C. ABCB5 expression shown in the cytoplasm of enterocytes and in lymphocytes (arrows) in the lamina propria mucosae. B. Staining is absent in a consecutive section stained with a non-reacting polyclonal antibody. C. Closer view of a villus: within enterocytes, there is intense focal perinuclear ABCB5 expression, consistent with Golgi apparatus (arrowheads). Strongest ABCB5 staining observed along the apical surface, consistent with ABCB5 expression along microvilli. D.ABCB5 expression shown in cutaneous melanoma used as positive control. Left: ABCB5 expressed in the cytoplasm of neoplastic cells, with intense focal perinuclear accumulation (arrows). Right: intense cytoplasmic ABCB5 expression in all cell layers in the overlying epidermis and in dermal leukocytes (arrow). Peroxidase anti-peroxidase method, Papanicolaou's hematoxylin counterstain. Bars = 10μm.



The aim of this chapter was to explore ABCB1 and ABCB5 expression in the small intestine (ileum) and colon (sigmoid) of the ABC study participants, to compare how this may vary between cases and controls, and how it correlates with respective gene expression and clinical phenotypes. Correlation between treatment response to CS at 3 months post diagnosis and ABCB1 protein expression in small intestine and colon have also been assessed for included UC patients that received CS at diagnosis.

6.2 Materials and Methods

6.2.1. Materials and reagents

During diagnostic gastrointestinal endoscopy intestinal biopsies were obtained and placed in 10% neutral buffered formalin, to ensure effective tissue fixation prior to further analysis. This is a widely used technique followed by staining with hematoxylin/eosin and/or tissue specific antibodies, implemented alongside negative (or isotype) and positive controls, for the purpose of detection of proteins of interest expressed in tissue sections (Malik and Daymon 1982).

The ABCB1 antibody used for the purpose of IHC staining protocol in this study was the monoclonal mouse anti-human LS-b5570 (clone name JSB-1) from Life Span Biosciences (2012). The antibody was diluted using Thermo-Fischer Scientific antibody diluent at a final concentration of 1/500.

The ABCB5 rabbit anti-human polyclonal antibody LS-B3454 from Life Span Biosciences (2012), raised against keyhole limpet hemocyanin (kLH) conjugated synthetic peptide selected for the N-terminal region of ABCB5, was diluted with Thermo-Fischer Scientific antibody diluent at a final concentration of 1/250. The ABCB5 antibody specificity was tested on a panel of 21 FFPE human tissues following heat induced antigen retrieval in citrate buffer by the manufacturer.

Reagents purchased from Thermo-Fisher Scientific included the PT Module Buffer 1 (10μM Sodium citrate, 0.05%, Tween 20, pH 6), the Tris Buffered Saline & Tween and the Ultra Clean antibody Diluent. The Ultra Vision Quanto Detection System HRP DAB (Thermo-Scientific catalogue number TL-060-QHD) was used due to its properties of enhancing antigen sensitivity with signal amplification and avoidance of

background ‘noise’, with the use of an innovative micro-polymer technology, which provides increased sensitivity and detection simplicity. This kit contains the universal antibody conjugated to an enzyme-labelled polymer that recognizes IgG mouse and rabbit antibodies of interest (bound to an antigen in tissue sections), Thermo Fisher Scientific Hydrogen Peroxide Block (horseradish peroxidase) and diaminobenzidine (DAB) Quanto Chromogen/substrate, with which the enzyme-labelled polymer complex is visualized. Other reagents used included Harris’s Hematoxylin, acid Alcohol 0.5% Aqueous Solution in 70% Industrial Methylated Spirits and Scott’s Tap Water Substitute. Industrial Methylated Spirits (IMS) were used for counterstaining, Xylene for washing and Pertex for slide mounting.

6.2.2. IHC protocol

IHC is a versatile diagnostic tool used in clinical practice for elucidation of differential diagnoses; it detects infectious agents as well as individual cell populations, through the application of specific stains and antibodies in FFPE tissue sections, mounted on slides and visualized under a light microscope. IHC facilitates exploration of in vivo function but findings depend on the rigor of execution of the experiment and interpretation of results (Actis and Pellicano 2016). IHC can detect inflammation, differentiate neoplastic lesions in tissues and identify structures and organisms secreted by cells (Leong and Wright 1987). Staining correlates with protein expression levels (Brey, Lalani et al. 2003). Automated or manual image analyses can be implemented for semi-quantification of protein expression.

ABCB1 and ABCB5 antibody staining was carried out in FFPE tissue sections; 4μm transverse sections were cut with the use of a microtome, and samples were mounted on poly-l-lysine coated slides. Two separate slides were stained with

ABCB1 and ABCB5 for each ABC study participant, with sections from small intestine (ileum) and colon (sigmoid). Positive and negative intestinal tissue controls were included in each run for ABCB1 and ABCB5, as well as a cutaneous melanoma control for ABCB5. Mounted sections from each participant served as individual negative controls.

The IHC protocol was optimized, as per standard practice, by senior biomedical scientists at the Department of Histopathology Alder Hey Children's hospital, with the use of commercially available antibodies, negative and positive controls. Staining specificity was qualitatively evaluated and compared with negative and positive controls by Dr Rajeev Shukla, Consultant Pathologist at Alder Hey Children's Hospital.

Sections were dried overnight at 37°C. Slides were heated for a minimum of 30 minutes at 60°C to ensure maximum section adhesion. Sections were de-paraffinated with xylene and subsequently dehydrated with Thermo PT Module Buffer 1 (sodium citrate ph6) using the Pre-treatment module (90°C for 20 minutes). Following pre-treatment, sections were washed in wash buffer for 10 minutes in preparation for heat induced epitope retrieval; drying was not allowed during this step to avoid background nonspecific antibody binding. Thermo Ultra V Protein Block was applied for 10 minutes in order to block non-specific background staining.

The respective ABCB1 and ABCB5 antibodies were applied at above dilutions to study participants' samples mounted on separate slides for each transporter protein; these were incubated according to the manufacturer's recommended protocols. Wash buffer only was applied to negative controls. Slides were incubated at room temperature in a humid incubation chamber for 20 minutes.

Primary antibodies were rinsed off and sections were washed twice in wash buffer for 5 minutes; application of Thermo Primary Antibody Amplifier Quanto followed for 10 minutes, in order to achieve amplification with both mouse and rabbit primary antibodies and to avoid non-specific binding with endogenous biotin molecules. Slides were washed twice for 5 minutes in wash buffer. Thermo Hydrogen Peroxide Block was applied for 20 minutes in order to quench endogenous peroxidase (which can also cause non-specific background staining), and was washed twice in wash buffer for 5 minutes. Thermo-Fischer Scientific HRP Polymer Quanto (light sensitive) was applied for 10 minutes followed by washing twice in wash buffer for 5 minutes at a time. The Quanto DAB containing Substrate Working Solution was applied at 1 drop of DAB per 1 ml of substrate and slides were incubated for 10 minutes and washed for 2 minutes in running tap water. Slides were counterstained with Harris Haematoxylin for 30 seconds and differentiated using 0.5% acid alcohol Aqueous Solution in 70% Industrial Methylated Spirits (IMS) and Scott's Tap Water Substitute. Slides were dehydrated in IMS, cleared with xylene, and mounted with cover slips with the use of pertex as permanent mounting medium.

6.2.3. Semi-quantitative evaluation of ABCB1 and ABCB5 protein expression with IHC

Intestinal antigen expression was evaluated with the use of the Nikon Eclipse Ni light microscope and visualization at 40-fold, 100/200-fold and 400-fold magnification under bright field illumination. Staining intensity scoring was assessed by the author and two pathologists at Alder Hey Hospital, according to pre-defined visual scores of expression intensity, varying from negative (grade 0) to weak (grade 1), moderate (grade 2) and high signal intensity (grade 3). The extension of immunostaining

depended on meticulous counting of the percentage of labelled cells and implementation of predetermined visual analogue scores. ABCB1 is a membrane protein of the ABC transporter superfamily. There is no validated ABCB1 IHC score, therefore the expression scoring methodology utilized was extrapolated from ABCG2, another ABC membrane transporter implicated in drug efflux. Standardized approach to ABCG2 protein expression by IHC in the apical membrane of luminal epithelial cells of the colon was used, as previously described and validated by Cederbye et al (Cederbye, Palshof et al. 2016).

A score 0 was assigned if no apical membrane staining was observed or if the apical membrane staining was observed in less than 10% of the cells at 400x magnification. Weak apical membrane staining in ≥ 10% of the cells visible only at 400x magnification, was scored as grade 1, whereas weak to moderate apical membrane staining in ≥ 10% of the cells visible at 100/200 x magnification was scored as grade 2. Strong apical membrane staining in ≥ 10 % of the cells visible at 40 x magnification was scored as grade 3. If slide scoring was discordant, the opinion of the senior pathologist was sought and their opinion was final. Assessors were 'blinded' as study samples had been anonymized at the point of collection. Comparisons of staining intensity with samples treated with secondary antibody, horseradish peroxidase (HRP) and DAB only without primary antibody were made. The specificity of staining was determined using the following controls: negative control for each study participant without primary antibody, respective slide from same tissue section for each study participant prepared with primary antibody, positive controls for detection of ABCB1/ABCB5 in intestinal/liver tissue and cutaneous melanoma respectively, each treated with respective primary antibody.

Digital pictures were taken with a microscope integrated Nikon digital sight DS-U3 camera, with use of NIS-elements imaging software version 4.13 (32 bit) and constant exposure time at 400x magnification. Representative pictures were taken depicting ABCB1 negative and positive controls and different staining intensity scores for grades 0-3, as well as ABCB5 stained enterocyte samples and the respective negative and positive tissue controls.

6.2.4. ABCB1 gene and protein expression in relation to clinical phenotype and treatment response to CS at 3 months post diagnosis

Descriptive statistics of ABCB1 gene expression and semi-quantified protein expression in small intestine and colon of IBD patients and controls was undertaken. ABCB1 protein expression and gene expression in the intestinal epithelium (terminal ileum and sigmoid colon) were compared with use of the Kruskal Wallis test. Differences in ABCB1 protein expression in terminal ileum and sigmoid colon of IBD patients versus healthy controls were investigated with the use of Pearson Chi square test. Analysis was performed with the use of statistical software package SPSS version 22. Fisher's exact test was implemented to check how the response to CS in the 8 patients diagnosed with UC related to ABCB1 protein expression by IHC. The critical time point of 3 months was selected, as this timepoint bears good prognostic value for treatment response to CS 12 months later (Schechter, Griffiths et al. 2015). Due to the relatively small sample size of patients who received CS in the study-i.e. a group of 8 patients, 3 steroid dependent and 5 steroid responsive patients- the Fisher's exact test was selected so as to avoid type II error. ABCB1 protein expression in small intestine and colon was categorized as 0-1 and 2-3,

reflecting no or weak staining, versus moderate to strong intensity staining scores respectively, as assessed by IHC protocol described above.

6.3 Results

6.3.1. ABCB1 protein expression

Clear brown staining was restricted to the cytoplasm, nuclei and apical cell membrane, indicating a positive result for ABCB1 expression as shown in figures 6.2 and 6.3 below, where ABCB1 negative and positive intestinal and liver controls, and grading scores 0-3 are depicted respectively. Images of grade 3 scoring are shown in figure 5.4. Strong ABCB1 immunoreactivity in the apical membrane of the luminal epithelial cells was observed with use of LS-C58240 IgG2a monoclonal mouse antibody (Abcam). The latter recognized the extracellular conformational epitope of CD243 or ABCB1, constitutively expressed on the apical plasma membrane of excretory epithelial cells in the small intestine. The apical membrane staining was reproduced with a different ABCB1 antibody, the monoclonal mouse anti-human LS-b5570 (clone name JSB-1) from Life Span Biosciences (2012). ABCB1 and ABCB5 expression intensity were ascertained by two blinded observers in a semi-quantitative manner and with the use of predefined criteria. 90 % consensus rate was noted between the two observers; the opinion of the more senior pathologist prevailed in 10% of the cases where disagreement about grade of staining intensity occurred.

Figure 6.2. JPEG digital images of slide sections taken with integrated Nikon digital sight DS-U3 camera at 400xmagnification using NIS-elements version 4.13 imaging software. A-B: Positive and negative ABCB1 staining on enterocyte surface. C-D: positive and negative ABCB1 staining in the surface of the canalicular cells of liver.

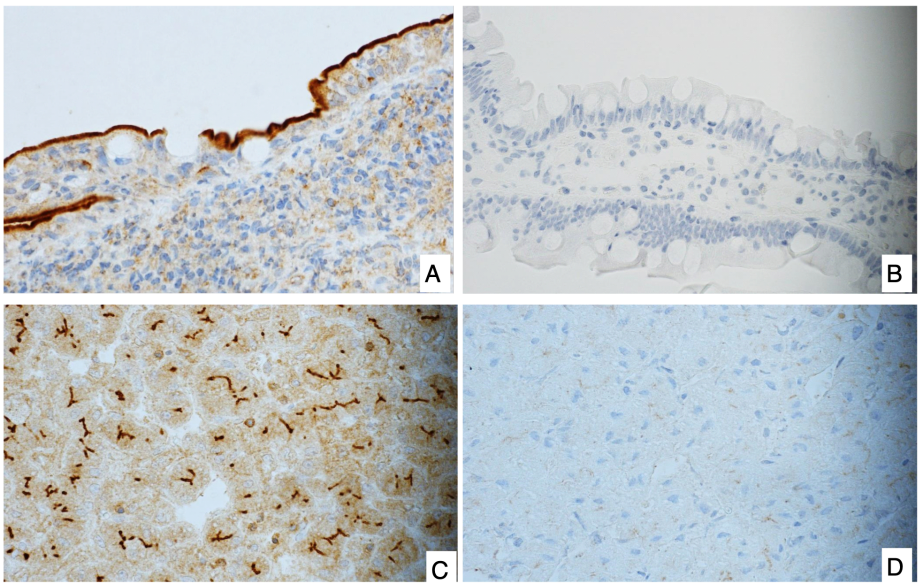


Figure 6.3. Grades of ABCB1 positivity on enterocyte surface as viewed at 400x magnification.

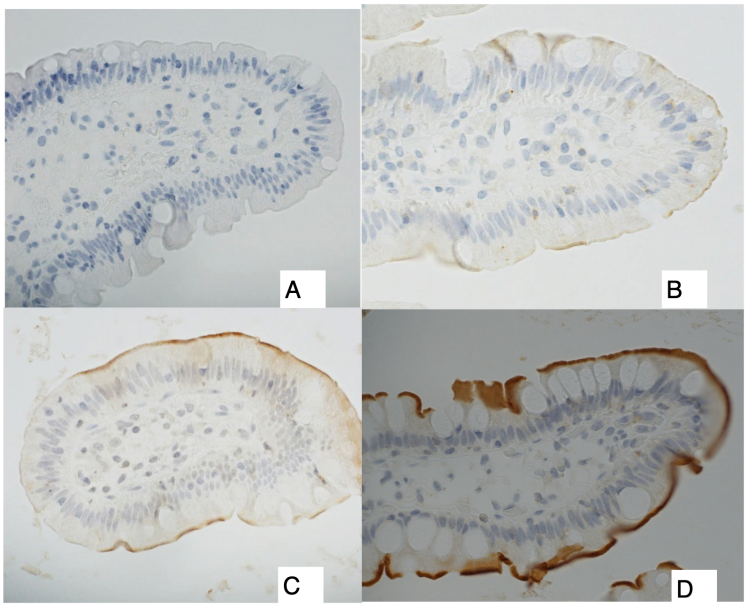


Figure 6.4. ABCB1 grade 3 staining positivity in the apical surface of enterocytes easily visualized at 40x and 400x magnification.

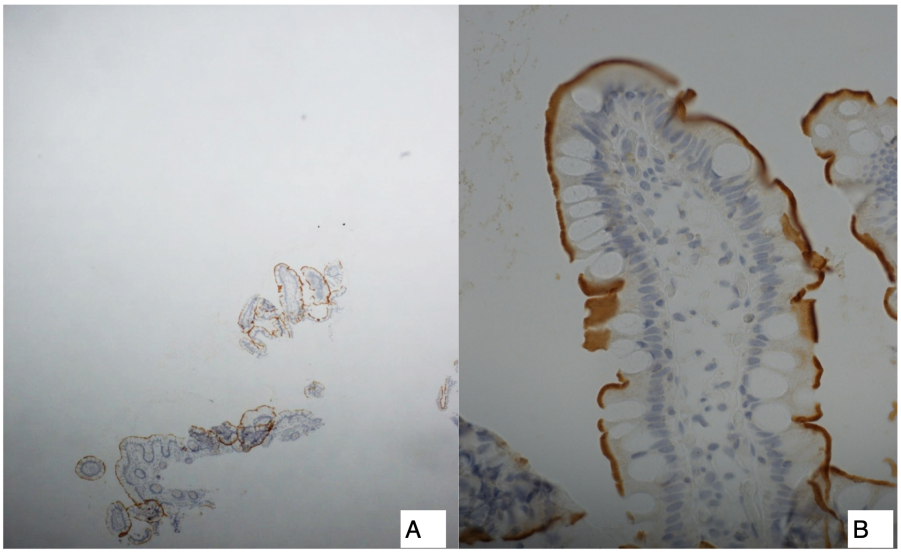


Table 6.1 below shows the number of cases and controls with relevant IHC scores in terminal ileum and sigmoid colon of the ABC study participants.

Table 6.1. Visual analogue grading scores of ABCB1 expression by IHC in terminal ileum and sigmoid colon of healthy controls and IBD patients.

ABCB1 IHC	Terminal ileum-healthy	Terminal ileum-IBD	Sigmoid colon-healthy	Sigmoid colon-IBD
Grade 0	N=9	N=6	N=8	N=6
Grade 1	N=3	N=3	N=1	N=4
Grade 2	N=3	N=2	N=6	N=6
Grade 3	N=4	N=6	N=1	N=4

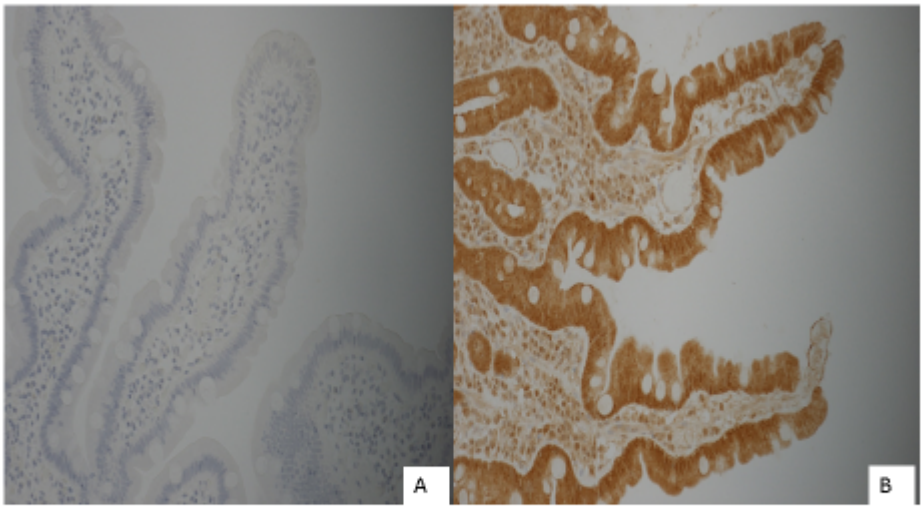
The Kruskal Wallis test was applied to compare between gene expression by RT q PCR and protein expression in terminal ileum by IHC and the distribution of ABCB1 gene expression did not vary across different scoring grades of protein expression by IHC ($p=0.48$). Pearson chi square was performed to compare ABCB1 protein expression by IHC between cases and controls and this was not significantly different ($p=0.34$). Gene expression of ABCB1 in colon by RT q PCR varied accordingly to grades of IHC scoring for ABCB1 protein expression in the sigmoid colon (Kruskal Wallis $p=0.007$); relative gene expression was increased in patients with high IHC grading scores. This finding is however based on small numbers and further work is necessary. Pearson's chi square test showed no difference in ABCB1 protein expression by IHC between cases and controls ($p=0.39$). Fisher's exact test showed no difference in ABCB1 protein expression in the small bowel and colon between steroid responders and steroid dependent UC patients; treatment response to CS given at diagnosis was assessed at 3 months post diagnosis ($p<0.05$).

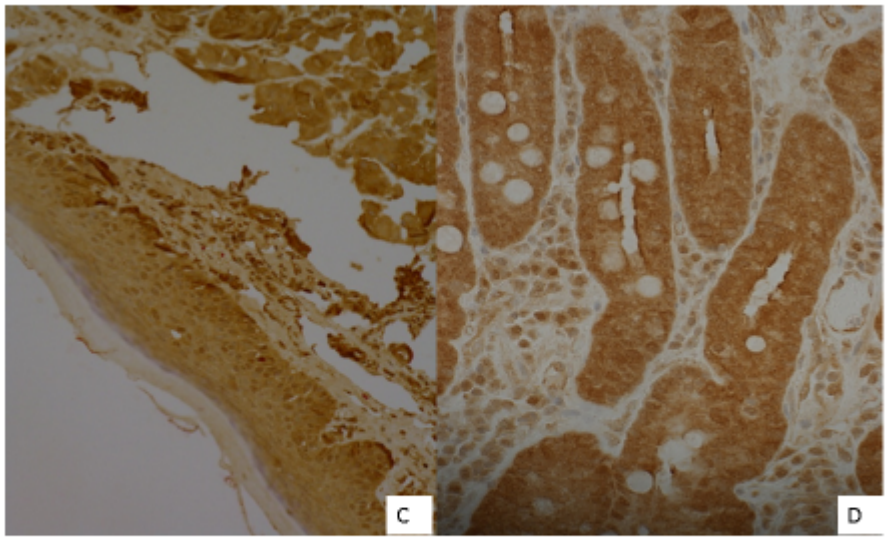
6.3.2. ABCB5 protein expression

ABCB5 protein was not reliably detectable by IHC as decided unanimously by assessors. The staining was non-specific as the cytoplasm of epithelial cells, plasma cells, stromal cells and lymphocytes in the lamina propria of the small intestine and colon all stained positively. ABCB5 staining alongside negative enterocyte and positive skin controls is shown in Figure 6.5 below. Observed

membrane accentuation suggested possible cross reactivity with homologous membrane transporters such as ABCB1 (Figure 6.5). Definitive conclusions about ABCB5 expression could not be drawn despite multiple attempts to standardize commercially available ABCB5 polyclonal antibodies.

Figure 6.5. A-B: Nonspecific ABCB5 IHC staining in enterocyte and negative control. C: skin positive control ABCB5 IHC staining. D: membrane surface accentuation of ABCB5 staining in deep colonic crypts.





6.4 Discussion

6.4.1. Key results and interpretation

This study is the first to explore ABCB1 protein expression by IHC in the gastrointestinal tract of newly diagnosed children with IBD and healthy controls and to report the association between gene, protein expression and clinical phenotype. This study showed significant correlation between ABCB1 protein and gene expression in the sigmoid colon (Kruskal Wallis, P=0.007).

In conclusion, there was no differences in the intestinal ABCB1 protein expression in children with and without IBD investigated in this study. This study was able to identify statistically significant correlation between ABCB1 protein and gene expression in the sigmoid colon only. This could reflect post-transcriptional modifications that take place between mRNA production and protein expression, or the lack of specificity of the antibodies used in IHC, or the TaqMan gene expression assays used in RT qPCR. Intra-individual and inter-individual variability in ABCB1

expression in small intestine and colon was noted in cases and controls, without distinct expression pattern across the clinical phenotypes of the study participants.

There was no statistically significant difference in ABCB1 protein expression in small intestine and colon between steroid responders and steroid dependent UC patients at 3 months post diagnosis. Due to the small sample size of 8 patients, the statistical test used for correlation between ABCB1 expression and treatment response, was selected so as to minimize type II error. PUCAI at 3 months is a standardized, reliable index reflective of treatment response and has good predictive value for clinical status or treatment response at 12 months post diagnosis (Turner, Otley et al. 2007, Schechter, Griffiths et al. 2015).

A similar approach to the expression scoring implemented in the ABC study was used by Trumpi et al for semi-quantification of the intestinal expression of membrane transporters ABCB1 and ABCG2. The positively stained percentage of the luminal surface was used for grade determination as follows: grade 0=no staining, grade 1=weak, grade 2=moderate and grade 3=strong expression (Trumpi, Emmink et al. 2015). Gutmann et al described similar grading scores 0-3 for negative, low, intermediate and high expression of ABCB1 in normal and inflamed human duodenum, however no exact details are reported in their paper with regards to the objective definition of these scores (Gutmann, Hruz et al. 2008). De Oliveira graded ABCB1 expression semi-quantitatively according to the percentage of stained cells and their staining intensity. A combined numerical scoring system was used to assess the observed ABCB1 protein expression by IHC in gastric cancer patients (de Oliveira, Felipe et al. 2014).

There is lack of published IHC data on ABCB5 immunohistochemistry in human intestine. ABC study did not show intestinal ABCB5 protein expression by IHC. Other researchers have looked at different tissues or organs, for example, Volpicelli et al observed ABCB5 expression in villous trophoblasts of first trimester human placenta by IHC. ABCB5 immunoreactivity shown by IHC in the cytotrophoblast layer of the human placental trophoblast suggests that the protein could be localized in the cytoplasm, membrane or perinuclear areas; the precise distribution of ABCB5 protein requires further characterization (Volpicelli, Lezcano et al. 2014). ABCB5 is expressed in squamous cells of oral cancers, as noted in CD44 positive cells (Grimm, Krimmel et al. 2012). ABCB5 expression by IHC has been described in cutaneous melanoma where it is co-expressed in CD133 positive cells; ABCB5 is also expressed in primary malignant melanoma and lymph node metastases (Gambichler, Petig et al. 2016). Kleffel et al observed positive IHC staining in Merkel's cell carcinoma specimens in the majority of tumor cells (Kleffel, Lee et al. 2014).

Polyclonal antibodies, like the one used for ABCB5, can recognize more than one epitopes, and therefore by definition are less specific antibodies that bind to part of the epitope and can cross react with similar epitopes in other antigens (Gao, Huang et al. 2018). Antibodies bind to part of the epitope and can cross react with similar epitopes in other antigens, for example ABCB1 and ABCB5 which are highly homologous (Moitra, Scally et al. 2011). Polyclonal antibodies recognize multiple independent epitopes and therefore show enhanced affinity for epitopes available despite tissue fixation. Greater sensitivity of polyclonal antibodies for proteins with low expression has been reported (Ivell, Teerds et al. 2014). Non-specific background staining may result from the binding of the antibody's Fc region to the Fc

receptor in the sample. Polyclonal antibodies are less expensive and less time consuming to generate, in comparison to monoclonal antibodies; they also show tolerance of structural variation of primary antibodies and therefore can exhibit greater sensitivity in IHC (Ascoli and Aggeler 2018). On the other hand, the monoclonal antibodies (like the one used for ABCB1) are homogenous, offer reproducible results and are therefore in general more reliable for interpretation purposes. Monoclonal antibodies derive from known nucleic acid sequences cloned and expressed in single B lymphocyte clones and offer reproducibility as they have higher affinity to antigens. The specificity of an antibody refers to its ability to recognize a specific epitope in the presence of various epitopes. An antibody with high specificity has less cross-reactivity. In view of the data presented in this study, it is likely that the staining seen in relation to ABCB5 expression was most likely non-specific and related to cross-reactivity with other epitopes.

6.4.2. Limitations

IHC is a widely used technique, but can be subject to reaction and interpretation bias (Nicol, Fedoriw et al. 2014). Reproducibility of results and interpretation across laboratories may vary, and therefore various improvements in IHC protocols and computer assisted image analyses have been implemented over the years to minimize intra-and inter- observer bias. These include discovery of antigen retrieval methods (Huang, Minassian et al. 1976), use of secondary antibody detection (Hsu and Raine 1981) and the use of computer assisted image analysis software (Cross 2001). The latter has been implemented in estrogen receptor analysis in breast cancer research (Press, Pike et al. 1993) and in umbilical cord IHC analysis of ABCB1 and ABCC1 expression (Riches, Walia et al. 2016).

The quality and specificity of the antibodies, the protocol steps of fixation and staining and the implemented visual analogue score play an important role in the interpretation and semi-quantification of protein expression by IHC. The ABCB1 antibodies used in this study were monoclonal, whereas the ABCB5 antibody was polyclonal. No monoclonal ABCB5 antibodies were commercially available. Conformational changes in the protein influence antibody-antigen configuration and thereby can change the affinity of the binding antibody, which is reversible.

The highly variable processing of epitope and the fixation process can influence the outcome of IHC. Chemical fixatives ensure adequate fixation without altering the antigen. Formalin is a 4% v/v formaldehyde solution and the most common fixative used for fixation of any tissue for variable lengths of time; it shows broad specificity for most cellular targets in most tissues, preserves and stabilizes cell morphology and tissue architecture.

Different protein detection methods, such as Western Blot or mass spectrometry can be implemented in larger cohorts in order to explore ABCB5 transporter expression in the gut. Alternative methods of protein expression in the intestinal epithelium were outside the scope of this study, and therefore this is a limitation of this study, alongside the small number of study participants.

Despite the 10% rate of inter-observer discordance noted in this study, this semi-quantitative approach is a recognized and scientifically accepted methodology for protein expression. The degree of inter-observer discordance on the semi-quantification of transporter protein expression by IHC, is not commonly reported in above published studies. Visual analogue scoring can be subject to observer bias; there is no consensus on a standardized approach or validated automated image

analysis that can be implemented as reference for ascertaining intestinal expression of the large majority of drug transporter proteins.

The correlation between ABCBC5 expression and response to CS could not be assessed, due to the non-specific staining pattern noted by IHC for ABCB5.

6.4.3. Implications for future work

Determination of cut off values for negative and positive expression and consensus in slide scoring in large scale studies are necessary, so as to achieve optimal standardization and therefore reproducibility with minimal observer bias. Application specific antibody validation and the inception of validation framework in an assay-specific context is necessary. Collaborative studies and consensus around grades of expression of various molecules are therefore important to achieve this. Both manual and automated scoring need validation in various tissues. Tissue microarray technology is a relatively new and promising high throughput screening method in proteomics, which can allow for standardization and reproducibility of immunohistochemistry results in various tissues and the creation of reference tissue libraries (Kriegsmann, Longuespee et al. 2018).

This study is the first to explore ABCB1 protein expression by IHC in the gastrointestinal tract of newly diagnosed children with IBD and healthy controls and to report the association between gene, protein expression and clinical phenotype. Given the small number of the ABC study participants no generalizable conclusions can be drawn, prior to replication of findings in larger cohorts.

Further research is necessary to elucidate the variability of ABCB1 expression in health and disease across different age groups. Investigation of ABCB5 expression

in the gastrointestinal tract of children with IBD and healthy controls may be hampered because of the lack of commercially available specific monoclonal antibodies and the lack of detectable protein expression in the gastrointestinal tract, which would fit with the lack of detectable gene expression by RT q PCR described in chapter 5. Different protein detection methods, such as Western Blot or mass spectrometry can be implemented in larger cohorts in order to explore ABCB5 transporter expression in the gut. Alternative methods of protein expression in the intestinal epithelium were outside the scope of this study, and therefore this is a limitation of this study, alongside the small number of study participants.

Despite the 10% rate of inter-observer discordance noted in this study, this semi-quantitative approach is a recognized and scientifically accepted methodology for protein expression. The degree of inter-observer discordance on the semi-quantification of transporter protein expression by IHC, is not commonly reported in above published studies. Visual analogue scoring can be subject to observer bias; there is no consensus on a standardized approach or validated automated image analysis that can be implemented as reference for ascertaining intestinal expression of the large majority of drug transporter proteins.

Characterization of ABCB1/ABCB5 transporter expression in health and disease is of potential interest for understanding drug resistance and future drug development according to patient specific drug transporter profiles. IHC can be a valuable tool in this research, but standardization of IHC protocols, use of monoclonal antibodies, interpretation and reproducibility of results according to tissue reference libraries will be paramount to ensure avoidance of bias. Implementation of different scoring

methods would be required to be prospectively applied to large number of samples for comparison purposes.

Chapter 7

Final discussion

7.1 Summary of key results

IBD represents a steadily increasing disease burden with rising incidence in children and adults. Therapeutic challenges involve patient tailored treatment, maintaining clinical remission, achieving mucosal healing and overall optimizing the therapeutic response over time with least adverse drug reactions.

This thesis focused on the production of an overexpressing ABCB5 mammalian cell line and the characterization of ABCB5 role in CS efflux; description of specific ABCB1 and ABCB5 genotypes and their association with gene and protein expression in blood and intestinal biopsies from children with IBD and healthy controls has been investigated. A correlation of clinical response to CS at 12 months post diagnosis with ABCB1 gene and protein expression at diagnosis was made.

According to the findings of this thesis, specific *ABCB1* and *ABCB5* variant allele frequencies with resulting genotypic variations do not differ significantly across the study participants, irrespective of their clinical phenotype (healthy versus patients with IBD). ABCB1 protein and gene expression was variable without a specific pattern between cases and controls. There was weak association between ABCB1 gene expression and clinical response to CS (steroid responsive versus steroid dependent UC patients).

ABCB5 protein was not detectable in ABCB5 overexpressing mammalian cell lines, despite achieving high levels of gene expression in the respective clones. The drug uptake studies with the use of radiolabeled CS and overexpressing ABCB5 cell lines were negative. As no protein transcript was isolated from any of the ABCB5 overexpressing cell lines, including the melanoma cell line where budesonide efflux was tested, no conclusion can be safely drawn about CS efflux by ABCB5. Our

hypothesis that CS are putative ABCB5 substrates could not be confirmed, despite implementation of different strategies to detect protein expression; based on our in vitro testing, ABCB5 is unlikely to play a direct role in CS transport. ABCB5 mRNA transcripts and protein expression were not detected in the blood, small intestine and colon of healthy and treatment naïve children, newly diagnosed with IBD.

7.2 Interpretation of findings and limitations

In vitro cellular work and drug transporter assays were carried out with the use of validated laboratory protocols, according to current best practice. HEK 293 cell line was implemented as a recognized robust transfection vessel for drug uptake studies (Arena, Harms et al. 2018). Alternative cell lines such as Caco-2 cells, perhaps more relevant to the intestinal epithelium barrier, could have been used instead. Other cell lines derived from malignant leucocytes or skin melanoma cells may have been more appropriate for generation of ABCB5 overexpressing cell lines. Given the contradictory findings in the literature with regards to ABCB5 expression and function (Frank, Margaryan et al. 2005, Lin, Zhang et al. 2013, Schatton, Yang et al. 2015, Lutz, Banerjee et al. 2016, Guo, Grimmig et al. 2018), it would probably not have changed the outcome of the study, due to the concurrent implementation of positive and negative ABCB5 control cell lines used during the radio-labelled drug uptake studies. Furthermore, the collaboration established with basic scientists and experts in the field of molecular pharmacology with a track record of ABCB5 work, facilitated a broader screening approach with use of alternative cell lines -with emphasis on melanoma cell lines- as the more optimal vessel for ABCB5 expression and characterization.

Commercially available ABCB5 antibodies were used. ABCB5 antibodies produced by previous research groups had not been available for purchase at the time of request to allow for reproducibility testing using exactly same antibodies.

Positive ABCB5 gene expression and lack of detection of respective protein isoforms reported in this thesis raises the possibility that the ABCB5 gene encodes a long non-coding RNA. Studies offering new insight in the role and function of various long non coding RNAs have been recently reported; for example their involvement in colorectal cancer progression through cell proliferation inhibition and promotion of apoptosis, as well as their oncogenic role in laryngeal squamous cell carcinoma (Wei, Yang et al. 2018, Zhao, Cao et al. 2018). Long non-coding RNAs are part of the newly reported G4-RNA molecules, stem-loop spontaneously folded quadruplex RNA motifs, thought to be transiently expressed in live human cells and involved in the regulation of RNA processing and translation (Yang, Lejault et al. 2018). The oncogenic role of long non -coding RNAs has been more recognized in different types of human malignancies, including pancreatic, bladder, central nervous system cancers; their overexpression is considered a negative prognostic factor according to recent meta-analysis (Yang, Chen et al. 2018).

Gene and protein expression are regulated by circulating micro-RNA and transcription factors, which bind to either enhancing or promoter regions of DNA, resulting in up or down regulation of transcription with variable effect on protein translation; such an explanation could account for the lack of protein expression despite proven ABCB5 gene expression (Yang, Jiang et al. 2015). Functional redundancy of ABCB5 in health and specific diseases is also a biologically plausible likelihood.

Chapters 3-5 of this thesis investigate the genotypic variation, mRNA and protein expression of ABCB1 and ABCB5 in healthy children and in children with newly diagnosed IBD. Studies mainly in animal and adults have suggested that ABCB1 plays a role in susceptibility to colonic inflammation (UC) and colorectal cancer; Variant alleles for rs1045642 can confer increased risk for UC (Ho, Soranzo et al. 2006, Andersen, Svenningsen et al. 2015, Zhao, Wang et al. 2015).

The above taken together with the molecular homogeneity of ABCB1 with ABCB5 prompted the study design presented in this thesis. No significant differences were however detected between cases and controls, but as this was a pilot study, a limitation is the small sample size. Clearly, environmental factors such as diet, stress, concurrent drug administration and other epigenetic mechanisms such as DNA methylation (Howell, Kraiczy et al. 2018), may influence gene and protein expression but have not been the objective of this study. Sampling, transport, storage and possible alteration of quality of nucleic acids or cells over time prior to analysis, for instance the length of storage time prior to molecular studies, may have played a role. However, strict adherence was exercised to the study protocol and manufacturers' instructions followed; the length of time that samples had been kept in storage prior to analysis was variable and depended on the sequence of subject recruitment, but the samples were all stored at -80 ^{o}C.

The change in protocol after the second minor amendment allowed for acquisition of additional small bowel biopsies during the second part of the study. Considering the lack of studies on intestinal expression of ABCB5 and following the analysis of initial samples, this strategy allowed for protocol optimization in order to capture additional transporter expression information from different parts of the gastrointestinal tract.

IHC based semi-quantification was used for detection of ABCB1 and ABCB5 protein expression in intestinal biopsies from ABC study participants. FFPE sections with positive and negative ABCB1 intestinal controls were used. ABCB5 positive and negative skin/intestinal controls were also used for robust comparison with participants' samples. Clearly, the interpretation of IHC findings can be susceptible to potential inter-observer bias; no ABCB1/ABCB5 automated computer assisted scoring option has been implemented or validated. Future use of monoclonal, more specific, optimized antibodies may enhance protein detection; other protein detection methods, for example, fluorescence activated cell sorting (FACS), i.e. cell counting and concurrent real time analysis of physical and chemical parameters of up to thousands of particles per second could be alternatively implemented provided standardized antibodies can be developed. Alternative widely used method of protein detection in PBMCs and intestinal tissue could include tandem mass spectrometry, immunoblotting, however limitations in acquisition of tissue samples did not allow for further testing of participants' samples with additional protein detection methods for comparison purposes.

The exploration of the role of three common ABCB1 and ABCB5 genetic polymorphisms in IBD, showed no significant differences between cases and controls. However it is important to note that with respect to ABCB1 polymorphisms, there is a lot of controversy and contradictory data in the literature in many disease areas, as to whether the SNPs are functional, and whether they affect disease predisposition and drug response (Ieiri, Takane et al. 2004, Zhao, Wang et al. 2015, Mijac, Vukovic-Petrovic et al. 2018, Petryszyn and Wiela-Hojenska 2018). The large majority of Caucasian subjects recruited in the ABC study and the overall small sample size of recruited patients does not allow for generalizability of results,

certainly not to non-Caucasian populations. Allele and haplotype frequencies vary between different populations and therefore stratification of results according to population of origin is essential. For example, C allele and CC genotype of rs1045642 and G allele and GG genotype rs2032582 conferred a protective role in comparison to T allele carriage in all three common ABCB1 SNPs in a Serbian cohort of adult IBD patients. The TT genotype for rs1045642 was associated with increased UC risk in Moroccan patients. The CC genotypes for rs1128503 and rs1045642 were more frequent in CS dependent IBD Chinese patients. The genetic polymorphism for rs1045642 was not a risk factor for IBD susceptibility in a Polish adult patient cohort (Schwab, Schaeffeler et al. 2003, Ardizzone, Maconi et al. 2007, Farnood, Naderi et al. 2007, Dudarewicz, Baranska et al. 2012, Senhaji, Kassogue et al. 2015, Yang, Chen et al. 2015, Mijac, Vukovic-Petrovic et al. 2018).

As far as the selection of the ABCB5 SNPs included in the analysis is concerned, these had been extrapolated from GWAS undertaken in an adult inception cohort of IBD patients in Scotland, where initial signal indicated that ABCB5 could potentially play a role related to the observed clinical response of IBD patients to CS; this association did not however reach genome wide significance (Ho et al, personal communication). The three ABCB5 SNPs were intronic, therefore belonged to the 'non-coding' part of the human genome and were more likely to represent SNPs in LD with potential causal variants. While exonic SNPs are arguably more likely to influence gene and protein expression or a clinical phenotype, it is becoming increasingly evident that intronic SNPs can also be important in influencing disease through different mechanisms. For example, by exercising variable effect on mRNA translation, such as affecting gene promoter directionality, rate of transcription, or

transcript stability, thereby enhancing or suppressing gene expression (Agarwal and Ansari 2016, Bonnet, Grosso et al. 2017, Shaul 2017).

The role of ABCB1 gene and protein expression in determining CS response in children with IBD cannot be ascertained due to the small sample size and lack of power, however the correlation tests done in the ABC study, indicated weak correlation between m RNA and protein expression in sigmoid colon and responsiveness to (or efficacy of) CS.

ABCB1 expression has been shown to be downregulated in IBD, but upregulated following CS treatment (Farrell, Murphy et al. 2000, Hirano, Onda et al. 2004). In children with CD, rs2032582 polymorphism has been related to increased CS dependency (Krupoves, Mack et al. 2011); such effects need to be further investigated, replicated and interpreted in large cohorts over time as controversy exists in medical literature.

7.3 Future work

Deep transcriptomic and proteomic profiling of human tissues is necessary so as to direct in vitro studies in relevant patient cohorts, for instance recent studies showed ABCB5 expression in skin progenitor/stem cells and in melanoma skin cancer, hematological, ocular and colorectal malignancies (Frank, Pendse et al. 2003, Frank and Frank 2009, Koloyou, Giani et al. 2010, Wilson, Schatton et al. 2011, Borchers, Mabetalo et al. 2018). Further work is required in relation to ABCB5, in order to understand its role and tissue expression and to determine whether it does lead to the expression of a functional transporter in certain tissues and/or disease states, or

whether it is acting as a long noncoding RNA with alternative role. It is possible that ABCB5 plays no role in IBD susceptibility or treatment response, however further GWAS would be required to explore possible association or lack of it. The role of ABCB1 needs further investigation too with regards to elucidation of its role in IBD susceptibility and/or treatment response. Clinical studies exploring ABC transporter profiling as a potential target for drug efficacy or therapeutic intervention in IBD are very sparse, especially in children. Baseline data on expression profiling in humans is limited to ABCB1, ABCG2 and ABCC2.

The complexities of pharmacogenomics have been recognized over the recent years (Crews, Hicks et al. 2012); the individual profiling of ABC transporters would pose mechanistic, technical and financial challenges possibly similar to those of CYP450 enzyme guided treatment (Fleeman, McLeod et al. 2010). There are 48 known ABC transporters; evaluating the role of these transporters in health and disease will require time and resources. Recently updated recommendations by the International Transporter Consortium (Giacomini, Galetin et al. 2018) prioritize research on ABC transporters which are ubiquitously expressed or expressed at the four pharmacologically important barriers (blood brain barrier, intestinal barrier, bile ducts liver hepatocytes interface, tubule epithelial kidney cells), as they may potentially be more clinically relevant (International Transporter et al., 2010).

The importance of drug efflux transporters has been the focus of increasing attention over the last two decades (Estudante, Morais et al. 2013, Giacomini, Galetin et al. 2018). *In vitro* experimentation with implementation of cell lines and animal models has laid the foundation for understanding the function and role of drug transporters, including the transporters of the ABC superfamily. Drugs used in modern

therapeutics undergo complex transformation to their biologically active molecules in order to exert their action prior to elimination from the body (pharmacokinetics). Pharmacokinetics involves the processes of liberation of the active compound from the formulation, absorption (substance entering blood circulation), distribution (dissemination of the substance in body fluids and tissues), metabolism (irreversible transformation to metabolites) and excretion (the two latter processes can be called elimination). Drug metabolism has been studied since 20th century by R.T. Williams, who introduced the concept of phase I (oxidation, reduction, hydrolysis reactions) and phase II reactions (such as glucuronidation, acetylation, glutathione conjugation) (Bachmann 2009). New concepts of Phase 0 and Phase III reactions in drug metabolism have been proposed by Doring et al, referring to uptake and efflux of drugs across the plasma membranes by members of solute load carrier (SLC) and ABC drug transporter super-families respectively (Doring and Petzinger 2014).

The CYP450 enzymes have been found to be involved in the metabolism of many drugs (Gong, Zhang et al. 2017), for instance CS, and largely mediate multidrug interaction processes (Uno, Nakano et al. 2018). *In vitro* work can be used to predict '*in vivo'* responses, such as clinical outcomes and treatment response, however this would require validation from clinical studies in relevant patient groups. Indeed, the impact of important CYP enzymes forms part of routine evaluation for the development of investigational new drugs, starting with *in vitro* profiling, *ex vivo* mechanistic modelling and *in vivo* investigations (Johnson, Caudle et al. 2017).

ABC drug transporters and their potential effect on drug disposition has not yet received as much attention as CYP enzymes. ABCB1 has been the most studied efflux transporter to date and its biological significance has been acknowledged;

ABCB1, ABCC2 and ABCG2 have recently been included in the United States Food and Drug Administration (FDA) list of drug transporters, recommended for evaluation in new drug investigation initiatives (Giacomini, Balimane et al. 2013). The large number of uncharacterized ABC and SLC transporters could therefore make an exciting and growing field of research in Clinical Pharmacology and Therapeutics. ABC transporters are therefore an emerging and exciting field for exploration. In order to avoid methodological pitfalls and overcome technical and mechanistic difficulties, collaboration between expert basic and clinician scientists and clinical pharmacologists is paramount. Exploration of cell/tissue localization, investigation of transporter function, variability in expression due to common genetic polymorphisms and rare mutations, are important areas for further research, as well as investigation of associations with treatment outcomes. The evaluation of potential biomarkers of drug efficacy and their translational potential in clinical practice is paramount. Comprehensive understanding of the role of drug transporters in drug disposition requires a targeted approach with focus on the identification of clinically applicable transporter biomarkers, through robust, methodologically sound studies in various populations. These biomarkers would require validation in large cohorts of patients and appropriately matched controls.

ABCB1 transporter is the most studied member of the ABC superfamily to date in the context of IBD, whereas ABCB5 is far less known. This study is original in the context of exploring ABCB1/ABCB5 expression in health and childhood acquired IBD. Clinical studies in relevant patient cohorts can guide further in vitro work for identification of transporters' molecular and functional properties in health and disease. Paradigms from other chronic diseases such as rheumatoid arthritis can be useful so as to guide a multi-omics approach in IBD, with emphasis on exploration of

specific gene signatures and their potential for clinical usefulness using genomics and transcriptomics (Aterido, Canete et al. 2019). The feasibility of prospective studies in various populations and reproducibility in larger cohorts can be hampered by high costs (Haycox, Pirmohamed et al. 2014) and difficulties with tissue sampling in children. Acquisition of less invasive biological samples, for instance saliva or buccal swabs, needs to be considered as alternative sampling options, especially in children (Ensom, Chang et al. 2001), in order to inform GWAS which will facilitate expensive genotype-phenotype studies at baseline and post treatment. Investigation of gene and protein expression in clinically relevant tissues, exploration of potential association with common genetic polymorphisms and influence on drug efficacy follow the principle of pharmacogenomics and translational medicine (Relling and Evans 2015, Kaye, Schultz et al. 2017).

Appendices

Appendix 1: ABC STUDY PROTOCOL

ABC STUDY

Relative expression of ABCB1/ABCB5 membrane transporters in blood and gut biopsies of healthy and newly diagnosed paediatric patients with inflammatory bowel disease (IBD):

a pilot study in a single paediatric tertiary center

Personnel involved

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1.Background

1.1. Definition and types of IBD

Crohn's disease (CD) and ulcerative colitis (UC) are the two most common types of chronic inflammatory bowel disease (IBD), with lifelong relapsing and remitting phases. The aetiology of both conditions has not been defined. The burden of the disease is considerable in affected children. Understanding the multifactorial pathogenesis of IBD is a major research challenge. It is believed that elucidation of the genetic basis of the disease could uncover the primary mechanisms underlying its development. The application of genome scanning techniques to search for genes contributing to disease susceptibility and response to drug therapy has opened a new and exciting research avenue. As complex disorders, both UC and CD have some shared and many unique predisposing genes (1). In addition to providing clues on disease pathogenesis, knowledge of genetic polymorphisms in predisposing and modifying genes may explain variations in disease location, behavior, severity and responsiveness to therapies. Accurate phenotype classification, which encompasses disease type and severity, is also essential, so that the usefulness of genotyping data in predicting clinical variability can be fully developed (2, 3).

IBD has been showing increasing incidence in the Western World, particularly in the paediatric population over the last fifteen years. Further research is required in to the ontogeny and pathogenesis of inflammatory bowel disease in this age group

(4,5,6) The only prospective national survey of IBD in children aged <16 years in the UK during 1998-1999 showed the incidence to be 5.2 per 100,000 individuals per year (60% CD, 28% UC and 12% indeterminate colitis). It was shown to be slightly more common in boys, with a slightly higher rate of UC in Asian children than in other ethnic groups. The mean age at diagnosis was 11.9 years. For CD there were approximately equal proportions of ileitis, colitis and ileocolitis, and for UC almost 90% of children had a pancolitis (7,8).

1.2. Corticosteroids in IBD

Induction of remission is achieved with either exclusive enteral nutrition (polymeric or elemental feeds) or/and corticosteroids (CS). Insufficient data on remission induction with other medicines, other than aminosalicylates for ulcerative colitis, has limited paediatric pharmacotherapy induction agents, predominantly to these two in the first instance (8).

Remission rates of up to 60-80% have been attained with the use of exclusive enteral nutrition for 6 weeks in Crohn's patients. Steroids are administered either as oral prednisolone (from 6 up to 12 weeks)/ budesonide, or as parenteral methylprednisolone/hydrocortisone (8).

Use of the terms responsive and refractory to steroid treatment is generally used in a consistent manner between studies, as per Munkholm and Truelove (9,10):

Responsive: response to CS has been defined as clinical improvement after treatment with high-dose oral CS (40-60 mg prednisone or equivalent) within 30 days, or clinical improvement after treatment with high-dose intravenous CS within 7-10 days

Refractory: patients who fail to respond to CS within this timeframe have been defined as CS refractory

Another term that is also used is steroid dependency; however, the definition has varied with each study. This leads to various problems in analyzing therapeutic outcomes (11, 12). Nevertheless, there are a few definitions that are more frequently used in different studies, for example, the definition developed by Munkholm and Truelove (9,10):

Steroid dependent: Patients who initially respond to CS but then relapse during tapering or shortly after drug discontinuation of CS, and require re-introduction of CS therapy to maintain symptoms control, have been defined as CS dependent. These definitions allow us to define subgroups of patients based on objective clinical criteria. They have the advantage of incorporating time from first corticosteroid use, allowing for stratification of patients' steroid responsiveness at predefined time points following CS introduction (13,14,15).

1.3 Why research in steroid resistance is necessary for clinical practice

Medical evidence shows that the initial induction of remission after a first corticosteroid regime is quite successful, however subsequent relapses of disease are followed by increasing steroid resistance or steroid dependence. Both of these outcomes although by definition are completely different, translate into similar consequences for patients, which is either a need for alternative pharmacotherapies for induction of remission, or consideration of surgical management, with associated risks and comorbidities.

Up to one half of children with Crohn's disease, and most if not all (about 90%) with ulcerative colitis, will need treatment with steroids, with remission rates close to 85% and 80% respectively. One year later, around 60% of Crohn's patients and 40% of ulcerative colitis patients are steroid dependent or require surgery (13,14,15).

Continued corticosteroid treatment is therefore considered a predictor of relatively poor outcome after one year (16).

In an adult European Crohn's disease registry, 58% of patients received infliximab and 42% received non-biologic therapy. Over the 13-month follow-up period, treatment-emergent serious infections were seen significantly more often in patients receiving steroids (P = .009), but were not significantly increased in patients receiving infliximab (P = .30), (16). Another cohort study examined long term outcomes associated with steroid use in 266 IBD patients (158 with ulcerative colitis, 108 with Crohn's disease). Among ulcerative colitis patients, 82% demonstrated complete or partial response by 30 days, and at 1 year, 59% had prolonged response, 21.5% were steroid dependent,

and 19% required surgery. Among Crohn's disease patients, 84% demonstrated complete or partial response by 30 days, and at 1 year, 38% had prolonged response, 25% were steroid dependent, and 37% required surgery. There was no time-cohort effect noted, i.e. the findings supported a defined variability in corticosteroid response in IBD (14).

According to retrospective review, major intra- abdominal surgery may be required in about 15% of patients with either UC or CD within 2 years of diagnosis (15). Clinical research studies aiming to explore cellular/molecular mechanisms of steroid resistance/sensitivity in these patients, could have significant impact in day to day clinical management , as it could empower clinicians to tailor pharmacotherapy in relation to an individual genetic or biomarker profile. It would have a medium to long term impact, with regards to disease activity, morbidity and quality of life, as inflammatory bowel disease is a lifelong relapsing/remitting disease.

1.4 Pharmacogenetics

The ATPase dependent efflux transporters are expressed in tissues involved in absorption, detoxification and elimination. Members of ATP binding cassette transporters subgroup, briefly called (ABC)-transporters, such as the multidrug resistance gene MDR1 (also called ABCB1), the multidrug resistance associated proteins 1 and 2 (MRP1 or ABCC1 and MRP2 or ABCC2), and the breast cancer resistance protein (BCRP or ABCG2) have a large impact on the pharmacokinetics of numerous drugs. They may also modulate the effectiveness of drug therapy (17,18).

Research in IBD suggests that the phenomenon of glucocorticoid resistance occurs via T-lymphocytes and possibly other target inflammatory cells. According to recent review, research should be focused on three key molecular mechanisms of glucocorticoid resistance in IBD:(i) decreased cytoplasmic glucocorticoid concentration secondary to increased P-glycoprotein (PgP) mediated efflux of glucocorticoid from target cells due to overexpression of the multidrug resistance gene (MDR1); (ii) impaired glucocorticoid signaling because of dysfunction at the level of the glucocorticoid receptor; and (iii) constitutive epithelial activation of pro inflammatory mediators, including nuclear factor kappa B, resulting in inhibition of glucocorticoid receptor transcriptional activity (19).

Our work focuses on transporters: ABCB1 in particular has been implicated in poor response to corticosteroids in adult IBD patients (19,20,21). ABCB1 is upregulated in peripheral blood mononuclear cells from UC or CD patients who have been administered glucocorticoids (21,22). Inter-individual variability in expression of transporters, which has an effect on the overall bioavailability and thus efficacy of drugs, has been well documented (22,23).

ABCB5 (ATP-binding cassette subfamily B member 5), is the third and most recent member of the human MDR family to be identified. ABCB5 like ABCB1 and ABCB4 has been shown to mediate multi-drug resistance (MDR) in human cancer cells (24). ABCB5 provides doxorubicin resistance in melanoma cells by acting as a doxorubicin efflux pump , other substrates include rhodamine 123, camptothecin and 5-fluorouracil (25,26,27).

A recent retrospective cohort study conducted in two tertiary paediatric gastroenterology units in Canada, showed that ABCB1 gene may be associated with corticosteroid dependence in children with Crohn's. Thirteen tagging single nucleotide polymorphisms (tag-SNPs) and a synonymous variation (C3435T) in the ABCB1 gene were genotyped. Tag-SNP rs2032583 was statistically significantly associated with CS dependency. The rare C allele of this SNP (odds ratio [OR] = 0.56, 95% confidence interval [CI]: 0.34-0.95, P = 0.029) and heterozygous genotype TC (OR = 0.52, 95% CI: 0.28-0.95, P = 0.035) conferred protection from CS dependency (33).

In a different study, Weiss et al (34) have quantified the mRNA expression of the four ABC-transporters and of the Pregnane X receptor (PXR; nuclear hormone receptor for corticosteroids, which regulates induction of genes involved in response to xenobiotics metabolism, i.e. CYP3A4, ABC family genes), a key regulator in drug metabolism and efflux, in peripheral blood mononuclear cells (PBMCs), and corresponding liver or small intestine samples of humans by real-time reverse transcription-polymerase chain reaction (RT-PCR). The results obtained prove the absence of a correlation between the expression of four major ABC-transporters in PBMCs and in the intestine or liver. For all transporters (except MRP1/ABCC1 in the intestine), the mRNA concentration for

ABC-transporters was positively correlated with PXR expression in PBMCs and intestine.

In conclusion, this study suggests that basal expression levels of the transporters are directly influenced by PXR expression in liver and PBMCs and demonstrates that PBMCs do not qualify as surrogate tissue for the expression of the four ABC-transporters in small intestine and liver (23,34,35,36).

1.5 Rationale for planned research

While the molecular basis of glucocorticoid resistance has been widely assessed in other inflammatory conditions, the pathophysiology of the glucocorticoid resistance in IBD is poorly understood. ABC transporters have been implicated in the pathogenesis of steroid resistance, but the work has mostly focused on ABCB1 (P-glycoprotein; PgP) (24,30,31,32).

Dexamethasone, hydrocortisone, prednisone, methylprednisolone and budesonide are known to be ABCB1 substrates. Our aim is to investigate the role of ABCB5, a close homologue of ABCB1, in steroid resistance, in light of our recent, in vitro data in healthy adults (28). At the same time, we will also investigate ABCB1 to evaluate the comparative effects of these two transporters in health and disease.

Given the close homology between ABCB5 and ABCB1, we hypothesize that these two transporters have overlapping substrate specificities and tissue localization, and demonstrate inter-individual variability of expression and variability of expression between healthy and individuals with disease.

Interestingly, studies in adult patients have shown that ABCB1 and ABCB5 expression may influence response of IBD patients to steroid treatment (28). However, this has not yet been examined in relation to ABCB5. It is therefore

important to explore expression of above transporters in both blood and gut biopsies, so as to draw more informative results in healthy children and children with IBD. This could then enable us to investigate role of these transporters in steroid responsiveness in IBD. **This is a pilot study to answer some of these questions.**

Ultimately and as part of a further definitive study, we would like to investigate the correlation between genomic profile, clinical phenotype/disease progression and steroid responsiveness.

1.6 Research hypothesis

ABCB5 expression in PBMCs and enterocytes is not affected in children with IBD, compared with ABCB1 which, from adult studies, has been shown to undergo down regulation.

Research questions

1. How does ABCB1 and ABCB5 expression compare in blood and gut biopsies of newly diagnosed patients with IBD and healthy controls?
2. What is the relative expression of ABCB1 and ABCB5 expression in inflamed and non-inflamed gut mucosa in newly diagnosed IBD patients?
3. What is the relative importance ofABCB1 and ABCB5 expression in determining steroid resistance in newly diagnosed IBD patients?

1.7 Ethical and safety considerations

Consent to research

The study will require approval from a relevant local ethics committee. All participants will require parental / guardian informed consent so as to be recruited in the study. In addition, assent will be sought from children who are old enough to understand the implications of participating in the study. The ability of a

child to assent will be based on their ability to understand the information presented (including factors such as age and maturity), and will be judged on a case by case basis. Prior to the consent/ assent process, information will be provided to the parents/guardians and the participants about the nature of the study and the expected information it will produce. This information will be available in a variety of age appropriate formats. Every effort will be made to provide as much information as possible prior to endoscopy: this will be done either in clinic and/ or by post with appropriate contact details. However, in the majority of patients, endoscopy is performed on a day case basis and it is possible that a number of patients will be asked for consent on the day of the procedure itself, just after consent for endoscopy has been obtained. Consent for the research study can be withdrawn until full sample anonymization.

Venous blood (up to 6 milliliters) will be collected at initial diagnostic work up during induction of anesthesia in theatre, just prior to endoscopy, when patients will routinely have a cannula inserted. Blood will be analyzed for ABCB1 and ABCB5 genotyping, gene and protein expression. Microscopic (up to 3 millimeters in size) biopsies from inflamed and non- inflamed duodenum and ileum or colon will be collected, without additional risk or discomfort to the patient. Patients and parents will have been fully informed and consented about the procedure itself, by the clinician who will be undertaking the procedure. Usually between 10 and up to 18 biopsies are routinely taken during endoscopy; up to 10 of these will be snap frozen in liquid nitrogen and sent for RNA analysis. Immunohistochemistry will be used for protein analysis in paraffin embedded gut tissue, which will have been used in pathology department.

Sample storage and DNA testing

Patient samples will be clearly labelled with subject number, processed and safely transferred to the Wolfson Centre for Personalized Medicine (Department of Clinical Pharmacology and Therapeutics, University of Liverpool), where they will be safely stored. The consent/assent process and patient information leaflets will explain that samples will be retained in a coded form stored on university computers at the Wolfson Centre and/ or on National Health System(NHS) computers at Alder Hey Children's NHS Foundation Trust. They will be used for genotyping, gene expression and protein expression analysis, as per basic science protocols. Clinical data will be safely stored at the Research and Development Department in Alder Hey.

As the clinical implications of the study findings will not be known until the definitive study is completed, we will not disclose information to patients on their biopsy results. This information will also not be routinely disclosed to any third party. The results will not affect the ability of the patients or their families to obtain medical/ travel insurance. If a significant association is found in our study, it will need validation in another cohort; once this has been done and the findings published, the future clinical utility will need to be decided upon by other researchers, regulators and bodies that develop clinical guidelines.

2. Study Objectives, Design and Statistics

2.1 Study aims and objectives

1. Compare ABCB1 and ABCB5 expression in blood and gut of healthy and children newly diagnosed with IBD
2. Explore whether ABCB1 and ABCB5 expression differs in inflamed gut in comparison with non-inflamed in children newly diagnosed with IBD

3. Investigate the possible association between phenotype and genotype of newly diagnosed IBD patients, by genotyping for ABCB1 and ABCB5 SNPs (37).

This is a pilot study, so as to investigate these questions in a small number of IBD patients and healthy controls, which will provide us with valuable information on the design and size of a future definitive study. A pilot study is necessary since the work with ABCB5 is completely novel with no other research group having published on its role in steroid resistance.

2.2 Study design

This is a prospective observation cohort study over a nine-twelve month period in a single tertiary paediatric hospital, involving:

a) Paediatric patients undergoing scheduled diagnostic endoscopy and work up (based on clinical grounds only), for suspected IBD, or for exclusion of gastrointestinal (GI) tract pathology purposes.

b) Paediatric patients with histologically confirmed IBD (CD or UC), and healthy controls (histologically normal GI tract).

All participants and controls will be prospectively identified from scheduled endoscopy lists. The decision for a diagnostic endoscopy will have been made on clinical grounds only, and according to national and international guidelines. This will have been agreed beforehand between paediatric gastroenterology consultants and families, either in the clinic or on the ward. Subjects will be allocated to the IBD group or to healthy control group, depending on the findings in the tissue biopsies.

2.3 Phenotype assessment

Accurate phenotyping will be undertaken, as per the Paediatric Paris Modification of the Montreal Classification for Inflammatory Bowel Disease. This algorithm has

been devised in June 2011 by an international group of experts in paediatric gastroenterology. It is a constellation of the latest evidence which amalgamated and modified the Montreal Classification, so as to reflect more accurately the paediatric disease phenotype spectrum. Patients are classified in subgroups, depending on age, growth, disease location, extent and behavior in Crohn's (as informed by clinical evidence of growth delay, macroscopic endoscopic findings, radiological GI imaging), and disease severity in UC (as defined by Paediatric Ulcerative Colitis Activity Index, PUCAI score) (2).

3.Selection and withdrawal of participants

3.1. Participant selection

IBD group (inclusion criteria)

1) Children between 2 and 16 years of age who will undergo planned upper and lower GI diagnostic endoscopy in Alder Hey, because of high clinical index of suspicion for IBD disease (nine-twelve month period).

2) Written informed consent obtained from parent/ guardian (as <16 years at recruitment).

3) Assent obtained from competent young person (assessed on case by case basis)

Estimated number of patients to be recruited over a nine-twelve month period in Alder Hey is 35.

Healthy control group (inclusion criteria)

1) Children between 2 and 16 years of age, who will undergo planned upper and lower GI diagnostic endoscopy for variable GI symptomatology , with

histologically normal gut biopsy, recruited from the same specialist clinical area over same nine-twelve month period.

2) Written informed consent obtained from parent/guardian (as ≤ 16 years at recruitment)

3) Assent obtained from competent young person (assessed on case by case basis).

Estimated number of patients to be recruited over a nine-twelve month period in Alder Hey is 50.

Exclusion criteria

1) Parent guardian unwilling to take part or not understanding/unsure of the nature of the research study, in case of any communication issues (however every effort will be made for interpreter for example).

2) Competent older participant unwilling to assent (competence assessed on a case by case basis).

3) Patient not suitable to participate in the study, if for example alternative diagnosis accounting for GI symptomatology is made, such as coeliac disease , threadworms or cancer.

4)Patients with poor genotype data.

There is no restriction on medications in this study. All medications and any co morbidities at the time of recruitment will be noted in case record files (CRFs).

3.2 Participant Recruitment

Recruitment target

On average between 60 and 70 new patients are diagnosed with IBD on an annual basis in Alder Hey. There is seasonal variation and patients tend to be diagnosed in

clusters, with increased incidence during winter months, we therefore estimate that up to 35 new IBD patients will be diagnosed in nine-twelve months.

Furthermore, on average about 100 endoscopies are undertaken annually in Alder Hey, where patients have a histologically normal GI tract. Extrapolating this to the nine month recruitment period, we expect that up to 50 patients with normal gut biopsies will be seen over nine-twelve months.

Study population

The study population will be recruited from Alder Hey, where consenting will take place as well as the sampling for recruited individuals. There are four planned endoscopy lists per week. We aim to recruit at least on two of these lists per week, subject to parental consent, availability of relevant clinical and non-clinical staff, laboratory facilities and resources.

As this is a prospective study in a fixed time period and taking into account above figures, it is possible that our target sample of healthy controls could be reached earlier than estimated. In order to avoid over or under recruiting in any of the two cohorts, we will aim to have patients selected from the extreme ends of the clinical spectrum, working closely and as guided by consultant paediatric gastroenterologists, overseeing patient care. Consent will be sought appropriately from families.

Sample and Data Collection

Patients who are eligible to be recruited to the research project will provide a single sample of blood (up to 6 milliliters) for DNA, RNA and protein expression analysis, as well as up to 12 (up to 3 millimeters in size) microscopic gut biopsies from inflamed and non-inflamed tissue from upper and lower GI tract. Samples will

be initially processed for stabilization purposes and temporarily stored at the University Laboratory at the Institute of Child Health in Alder Hey. They will then be safely delivered at the Wolfson Center for Personalized Medicine, at the University of Liverpool, Department of Pharmacology. Samples will be further processed, DNA and RNA will be extracted. Protein expression analysis will be undertaken using immunohistochemistry to assess protein expression in the gut (this will require obtaining paraffin-embedded tissue from the Pathology Department, once it has been used for clinical diagnosis). All biological samples will be stored securely within the Wolfson Centre for Personalized Medicine, in keeping with the Human Tissue Act (license held by the University of Liverpool). A bar coding system will add additional security in terms of sample tracking and for confidentiality purposes.

Data to be collected from patients will include demographics, such as age, sex, weight, height, ethnicity and clinical data, including current symptoms, medical history, relevant family history, routine medication, investigational reports including routine blood tests, endoscopy, radiology and pathology reports. The patient's clinical care will not be affected by taking part in the study.

3.3 Withdrawal of participants

Participants will be informed that they are free to withdraw from the study at any time up to the point that the samples will be coded but not anonymized. When the samples are fully anonymized, this will not be possible. The investigator may remove a subject, if, in his/ her opinion, it is in the best interests of the subject. If a patient withdraws from the study, the reason will be recorded.

3.4 DNA/gene expression analysis

Laboratory work- DNA analysis/gene and protein expression

DNA/RNA will be extracted from whole blood using commercially available kits. Patients will be genotyped for ABCB1 and ABCB5 SNPs, which have been predicted to have a functional effect or have been previously identified to have an effect on ABCB1 and ABCB5 expression. RNA will be reverse transcribed to cDNA and gene expression will be quantified by quantitative PCR. For protein analysis, cell fix will be used to fix PBMCs in whole blood. Red blood cells will be lysed and separated from the fixed PBMCs.

As soon as possible upon collection, part of the gut tissue will be snap frozen in liquid nitrogen and stored at -80°C. Intestinal ABCB1 and ABCB5 gene expression will be prospectively determined by quantitative PCR. One cell thick sections from paraffin embedded gut biopsies will be incubated with ABCB1 and ABCB5 antibodies and secondary antibodies. Protein expression will be visualized with diaminobenzidintetrahydrochloride (DAB).

3.5 Statistical analysis

Given the novelty of our findings with ABCB5, and the fact that no such study has been undertaken in children, this should be regarded as a pilot study, and thus no sample size calculation is possible. Any statistical analysis that is required will be undertaken in collaboration with Dr Andrea Jorgensen in the department of Biostatistics in the University of Liverpool. We have extensive expertise in this area (38). Sample size calculation has been done and the recruitment target between 60-85 participants has been set.

4. Safety assessment

The study will be conducted with close attention to patient safety. Participants recruited will have donated blood and gut tissue. There is minimal risk of bruising or bleeding as far as the venepuncture is concerned. However, this will only be

undertaken as part of routine care during induction of anesthesia. No additional risk is associated with gut biopsy as such, other than the risks relevant to the endoscopy itself, which will have been explicitly explained to the parent/patient during routine consenting process by competent clinical staff, prior to undertaking the procedure.

5. Direct access to source data and documents

The case record files (CRFs) will be supplied by the investigator for recording all data collected during the study. All CRFs are to be completely filled out by personnel administering the study procedures and reviewed and signed by the Chief Investigator. All CRFs are to be completed in a clear, legible manner. Black ink must be used to ensure accurate interpretation of data. Any corrections are to be made by drawing a line through, and have to be initialed.

The CRFs will be securely stored in a filing cabinet in Research and Development Department at Alder Hey. This database will be classed as source documentation. Every effort should be made to have the CRFs completed as soon as possible following recruitment. All study documentation will be made available to Ethics Committee and to regulatory authorities for inspection on request. Any study relevant data produced will be stored on University of Liverpool computers and will be password protected and backed up. Data managers may liaise with researchers and relevant clinicians regarding data storing.

The following abbreviations may be applicable for use: NA=not applicable, NK=not known, ND=not done, NR not retrievable or not available.

Individual subject demographic /medical information obtained as a result of this study is considered confidential and disclosure to third parties other than those noted below is prohibited. Records identifying the subject will be kept confidential and, to

the extent permitted by the applicable laws and /or regulations will not be made publicly available. All study center personnel will comply with the privacy rules of their institutions and/or professional groups and with the Institute of Child Health Guideline for Good Clinical Practice.Individual subject medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited, except for trial-related inspection on request by one or more of the following:

Research Ethics Committee

Auditors (including those instructed by regulatory bodies)

Documents relating to the trial that contain personal data that may disclose the identity of the subject will remain in locked filing cabinet. The Investigator will not provide any personal data that may identify the subject to any third party at any time during or after the study. Subject confidentiality will be further assured by using unique participant identification code number.

The link between the patient's name and code will be broken and the sample completely anonymized when the study is completed and all clinical data have been obtained. We will maintain adequate records for the study including completed CRFs, laboratory reports signed consent/assent forms, adverse experience reports, information regarding subjects who discontinued, all correspondence with the Research Ethics Committee and other pertinent data. All records are to be retained by the Investigator for the period of 15 years.

6.Ethics

Potential subjects for the study will be prospectively identified from scheduled endoscopy lists undertaken by competent or supervised endoscopists. Potential participants will be given a detailed oral presentation of the study nature and may

receive detailed written information by post or in clinic prior to the procedure. However, due to the fact that endoscopies are performed on

a day case basis, other than children who may be inpatients, consent may be sought on the day of the procedure, during the immediate preoperative time allocated to patient consenting. A member of the research/clinical team will be available to discuss the project in detail for clarifications to be made as necessary. All subjects will be informed of the nature and the purpose of the study, its requirements and possible hazards, and their rights to withdraw at any time from the study without prejudice and without jeopardy to any future medical care. They will have adequate opportunity to ask the investigator or nominated designee about any aspect of the study and they will be supplied with contact details for further contact, if desired. Each subject must agree to co-operate in all aspects of the study, so as to be eligible and must give informed written consent to the researcher/ clinician for participation.

Three consent/assent forms will be signed, one for the researcher, one for the case notes and the other for the participant. Signed Consent Forms must remain in the study file and will be available for monitoring purposes at any time.

The study will comply will be conducted in compliance with the guidelines of the Declaration of Helsinki on biomedical research involving human volunteers (Hong Kong revision, 1989 and the 48th General Assembly, Somerset West, Republic of South Africa, October 1996, updated in October 2000), Institute of Child Health Good Clinical Practice guidelines, relevant regulatory guidelines, and the study protocol. The protocol and relevant substantive data will be submitted for consideration by the Local Ethics Research Committee and written approval from the Chair of the Ethics

Committee will be obtained before the study is initiated and clinical activities of the study can commence.

The Ethics Committee will be notified promptly by the Investigator about the following:

a) deviations from or changes of protocol to eliminate immediate hazards to the study volunteers

b) changes increasing the risk to volunteers and /or affecting significantly the conduct of the study

c) new information that may affect the safety of the volunteers or the conduct of this study

d) if any problem/adverse experience occurs during blood or tissue sampling, this will be recorded in the CRF and the patient/parent will be informed as soon as possible following the procedure

7. Quality assurance, Data Handling, Publication Policy and Finance

The principal Investigator will take overall responsibility for the internal monitoring of all CRFs, taking care to ensure all entries are complete and legible and to otherwise ensure compliance to the above mentioned protocols/guidelines. The Investigator will permit representatives of the regulatory authorities to inspect facilities and records relevant to this study.

The Chief Investigator will be sample and produced data custodian.

Principal and Chief Investigators will be responsible for preparing any interim and final study reports, with statistical support as necessary. All results generated from this study may be submitted for publication in peer reviewed medical journals or for presentation within the department, in scientific and medical conferences.

The study has been funded by Pfizer in partnership with the Department of Clinical Pharmacology and Therapeutics, University of Liverpool.

The study will be co-sponsored by the University of Liverpool and Alder Hey Children's Foundation NHS Trust.

Appendix 2: Case record file (CRF)

Data collected in the participant CRF

Age

Sex

Weight

Height

Ethnicity

Past medical history/current symptoms/quality of life

Current medication

Past medication relevant to IBD

Relevant family history

Investigational results including routine blood tests, pathology and radiology reports for barium meal and follow through/abdominal ultra sound scan/magnetic resonance enterography as well as endoscopy findings

Adverse experience/effect

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Appendix 2: Case record file (**CRF**)

Data to be collected in the participant CRF

Age

Sex

Weight

Height

Ethnicity

Past medical history/current symptoms/quality of life

Current medication

Past medication relevant to IBD

Relevant family history

Investigational results including routine blood tests, pathology and radiology reports for barium meal and follow through/abdominal ultra sound scan/magnetic resonance enterography as well as endoscopy findings

Adverse experience/effect

Appendix 3

NHS
Health Research Authority
National Research Ethics Service

NRES Committee North West - Greater Manchester East
3rd Floor, Barlow House
4 Minshull Street
Manchester
M1 3DZ

Telephone: 0161 625 7820
Facsimile: 0161 625 7299

26 April 2012

Professor Munir Pirmohamed, Professor of Clinical Pharmacology,
University of Liverpool
Waterhouse Buildings: Block A
1-5 Brownlow Street
Liverpool
L69 3GL

Dear Professor Pirmohamed

Study title:	**Relative expression of ABCB1/ABCB5 membrane transporters in blood and gut biopsies of healthy and newly diagnosed paediatric patients with inflammatory bowel disease(IBD): a pilot study in a single paediatric tertiary centre**
REC reference:	**12/NW/0207**

Thank you for your letter of 13 April 2012, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Appendices 4-6: Patient information leaflets (PILs)

ABC STUDY

Child info leaflet 6-10Y

What is this about ?

Children may have sore tummy especially when they eat or pass poo.

Why is my tummy sore?

There are many reasons. Your body is made of millions of cells, and DNA in the cell tells it what to do. Everybody's DNA is different. We want to study the DNA to see how we can improve the tummy pain.

Why me ?

Because you have sore tummy. We want to look at your DNA type and see how it relates to your tummy pain.

What will happen?

After your parents agree to your scope, we will ask you and your parents some questions about your health. This will take about 20 minutes. Then, we will look into what your doctor knows about your health. We also need to collect DNA samples.

How is DNA collected?

We will take a teaspoonful of blood when you will be put to sleep for your scope, and also take four extra tiny shavings from your gut lining through the camera.

No other tests are needed.

We do not expect any extra risks to taking part. If we learn more about tummy ache, we will tell people to help change things for children with sore tummy.

ABC study

INFO LEAFLET 11-16Y

What is the study about?

Some children and young people experience severe recurrent tummy pain, especially when eating or passing stool. This may be due to IBD (inflammatory bowel disease).

What is IBD?

It is an illness which requires frequent visits to doctor or hospital, because of sore tummy and problems with eating and passing stool. Various medications will be needed and also surgery.

version 3.0
13 April 2012

What is DNA?

We want to collect DNA to assess how you respond to the medicines used for IBD. DNA is the genetic make up of our body cells. Each person's DNA makes him/her special and different.

How will DNA be collected?

A teaspoonful of blood will be taken whilst you are put to sleep for your scope, and also four extra, tiny shavings from your gut lining will be taken through the camera. No other tests are needed.

What will happen if I take part?

On the day of your scope, provided your parents consent to the scope, we will ask you and your parents questions about your tummy pain and whether you wish to take part in the study, it will take about 20 minutes. Then, we will look into what your doctor knows about your health. DNA samples will be collected and kept safe until we look at them in the future.

Appendix 7: First Substantial amendment

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Health Research Authority
National Research Ethics Service

NRES Committee North West - Greater Manchester East

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03 December 2012

Professor Munir Pirmohamed, Professor of Clinical Pharmacology,
University of Liverpool
Waterhouse Buildings: Block A
1-5 Brownlow Street
Liverpool
L69 3GL

Dear Professor Pirmohamed

Study title:	**Relative expression of ABCB1/ABCB5 membrane transporters in blood and gut biopsies of healthy and newly diagnosed paediatric patients with inflammatory bowel disease(IBD): a pilot study in a single paediatric tertiary centre**
REC reference:	**12/NW/0207**
Amendment number:	**Substantial amendment 1**
Amendment date:	**19 November 2012**
Summary:	**To halt the study temporarily while necessary laboratory work is completed.**

Thank you for submitting the above amendment, which was received on 03 December 2012. This is a valid notice of a substantial amendment and will be reviewed by the Sub-Committee of the REC at its next meeting.

Documents received

The documents to be reviewed are as follows:

Document	Version	Date
Notice of Substantial Amendment (non-CTIMPs)	Substantial amendment 1	19 November 2012

Notification of the Committee's decision

The Committee will issue an ethical opinion on the amendment within a maximum of 35 days from the date of receipt.

Appendix 8: Second substantial amendment: ABC study

NHS
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22 April 2013

Dr A Konidari
Clinical Research Fellow in Paediatric Pharmacology / Paediatric Speciality Registrar
University of Liverpool
Wolfson Centre for Personalised Medicine
University of Liverpool
Waterhouse Buildings – Block A
1-5 Brownlow Street
Liverpool
L69 3GL

Dear Dr Konidari

Study title:	**Relative expression of ABCB1/ABCB5 membrane transporters in blood and gut biopsies of healthy and newly diagnosed paediatric patients with inflammatory bowel disease(IBD): a pilot study in a single paediatric tertiary centre**
REC reference:	**12/NW/0207**
Amendment number:	**Substantial Amendment 2**
Amendment date:	**25 March 2013**
IRAS project ID:	**101025**

The above amendment was reviewed by the Sub-Committee in correspondence.

Ethical opinion

The Sub Committee found no ethical issues with this amendment.

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Notice of Substantial Amendment (non-CTIMPs)	Substantial Amendment 2	25 March 2013
Participant Information Sheet: Child (11-16 years)		
Participant Information Sheet: Child (6-10 years)		

References

Abanda, N. N., et al. (2017). "Lobular Distribution and Variability in Hepatic ATP Binding Cassette Protein B1 (ABCB1, P-gp): Ontogenetic Differences and Potential for Toxicity." Pharmaceutics **9**(1).

Abdalla, M. I. and H. Herfarth (2016). "Budesonide for the treatment of ulcerative colitis." Expert Opin Pharmacother **17**(11): 1549-1559.

Abele, R. and R. Tampe (2011). "The TAP translocation machinery in adaptive immunity and viral escape mechanisms." Essays Biochem **50**(1): 249-264.

Abu-Qare, A. W., et al. (2003). "A role for P-glycoprotein in environmental toxicology." J Toxicol Environ Health B Crit Rev **6**(3): 279-288.

Actis, G. C. and R. Pellicano (2016). "The pathologic galaxy modulating the genotype and phenotype of inflammatory bowel disease: comorbidity, contiguity, and genetic and epigenetic factors." Minerva Medica **107**(6): 401-412.

The inflammatory bowel diseases (IBDs) are being seen as a gut inflammatory hub occurring: 1) with inflammatory spots in the eyes, skin, liver, joints (extra-intestinal

manifestations); 2) with functionally contiguous disorders such as psoriasis and lung disease (barrier organ diseases); 3) as the consequence of genetic loss of non-redundant cell functions that are critical for gut homeostasis and defense (monogenic IBD). Recent multidisciplinary analysis, fostered by the input of genomic search, has helped hypothesize two pathogenetic models for the main phenotypes of IBDs. In ulcerative colitis, an increased mucosal permeability would prevail, allowing arousal of inflammation from the hyper-reactive underneath lymphoid tissue; an impaired bacterial sensing by innate immunity cells would by contrast place Crohn's disease (CD) in the chapter of the immune deficiency disorders, with the activity phases (the actual target of traditional immune suppressive strategies) representing just "zenith" phases in the continuously waxing-and-waning course of the attempts of the blunted inflammatory machinery to clear the invaders. Studying such errors of innate immunity, a few open-minded investigators have observed that they might not be a CD exclusivity at all: the proven evidence of CD-like pictures in a plethora of granulomatous disorders has thus been consolidated. This scenario calls for a concept of "syndrome" to best be accounted for, and to encourage the envisaging of the future therapy for IBD.

Actis, G. C., et al. (2011). "Inflammatory bowel disease: beyond the boundaries of the bowel." Expert Review of Gastroenterology & Hepatology **5**(3): 401-410.

Dysregulated inflammation in the gut, designated clinically as inflammatory bowel disease (IBD), is manifested by the prototypic phenotypes of an Arthus-like reaction

restricted to the mucosa of the colon, as in ulcerative colitis, or a transmural granulomatous reaction, as in Crohn's disease, or an indeterminate form of the two polar types. That the inflammation of IBD can trespass the boundaries of the bowel has long been known, with articular, ophthalmologic, cutaneous, hepatobiliary or other complications/associations - some autoimmune and others not - affecting significant numbers of patients with IBD. Also notable is the frequency of diagnosis of IBD-type diseases on a background of systemic, (mostly myelo-hematological) disorders, associated with alterations of either (or both) innate or adaptive arms of the immune response. Finally, cases of IBD are reported to occur as an adverse effect of TNF inhibitors. Bone marrow transplant has been proven to be the only curative measure for some of the above cases. Thus, in effect, the IBDs should now be regarded as a systemic, rather than bowel-localized, disease. Genome-wide association studies have been informative in consolidating the view of three phenotypes of IBD (ulcerative colitis, Crohn's disease and mixed) and, notably, are revealing that the onset of IBD can be linked to polymorphisms in regulatory miRNAs, or to nucleotide sequences coding for regulatory lymphokines and/or their receptors. At the effector level, we emphasize the major role of the Th17/IL-23 axis in dictating the perpetuation of intestinal inflammation, augmented by a failure of physiological control by regulatory T-cells. In conclusion, there is a central genesis of the defects underlying IBD, which therefore, in our opinion, is best accommodated by the concept of IBD as more of a syndrome than an autonomous disease. This altered mindset should upgrade our knowledge of IBD, influence its medical care and provide a platform for further advances.

Adamiak, T., et al. (2013). "Incidence, clinical characteristics, and natural history of pediatric IBD in Wisconsin: a population-based epidemiological study." Inflamm Bowel Dis **19**(6): 1218-1223.

BACKGROUND: Epidemiological studies of pediatric inflammatory bowel diseases (IBD) are needed to generate etiological hypotheses and inform public policy; yet, rigorous population-based studies of the incidence and natural history of Crohn's disease (CD) and ulcerative colitis (UC) in the United States are limited. METHODS: We developed a field-tested prospective system for identifying all new cases of IBD among Wisconsin children over an 8-year period (2000-2007). Subsequently, at the end of the study period, we retrospectively reconfirmed each case and characterized the clinical course of this incident cohort. RESULTS: The annual incidence of IBD among Wisconsin children was 9.5 per 100,000 (6.6 per 100,000 for CD and 2.4 per 100,000 for UC). Approximately 19% of incident cases occurred in the first decade of life. Over the 8-year study period, the incidence of both CD and UC remained relatively stable. Additionally, (1) childhood IBD affected all racial groups equally, (2) over a follow-up of 4 years, 17% of patients with CD and 13% of patients with patients with UC required surgery, and (3) 85% and 40% of children with CD were treated with immunosuppressives and biologics, respectively, compared with 62% and 30% of patients with UC. CONCLUSIONS: As in other North American populations, these data confirm a high incidence of pediatric-onset IBD. Importantly, in this Midwestern U.S. population, the incidence of CD and UC seems to be relatively stable over the last decade. The proportions of children requiring surgery and

undergoing treatment with immunosuppressive and biological medications underscore the burden of these conditions.

Adcock, I. M., et al. (1999). "Ligand-induced differentiation of glucocorticoid receptor (GR) trans-repression and transactivation: preferential targetting of NF-kappaB and lack of I-kappaB involvement." Br J Pharmacol **127**(4): 1003-1011.

Agarwal, N. and A. Ansari (2016). "Enhancement of Transcription by a Splicing-Competent Intron Is Dependent on Promoter Directionality." PLoS Genet **12**(5): e1006047.

Ahmad, T., et al. (2002). "The molecular classification of the clinical manifestations of Crohn's disease." Gastroenterology **122**(4): 854-866.

BACKGROUND & AIMS: Crohn's disease is a common inflammatory disorder of the gut characterized by variation in both location and behavior. Chromosome 16 and the HLA region on chromosome 6 have been implicated in susceptibility to disease. Mutations in the NOD2/CARD15 gene, recently identified on chromosome 16, have been associated with disease overall but are found in only 25% of patients. No data regarding their contribution to specific disease subtypes exist. Here we report a detailed genotype-phenotype analysis of 244 accurately characterized patients. METHODS: A total of 244 white patients with Crohn's disease recruited from a single center in the United Kingdom were studied. All patients were rigorously phenotyped

and followed-up for a median time of 16 years. By using linkage disequilibrium mapping we studied 340 polymorphisms in 24 HLA genes and 3 NOD2/CARD15 polymorphisms. RESULTS: We show that NOD2/CARD15 mutations determine ileal disease only. We confirm that alleles on specific long-range HLA haplotypes determine overall susceptibility and describe novel genetic associations with susceptibility, location, and behavior of Crohn's disease. CONCLUSIONS: The clinical pattern of Crohn's disease may be defined by specific genotypes. This study may provide the basis for a future molecular classification of disease.

Ahmed, I., et al. (2016). "Microbiome, Metabolome and Inflammatory Bowel Disease." Microorganisms **4**(2).

Inflammatory Bowel Disease (IBD) is a multifactorial disorder that conceptually occurs as a result of altered immune responses to commensal and/or pathogenic gut microbes in individuals most susceptible to the disease. During Crohn's Disease (CD) or Ulcerative Colitis (UC), two components of the human IBD, distinct stages define the disease onset, severity, progression and remission. Epigenetic, environmental (microbiome, metabolome) and nutritional factors are important in IBD pathogenesis. While the dysbiotic microbiota has been proposed to play a role in disease pathogenesis, the data on IBD and diet are still less convincing. Nonetheless, studies are ongoing to examine the effect of pre/probiotics and/or FODMAP reduced diets on both the gut microbiome and its metabolome in an effort to define the healthy diet in

patients with IBD. Knowledge of a unique metabolomic fingerprint in IBD could be useful for diagnosis, treatment and detection of disease pathogenesis.

Al-Mofarreh, M. A. and I. A. Al-Mofleh (2013). "Emerging inflammatory bowel disease in saudi outpatients: a report of 693 cases." Saudi J Gastroenterol **19**(1): 16-22.

BACKGROUND/AIM: Inflammatory bowel disease (IBD) is a chronic disease of unknown etiology and considered traditionally as a disease of the western world. Recently, rising trends have been observed in countries previously known to have a low prevalence and incidence. The aim of this study is to collect epidemiological data on IBD outpatients and to add data from the Kingdom of Saudi Arabia (KSA) to the available IBD literature. PATIENTS AND METHODS: The medical records of 693 Saudi patients with IBD over a period of 17 years, between 1993 and 2009, were reviewed. The demographic and clinical data and methods of diagnosis were retrieved. RESULTS: The total number of patients in this cohort was 693. It constituted 238 (34.3%) ulcerative colitis (UC) and 455 (65.7%) Crohn's disease (CD) patients. UC was steady throughout the years, whereas only 1.2 CD patients were diagnosed per year in the first 11 years, and 73.7 per year in the last six years. The median age of UC patients was 34 years, ranging from 10 to 80 years with a peak between 21 and 40 years and in CD it was 27 years, ranging from 11 to 73 years with a peak between 11 and 30 years. There was a male preponderance of 1.5:1 and 2:1, respectively. The rest of the data is discussed in this study. CONCLUSION: IBD is no

longer a rare disease in KSA. UC is in a steady state, whereas CD is increasing significantly and far outnumbering UC.

Aldhous, M. C., et al. (2007). "Does cigarette smoking influence the phenotype of Crohn's disease? Analysis using the Montreal classification." Am J Gastroenterol **102**(3): 577-588.

OBJECTIVES: The clinical subclassification of Crohn's disease by phenotype has recently been reevaluated. We have investigated the relationships between smoking habit, age at diagnosis, disease location, and progression to stricturing or penetrating complications using the Montreal classification. METHODS: 408 patients (157 male, median age 29.4 yr) were assessed. Data were collected on smoking habit, age at diagnosis, anatomical distribution, and disease behavior. Follow-up data were available on all patients (median 10 yr). RESULTS: At diagnosis, ex-smokers (N = 53) were older than nonsmokers (N = 177) or current smokers (N = 178, medians 43.2 vs 28.3 or 28.9 yr, respectively, P < 0.001). Disease location differed according to smoking habit at diagnosis (chi(2)= 24.1, P= 0.02) as current smokers had less colonic (L2) disease than nonsmokers or ex-smokers (30%vs 45%, 50%, respectively). In univariate Kaplan-Meier survival analysis, smoking habit at diagnosis was not associated with time to development of stricturing disease, internal penetrating disease, perianal penetrating disease, or time to first surgery. Patients with isolated colonic (L2) disease were slower to develop strictures (P < 0.001) or internal penetrating disease (P= 0.001) and to require surgery (P < 0.001). Cox models with smoking habit as time-dependent covariates showed that, relative to ileal (L1)

location of disease, progression to stricturing disease was less rapid for patients with colonic (L2) disease (HR 0.140, P < 0.001), but not independently affected by smoking habit. Progression to surgery was also slower for colonic (L2) than ileal (L1) disease location (HR 0.273, P < 0.001), but was independent of smoking habit. CONCLUSIONS: Smoking habit was associated with age at diagnosis and disease location in Crohn's disease, while disease location was associated with the rate of development of stricturing complications and requirement for surgery. The pathogenic basis of these observations needs to be explained.

Alexander, S., Kelly E, Marion NV, Peters JA., Faccenda E., Harding SD., Pawson AJ, Sharman JL, Southan C, Davies JA, CGTP Collaborators (2017) (2017-2018). "The concise guide to Pharmacology: Transporters." Br J Pharmaco.l **174**(Suppl 1): S360-S446.

Allez, M., et al. (2010). "Report of the ECCO pathogenesis workshop on anti-TNF therapy failures in inflammatory bowel diseases: definitions, frequency and pharmacological aspects." J Crohns Colitis **4**(4): 355-366.

The first ECCO pathogenesis workshop focused on anti-TNF therapy failures in inflammatory bowel diseases (IBDs). The overall objective was to better understand and explore primary non response and loss of response to anti-TNF agents in IBD. The outcome of this workshop is presented into two parts. This first section addresses definitions, frequency and pharmacological aspects of anti-TNF therapy failure, including pharmacokinetics of anti-TNF monoclonal antibodies and immune and non-

immune mediated clearance of anti-TNF mAbs. The second section concerns the biological roles of TNF and TNF antagonists, including mechanisms of action of anti-TNF agents, and discuss hypothesis regarding their failures and phenomenon of paradoxical inflammation, including the potential role of TNF independent inflammatory pathways.

Ananthakrishnan, A. N. (2015). "Epidemiology and risk factors for IBD." Nat Rev Gastroenterol Hepatol **12**(4): 205-217.

IBD, comprising Crohn's disease and ulcerative colitis, is a chronic immunologically mediated disease at the intersection of complex interactions between genetics, environment and gut microbiota. Established high-prevalence populations of IBD in North America and Europe experienced the steepest increase in incidence towards the second half of the twentieth century. Furthermore, populations previously considered 'low risk' (such as in Japan and India) are witnessing an increase in incidence. Potentially relevant environmental influences span the spectrum of life from mode of childbirth and early-life exposures (including breastfeeding and antibiotic exposure in infancy) to exposures later on in adulthood (including smoking, major life stressors, diet and lifestyle). Data support an association between smoking and Crohn's disease whereas smoking cessation, but not current smoking, is associated with an increased risk of ulcerative colitis. Dietary fibre (particularly fruits and vegetables), saturated fats, depression and impaired sleep, and low vitamin D levels have all been associated with incident IBD. Interventional studies assessing the effects of modifying these risk

factors on natural history and patient outcomes are an important unmet need. In this Review, the changing epidemiology of IBD, mechanisms behind various environmental associations and interventional studies to modify risk factors and disease course are discussed.

Andersen, V., et al. (2015). "Novel understanding of ABC transporters ABCB1/MDR/P-glycoprotein, ABCC2/MRP2, and ABCG2/BCRP in colorectal pathophysiology." World J Gastroenterol **21**(41): 11862-11876.

AIM: To evaluate ATP-binding cassette (ABC) transporters in colonic pathophysiology as they had recently been related to colorectal cancer (CRC) development. METHODS: Literature search was conducted on PubMed using combinations of the following terms: ABC transporters, ATP binding cassette transporter proteins, inflammatory bowel disease, ulcerative, colitis, Crohns disease, colorectal cancer, colitis, intestinal inflammation, intestinal carcinogenesis, ABCB1/ P-glycoprotein (P-gp/CD243/MDR1), ABCC2/multidrug resistance protein 2 (MRP2) and ABCG2/breast cancer resistance protein (BCRP), Abcb1/Mdr1a, abcc2/Mrp2, abcg2/Bcrp, knock-out mice, tight junction, membrane lipid function. RESULTS: Recently, human studies reported that changes in the levels of ABC transporters were early events in the adenoma-carcinoma sequence leading to CRC. A link between ABCB1, high fat diet and gut microbes in relation to colitis was suggested by the animal studies. The finding that colitis was preceded by altered gut bacterial composition suggests that deletion of Abcb1 leads to fundamental changes of host-

microbiota interaction. Also, high fat diet increases the frequency and severity of colitis in specific pathogen-free Abcb1 KO mice. The Abcb1 KO mice might thus serve as a model in which diet/environmental factors and microbes may be controlled and investigated in relation to intestinal inflammation. Potential molecular mechanisms include defective transport of inflammatory mediators and/or phospholipid translocation from one side to the other of the cell membrane lipid bilayer by ABC transporters affecting inflammatory response and/or function of tight junctions, phagocytosis and vesicle trafficking. Also, diet and microbes give rise to molecules which are potential substrates for the ABC transporters and which may additionally affect ABC transporter function through nuclear receptors and transcriptional regulation. Another critical role of ABCB1 was suggested by the finding that ABCB1 expression identifies a subpopulation of pro-inflammatory Th17 cells which were resistant to treatment with glucocorticoids. The evidence for the involvement of ABCC2 and ABCG2 in colonic pathophysiology was weak. CONCLUSION: ABCB1, diet, and gut microbes mutually interact in colonic inflammation, a well-known risk factor for CRC. Further insight may be translated into preventive and treatment strategies.

Andersen, V., et al. (2013). "Low ABCB1 gene expression is an early event in colorectal carcinogenesis." PLoS One **8**(8): e72119.

The ABCB1/MDR1 gene product ABCB1/P-glycoprotein is implicated in the development of colorectal cancer (CRC). NFKB1 encodes transcription factors

regulating expression of a number of genes including ABCB1. We have previously found association between the ABCB1 C-rs3789243-T polymorphism and CRC risk and interactions between the ABCB1 C-rs3789243-T and C3435T polymorphisms and meat intake in relation to CRC risk (Andersen, BMC Cancer, 2009, 9, 407). ABCB1 and NFKB1 mRNA levels were assessed in intestinal tissue from 122 CRC cases, 101 adenoma cases (12 with severe dysplasia, 89 with mild-moderate dysplasia) and from 18 healthy individuals, together with gene polymorphisms in ABCB1 and NFKB1. ABCB1 mRNA levels were highest in the healthy individuals and significantly lower in mild/moderate and severe dysplasia tissue ($P<0.05$ for both), morphologically normal tissues close to the tumour ($P<0.05$), morphologically normal tissue at a distance from the tumour ($P<0.05$) and CRC tissue ($P<0.001$). Furthermore, ABCB1 mRNA levels were lower in adenomas and carcinomas compared to morphologically normal tissue from the same individuals ($P<0.01$). The ABCB1 C-rs3789243-T and NFKB1 -94ins/del homozygous variant genotypes were associated with low ABCB1 mRNA levels in morphologically normal sigmoid tissue from adenoma cases ($P<0.05$ for both). NFKB1 mRNA levels were lower in both tumour and normal tissue from cancer patients ($P<0.001$) as compared to healthy individuals but we were unable to show association between NFKB1 -94ins/del genotype and NFKB1 mRNA levels. This study suggests that low ABCB1 mRNA levels are an early event in CRC development and that the two polymorphisms affect ABCB1 mRNA levels whereas low NFKB1 mRNA levels occur later in carcinogenesis. Low ABCB1 protein levels may promote colorectal carcinogenesis through increasing intracellular exposure to carcinogenic ABCB1 substrates.

Annese, V. (2019). "A Review of Extraintestinal Manifestations and Complications of Inflammatory Bowel Disease." Saudi J Med Med Sci **7**(2): 66-73.

Extraintestinal manifestations (EIMs) are common in inflammatory bowel disease (IBD), in both Crohn's disease and ulcerative colitis. Almost any organ system can be affected, including the musculoskeletal, dermatologic, renal, hepatopancreatobiliary, pulmonary and ocular systems. However, the musculoskeletal and dermatologic systems are the most commonly involved sites of manifestations. While some manifestations such as peripheral arthritis and erythema nodosum have an association with IBD activity, others such as axial arthropathy, pyoderma gangrenosum and primary sclerosing cholangitis have an independent disease course. This review provides a summary of the most common EIMs in IBD and their prevalence and management.

Annese, V., et al. (2006). "Multidrug resistance 1 gene in inflammatory bowel disease: a meta-analysis." World J Gastroenterol **12**(23): 3636-3644.

The MDR1 gene is an attractive candidate gene for the pathogenesis of inflammatory bowel disease (IBD) and perhaps response to therapy, with evidences at both functional and genetic levels. Its product, the P-glycoprotein (P-gp) functions as a transmembrane efflux pump thus influencing disposition and response of many drugs, some of whom (i.e. glucocorticoids) central to IBD therapy. In addition P-gp is highly expressed in many epithelial surfaces, included gastrointestinal tract (G-I) with a

putative role in decreasing the absorption of endogenous or exogenous toxins, and perhaps host-bacteria interaction. Many genetic variations of MDR1 gene has been described and in some instances evidences for different P-gp expression as well drugs metabolism have been provided. However data are often conflicting due to genetic heterogeneity and different methodologies employed. Perhaps the greatest piece of evidence of the physiological importance of P-gp in the G-I tract has come from the description of the mdr1 knock-out mice model, which develops a spontaneous colitis in a specific pathogen-free environment. Studies investigating MDR1 gene polymorphism and predisposition to IBD have also shown conflicting results, owing to the known difficulties in complex diseases, especially when the supposed genetic contribution is weak. In this study we have undertaken a meta-analysis of the available findings obtained with two SNPs polymorphism (C3435T and G2677T/A) in IBD; a significant association of 3435T allele and 3435TT genotype has been found with UC (OR = 1.17, P = 0.003 and OR = 1.36, P = 0.017, respectively). In contrast no association with CD and the G2677T/A polymorphism could be demonstrated.

Archanioti, P., et al. (2011). "Micro-RNAs as regulators and possible diagnostic bio-markers in inflammatory bowel disease." J Crohns Colitis **5**(6): 520-524.

Ardizzone, S., et al. (2007). "Multidrug resistance 1 gene polymorphism and susceptibility to inflammatory bowel disease." Inflamm Bowel Dis **13**(5): 516-523.

Ardlie, K. G., et al. (2015). "The Genotype-Tissue Expression (GTEx) pilot analysis: Multitissue gene regulation in humans." Science **348**(6235): 648-660.

Understanding the functional consequences of genetic variation, and how it affects complex human disease and quantitative traits, remains a critical challenge for biomedicine. We present an analysis of RNA sequencing data from 1641 samples across 43 tissues from 175 individuals, generated as part of the pilot phase of the Genotype-Tissue Expression (GTEx) project. We describe the landscape of gene expression across tissues, catalog thousands of tissue-specific and shared regulatory expression quantitative trait loci (eQTL) variants, describe complex network relationships, and identify signals from genome-wide association studies explained by eQTLs. These findings provide a systematic understanding of the cellular and biological consequences of human genetic variation and of the heterogeneity of such effects among a diverse set of human tissues.

Arena, T. A., et al. (2018). "High Throughput Transfection of HEK293 Cells for Transient Protein Production." Methods Mol Biol **1850**: 179-187.

Arijs, I., et al. (2009). "Mucosal gene signatures to predict response to infliximab in patients with ulcerative colitis." Gut **58**(12): 1612-1619.

Arisawa, T., et al. (2017). "Genetic polymorphisms of MAFK, encoding a small Maf protein, are associated with susceptibility to ulcerative colitis in Japan." World J Gastroenterol **23**(29): 5364-5370.

Ascoli, C. A. and B. Aggeler (2018). "Overlooked benefits of using polyclonal antibodies." Biotechniques **65**(3): 127-136.

Asl Baakhtari, S., et al. (2017). "Observational Study of Perspectives of Inflammatory Bowel Disease Patients Concerning the Use of Corticosteroids." Dig Dis.

AIM: We aimed to investigate the factors that make inflammatory bowel disease (IBD) patients more or less likely to be willing to take corticosteroids. METHODS: Respondents completed a questionnaire. The primary outcome was whether the respondents would or would not use corticosteroids again to treat their IBD. Three separate univariate and multivariate analyses were performed to examine which variables predicted willingness to take steroids, including specific side effects. RESULTS: Four hundred fifty three respondents (321 with Crohn's disease, 115 with ulcerative colitis; mean age 40 years, 297 [66%] female) completed the questionnaire. Corticosteroid efficacy (OR 6.83, 95% CI 3.67-12.7), lack of previous negative side effects (OR 0.11, 95% CI 0.04-0.32), and positive side effects (OR 2.96, 95% CI 1.63-5.40) were associated with a willingness to use corticosteroids in the future. In multivariate analysis, weight gain (OR 0.53, 95% CI 0.29-0.98) and hallucinations (OR 0.28, CI 0.09-0.89) were associated with an unwillingness to use corticosteroids

again, whereas increased energy (OR 2.30, 95% CI 1.20-4.42) was the only significant positive side effect in a multivariate model. CONCLUSIONS: Past experiences with corticosteroids influence whether patients will take corticosteroids again. Clinicians should enquire about side effects and positive psychological symptoms associated with corticosteroid use.

Aterido, A., et al. (2019). "A Combined Transcriptomic and Genomic Analysis Identifies a Gene Signature Associated With the Response to Anti-TNF Therapy in Rheumatoid Arthritis." Frontiers in Immunology **10**: 1459.

Background: Rheumatoid arthritis (RA) is the most frequent autoimmune disease involving the joints. Although anti-TNF therapies have proven effective in the management of RA, approximately one third of patients do not show a significant clinical response. The objective of this study was to identify new genetic variation associated with the clinical response to anti-TNF therapy in RA. Methods: We performed a sequential multi-omic analysis integrating different sources of molecular information. First, we extracted the RNA from synovial biopsies of 11 RA patients starting anti-TNF therapy to identify gene coexpression modules (GCMs) in the RA synovium. Second, we analyzed the transcriptomic association between each GCM and the clinical response to anti-TNF therapy. The clinical response was determined at week 14 using the EULAR criteria. Third, we analyzed the association between the GCMs and anti-TNF response at the genetic level. For this objective, we used genome-wide data from a cohort of 348 anti-TNF treated patients from Spain. The

GCMs that were significantly associated with the anti-TNF response were then tested for validation in an independent cohort of 2,706 anti-TNF treated patients. Finally, the functional implication of the validated GCMs was evaluated via pathway and cell type epigenetic enrichment analyses. Results: A total of 149 GCMs were identified in the RA synovium. From these, 13 GCMs were found to be significantly associated with anti-TNF response ($P < 0.05$). At the genetic level, we detected two of the 13 GCMs to be significantly associated with the response to adalimumab ($P = 0.0015$) and infliximab ($P = 0.021$) in the Spain cohort. Using the independent cohort of RA patients, we replicated the association of the GCM associated with the response to adalimumab ($P = 0.0019$). The validated module was found to be significantly enriched for genes involved in the nucleotide metabolism ($P = 2.41e-5$) and epigenetic marks from immune cells, including CD4+ regulatory T cells ($P = 0.041$). Conclusions: These findings show the existence of a drug-specific genetic basis for anti-TNF response, thereby supporting treatment stratification in the search for response biomarkers in RA.

Auphan, N., et al. (1995). "Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis." Science **270**(5234): 286-290.

Aye, I. L., et al. (2009). "Transport of lipids by ABC proteins: interactions and implications for cellular toxicity, viability and function." Chem Biol Interact **180**(3): 327-339.

Bachmann, K. (2009). Chapter 8: Drug metabolism in Pharmacology**:** 131-173.

Barnes, P. J. and I. M. Adcock (2009). "Glucocorticoid resistance in inflammatory diseases." Lancet **373**(9678): 1905-1917.

Glucocorticoid resistance or insensitivity is a major barrier to the treatment of several common inflammatory diseases-including chronic obstructive pulmonary disease and acute respiratory distress syndrome; it is also an issue for some patients with asthma, rheumatoid arthritis, and inflammatory bowel disease. Several molecular mechanisms of glucocorticoid resistance have now been identified, including activation of mitogen-activated protein (MAP) kinase pathways by certain cytokines, excessive activation of the transcription factor activator protein 1, reduced histone deacetylase-2 (HDAC2) expression, raised macrophage migration inhibitory factor, and increased P-glycoprotein-mediated drug efflux. Patients with glucocorticoid resistance can be treated with alternative broad-spectrum anti-inflammatory treatments, such as calcineurin inhibitors and other immunomodulators, or novel anti-inflammatory treatments, such as inhibitors of phosphodiesterase 4 or nuclear factor kappaB, although these drugs are all likely to have major side-effects. An alternative treatment strategy is to reverse glucocorticoid resistance by blocking its underlying mechanisms. Some examples of this approach are inhibition of p38 MAP kinase, use of vitamin D to restore interleukin-10 response, activation of HDAC2 expression by use of theophylline, antioxidants, or phosphoinositide-3-kinase-delta inhibitors, and inhibition of macrophage migration inhibitory factor and P-glycoprotein.

Barretina, J., et al. (2012). "The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity." Nature **483**(7391): 603-607.

Barretina, J., et al. (2011). "Integrative analysis of the Cancer Cell Line Encyclopedia reveals genetic and transcriptional predictors of compound sensitivity." Cancer Research **71**.

Barrett, J. C., et al. (2005). "Haploview: analysis and visualization of LD and haplotype maps." Bioinformatics **21**(2): 263-265.

Barrett, J. C., et al. (2008). "Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease." Nature Genetics **40**(8): 955-962.

Several risk factors for Crohn's disease have been identified in recent genome-wide association studies. To advance gene discovery further, we combined data from three studies on Crohn's disease (a total of 3,230 cases and 4,829 controls) and carried out replication in 3,664 independent cases with a mixture of population-based and family-based controls. The results strongly confirm 11 previously reported loci and provide genome-wide significant evidence for 21 additional loci, including the regions containing STAT3, JAK2, ICOSLG, CDKAL1 and ITLN1. The expanded molecular

understanding of the basis of this disease offers promise for informed therapeutic development.

Baumgart, D. C. and S. R. Carding (2007). "Inflammatory bowel disease: cause and immunobiology." Lancet **369**(9573): 1627-1640.

Crohn's disease and ulcerative colitis are idiopathic inflammatory bowel disorders. In this paper, we discuss how environmental factors (eg, geography, cigarette smoking, sanitation and hygiene), infectious microbes, ethnic origin, genetic susceptibility, and a dysregulated immune system can result in mucosal inflammation. After describing the symbiotic interaction of the commensal microbiota with the host, oral tolerance, epithelial barrier function, antigen recognition, and immunoregulation by the innate and adaptive immune system, we examine the initiating and perpetuating events of mucosal inflammation. We pay special attention to pattern-recognition receptors, such as toll-like receptors and nucleotide-binding-oligomerisation-domains (NOD), NOD-like receptors and their mutual interaction on epithelial cells and antigen-presenting cells. We also discuss the important role of dendritic cells in directing tolerance and immunity by modulation of subpopulations of effector T cells, regulatory T cells, Th17 cells, natural killer T cells, natural killer cells, and monocyte-macrophages in mucosal inflammation. Implications for novel therapies, which are discussed in detail in the second paper in this Series, are covered briefly.

Beato, M. and J. Klug (2000). "Steroid hormone receptors: an update." Hum Reprod Update **6**(3): 225-236.

Benchimol, E. I., et al. (2009). "Increasing incidence of paediatric inflammatory bowel disease in Ontario, Canada: evidence from health administrative data." Gut **58**(11): 1490-1497.

Objective: Health administrative databases can be used to track chronic diseases. The aim of this study was to validate a case ascertainment definition of paediatric-onset inflammatory bowel disease (IBD) using administrative data and describe its epidemiology in Ontario, Canada.

Methods: A population-based clinical database of patients with IBD aged,15 years was used to define cases, and patient information was linked to health administrative data to compare the accuracy of various patterns of healthcare use. The most accurate algorithm was validated with chart data of children aged,18 years from 12 medical practices. Administrative data from the period 1991-2008 were used to describe the incidence and prevalence of IBD in Ontario children. Changes in incidence were tested using Poisson regression.

Results: Accurate identification of children with IBD required four physician contacts or two hospitalisations (with International Classification of Disease (ICD) codes for IBD) within 3 years if they underwent colonoscopy and seven contacts or three hospitalisations within 3 years in those without colonoscopy (children < 12 years old, sensitivity 90.5%, specificity > 99.9%; children < 15 years old, sensitivity 89.6%,

specificity > 99.9%; children < 18 years old, sensitivity 91.1%, specificity 99.5%). Age-and sex-standardised prevalence per 100 000 population of paediatric IBD has increased from 42.1 (in 1994) to 56.3 (in 2005). Incidence per 100 000 has increased from 9.5 (in 1994) to 11.4 (in 2005). Statistically significant increases in incidence were noted in 0-4 year olds (5.0%/year, p = 0.03) and 5-9 year olds (7.6%/year, p < 0.0001), but not in 10-14 or 15-17 year olds.

Conclusion: Ontario has one of the highest rates of childhood-onset IBD in the world, and there is an accelerated increase in incidence in younger children.

Benoni, C. and H. Prytz (1998). "Effects of smoking on the urine excretion of oral Cr-51 EDTA in ulcerative colitis." Gut **42**(5): 656-658.

Berg, D. R., et al. (2019). "The Role of Early Biologic Therapy in Inflammatory Bowel Disease." Inflamm Bowel Dis.

The goals for treatment of inflammatory bowel diseases (IBDs) are changing from elimination of symptoms toward complete disease control-a process that demands both clinical and endoscopic remission. This new IBD treatment paradigm has been shifting from a conventional "step-up" approach toward a more "top-down" early intervention treatment strategy. Recent studies suggest that the use of biologic agents, specifically those targeting tumor necrosis factor alpha, earlier in the treatment course improves patient outcomes and can prevent progression to irreversible bowel damage.

Although the strategy of early intervention has accumulating evidence in Crohn's disease, there is less evidence supporting its impact in ulcerative colitis.

Bianchi Porro, G., et al. (2007). "Review article: the management of steroid dependency in ulcerative colitis." Aliment Pharmacol Ther **26**(6): 779-794.

BACKGROUND: Approximately 20% of patients with ulcerative colitis have a chronic active disease often requiring several courses of systemic steroids in order to achieve remission, but followed by relapse of symptoms during steroid tapering or soon after their discontinuation. Although short term control of symptoms can be achieved with steroid treatment, this pattern of drug response, known as steroid-dependency, leads to important complications of the treatment, while a significant proportion of patients requires colectomy. AIM: To review the studies currently available specifically evaluating the management of steroid-dependent ulcerative colitis. RESULTS: The clinical and biological mechanisms of steroid-dependency are not well understood compared with those determining steroid-refractoriness. Very few evidence-based data are available concerning the management of patients with steroid-dependent ulcerative colitis. The therapeutic role of aminosalicylates, thiopurines, methotrexate, infliximab, leukocyte apheresis and other drugs in the treatment of steroid-dependent ulcerative colitis are evaluated. CONCLUSIONS: Outcomes of studies in steroid-refractory patients may not be applicable to steroid-dependency. Trials are needed to define the correct approaches and new strategies to ameliorate the therapy of steroid-dependent ulcerative colitis.

Billiet, T., et al. (2015). "Drug Concentrations and Antibodies to Infliximab Are Inferior to the Impact of Disease Burden in Primary Non-Response to Infliximab in Crohn's Disease Patients." Gastroenterology **148**(4): S62-S62.

Birimberg-Schwartz, L., et al. (2017). "Development and Validation of Diagnostic Criteria for IBD Subtypes Including IBD-unclassified in Children: a Multicentre Study From the Pediatric IBD Porto Group of ESPGHAN." J Crohns Colitis **11**(9): 1078-1084.

Background: The revised Porto criteria identify subtypes of paediatric inflammatory bowel diseases: ulcerative colitis [UC], atypical UC, inflammatory bowel disease unclassified [IBDU], and Crohn's disease [CD]. Others have proposed another subclassifiction of Crohn's colitis. In continuation of the Porto criteria, we aimed to derive and validate criteria, termed "PIBD-classes," for standardising the classification of the different IBD subtypes. Methods: This was a multicentre retrospective longitudinal study from 23 centres affiliated with the Port -group of ESPGHAN. Both a hypothesis-driven judgmental approach and mathematical classification and regression tree [CART] modelling were used for creating a diagnostic algorithm. Since small bowel inflammation is easily recognised as CD, we focused here primarily on the phenotype of colitis. Results: In all, 749 IBD children were enrolled: 236 [32%] Crohn's colitis, 272 [36%] UC and 241 [32%] IBDU [age 10.9 +/- 3.6 years] with a median follow-up of 2.8 years (interquartile range [IQR] 1.7-4.3). A total of 23 features were clustered in three classes according to their

prevalence in UC: six class-1 features [0% prevalence in UC], 12 class-2 features [< 5% prevalence], and five class-3 features [5-10% prevalence]. According to the algorithm, the disease should be classified as UC if no features exist in any of the classes. When at least one feature exists, different combinations classify the disease into atypical UC, IBDU or CD. The algorithm differentiated UC from CD and IBDU with 80% sensitivity (95% confidence interval [CI] 71-88%) and 84% specificity [77-89%], and CD from IBDU and UC with 78% sensitivity [67-87%] and 94% specificity [89-97%]. Conclusions: The validated PIBD-classes algorithm can adequately classify children with IBD into small bowel CD, colonic CD, IBDU, atypical UC, and UC.

Bonnet, A., et al. (2017). "Introns Protect Eukaryotic Genomes from Transcription-Associated Genetic Instability." Mol Cell **67**(4): 608-621 e606.

Borchers, S., et al. (2018). "Detection of ABCB5 tumour antigen-specific CD8(+) T cells in melanoma patients and implications for immunotherapy." Clin Exp Immunol **191**(1): 74-83.

Bouguen, G., et al. (2015). "Intestinal steroidogenesis." Steroids **103**: 64-71.

Bouguen, G., et al. (2015). "Intestinal steroidogenesis controls PPARgamma expression in the colon and is impaired during ulcerative colitis." Gut **64**(6): 901-910.

Bousvaros, A., et al. (2006). "Challenges in pediatric inflammatory bowel disease." Inflamm Bowel Dis **12**(9): 885-913.

Braker, P. (2014). ABCB5 and the regulation of p16INK4a by non-coding RNA, Queen Mary University of London. **PhD**.

Brey, E. M., et al. (2003). "Automated selection of DAB-labeled tissue for immunohistochemical quantification." J Histochem Cytochem **51**(5): 575-584.

Brouwer, K. L., et al. (2013). "In vitro methods to support transporter evaluation in drug discovery and development." Clin Pharmacol Ther **94**(1): 95-112.

Brzozowski, B., et al. (2016). "Mechanisms by which Stress Affects the Experimental and Clinical Inflammatory Bowel Disease (IBD): Role of Brain-Gut Axis." Current Neuropharmacology **14**(8): 892-900.

Background: Stress of different origin is known to alter so called "braingut axis" and contributes to a broad array of gastrointestinal disorders including inflammatory bowel disease (IBD), irritable bowel syndrome (IBS) and other functional gastrointestinal diseases. The stressful situations and various stressors including psychosocial events, heat, hypo- and hyperthermia may worsen the course of IBD via unknown mechanism. The aims of this paper were to provide an overview of experimental and clinical evidences that stress activates the brain-gut axis which results in a mucosal mast cells activation and an increase in the production of proinflammatory cytokines and other endocrine and humoral mediators.

Methods: Research and online content related to effects of stress on lower bowel disorders are reviewed and most important mechanisms are delineated.

Results: Brain conveys the neural, endocrine and circulatory messages to the gut via brain-gut axis reflecting changes in corticotrophin releasing hormone, mast cells activity, neurotransmission at the autonomic nerves system and intestinal barrier function all affecting the pathogenesis of animal colitis and human IBD. Stress triggers the hypothalamus-pituitary axis and the activation of the autonomic nervous system, an increase in cortisol levels and proinflammatory cytokines such as tumor necrosis factor-alpha, interleukin-8, interleukin-1beta and interleukin-6.

Conclusion: The acute or chronic stress enhances the intestinal permeability weakening of the tight junctions and increasing bacterial translocation into the intestinal wall. An increased microbial load in the colonic tissue, excessive cytokine release and a

partially blunted immune reactivity in response to stress result in its negative impact on IBD.

Butwicka, A., et al. (2019). "Association of Childhood-Onset Inflammatory Bowel Disease With Risk of Psychiatric Disorders and Suicide Attempt." JAMA Pediatr.

Importance: Inflammatory bowel disease (IBD) has been associated with psychiatric morbidity in adults, although previous studies have not accounted for familial confounding. In children, IBD has an even more severe course, but the association between childhood-onset IBD and psychiatric morbidity remains unclear. Objective: To examine the risk of psychiatric morbidity in individuals with childhood-onset IBD, controlling for potential confounding shared between siblings. Design, Setting, and Participants: A population-based cohort study was conducted using data from the Swedish national health care and population registers of all children younger than 18 years born from 1973 to 2013. The study included 6464 individuals with a diagnosis of childhood-onset IBD (3228 with ulcerative colitis, 2536 with Crohn disease, and 700 with IBD unclassified) who were compared with 323200 matched reference individuals from the general population and 6999 siblings of patients with IBD. Cox proportional hazards regression was used to estimate hazard ratios (HRs) with 95% CIs. Statistical analysis was performed from January 1, 1973, to December 1, 2013. Main Outcomes and Measures: The primary outcome was any psychiatric disorder and suicide attempt. Secondary outcomes were the following specific psychiatric disorders: psychotic, mood, anxiety, eating, personality, and behavioral disorders;

substance misuse; attention-deficit/hyperactivity disorder; autism spectrum disorders; and intellectual disability. Results: The study included 6464 individuals with a diagnosis of childhood-onset IBD (2831 girls and 3633 boys; mean [SD] age at diagnosis of IBD, 13 [4] years). During a median follow-up time of 9 years, 1117 individuals with IBD (17.3%) received a diagnosis of any psychiatric disorder (incidence rate, 17.1 per 1000 person-years), compared with 38044 of 323200 individuals (11.8%) in the general population (incidence rate, 11.2 per 1000 person-years), corresponding to an HR of 1.6 (95% CI, 1.5-1.7), equaling 1 extra case of any psychiatric disorder per 170 person-years. Inflammatory bowel disease was significantly associated with suicide attempt (HR, 1.4; 95% CI, 1.2-1.7) as well as mood disorders (HR, 1.6; 95% CI, 1.4-1.7), anxiety disorders (HR, 1.9; 95% CI, 1.7-2.0) eating disorders (HR, 1.6; 95% CI, 1.3-2.0), personality disorders (HR, 1.4; 95% CI, 1.1-1.8), attention-deficit/hyperactivity disorder (HR, 1.2; 95% CI, 1.1-1.4), and autism spectrum disorders (HR, 1.4; 95% CI, 1.1-1.7) Results were similar for boys and girls. Hazard ratios for any psychiatric disorder were highest in the first year of follow-up but remained statistically significant after more than 5 years. Psychiatric disorders were particularly common for patients with very early-onset IBD (<6 years) and for patients with a parental psychiatric history. Results were largely confirmed by sibling comparison, with similar estimates noted for any psychiatric disorder (HR, 1.6; 95% CI, 1.5-1.8) and suicide attempt (HR, 1.7; 95% CI, 1.2-2.3). Conclusions and Relevance: Overall, childhood-onset IBD was associated with psychiatric morbidity, confirmed by between-sibling results. Particularly concerning is the increased risk of suicide attempt, suggesting that long-term psychological support be considered for patients with childhood-onset IBD.

Cascorbi, I. (2017). "Inflammation: Treatment Progress and Limitations." Clin Pharmacol Ther **102**(4): 564-567.

There is an increasing understanding on the etiology of chronic immune-mediated inflammatory diseases such as inflammatory bowel disease (IBD), psoriasis, or rheumatoid arthritis. Large consortia contributed to the elucidation of the genetics, for instance, of IBD identifying a number of genes involved in innate mucosal defense and immune tolerance (most prominent, e.g., NOD2) and other related processes. For a number of such diseases, common genetic susceptibility loci were identified, suggesting overlapping immune response pathways, although there is no causality of single genetic traits.2 In particular, the elucidation of main triggers of inflammation like tumor necrosis factor alpha (TNFalpha), integrins, specific cytokines like interleukin (IL)-6 or IL-23 launched the successful development of new pharmacological approaches, leading to a tremendous improvement of therapeutic outcomes.

Cascorbi, I. (2018). "The Pharmacogenetics of Immune-Modulating Therapy." Adv Pharmacol **83**: 275-296.

Castano-Rodriguez, N., et al. (2017). "Dual role of Helicobacter and Campylobacter species in IBD: a systematic review and meta-analysis." Gut **66**(2): 235-249.

OBJECTIVE: To conduct a comprehensive global systematic review and meta-analysis on the association between Helicobacter pylori infection and IBD. As bacterial antigen cross-reactivity has been postulated to be involved in this association, published data on enterohepatic Helicobacter spp (EHS) and Campylobacter spp and IBD was also analysed. DESIGN: Electronic databases were searched up to July 2015 for all case-control studies on H. pylori infection/EHS/ Campylobacter spp and IBD. Pooled ORs (P-OR) and 95% CIs were obtained using the random effects model. Heterogeneity, sensitivity and stratified analyses were performed. RESULTS: Analyses comprising patients with Crohn's disease (CD), UC and IBD unclassified (IBDU), showed a consistent negative association between gastric H. pylori infection and IBD (P-OR: 0.43, p value <1e-10). This association appears to be stronger in patients with CD (P-OR: 0.38, p value <1e-10) and IBDU (P-OR: 0.43, p value=0.008) than UC (P-OR: 0.53, p value <1e-10). Stratification by age, ethnicity and medications showed significant results. In contrast to gastric H. pylori, non H. pylori-EHS (P-OR: 2.62, p value=0.001) and Campylobacter spp, in particular C. concisus (P-OR: 3.76, p value=0.006) and C. showae (P-OR: 2.39, p value=0.027), increase IBD risk. CONCLUSIONS: H. pylori infection is negatively associated with IBD regardless of ethnicity, age, H. pylori detection methods and previous use of aminosalicylates and corticosteroids. Antibiotics influenced the magnitude of this association. Closely related bacteria including EHS and Campylobacter spp increase the risk of IBD. These results infer that H. pylori might exert an immunomodulatory effect in IBD.

Castellaneta, S. P., et al. (2004). "Diagnostic role of upper gastrointestinal endoscopy in pediatric inflammatory bowel disease." J Pediatr Gastroenterol Nutr **39**(3): 257-261.

BACKGROUND: Discrimination between ulcerative colitis (UC) and Crohn disease (CD) may be difficult on ileo-colonoscopy alone because of a lack of definitive lesions. Retrospective studies show upper gastrointestinal endoscopy may be helpful in confirming diagnosis in such cases. AIMS: To prospectively determine importance of upper gastrointestinal endoscopy in diagnosis of inflammatory bowel disease (IBD) and assess factors predictive of upper gastrointestinal involvement in IBD. METHODS: All pediatric patients were enrolled prospectively and consecutively over a 2-year period and investigated with an ileo-colonoscopy and barium meal follow-through. Children with procto-sigmoiditis, later confirmed histologically to be typical of UC, were excluded from the study. The remainder underwent upper gastrointestinal endoscopy. The protocol and methodology were determined a priori. RESULTS: 65 children suspected of IBD underwent colonoscopy. Of the total, 11 had recto-sigmoiditis with typical macroscopic appearances of UC; once this was confirmed on histology these patients were excluded from the study. Of the 54 children (males, 31; median age, 11.1 years) remaining, 23 were initially diagnosed with CD on ileo-colonoscopy and 18 (33%) were diagnosed with UC. The diagnosis remained ambiguous in 13 (six colonic, four ileo-colonic, three normal colon) on clinical, radiologic and histologic grounds. Upper GI endoscopy helped to confirm CD in a further 11 (20.4%). Two patients were diagnosed with indeterminate colitis. Upper gastrointestinal inflammation was seen in 29 of 54 (22 CD; 7 UC). Epigastric and abdominal pain, nausea and vomiting, weight loss and pan-ileocolitis were predictive

of upper gastrointestinal involvement ($P < 0.05$). However, 9 children with upper gastrointestinal involvement were asymptomatic at presentation (31%). Overall upper gastrointestinal tract inflammation was most common in the stomach (67%), followed by the esophagus (54%) and duodenum (22%). CONCLUSIONS: Upper gastrointestinal tract endoscopy should be part of the first-line investigation in all new cases suspected of IBD. Absence of specific upper gastrointestinal symptoms do not preclude presence of upper gastrointestinal inflammation.

Cederbye, C. N., et al. (2016). "Antibody validation and scoring guidelines for ABCG2 immunohistochemical staining in formalin-fixed paraffin-embedded colon cancer tissue." Sci Rep **6**: 26997.

Chandradevan, R., et al. (2018). "Evolution of Pediatric Inflammatory Bowel Disease Unclassified (IBD-U): Incorporated With Serological and Gene Expression Profiles." Inflamm Bowel Dis.

Charbit-Henrion, F., et al. (2018). "Diagnostic Yield of Next-Generation Sequencing in Very Early-Onset Inflammatory Bowel Diseases: A Multicenter Study." J Crohns Colitis.

Chartrain, M., et al. (2012). "Melanoma Chemotherapy Leads to the Selection of ABCB5-Expressing Cells." PLoS One **7**(5).

Metastatic melanoma is the most aggressive skin cancer. Recently, phenotypically distinct subpopulations of tumor cells were identified. Among them, ABCB5-expressing cells were proposed to display an enhanced tumorigenicity with stem cell-like properties. In addition, ABCB5(+) cells are thought to participate to chemoresistance through a potential efflux function of ABCB5. Nevertheless, the fate of these cells upon drugs that are used in melanoma chemotherapy remains to be clarified. Here we explored the effect of anti-melanoma treatments on the ABCB5-expressing cells. Using a melanoma xenograft model (WM266-4), we observed in vivo that ABCB5-expressing cells are enriched after a temozolomide treatment that induces a significant tumor regression. These results were further confirmed in a preliminary study conducted on clinical samples from patients that received dacarbazine. In vitro, we showed that ABCB5-expressing cells selectively survive when exposed to dacarbazine, the reference treatment of metastatic melanoma, but also to vemurafenib, a new inhibitor of the mutated kinase V600E BRAF and other various chemotherapeutic drugs. Our results show that anti-melanoma chemotherapy might participate to the chemoresistance acquisition by selecting tumor cell subpopulations expressing ABCB5. This is of particular importance in understanding the relapses observed after anti-melanoma treatments and reinforces the interest of ABCB5 and ABCB5-expressing cells as potential therapeutic targets in melanoma.

Chen, K. G., et al. (2005). "Principal expression of two mRNA isoforms (ABCB5 alpha and ABCB5 beta) of the ATP-binding cassette transporter gene ABCB5 in melanoma cells and melanocytes." Pigment Cell Research **18**(2): 102-112.

ATP-binding cassette (ABC) transporters play a pivotal role in physiology and pathology. We identified and cloned two novel mRNA isoforms (ABCB 5 alpha and ABCB 5 beta) of the ABC transporter ABCB 5 in human melanoma cells. The deduced ABCB 5 alpha protein appears to be an altered splice variant containing only a putative ABC, whereas the ABCB 5 beta isoform shares approximately 70% similarity with ABCB1 (MDR1) and has a deduced topological arrangement similar to that of the whole carboxyl terminal half of the ABCB1 gene product, P-glycoprotein, including an intact ABC. Northern blot, real-time PCR, and conventional RT-PCR were used to verify the expression profiles of ABCB 5 alpha/beta. We found that the melanomas included among the NCI-60 panel of cell lines preferentially expressed both ABCB 5 alpha and ABCB 5 beta. However, ABCB 5 alpha/beta expression was undetectable in two amelanotic melanomas (M14 and LOX-IMVI). The expression profile of ABCB 5 alpha/beta in all of the other melanomas of the panel was confirmed both by RT-PCR and by sequencing. Neither ABCB 5 alpha nor ABCB 5 beta expression was found in normal tissues such as liver, spleen, thymus, kidney, lung, colon, small intestines or placenta. ABCB 5 alpha/beta mRNAs were also expressed in normal melanocytes and in retinal pigment epithelial cells, suggesting that ABCB 5 alpha/beta expression is pigment cell-specific and might be involved in melanogenesis. Our findings indicate that expression of ABCB 5 alpha/beta might

possibly provide two novel molecular markers for differential diagnosis of melanomas and constitute potential molecular targets for therapy of melanomas.

Chen, K. G., et al. (2005). "Principal expression of two mRNA isoforms (ABCB 5alpha and ABCB 5beta) of the ATP-binding cassette transporter gene ABCB 5 in melanoma cells and melanocytes." Pigment Cell Res **18**(2): 102-112.

ATP-binding cassette (ABC) transporters play a pivotal role in physiology and pathology. We identified and cloned two novel mRNA isoforms (ABCB 5alpha and ABCB 5beta) of the ABC transporter ABCB 5 in human melanoma cells. The deduced ABCB 5alpha protein appears to be an altered splice variant containing only a putative ABC, whereas the ABCB 5beta isoform shares approximately 70% similarity with ABCB1 (MDR1) and has a deduced topological arrangement similar to that of the whole carboxyl terminal half of the ABCB1 gene product, P-glycoprotein, including an intact ABC. Northern blot, real-time PCR, and conventional RT-PCR were used to verify the expression profiles of ABCB 5alpha/beta. We found that the melanomas included among the NCI-60 panel of cell lines preferentially expressed both ABCB 5alpha and ABCB 5beta. However, ABCB 5alpha/beta expression was undetectable in two amelanotic melanomas (M14 and LOX-IMVI). The expression profile of ABCB 5alpha/beta in all of the other melanomas of the panel was confirmed both by RT-PCR and by sequencing. Neither ABCB 5alpha nor ABCB 5beta expression was found in normal tissues such as liver, spleen, thymus, kidney, lung, colon, small intestines or placenta. ABCB 5alpha/beta mRNAs were also

expressed in normal melanocytes and in retinal pigment epithelial cells, suggesting that ABCB 5alpha/beta expression is pigment cell-specific and might be involved in melanogenesis. Our findings indicate that expression of ABCB 5alpha/beta might possibly provide two novel molecular markers for differential diagnosis of melanomas and constitute potential molecular targets for therapy of melanomas.

Cheung, S. T., et al. (2011). "Granulin-epithelin precursor and ATP-dependent binding cassette (ABC)B5 regulate liver cancer cell chemoresistance." Gastroenterology **140**(1): 344-355.

BACKGROUND & AIMS: Chemotherapy is used to treat unresectable liver cancer with marginal efficacy; this might result from hepatic cancer cells with stem cell and chemoresistant features. Gene expression profiling studies have shown that hepatic cancer cells express granulin-epithelin precursor (GEP); we investigated its role in hepatic cancer stem cell functions and chemoresistance. METHODS: The effects of GEP and drug transporter signaling on chemoresistance were investigated in hepatic cancer stem cells. We analyzed the expression patterns of 142 clinical samples from liver tumors, adjacent nontumorous liver tissue, and liver tissue from patients who did not have cancer. RESULTS: GEP regulated the expression of the adenosine triphosphate-dependent binding cassette (ABC)B5 drug transporter in liver cancer cells. Chemoresistant cells that expressed GEP had increased levels of ABCB5; suppression of ABCB5 sensitized the cells to doxorubicin uptake and apoptosis. Most cells that expressed GEP and ABCB5 also expressed the hepatic cancer stem cell

markers CD133 and EpCAM; blocking ABCB5 reduced their expression. Expression levels of GEP and ABCB5 were correlated in human liver tumor samples. ABCB5 levels were increased in liver cancer cells compared with nontumor liver tissue from patients with cirrhosis or hepatitis, or normal liver tissue. ABCB5 expression was associated with the recurrence of hepatocellular carcinoma after partial hepatectomy. CONCLUSIONS: Expression of GEP and ABCB5 in liver cancer stem cells is associated with chemoresistance and reduced survival times of patients with hepatocellular carcinoma. Reagents designed to target these proteins might be developed as therapeutics and given in combination with chemotherapy to patients with liver cancer.

Chiba, H., et al. (2018). "Allele-specific DNA methylation of disease susceptibility genes in Japanese patients with inflammatory bowel disease." PLoS One **13**(3): e0194036.

Chikanza, I. C. and D. L. Kozaci (2004). "Corticosteroid resistance in rheumatoid arthritis: molecular and cellular perspectives." Rheumatology (Oxford) **43**(11): 1337-1345.

Cho, J. H. (2008). "The genetics and immunopathogenesis of inflammatory bowel disease." Nat Rev Immunol **8**(6): 458-466.

Genome-wide association studies efficiently and powerfully assay common genetic variation. The application of these studies to Crohn's disease has provided insight into

the immunopathogenesis of this disease, implicating a role for genes of the innate and adaptive immune systems. In this Review, I discuss our current understanding of the genetics and immunopathogenesis of Crohn's disease and ulcerative colitis. Crohn's disease, but not ulcerative colitis, is associated with genetic variation in NOD2 and an autophagy gene, ATG16L1, both of which affect the intracellular processing of bacterial components. By contrast, variation in the gene encoding the interleukin-23 (IL-23) receptor subunit, as well as in the IL12B, STAT3 and NKX2-3 gene regions, is associated with both Crohn's disease and ulcerative colitis. Comparative analyses of gene associations between these two inflammatory bowel diseases reveal common and unique mechanisms of their immunopathogenesis.

Cleynen, I., et al. (2015). "Inherited determinants of Crohn's disease and ulcerative colitis phenotypes: a genetic association study." Lancet.

BACKGROUND: Crohn's disease and ulcerative colitis are the two major forms of inflammatory bowel disease; treatment strategies have historically been determined by this binary categorisation. Genetic studies have identified 163 susceptibility loci for inflammatory bowel disease, mostly shared between Crohn's disease and ulcerative colitis. We undertook the largest genotype association study, to date, in widely used clinical subphenotypes of inflammatory bowel disease with the goal of further understanding the biological relations between diseases. METHODS: This study included patients from 49 centres in 16 countries in Europe, North America, and Australasia. We applied the Montreal classification system of inflammatory bowel

disease subphenotypes to 34 819 patients (19 713 with Crohn's disease, 14 683 with ulcerative colitis) genotyped on the Immunochip array. We tested for genotype-phenotype associations across 156 154 genetic variants. We generated genetic risk scores by combining information from all known inflammatory bowel disease associations to summarise the total load of genetic risk for a particular phenotype. We used these risk scores to test the hypothesis that colonic Crohn's disease, ileal Crohn's disease, and ulcerative colitis are all genetically distinct from each other, and to attempt to identify patients with a mismatch between clinical diagnosis and genetic risk profile. FINDINGS: After quality control, the primary analysis included 29 838 patients (16 902 with Crohn's disease, 12 597 with ulcerative colitis). Three loci (NOD2, MHC, and MST1 3p21) were associated with subphenotypes of inflammatory bowel disease, mainly disease location (essentially fixed over time; median follow-up of 10.5 years). Little or no genetic association with disease behaviour (which changed dramatically over time) remained after conditioning on disease location and age at onset. The genetic risk score representing all known risk alleles for inflammatory bowel disease showed strong association with disease subphenotype (p=1.65 x 10-78), even after exclusion of NOD2, MHC, and 3p21 (p=9.23 x 10-18). Predictive models based on the genetic risk score strongly distinguished colonic from ileal Crohn's disease. Our genetic risk score could also identify a small number of patients with discrepant genetic risk profiles who were significantly more likely to have a revised diagnosis after follow-up (p=6.8 x 10-4). INTERPRETATION: Our data support a continuum of disorders within inflammatory bowel disease, much better explained by three groups (ileal Crohn's disease, colonic Crohn's disease, and ulcerative colitis) than by Crohn's disease and ulcerative colitis

as currently defined. Disease location is an intrinsic aspect of a patient's disease, in part genetically determined, and the major driver to changes in disease behaviour over time. FUNDING: International Inflammatory Bowel Disease Genetics Consortium members funding sources (see Acknowledgments for full list).

Colman, R. J., et al. (2018). "Methotrexate for the Treatment of Pediatric Crohn's Disease: A Systematic Review and Meta-analysis." Inflamm Bowel Dis.

Colman, R. J. and D. T. Rubin (2015). "Optimal doses of methotrexate combined with anti-TNF therapy to maintain clinical remission in inflammatory bowel disease." J Crohns Colitis **9**(4): 312-317.

BACKGROUND AND AIMS: Methotrexate (MTX) is sometimes used as part of combination therapy for the treatment of inflammatory bowel disease [IBD]; however, the optimal MTX dose for combination therapy has not been established. This study compared the efficacy of lower-dose and higher-dose MTX with anti tumor necrosis factor alpha (anti-TNF) therapy among IBD patients. METHODS: Retrospective chart review was performed of 88 IBD patients at our center between 2010 and 2013. Low-dose MTX was defined as </= 12.5mg/week and high-dose MTX as 15-25mg/week. Patients who met the criteria for clinical remission [Harvey-Bradshaw Index </= 4, Simple Clinical Colitis Activity Index </= 2] at baseline were followed for up to 42 months. Chart review occurred in 6-month intervals. The primary outcome was consecutive months in remission prior to relapse. Secondary

outcomes included other indicators of worsening disease [endoscopic inflammation, steroid use, therapy escalation/addition, or surgery] and adverse events. Regression analysis and Kaplan-Meier survival analysis were completed. RESULTS: We identified 73 [83%] dual-therapy patients, of whom 32 low-dose and 14 high-dose individuals achieved remission. When compared with high-dose patients, low-dose patients were more likely to relapse [log-rank test, $p < 0.01$]. Secondary indicators of worsening disease occurred during 34.4% of low-dose review periods and 31.4% of high-dose review periods [$p = 0.67$]; 3/52 [6%] low-dose patients and 3/21 [14%] high-dose patients [$p = 0.34$] discontinued MTX therapy due to adverse events. CONCLUSIONS: When combined with anti-TNF therapy, MTX at doses of >12.5mg/week was more effective at maintaining clinical remission than lower doses. These findings will guide management of combination therapy in IBD patients.

Colombel, J. F., et al. (2018). "Effect of tight control management on Crohn's disease (CALM): a multicentre, randomised, controlled phase 3 trial." Lancet **390**(10114): 2779-2789.

BACKGROUND: Biomarkers of intestinal inflammation, such as faecal calprotectin and C-reactive protein, have been recommended for monitoring patients with Crohn's disease, but whether their use in treatment decisions improves outcomes is unknown. We aimed to compare endoscopic and clinical outcomes in patients with moderate to severe Crohn's disease who were managed with a tight control algorithm, using clinical symptoms and biomarkers, versus patients managed with a clinical

management algorithm. METHODS: CALM was an open-label, randomised, controlled phase 3 study, done in 22 countries at 74 hospitals and outpatient centres, which evaluated adult patients (aged 18-75 years) with active endoscopic Crohn's disease (Crohn's Disease Endoscopic Index of Severity [CDEIS] >6; sum of CDEIS subscores of >6 in one or more segments with ulcers), a Crohn's Disease Activity Index (CDAI) of 150-450 depending on dose of prednisone at baseline, and no previous use of immunomodulators or biologics. Patients were randomly assigned at a 1:1 ratio to tight control or clinical management groups, stratified by smoking status (yes or no), weight (<70 kg or >/=70 kg), and disease duration (</=2 years or >2 years) after 8 weeks of prednisone induction therapy, or earlier if they had active disease. In both groups, treatment was escalated in a stepwise manner, from no treatment, to adalimumab induction followed by adalimumab every other week, adalimumab every week, and lastly to both weekly adalimumab and daily azathioprine. This escalation was based on meeting treatment failure criteria, which differed between groups (tight control group before and after random assignment: faecal calprotectin >/=250 mug/g, C-reactive protein >/=5mg/L, CDAI >/=150, or prednisone use in the previous week; clinical management group before random assignment: CDAI decrease of <70 points compared with baseline or CDAI >200; clinical management group after random assignment: CDAI decrease of <100 points compared with baseline or CDAI >/=200, or prednisone use in the previous week). De-escalation was possible for patients receiving weekly adalimumab and azathioprine or weekly adalimumab alone if failure criteria were not met. The primary endpoint was mucosal healing (CDEIS <4) with absence of deep ulcers 48 weeks after randomisation. Primary and safety analyses were done in the intention-to-treat

population. This trial has been completed, and is registered with ClinicalTrials.gov, number NCT01235689. FINDINGS: Between Feb 11, 2011, and Nov 3, 2016, 244 patients (mean disease duration: clinical management group, 0.9 years [SD 1.7]; tight control group, 1.0 year [2.3]) were randomly assigned to monitoring groups (n=122 per group). 29 (24%) patients in the clinical management group and 32 (26%) patients in the tight control group discontinued the study, mostly because of adverse events. A significantly higher proportion of patients in the tight control group achieved the primary endpoint at week 48 (56 [46%] of 122 patients) than in the clinical management group (37 [30%] of 122 patients), with a Cochran-Mantel-Haenszel test-adjusted risk difference of 16.1% (95% CI 3.9-28.3; p=0.010). 105 (86%) of 122 patients in the tight control group and 100 (82%) of 122 patients in the clinical management group reported treatment-emergent adverse events; no treatment-related deaths occurred. The most common adverse events were nausea (21 [17%] of 122 patients), nasopharyngitis (18 [15%]), and headache (18 [15%]) in the tight control group, and worsening Crohn's disease (35 [29%] of 122 patients), arthralgia (19 [16%]), and nasopharyngitis (18 [15%]) in the clinical management group. INTERPRETATION: CALM is the first study to show that timely escalation with an anti-tumour necrosis factor therapy on the basis of clinical symptoms combined with biomarkers in patients with early Crohn's disease results in better clinical and endoscopic outcomes than symptom-driven decisions alone. Future studies should assess the effects of such a strategy on long-term outcomes such as bowel damage, surgeries, hospital admissions, and disability. FUNDING: AbbVie.

Colombel, J. F., et al. (2015). "Randomised clinical trial: deep remission in biologic and immunomodulator naive patients with Crohn's disease - a SONIC post hoc analysis." Aliment Pharmacol Ther **41**(8): 734-746.

BACKGROUND: As treatment goals in Crohn's disease (CD) evolve, targets now include clinical remission (CR), mucosal healing (MH) and biological remission [C-reactive protein normalisation (CRPnorm)]. AIMS: To evaluate the association of baseline factors and treatment with the achievement of different composite remission parameters at week 26. METHODS: This post hoc analysis of the SONIC trial evaluated different composite remission measures at week 26 in a subgroup of patients with Crohn's disease activity index (CDAI) scores, CRP, and endoscopic data available at baseline and week 26 (N = 188). Assessed composite remission measures were: CR (CDAI < 150) and MH (absence of any mucosal ulcerations), previously referred to as 'deep remission;' and alternative composite endpoints: CR + CRPnorm (CRP < 0.8 mg/dL); CRPnorm + MH; and CR + CRPnorm + MH. RESULTS: Among analysed patients, 136/188 (72.3%) achieved CR and 90/188 (47.9%) achieved MH at week 26. All composite outcomes were significantly greater (Bonferroni significance level, P </= 0.016) with combination therapy (i.e. infliximab and azathioprine; 52.3-63.6%) vs. azathioprine monotherapy (12.9-29.0%; p </= 0.005 for all comparisons). Composite remission rates including MH were significantly greater with combination therapy (52.3-56.9%) vs. infliximab (25.6-32.3%; P </= 0.015 for all comparisons except CRPnorm + MH, P = 0.017) and vs. azathioprine monotherapy (12.9-20.4%; P </= 0.002 for all comparisons). Median serum trough infliximab concentrations among patients who achieved MH or CR + MH were greater when

compared with those among patients who did not achieve MH (P = 0.018) or CR + MH (P = 0.053). Among the subgroup of patients with early Crohn's disease, MH alone or in combination with composite remission criteria significantly improved clinical outcomes of patients who received combination therapy. CONCLUSIONS: Combination therapy was more effective in achieving various composite remission measures vs. azathioprine or infliximab monotherapy. These data illustrate that 'deep remission' is achievable with combination therapy in a high percentage of patients with early Crohn's disease. ClinicalTrials.gov number: NCT00094458.

Connors, J., et al. (2017). "Exclusive Enteral Nutrition Therapy in Paediatric Crohn's Disease Results in Long-term Avoidance of Corticosteroids: Results of a Propensity-score Matched Cohort Analysis." J Crohns Colitis **11**(9): 1063-1070.

Background and Aims: Exclusive enteral nutrition [EEN] is recommended as a first-line induction therapy for paediatric Crohn's disease [CD] although corticosteroids [CS] are still used commonly. Our aim was to compare short- and long-term disease outcomes of paediatric CD patients initially managed with either EEN or CS. Methods: Medical records of newly diagnosed paediatric CD patients treated with EEN or CS as induction therapy were retrospectively reviewed. To minimise selection bias inherent in observational cohort studies, propensity analysis was carried out. Data on anthropometrics, medical history, and presenting phenotype were collected at time of diagnosis [baseline]; outcomes of interest, including medication use, hospitalisation, surgical procedures, and disease progression were assessed up to 6

years following diagnosis. Results: Of 127 patients reviewed, a total of 111 propensity-score matched CD patients receiving EEN [n = 76] or CS [n = 35] were analysed. By 4-12 weeks of induction therapy, 86.6% of EEN-treated patients achieved remission (Paediatric Crohn's Disease Activity Index [PCDAI] </= 7.5) compared with 58.1% of patients in the CS-treated group [p < 0.01]. Choice of EEN over CS for induction was associated with avoidance of corticosteroids over a 6-year follow-up period. Analysis of long-term linear growth, hospitalisation, need for biologic therapy, or surgical intervention did not reveal any significant differences. Conclusions: These findings suggest that EEN induction therapy is more effective in achieving early remission and is associated with long-term steroid avoidance without increased use of biologics or need for surgery.

Correia, M. A. and M. Liao (2007). "Cellular proteolytic systems in P450 degradation: evolutionary conservation from Saccharomyces cerevisiae to mammalian liver." Expert Opin Drug Metab Toxicol **3**(1): 33-49.

Crews, K. R., et al. (2012). "Pharmacogenomics and individualized medicine: translating science into practice." Clin Pharmacol Ther **92**(4): 467-475.

Cross, R. K. (2017). "Safety Considerations with the Use of Corticosteroids and Biologic Therapies in Mild-to-Moderate Ulcerative Colitis." Inflamm Bowel Dis **23**(10): 1689-1701.

BACKGROUND: The risk of corticosteroid-associated adverse events can limit the use of systemic corticosteroids. Oral, topically acting, second-generation corticosteroids that deliver drug to the site of inflammation, and biologic therapies, are effective treatment alternatives. The aim of this review was to evaluate the safety and tolerability of topically acting corticosteroids and biologic therapies versus oral systemic corticosteroids for ulcerative colitis (UC). METHODS: The PubMed database was searched for clinical and observational trials, systematic reviews, and case reports/series published between January 1950 and September 30, 2016. Search terms used included "corticosteroids," "beclomethasone dipropionate," "budesonide," "infliximab," "adalimumab," "golimumab," and "vedolizumab" in combination with "ulcerative colitis" or "inflammatory bowel disease." RESULTS: A total of 582 studies were identified from PubMed searches. Only 1 direct comparative trial for oral topically acting corticosteroids and systemic corticosteroids was available, and no comparative trials versus biologic therapies were identified. In patients with mild-to-moderate UC, short-term (4-8 wk) oral beclomethasone dipropionate or oral budesonide multimatrix system demonstrated safety profiles comparable with placebo with few corticosteroid-related adverse events reported. Based on long-term data in patients with moderate-to-severe UC, biologics have a generally tolerable adverse event profile, although infections, infusion reactions, and autoimmune disorders were frequently reported. CONCLUSIONS: Second-generation corticosteroids, beclomethasone dipropionate and budesonide multimatrix system, exhibited a favorable safety profile in patients with mild-to-moderate UC. For biologics, which are only indicated in moderate-to-severe UC, additional studies are needed to further ascertain the benefit to risk profile of these agents in patients with mild-to-moderate

disease (see Video Abstract, Supplemental Digital Content, http://links.lww.com/IBD/B653).

Cross, S. S. (2001). "Observer accuracy in estimating proportions in images: implications for the semiquantitative assessment of staining reactions and a proposal for a new system." J Clin Pathol **54**(5): 385-390.

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Crowe, A. and A. M. Tan (2012). "Oral and inhaled corticosteroids: differences in P-glycoprotein (ABCB1) mediated efflux." Toxicol Appl Pharmacol **260**(3): 294-302.

Cutler, D. J., et al. (2015). "Dissecting Allele Architecture of Early Onset IBD Using High-Density Genotyping." PLoS One **10**(6): e0128074.

BACKGROUND: The inflammatory bowel diseases (IBD) are common, complex disorders in which genetic and environmental factors are believed to interact leading to chronic inflammatory responses against the gut microbiota. Earlier genetic studies performed in mostly adult population of European descent identified 163 loci

affecting IBD risk, but most have relatively modest effect sizes, and altogether explain only ~20% of the genetic susceptibility. Pediatric onset represents about 25% of overall incident cases in IBD, characterized by distinct disease physiology, course and risks. The goal of this study is to compare the allelic architecture of early onset IBD with adult onset in population of European descent. METHODS: We performed a fine mapping association study of early onset IBD using high-density Immunochip genotyping on 1008 pediatric-onset IBD cases (801 Crohn's disease; 121 ulcerative colitis and 86 IBD undetermined) and 1633 healthy controls. Of the 158 SNP genotypes obtained (out of the 163 identified in adult onset), this study replicated 4% (5 SNPs out of 136) of the SNPs identified in the Crohn's disease (CD) cases and 0.8% (1 SNP out of 128) in the ulcerative colitis (UC) cases. Replicated SNPs implicated the well known NOD2 and IL23R. The point estimate for the odds ratio (ORs) for NOD2 was above and outside the confidence intervals reported in adult onset. A polygenic liability score weakly predicted the age of onset for a larger collection of CD cases ($p < 0.03$, $R2 = 0.007$), but not for the smaller number of UC cases. CONCLUSIONS: The allelic architecture of common susceptibility variants for early onset IBD is similar to that of adult onset. This immunochip genotyping study failed to identify additional common variants that may explain the distinct phenotype that characterize early onset IBD. A comprehensive dissection of genetic loci is necessary to further characterize the genetic architecture of early onset IBD.

D'Haens, G., et al. (2008). "Corticosteroids pose an increased risk for serious infection: An interim safety analysis of the ENCORE registry." Gastroenterology **134**(4): A140-A140.

D'Haens, G., et al. (2017). "Five-year Safety Data From ENCORE, a European Observational Safety Registry for Adults With Crohn's Disease Treated With Infliximab [Remicade(R)] or Conventional Therapy." J Crohns Colitis **11**(6): 680-689.

Davenport, M., et al. (2014). "Metabolic alterations to the mucosal microbiota in inflammatory bowel disease." Inflamm Bowel Dis **20**(4): 723-731.

BACKGROUND: Inflammation during inflammatory bowel disease may alter nutrient availability to adherent mucosal bacteria and impact their metabolic function. Microbial metabolites may regulate intestinal CD4 T-cell homeostasis. We investigated the relationship between inflammation and microbial function by inferred metagenomics of the mucosal microbiota from colonic pinch biopsies of patients with inflammatory bowel disease. METHODS: Paired pinch biopsy samples of known inflammation states were analyzed from ulcerative colitis (UC) (23), Crohn's disease (CD) (21), and control (24) subjects by 16S ribosomal sequencing, histopathologic assessment, and flow cytometry. PICRUSt was used to generate metagenomic data and derive relative Kyoto Encyclopedia of Genes and Genomes Pathway abundance information. Leukocytes were isolated from paired biopsy samples and analyzed by multicolor flow cytometry. Active inflammation was defined by neutrophil infiltration into the epithelium. RESULTS: Carriage of metabolic pathways in the mucosal microbiota was relatively stable among patients with inflammatory bowel disease, despite large variations in individual bacterial community structures. However,

microbial function was significantly altered in inflamed tissue of UC patients, with a reduction in carbohydrate and nucleotide metabolism in favor of increased lipid and amino acid metabolism. These differences were not observed in samples from CD patients. In CD, microbial lipid, carbohydrate, and amino acid metabolism tightly correlated with the frequency of CD4Foxp3 Tregs, whereas in UC, these pathways correlated with the frequency of CD4IL-22 (TH22) cells. CONCLUSIONS: Metabolic pathways of the mucosal microbiota in CD do not vary as much as UC with inflammation state, indicating a more systemic perturbation of host-bacteria interactions in CD compared with more localized dysfunction in UC.

De Iudicibus, S., et al. (2018). "High-Throughput Sequencing of microRNAs in Glucocorticoid Sensitive Paediatric Inflammatory Bowel Disease Patients." Int J Mol Sci **19**(5).

de Meij, T. G. J., et al. (2018). "Variability of core microbiota in newly diagnosed treatment-naive paediatric inflammatory bowel disease patients." PLoS One **13**(8): e0197649.

de Oliveira, J., et al. (2014). "Association between ABCB1 immunohistochemical expression and overall survival in gastric cancer patients." Asian Pac J Cancer Prev **15**(16): 6935-6938.

de Rie, D., et al. (2017). "An integrated expression atlas of miRNAs and their promoters in human and mouse." Nat Biotechnol **35**(9): 872-878.

de Souza, H. S. P. and C. Fiocchi (2018). "Network Medicine: A Mandatory Next Step for Inflammatory Bowel Disease." Inflamm Bowel Dis **24**(4): 671-679.

de Souza, P. R., et al. (2016). "Adrenal-Derived Hormones Differentially Modulate Intestinal Immunity in Experimental Colitis." Mediators Inflamm **2016**: 4936370.

de Waard, N. E., et al. (2015). "Expression of Multidrug Resistance Transporter ABCB5 in a Murine Model of Human Conjunctival Melanoma." Ocul Oncol Pathol **1**(3): 182-189.

Conjunctival melanoma (CM) is a rare ocular malignancy with a high tendency to reoccur locally and with a high risk of metastatic disease. Metastases are often unresponsive to conventional treatment. Recently, an animal model was set up using human CM cells. Orthotopic xenografts from human CM were created by subconjunctival injection of three different CM cell lines into NOD.Cg-Prkdcscid IL2rgtm1Wjl /SzJ (NSG) mice. Subconjunctival injection of cultured CM cells led to excellent subconjunctival growth, but no metastases were found. When single-cell suspensions were obtained from the subconjunctival xenografts and passaged in vivo, all mice developed metastases. As recent findings indicate that cancer stem cells are linked to tumor recurrences, we used this new murine model to determine the

expression of the stem cell marker ABCB5 during tumor progression. Expression of the ABCB5 protein was determined in three cell lines and during different stages of tumor development as observed in our model. All three cell lines contained a subpopulation of cells positive for ABCB5. During tumor development, expression of ABCB5 increased during phases of tumor expansion. Furthermore, expression of ABCB5 was increased in metastases. Using this model for CM, we were able to initiate metastatic spread and determine the expression of the stem cell marker ABCB5 during different stages of tumor development, identifying ABCB5 as a potential novel therapeutic target. This study illustrates the potential of our newly established murine model.

Denson, L. A., et al. (2018). "Genetic and Transcriptomic Variation Linked to Neutrophil Granulocyte-Macrophage Colony-Stimulating Factor Signaling in Pediatric Crohn's Disease." Inflamm Bowel Dis.

Denson, L. A., et al. (2018). "Clinical and Genomic Correlates of Neutrophil Reactive Oxygen Species Production in Pediatric Patients With Crohn's Disease." Gastroenterology.

Deshpande, G., et al. (2010). "Updated meta-analysis of probiotics for preventing necrotizing enterocolitis in preterm neonates." Pediatrics **125**(5): 921-930.

OBJECTIVE: Systematic reviews of randomized, controlled trials (RCTs) indicate lower mortality and necrotizing enterocolitis (NEC) and shorter time to full feeds after probiotic supplementation in preterm (<34 weeks' gestation) very low birth weight (VLBW; birth weight <1500 g) neonates. The objective of this study was to update our 2007 systematic review of RCTs of probiotic supplementation for preventing NEC in preterm VLBW neonates. METHODS: We searched in March 2009 the Cochrane Central register; Medline, Embase, and Cinahl databases; and proceedings of the Pediatric Academic Society meetings and gastroenterology conferences. Cochrane Neonatal Review Group search strategy was followed. Selection criteria were RCTs of any enteral probiotic supplementation that started within first 10 days and continued for > or =7 days in preterm VLBW neonates and reported on stage 2 NEC or higher (Modified Bell Staging). RESULTS: A total of 11 (N = 2176), including 4 new (n = 783), trials were eligible for inclusion in the meta-analysis by using a fixed-effects model. The risk for NEC and death was significantly lower. Risk for sepsis did not differ significantly. No significant adverse effects were reported. Trial sequential analysis) showed 30% reduction in the incidence of NEC (alpha = .05 and .01; power: 80%). CONCLUSIONS: The results confirm the significant benefits of probiotic supplements in reducing death and disease in preterm neonates. The dramatic effect sizes, tight confidence intervals, extremely low P values, and overall evidence indicate that additional placebo-controlled trials are unnecessary if a suitable probiotic product is available.

Deuring, J. J., et al. (2012). "Absence of ABCG2-mediated mucosal detoxification in patients with active inflammatory bowel disease is due to impeded protein folding." Biochem J **441**(1): 87-93.

Deuring, J. J., et al. (2011). "Impeded protein folding and function in active inflammatory bowel disease." Biochem Soc Trans **39**(4): 1107-1111.

Deveson, I. W., et al. (2017). "The Dimensions, Dynamics, and Relevance of the Mammalian Noncoding Transcriptome." Trends Genet **33**(7): 464-478.

Devkota, S., et al. (2012). "Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in Il10-/- mice." Nature **487**(7405): 104-108.

The composite human microbiome of Western populations has probably changed over the past century, brought on by new environmental triggers that often have a negative impact on human health. Here we show that consumption of a diet high in saturated (milk-derived) fat, but not polyunsaturated (safflower oil) fat, changes the conditions for microbial assemblage and promotes the expansion of a low-abundance, sulphite-reducing pathobiont, Bilophila wadsworthia. This was associated with a pro-inflammatory T helper type 1 (T(H)1) immune response and increased incidence of colitis in genetically susceptible Il10(-/-), but not wild-type mice. These effects are mediated by milk-derived-fat-promoted taurine conjugation of hepatic bile acids,

which increases the availability of organic sulphur used by sulphite-reducing microorganisms like B. wadsworthia. When mice were fed a low-fat diet supplemented with taurocholic acid, but not with glycocholic acid, for example, a bloom of B. wadsworthia and development of colitis were observed in Il10(-/-) mice. Together these data show that dietary fats, by promoting changes in host bile acid composition, can markedly alter conditions for gut microbial assemblage, resulting in dysbiosis that can perturb immune homeostasis. The data provide a plausible mechanistic basis by which Western-type diets high in certain saturated fats might increase the prevalence of complex immune-mediated diseases like inflammatory bowel disease in genetically susceptible hosts.

Dickens, D., et al. (2013). "Transport of gabapentin by LAT1 (SLC7A5)." Biochem Pharmacol **85**(11): 1672-1683.

Dilger, K., et al. (2004). "Identification of budesonide and prednisone as substrates of the intestinal drug efflux pump P-glycoprotein." Inflamm Bowel Dis **10**(5): 578-583.

do Imperio, G. E., et al. (2018). "Chorioamnionitis Induces a Specific Signature of Placental ABC Transporters Associated with an Increase of miR-331-5p in the Human Preterm Placenta." Cell Physiol Biochem **45**(2): 591-604.

Doecke, J. D., et al. (2013). "Genetic susceptibility in IBD: overlap between ulcerative colitis and Crohn's disease." Inflamm Bowel Dis **19**(2): 240-245.

Doring, B. and E. Petzinger (2014). "Phase 0 and phase III transport in various organs: combined concept of phases in xenobiotic transport and metabolism." Drug Metab Rev **46**(3): 261-282.

Dreesen, E., et al. (2017). "Anti-Infliximab Antibody Concentrations Guide Therapeutic Decision-Making in Patients with Crohn's Disease Losing Clinical Response." Gastroenterology **152**(5): S392-S393.

Dubinsky, M. C., et al. (2010). "Genome wide association (GWA) predictors of anti-TNFalpha therapeutic responsiveness in pediatric inflammatory bowel disease." Inflamm Bowel Dis **16**(8): 1357-1366.

BACKGROUND: Interindividual variation in response to anti-TNFalpha therapy may be explained by genetic variability in disease pathogenesis or mechanism of action. Recent genome-wide association studies (GWAS) in inflammatory bowel disease (IBD) have increased our understanding of the genetic susceptibility to IBD. The aim was to test associations of known IBD susceptibility loci and novel "pharmacogenetic" GWAS identified loci with primary nonresponse to anti-TNFalpha in pediatric IBD patients and develop a predictive model of primary nonresponse.

METHODS: Primary nonresponse was defined using the Harvey Bradshaw Index (HBI) for Crohn's disease (CD) and partial Mayo score for ulcerative colitis (UC). Genotyping was performed using the Illumina Infinium platform. Chi-square analysis tested associations of phenotype and genotype with primary nonresponse. Genetic associations were identified by testing known IBD susceptibility loci and by performing a GWAS for primary nonresponse. Stepwise multiple logistic regression was performed to build predictive models. RESULTS: Nonresponse occurred in 22 of 94 subjects. Six known susceptibility loci were associated with primary nonresponse ($P < 0.05$). Only the 21q22.2/BRWDI loci remained significant in the predictive model. The most predictive model included 3 novel "pharmacogenetic" GWAS loci, the previously identified BRWD1, pANCA, and a UC diagnosis ($R(2) = 0.82$ and area under the curve [AUC] = 0.98%). The relative risk of nonresponse increased 15-fold when number of risk factors increased from 0-2 to > or =3. CONCLUSIONS: The combination of phenotype and genotype is most predictive of primary nonresponse to anti-TNFalpha in pediatric IBD. Defining predictors of response to anti-TNFalpha may allow the identification of patients who will not benefit from this class of therapy.

Dudarewicz, M., et al. (2012). "C3435T polymorphism of the ABCB1/MDR1 gene encoding P-glycoprotein in patients with inflammatory bowel disease in a Polish population." Pharmacol Rep **64**(2): 343-350.

Duerr, R. H., et al. (2006). "A genome-wide association study identifies IL23R as an inflammatory bowel disease gene." Science **314**(5804): 1461-1463.

Dulai, P. S., et al. (2014). "Systematic review: Monotherapy with antitumour necrosis factor alpha agents versus combination therapy with an immunosuppressive for IBD." Gut **63**(12): 1843-1853.

Duricova, D., et al. (2014). "Age-related differences in presentation and course of inflammatory bowel disease: an update on the population-based literature." J Crohns Colitis **8**(11): 1351-1361.

Current data indicate a change in the epidemiology of inflammatory bowel diseases. The disease has become more widespread and the rise in the incidence has been reported in all age groups including early childhood and according to recent data also the elderly population. Some earlier studies have suggested that the phenotype and natural history of the disease may be different according to age of onset. Recently the importance of age at onset was reported in two population-based studies from France and Hungary including both paediatric and adult onset inception cohorts. Early onset disease was associated with more frequent disease extension in both Crohn's disease and ulcerative colitis and in most but not all studies with higher frequency of complicated disease behaviour. This is also accompanied by striking differences in the medical management with earlier and more prevalent (2-3-fold) use of immunosuppressives and to some extent biologicals in patients with early compared

to elderly-onset disease, especially in Crohn's disease. However, the results of population-based studies on impact of age on surgery rates in Crohn s disease as well as ulcerative colitis are conflicting. Furthermore, published data indicate that relative but not absolute risk of developing cancer and mortality is higher in patients with an early onset disease. Critical reviews that focus on the importance of age at onset in inflammatory bowel disease are rare. Therefore, the aim of this review is to describe the differences in epidemiology, clinical characteristics, and natural history of paediatric and elderly-onset inflammatory bowel disease based on studies performed in general population.

Duricova, D., et al. (2011). "The clinical implication of drug dependency in children and adults with inflammatory bowel disease: a review." J Crohns Colitis **5**(2): 81-90.

Drug dependency in adult and paediatric patients with inflammatory bowel disease (IBD) is described and the significance of this response pattern in clinical practice discussed in this review. Dependent patients maintain remission while on the treatment, but they relapse shortly after drug cessation or dose decrease. However, a quick restoration of remission and sustained response is achieved when the therapy is re-introduced or dose increased. Population-based studies have demonstrated that 22-36% of adults and 14-50% of children become corticosteroid dependent. Approximately 1/4-1/3 of treated patients undergo surgery </=1 year after treatment start, although newer paediatric studies reported lower risk of surgery (5-11%), including dependent patients. The frequent use of immunosuppressants (68-80% of

children) might explain this favourable outcome and thus reduce importance of the term corticosteroid dependency. Infliximab dependency was described in 42-66% of children and 29% of adults with Crohn's disease. The risk of surgery 50 and 40 months after treatment start was 10% and 23% in infliximab dependent children and adults, respectively. Maintenance of infliximab in dependent patients was suggested to postpone if not avoid the need of surgery. Lastly, mesalazine dependency was identified in 23% of adults with Crohn's disease. These patients were characterized by mild disease course and lower surgical risk compared to non-responders to mesalazine (32 vs. 61%). Identification of drug dependency is useful for prediction of a certain disease course and surgery. An adjustment of medical therapy may alter the prognosis and disease course.

Economou, M., et al. (2004). "Differential effects of NOD2 variants on Crohn's disease risk and phenotype in diverse populations: A metaanalysis." American Journal of Gastroenterology **99**(12): 2393-2404.

OBJECTIVES: Three variants of the CARD15/NOD2 gene (SNP8, SNP12, and SNP13) have been associated with Crohn's disease (CD). We assessed the impact of NOD2 variants on the CD risk across diverse populations and examined possible associations with disease phenotype.

METHODS: We performed a metaanalysis searching MEDLINE and EMBASE (last search 05/2004) and contacting field experts.

RESULTS: Forty-two eligible studies contributed data on 206 comparisons. No variants were detected in Asians. In non-Jewish descent Caucasians carriage of SNP8, SNP12, or SNP13 had an odds ratio (OR) for CD of 2.20 (95% CI: 1.84-2.62), 2.99 (95% CI: 2.38-3.74), and 4.09 (95% CI: 3.23-5.18), respectively. For Jewish descent patients the corresponding ORs were 1.74, 1.93, and 2.45, respectively. The OR in carriers of at least two alleles was 17.1 (95% CI: 10.7-27.2). Large studies tended to yield more conservative estimates than smaller studies, so publication or other bias cannot be excluded. Among CD patients, carrying at least one high-risk variant increased slightly the risk for familial disease (OR = 1.49, (95% CI: 1.18-1.87)), modestly the risk of stenosing CD (OR = 1.94, (95% CI: 1.61-2.34)), and more prominently the risk of small bowel involvement (OR 2.53, (95% CI: 2.01-3.16)).

CONCLUSIONS: SNP8, SNP12, and SNP13 have differential effects on CD risk, with SNP13 having the strongest genetic effect. These NOD2 variants are also significant risk factors for CD phenotype, in particular ileal location.

Eliakim, R., et al. (2001). "Dual effect of chronic nicotine administration: augmentation of jejunitis and amelioration of colitis induced by iodoacetamide in rats." Int J Colorectal Dis **16**(1): 14-21.

Englund, G., et al. (2007). "Efflux transporters in ulcerative colitis: decreased expression of BCRP (ABCG2) and Pgp (ABCB1)." Inflamm Bowel Dis **13**(3): 291-297.

BACKGROUND: Efflux transport proteins are important components of the intestinal barrier against bacterial toxins, carcinogens, and drugs. This investigation was conducted to determine the expression of Breast Cancer Resistance Protein (BCRP/ ABCG2), P-glycoprotein (Pgp/MDR1/ABCB1), and Multidrug Resistance Protein 2 (MRP2/ABCC2) in the gut mucosa of patients with ulcerative colitis (UC). METHODS: Patients were thoroughly diagnosed according to well-established clinical, endoscopic, and histologic criteria to be included in the group of patients with active UC (n = 16) or UC in remission (n = 17). Colonic and rectal mucosa from patients with UC were compared with tissues from control subjects (n = 15). The mRNA expression (TaqMan) of the efflux transporters and the proinflammatory cytokines interleukin (IL)-1beta and IL-6 was determined. Western blot was used in the analysis of protein expression and the tissue localization of BCRP was determined with confocal microscopy. RESULTS: BCRP and Pgp expression was strongly reduced in individuals with active inflammation compared with controls and was negatively correlated with the levels of IL-6 mRNA. The BCRP staining of colonic epithelium seen in healthy mucosa was diminished in inflamed tissues, with concurrent disruption of epithelial F-actin structure. CONCLUSIONS: Two of the efflux transporters of importance for the barrier function of the gut mucosa, Pgp and BCRP, are expressed at strongly reduced levels during active inflammation in patients with UC. Investigations are warranted to determine whether the low levels of efflux transporters during active UC contribute to altered transport and tissue exposure of carcinogens, bacterial toxins, and drugs.

Ensom, M. H., et al. (2001). "Pharmacogenetics: the therapeutic drug monitoring of the future?" Clin Pharmacokinet **40**(11): 783-802.

Estudante, M., et al. (2013). "Intestinal drug transporters: an overview." Adv Drug Deliv Rev **65**(10): 1340-1356.

The importance of drug transporters as one of the determinants of pharmacokinetics has become increasingly evident. While much research has been conducted focusing the role of drug transporters in the liver and kidney less is known about the importance of uptake and efflux transporters identified in the intestine. Over the past years the effects of intestinal transporters have been studied using in vivo models, in situ organ perfusions, in vitro tissue preparations and cell lines. This review aims to describe up to date findings regarding the importance of intestinal transporters on drug absorption and bioavailability, highlighting areas in need of further research. Wu and Benet proposed a Biopharmaceutics Drug Disposition Classification System (BDDCS) that allows the prediction of transporter effects on the drug disposition of orally administered drugs. This review also discusses BDDCS predictions with respect to the role of intestinal transporters and intestinal transporter-metabolizing enzyme interplay on oral drug pharmacokinetics.

Fabisiak, N., et al. (2017). "Fat-soluble Vitamin Deficiencies and Inflammatory Bowel Disease: Systematic Review and Meta-Analysis." J Clin Gastroenterol.

BACKGROUND: Vitamin deficiency is frequently associated with inflammatory bowel disease (IBD). Supplementation of vitamins could thus serve as an adjunctive therapy. The present meta-analysis reviews the deficiencies and alterations in serum fat-soluble vitamins (A, D, E, and K) reported in IBD patients. MATERIALS AND METHODS: PubMed database search was performed to identify all primary studies up to January 2015 that evaluated the serum concentrations of fat-soluble vitamin levels in IBD patients compared with healthy individuals. We estimated pooled mean differences between groups and estimated their relations with some compounding variables (age, disease duration, C-reactive protein, albumin), using a meta-regression analysis. RESULTS: Nineteen case-control studies met selection criteria. In patients with Crohn's disease (CD), vitamin A, D, E, K status was lower than in controls [D=212 mug/L.92; 95% confidence interval (CI), 95.36-330.48 mug/L, P=0.0002; D=6.97 nmol/L, 95% CI, 1.61-12.32 nmol/L, P=0.01; D=4.72 mumol/L, 95% CI, 1.60-7.84 mumol/L, P=0.003; D=1.46 ng/mL, 95% CI, 0.48-2.43 ng/mL, P=0.003, respectively]. Patients with ulcerative colitis had lower levels of vitamin A than controls (D=223.22 mug/L, 95% CI, 44.32-402.12 mug/L, P=0.01). Patients suffering from CD for a longer time had lower levels of vitamins A (95% CI=7.1-67.58 y, P=0.02) and K (95% CI, 0.09-0.71 y, P=0.02). Meta-regression analysis demonstrated statistically significant associations between the levels of inflammatory biomarkers: C-reactive protein (P=0.03, 95% CI, -9.74 to -0.6 mgl/L) and albumin (P=0.0003, 95% CI, 402.76-1361.98 g/dL), and vitamin A status in CD patients. CONCLUSION: Our meta-analysis shows that the levels of fat-soluble vitamins are generally lower in patients with inflammatory bowel diseases and their supplementation is undoubtedly indicated.

Fabre, A., et al. (2014). "Syndromic (phenotypic) diarrhoea of infancy/tricho-hepato-enteric syndrome." Arch Dis Child **99**(1): 35-38.

Faitova, J., et al. (2006). "Endoplasmic reticulum stress and apoptosis." Cell Mol Biol Lett **11**(4): 488-505.

Fakhoury, M., et al. (2005). "Localization and mRNA expression of CYP3A and P-glycoprotein in human duodenum as a function of age." Drug Metabolism and Disposition **33**(11): 1603-1607.

Farawela, H. M., et al. (2014). "The clinical relevance and prognostic significance of adenosine triphosphate ATP-binding cassette (ABCB5) and multidrug resistance (MDR1) genes expression in acute leukemia: an Egyptian study." J Cancer Res Clin Oncol **140**(8): 1323-1330.

Farnood, A., et al. (2007). "The frequency of C3435T MDR1 gene polymorphism in Iranian patients with ulcerative colitis." Int J Colorectal Dis **22**(9): 999-1003.

Farrell, R. J. and D. Kelleher (2003). "Glucocorticoid resistance in inflammatory bowel disease." Journal of Endocrinology **178**(3): 339-346.

Glucocorticoids are potent inhibitors of T cell activation and proinflammatory cytokines and are highly effective treatment for active inflammatory bowel disease (IBD). However, failure to respond, acutely or chronically, to glucocorticoid therapy is a common indication for surgery in IBD, with as many as 50% of patients with Crohn's disease (CD) and approximately 20% of patients with ulcerative colitis (UC) requiring surgery in their lifetime as a result of poor response to glucocorticoids. Studies report that approximately one-third of patients with CD are steroid dependent and one-fifth are steroid resistant while approximately one-quarter of patients with UC are steroid dependent and one-sixth are steroid resistant. While the molecular basis of glucocorticoid resistance has been widely assessed in other inflammatory conditions, the pathophysiology of the glucocorticoid resistance in IBD is poorly understood. Research in IBD suggests that the phenomenon of glucocorticoid resistance is compartmentalised to T-lymphocytes and possibly other target inflammatory cells. This review focuses on three key molecular mechanisms of glucocorticoid resistance in IBD: (i) decreased cytoplasmic glucocorticoid concentration secondary to increased P-glycoprotein-mediated efflux of,glucocorticoid from target cells due to overexpression of the multidrug resistance gene (MDR1); (ii) impaired glucocorticoid signaling because of dysfunction at the level of the glucocorticoid receptor; and (iii) constitutive epithelial activation of proinflammatory mediators, including nuclear factor kappa 13, resulting in inhibition of glucocorticoid receptor transcriptional activity. In addition, the impact of disease

heterogeneity on glucocorticoid responsiveness and recent advances in IBD pharmacogenetics are discussed.

Farrell, R. J., et al. (2000). "High multidrug resistance (P-glycoprotein 170) expression in inflammatory bowel disease patients who fail medical therapy." Gastroenterology **118**(2): 279-288.

BACKGROUND & AIMS: The multidrug resistance (MDR) gene codes for a drug efflux pump P-glycoprotein 170 (Pgp-170) expressed on the surface of lymphocytes and intestinal epithelial cells. Inflammatory bowel disease (IBD) poorly responsive to medical therapy may relate to MDR expression because glucocorticoids are known Pgp-170 substrates. METHODS: Using flow cytometry, we measured peripheral blood lymphocyte (PBL) MDR in 153 IBD patients and 50 healthy volunteers, and assessed the relationship between PBL, mucosal intraepithelial lymphocyte (IEL), and mucosal epithelial cell (EC) MDR expression in a further 20 IBD patients and 19 controls. RESULTS: Compared with controls, PBL MDR was significantly elevated in patients with Crohn's disease who required bowel resection for failed medical therapy (mean +/- SEM, 26.7 +/- 2.8 vs. 11.9 +/- 1.0; P <0.0001) and patients with ulcerative colitis who required proctocolectomy for failed medical therapy (20.3 +/- 2.5 vs. 11.9 +/- 1.0; P = 0.001). PBL MDR remained stable over time and was not influenced by disease activity or glucocorticoid therapy. Both PBL and mucosal MDR expression appeared independent of disease activity, and there was a significant correlation between PBL MDR expression and both IEL expression (r = 0.92; P <

0.0001) and EC expression (r = 0.54; P < 0.001). CONCLUSIONS: PBL and mucosal MDR expression may play an important role in determining the response of IBD patients to glucocorticoid therapy.

Faubion, W. A., Jr., et al. (2001). "The natural history of corticosteroid therapy for inflammatory bowel disease: a population-based study." Gastroenterology **121**(2): 255-260.

BACKGROUND & AIMS: The aim of this study was to determine the 1-year outcome after the first course of corticosteroids in an inception cohort of patients with inflammatory bowel disease. METHODS: All patients in Olmsted County, Minnesota, diagnosed with Crohn's disease (n = 173) or ulcerative colitis (n = 185) from 1970 to 1993 who were treated with systemic corticosteroids were identified (4 denied research authorization). Immediate outcome (30 days) and 1-year outcome after the first course of corticosteroids were determined. RESULTS: Seventy-four (43%) patients with Crohn's disease and 63 (34%) with ulcerative colitis were treated with corticosteroids. Immediate outcomes for Crohn's disease were complete remission in 43 (58%), partial remission in 19 (26%), and no response in 12 (16%). Immediate outcomes for ulcerative colitis were complete remission in 34 (54%), partial remission in 19 (30%), and no response in 10 (16%). One-year outcomes for Crohn's disease were prolonged response in 24 (32%), corticosteroid dependence in 21 (28%), operation in 28 (38%), and lost to follow-up in 1 (1%). One-year outcomes for ulcerative colitis were prolonged response in 31 (49%), corticosteroid dependence in 14 (22%), and operation in 18 (29%). CONCLUSIONS: Most patients with Crohn's

disease and ulcerative colitis initially respond to corticosteroids. At 1 year, 32% of patients with Crohn's disease and 48% with ulcerative colitis are corticosteroid free without operation.

Feagan, B. G., et al. (2013). "Vedolizumab as Induction and Maintenance Therapy for Ulcerative Colitis." New England Journal of Medicine **369**(8): 699-710.

BACKGROUND

Gut-selective blockade of lymphocyte trafficking by vedolizumab may constitute effective treatment for ulcerative colitis.

METHODS

We conducted two integrated randomized, double-blind, placebo-controlled trials of vedolizumab in patients with active disease. In the trial of induction therapy, 374 patients (cohort 1) received vedolizumab (at a dose of 300 mg) or placebo intravenously at weeks 0 and 2, and 521 patients (cohort 2) received open-label vedolizumab at weeks 0 and 2, with disease evaluation at week 6. In the trial of maintenance therapy, patients in either cohort who had a response to vedolizumab at week 6 were randomly assigned to continue receiving vedolizumab every 8 or 4 weeks or to switch to placebo for up to 52 weeks. A response was defined as a reduction in the Mayo Clinic score (range, 0 to 12, with higher scores indicating more active disease) of at least 3 points and a decrease of at least 30% from baseline, with

an accompanying decrease in the rectal bleeding subscore of at least 1 point or an absolute rectal bleeding subscore of 0 or 1.

RESULTS

Response rates at week 6 were 47.1% and 25.5% among patients in the vedolizumab group and placebo group, respectively (difference with adjustment for stratification factors, 21.7 percentage points; 95% confidence interval [CI], 11.6 to 31.7; P<0.001). At week 52, 41.8% of patients who continued to receive vedolizumab every 8 weeks and 44.8% of patients who continued to receive vedolizumab every 4 weeks were in clinical remission (Mayo Clinic score 2 and no subscore >1), as compared with 15.9% of patients who switched to placebo (adjusted difference, 26.1 percentage points for vedolizumab every 8 weeks vs. placebo [95% CI, 14.9 to 37.2; P<0.001] and 29.1 percentage points for vedolizumab every 4 weeks vs. placebo [95% CI, 17.9 to 40.4; P<0.001]). The frequency of adverse events was similar in the vedolizumab and placebo groups.

CONCLUSIONS

Vedolizumab was more effective than placebo as induction and maintenance therapy for ulcerative colitis.

Finnie, I. A., et al. (1996). "Stimulation of colonic mucin synthesis by corticosteroids and nicotine." Clin Sci (Lond) **91**(3): 359-364.

Fischer, S., et al. (2007). "The ATP-binding cassette transporter ABCG2 (BCRP) and ABCB1 (MDR1) variants are not associated with disease susceptibility, disease phenotype response to medical therapy or need for surgery in Hungarian patients with inflammatory bowel diseases." Scandinavian Journal of Gastroenterology **42**(6): 726-733.

Fleeman, N., et al. (2010). "The clinical effectiveness and cost-effectiveness of testing for cytochrome P450 polymorphisms in patients with schizophrenia treated with antipsychotics: a systematic review and economic evaluation." Health Technol Assess **14**(3): 1-157, iii.

Frank, D. N., et al. (2011). "Disease phenotype and genotype are associated with shifts in intestinal-associated microbiota in inflammatory bowel diseases." Inflamm Bowel Dis **17**(1): 179-184.

BACKGROUND: Abnormal host-microbe interactions are implicated in the pathogenesis of inflammatory bowel diseases. Previous 16S rRNA sequence analysis of intestinal tissues demonstrated that a subset of Crohn's disease (CD) and ulcerative colitis (UC) samples exhibited altered intestinal-associated microbial compositions characterized by depletion of Bacteroidetes and Firmicutes (particularly Clostridium taxa). We hypothesize that NOD2 and ATG16L1 risk alleles may be associated with these alterations. METHODS: To test this hypothesis, we genotyped 178 specimens collected from 35 CD, 35 UC, and 54 control patients for the three major NOD2 risk alleles (Leu 1007fs, R702W, and G908R) and the ATG16L1T300A risk allele, that had undergone previous 16S rRNA sequence analysis. Our statistical models

incorporated the following independent variables: 1) disease phenotype (CD, UC, non-IBD control); 2) NOD2 composite genotype (NOD2(R) = at least one risk allele, NOD2(NR) = no risk alleles); 3) ATG16L1T300A genotype (ATG16L1(R/R), ATG16L1(R/NR), ATG16L1(NR/NR)); 4) patient age at time of surgery and all first-order interactions. The dependent variable(s) were the relative frequencies of bacterial taxa classified by applying the RDP 2.1 classifier to previously reported 16S rRNA sequence data. RESULTS: Disease phenotype, NOD2 composite genotype and ATG16L1 genotype were significantly associated with shifts in microbial compositions by nonparametric multivariate analysis of covariance (MANCOVA). Shifts in the relative frequencies of Faecalibacterium and Escherichia taxa were significantly associated with disease phenotype by nonparametric ANCOVA. CONCLUSIONS: These results support the concept that disease phenotype and genotype are associated with compositional changes in intestinal-associated microbiota.

Frank, N. Y. and M. H. Frank (2009). "ABCB5 gene amplification in human leukemia cells." Leuk Res **33**(10): 1303-1305.

Frank, N. Y., et al. (2006). "Regulation of myogenic progenitor proliferation in human fetal skeletal muscle by BMP4 and its antagonist Gremlin." J Cell Biol **175**(1): 99-110.

Skeletal muscle side population (SP) cells are thought to be "stem"-like cells. Despite reports confirming the ability of muscle SP cells to give rise to differentiated progeny

in vitro and in vivo, the molecular mechanisms defining their phenotype remain unclear. In this study, gene expression analyses of human fetal skeletal muscle demonstrate that bone morphogenetic protein 4 (BMP4) is highly expressed in SP cells but not in main population (MP) mononuclear muscle-derived cells. Functional studies revealed that BMP4 specifically induces proliferation of BMP receptor 1a-positive MP cells but has no effect on SP cells, which are BMPR1a-negative. In contrast, the BMP4 antagonist Gremlin, specifically up-regulated in MP cells, counteracts the stimulatory effects of BMP4 and inhibits proliferation of BMPR1a-positive muscle cells. In vivo, BMP4-positive cells can be found in the proximity of BMPR1a-positive cells in the interstitial spaces between myofibers. Gremlin is expressed by mature myofibers and interstitial cells, which are separate from BMP4-expressing cells. Together, these studies propose that BMP4 and Gremlin, which are highly expressed by human fetal skeletal muscle SP and MP cells, respectively, are regulators of myogenic progenitor proliferation.

Frank, N. Y., et al. (2005). "ABCB5-mediated doxorubicin transport and chemoresistance in human malignant melanoma." Cancer Research **65**(10): 4320-4333.

Enhanced drug efflux mediated by ABCB1 P-glycoprotein and related ATP-binding cassette transporters is one of several mechanisms of multidrug resistance thought to impair chemotherapeutic success in human cancers. In malignant melanoma, its potential contribution to chemoresistance is uncertain. Here, we show that ABCB5, which functions as a determinant of membrane potential and regulator of cell fusion

in physiologic skin progenitor cells, is expressed in clinical malignant melanoma tumors and preferentially marks a subset of hyperpolarized, CD133+ stem cell phenotype-expressing tumor cells in malignant melanoma cultures and clinical melanomas. We found that ABCB5 blockade significantly reversed resistance of G3361 melanoma cells to doxorubicin, an agent to which clinical melanomas have been found refractory, resulting in a 43% reduction in the LD50 from 4 to 2.3 micromol/L doxorubicin ($P < 0.05$). Our results identified ABCB5-mediated doxorubicin efflux transport as the underlying mechanism of resistance, because ABCB5 blockade significantly enhanced intracellular drug accumulation. Consistent with this novel ABCB5 function and mechanism in doxorubicin resistance, gene expression levels of the transporter across a panel of human cancer cell lines used by the National Cancer Institute for drug screening correlated significantly with tumor resistance to doxorubicin ($r = 0.44$; $P = 0.016$). Our results identify ABCB5 as a novel drug transporter and chemoresistance mediator in human malignant melanoma. Moreover, our findings show that ABCB5 is a novel molecular marker for a distinct subset of chemoresistant, stem cell phenotype-expressing tumor cells among melanoma bulk populations and indicate that these chemoresistant cells can be specifically targeted via ABCB5 to enhance cytotoxic efficacy.

Frank, N. Y., et al. (2003). "Regulation of progenitor cell fusion by ABCB5 P-glycoprotein, a novel human ATP-binding cassette transporter." J Biol Chem **278**(47): 47156-47165.

Franke, A., et al. (2010). "Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci." Nature Genetics **42**(12): 1118-1125.

We undertook a meta-analysis of six Crohn's disease genome-wide association studies (GWAS) comprising 6,333 affected individuals (cases) and 15,056 controls and followed up the top association signals in 15,694 cases, 14,026 controls and 414 parent-offspring trios. We identified 30 new susceptibility loci meeting genome-wide significance (P < 5 x 10(-)(8)). A series of in silico analyses highlighted particular genes within these loci and, together with manual curation, implicated functionally interesting candidate genes including SMAD3, ERAP2, IL10, IL2RA, TYK2, FUT2, DNMT3A, DENND1B, BACH2 and TAGAP. Combined with previously confirmed loci, these results identify 71 distinct loci with genome-wide significant evidence for association with Crohn's disease.

Friend, D. R. (1998). "Review article: issues in oral administration of locally acting glucocorticosteroids for treatment of inflammatory bowel disease." Aliment Pharmacol Ther **12**(7): 591-603.

Fujimoto, Y., et al. (2011). "Transporter associated with antigen processing-like (ABCB9) stably expressed in Chinese hamster ovary-K1 cells is sorted to the microdomains of lysosomal membranes." Biol Pharm Bull **34**(1): 36-40.

Fukuda, Y., et al. (2011). "Conserved intramolecular disulfide bond is critical to trafficking and fate of ATP-binding cassette (ABC) transporters ABCB6 and sulfonylurea receptor 1 (SUR1)/ABCC8." J Biol Chem **286**(10): 8481-8492.

The ATP-binding cassette (ABC) transporter ABCB6 is a mitochondrial porphyrin transporter that activates porphyrin biosynthesis. ABCB6 lacks a canonical mitochondrial targeting sequence but reportedly traffics to other cellular compartments such as the plasma membrane. How ABCB6 reaches these destinations is unknown. In this study, we show that endogenous ABCB6 is glycosylated in multiple cell types, indicating trafficking through the endoplasmic reticulum (ER), and has only one atypical site for glycosylation (NXC) in its amino terminus. ABCB6 remained glycosylated when the highly conserved cysteine (Cys-8) was substituted with serine to make a consensus site, NXS. However, this substitution blocked ER exit and produced ABCB6 degradation, which was mostly reversed by the proteasomal inhibitor MG132. The amino terminus of ABCB6 has an additional highly conserved ER luminal cysteine (Cys-26). When Cys-26 was mutated alone or in combination with Cys-8, it also resulted in instability and ER retention. Further analysis revealed that these two cysteines form a disulfide bond. We discovered that other ABC transporters with an amino terminus in the ER had similarly configured conserved cysteines. This analysis led to the discovery of a disease-causing mutation in the sulfonylurea receptor 1 (SUR1)/ABCC8 from a patient with hyperinsulinemic hypoglycemia. The mutant allele only contains a mutation in a conserved amino-terminal cysteine, producing SUR1 that fails to reach the cell surface. These results

suggest that for ABC transporters the propensity to form a disulfide bond in the ER defines a unique checkpoint that determines whether a protein is ER-retained.

Fumery, M., et al. (2016). "Review article: the natural history of paediatric-onset ulcerative colitis in population-based studies." Aliment Pharmacol Ther **43**(3): 346-355.

BACKGROUND: A better knowledge of the natural history of disabling chronic diseases is essential to improve patient management, evaluate the impact of treatment strategies and provide predictors for disabling disease and comprehensive information for patients. AIM: To summarise our current knowledge issued from population-based studies of the natural history of ulcerative colitis (UC) in children. METHODS: We searched MEDLINE (source PubMed) and international conference abstracts, and included all population-based studies that evaluated long-term outcome of paediatric-onset (<17 years at diagnosis) UC. RESULTS: A total of 26 population-based studies were considered in this review from the total of 61 articles or abstracts screened. Most patients presented disease extension and about two-thirds of patients had pancolitis at the end of follow-up. One-half of patients experienced extra-intestinal manifestations and primary sclerosing cholangitis was observed in 5-10% of patients. Overall, patients did not appear to have any significant growth retardation or delayed puberty. About two-thirds of patients required corticosteroid therapy and up to 25% were steroid dependent. An increased use of thiopurines was observed and the most recent data indicate that up to one-half of patients were exposed to thiopurines and 10-30% were exposed to anti-tumour necrosis factor. One-half of patients required

hospitalisations and 20% of patients required colectomy after a follow-up of 10 years. CONCLUSIONS: Paediatric-onset UC is characterised by a high rate of disease extension. About 20% of patients had been operated at 10-year follow-up. New population-based studies are needed to evaluate the impact of new treatment strategies comprising immunosuppressants and biologics.

Gabryel, M., et al. (2016). "The impact of genetic factors on response to glucocorticoids therapy in IBD." Scand J Gastroenterol **51**(6): 654-665.

Gajendran, M., et al. (2017). "A comprehensive review and update on Crohn's disease." Dis Mon.

The term inflammatory bowel disease (IBD) refers principally to two major categories of chronic relapsing inflammatory intestinal disorders: Crohn's disease (CD) and ulcerative colitis (UC). In the United States, it is currently estimated that about 1.5 million people suffer from IBD, causing considerable suffering, mortality and economic loss every year. Yet the cause of IBD is unknown, and until we understand more, prevention or cure will not be possible. There is a lot of variation in the incidence and prevalence of CD based on geographic region, environment, immigrant population, and ethnic groups. The annual incidence of CD in North America is reported to be 3.1-20.2 per 100,000 with a prevalence of 201 per 100,000 population. Based on the epidemiological, genetic and immunological data, CD is considered to be a heterogeneous disorder with multifactorial etiology in which genetics and

environment interact to manifest the disease. Several genes have been studied so for with respect to CD, but thus far the strong and replicated associations have been identified with NOD2, IL23R and ATG16L1 genes. The risk factors implicated with CD include smoking, low fiber- high carbohydrate diet, altered microbiome and medications such as non-steroidal anti-inflammatory drugs. CD is typically characterized by transmural inflammation of the intestine and could affect any part of the gastrointestinal tract from mouth to perianal area. In terms of distribution of the disease 25% of the patients have colitis only, 25% is ileitis only and 50% have ileocolitis. The Montreal classification is based on the age at diagnosis (<16, 17-40, > 40), disease location (Ileal, colonic, Ileocolonic) and the disease behavior (nonstricturing/nonpenetrating, stricturing, penetrating). The key features for diagnosing CD comprises a combination of radiographic, endoscopic and pathological findings demonstrating focal, asymmetric, transmural or granulomatous features. Abdominal Computed tomography (CT) enterography is the most preferred first-line radiologic study used in the assessment of small bowel CD. The diagnostic accuracy of magnetic resonance enterography/enteroclysis is similar to that of CT scans and also prevents exposure to ionizing radiation. Endoscopic scores are considered to be the gold standard tool to measure the activity of CD and they are used more commonly in the clinical trials to measure the efficacy of various drugs on inducing and maintaining mucosal healing. The most common scoring systems used to measure clinical disease activity include Crohn's Disease Activity Index (CDAI), HBI-Harvey-Bradshaw index (HBI), short inflammatory bowel disease questionnaire (SIBDQ) and Lehmann score. Management of Crohn's disease has been seen as an evolving challenge owing to its widely heterogeneous manifestations, overlapping

characteristics with other inflammatory disorders, often elusive extraintestinal manifestations and uncertain etiology. Therapeutic interventions are tailored to address symptomatic response and subsequent tolerance of the intervention. Chronology of treatment should favor treatment dose acute disease or "induction therapy", followed by maintenance of adequate response or remission, i.e. "maintenance therapy". The medications which are highly effective in inducing remission include steroids and Tumor Necrosis Factor (TNF) inhibitors. Medications used to maintain remission include 5-aminosalicyclic acid products, immunomodulators (Azathioprine, 6-mercaptopurine, methotrexate) and TNF inhibitors (infliximab, adalimumab, certolizumab and golimumab). Surgical interventions like bowel resection, stricturoplasty or drainage of abscess is required in up to two thirds of CD patients during their lifetime. The most common indications for surgical resection are medically refractory disease, perforation, persisting or recurrent obstruction, abscess not amenable to percutaneous drainage, intractable hemorrhage, dysplasia or cancer. Endoscopic recurrence in postoperative CD patients, as defined by Rutgeers score i2-i4 occur in 30-90% of the patients at the neoterminal ileum within 12 months of surgery and almost universally by 5 years. Treating CD requires a comprehensive care team including the patient, primary care provider, and gastroenterologist. In summary CD is a chronic inflammatory condition with a remitting and relapsing course primarily affecting relatively younger population with significant socioeconomic effects.

Gambichler, T., et al. (2016). "Expression of SOX10, ABCB5 and CD271 in melanocytic lesions and correlation with survival data of patients with melanoma." Clin Exp Dermatol **41**(7): 709-716.

Gao, Y., et al. (2018). "A brief review of monoclonal antibody technology and its representative applications in immunoassays." J Immunoassay Immunochem **39**(4): 351-364.

Garcia-Carrasco, M., et al. (2017). "Clinical relevance of P-glycoprotein activity on peripheral blood mononuclear cells and polymorphonuclear neutrophils to methotrexate in systemic lupus erythematosus patients." Clinical Rheumatology **36**(10): 2267-2272.

Gasparetto, M., et al. (2018). "Early Treatment Response Predicts Outcome in Paediatric Ulcerative Colitis." J Pediatr Gastroenterol Nutr.

Geirnaert, A., et al. (2017). "Butyrate-producing bacteria supplemented in vitro to Crohn's disease patient microbiota increased butyrate production and enhanced intestinal epithelial barrier integrity." Sci Rep **7**(1): 11450.

The management of the dysbiosed gut microbiota in inflammatory bowel diseases (IBD) is gaining more attention as a novel target to control this disease. Probiotic treatment with butyrate-producing bacteria has therapeutic potential since these

bacteria are depleted in IBD patients and butyrate has beneficial effects on epithelial barrier function and overall gut health. However, studies assessing the effect of probiotic supplementation on microbe-microbe and host-microbe interactions are rare. In this study, butyrate-producing bacteria (three mono-species and one multispecies mix) were supplemented to the fecal microbial communities of ten Crohn's disease (CD) patients in an in vitro system simulating the mucus- and lumen-associated microbiota. Effects of supplementation in short-chain fatty acid levels, bacterial colonization of mucus environment and intestinal epithelial barrier function were evaluated. Treatment with F. prausnitzii and the mix of six butyrate-producers significantly increased the butyrate production by 5-11 mol%, and colonization capacity in mucus- and lumen-associated CD microbiota. Treatments with B. pullicaecorum 25-3T and the mix of six butyrate-producers improved epithelial barrier integrity in vitro. This study provides proof-of-concept data for the therapeutic potential of butyrate-producing bacteria in CD and supports the future preclinical development of a probiotic product containing butyrate-producing species.

Geirnaert, A., et al. (2012). "In vitro evaluation of the upper gastrointestinal passage of a novel butyrate producing isolate to counterbalance dysbiosis in inflammatory bowel disease." Commun Agric Appl Biol Sci **77**(1): 195-199.

Giacomini, K. M., et al. (2013). "International Transporter Consortium commentary on clinically important transporter polymorphisms." Clin Pharmacol Ther **94**(1): 23-26.

Giacomini, K. M., et al. (2018). "The International Transporter Consortium: Summarizing advances in the role of transporters in drug development." Clin Pharmacol Ther.

Giacomini, K. M. and S. M. Huang (2013). "Transporters in drug development and clinical pharmacology." Clin Pharmacol Ther **94**(1): 3-9.

Ginestier, C., et al. (2010). "CXCR1 blockade selectively targets human breast cancer stem cells in vitro and in xenografts." J Clin Invest **120**(2): 485-497.

Recent evidence suggests that breast cancer and other solid tumors possess a rare population of cells capable of extensive self-renewal that contribute to metastasis and treatment resistance. We report here the development of a strategy to target these breast cancer stem cells (CSCs) through blockade of the IL-8 receptor CXCR1. CXCR1 blockade using either a CXCR1-specific blocking antibody or repertaxin, a small-molecule CXCR1 inhibitor, selectively depleted the CSC population in 2 human breast cancer cell lines in vitro. Furthermore, this was followed by the induction of massive apoptosis in the bulk tumor population via FASL/FAS signaling. The effects of CXCR1 blockade on CSC viability and on FASL production were mediated by the FAK/AKT/FOXO3A pathway. In addition, repertaxin was able to specifically target the CSC population in human breast cancer xenografts, retarding

tumor growth and reducing metastasis. Our data therefore suggest that CXCR1 blockade may provide a novel means of targeting and eliminating breast CSCs.

Glymenaki, M., et al. (2017). "Stability in metabolic phenotypes and inferred metagenome profiles before the onset of colitis-induced inflammation." Sci Rep **7**(1): 8836.

Inflammatory bowel disease (IBD) is associated with altered microbiota composition and metabolism, but it is unclear whether these changes precede inflammation or are the result of it since current studies have mainly focused on changes after the onset of disease. We previously showed differences in mucus gut microbiota composition preceded colitis-induced inflammation and stool microbial differences only became apparent at colitis onset. In the present study, we aimed to investigate whether microbial dysbiosis was associated with differences in both predicted microbial gene content and endogenous metabolite profiles. We examined the functional potential of mucus and stool microbial communities in the mdr1a -/- mouse model of colitis and littermate controls using PICRUSt on 16S rRNA sequencing data. Our findings indicate that despite changes in microbial composition, microbial functional pathways were stable before and during the development of mucosal inflammation. LC-MS-based metabolic phenotyping (metabotyping) in urine samples confirmed that metabolite profiles in mdr1a -/- mice were remarkably unaffected by development of intestinal inflammation and there were no differences in previously published metabolic markers of IBD. Metabolic profiles did, however, discriminate the colitis-prone mdr1a -/- genotype from controls. Our results indicate resilience of the

metabolic network irrespective of inflammation. Importantly as metabolites differentiated genotype, genotype-differentiating metabolites could potentially predict IBD risk.

Gong, L., et al. (2017). "Polymorphisms in cytochrome P450 oxidoreductase and its effect on drug metabolism and efficacy." Pharmacogenet Genomics **27**(9): 337-346.

Govindan, R., et al. (2012). "Genomic Landscape of Non-Small Cell Lung Cancer in Smokers and Never-Smokers." Cell **150**(6): 1121-1134.

We report the results of whole-genome and transcriptome sequencing of tumor and adjacent normal tissue samples from 17 patients with non-small cell lung carcinoma (NSCLC). We identified 3,726 point mutations and more than 90 indels in the coding sequence, with an average mutation frequency more than 10-fold higher in smokers than in never-smokers. Novel alterations in genes involved in chromatin modification and DNA repair pathways were identified, along with DACH1, CFTR, RELN, ABCB5, and HGF. Deep digital sequencing revealed diverse clonality patterns in both never-smokers and smokers. All validated EFGR and KRAS mutations were present in the founder clones, suggesting possible roles in cancer initiation. Analysis revealed 14 fusions, including ROS1 and ALK, as well as novel metabolic enzymes. Cell-cycle and JAK-STAT pathways are significantly altered in lung cancer, along with perturbations in 54 genes that are potentially targetable with currently available drugs.

Greenbaum, D., et al. (2003). "Comparing protein abundance and mRNA expression levels on a genomic scale." Genome Biology **4**(9): 117.

Grimm, M., et al. (2012). "ABCB5 expression and cancer stem cell hypothesis in oral squamous cell carcinoma." European Journal of Cancer **48**(17): 3186-3197.

Introduction: The vast majority of oral cancers are squamous cell carcinomas (OSCC). The effectiveness of adjuvant cytostatic chemotherapy for OSCC is frequently restricted due to an inducible cellular mechanism called multidrug resistance (MDR) and a putative cancer stem cell (CSC) compartment in human carcinogenesis expressing multidrug efflux pumps. The novel human ATP-binding cassette (ABC) transporter ABCB5 [subfamily B (MDR/TAP) member 5] acts as an energy-dependent drug efflux transporter and marks tumour cells of a putative CSC compartment. However, to date, there is no link between ABCB5 expression and OSCC.

Materials and methods: Expression of ABCB5 was analysed in OSCC specimen (n = 191) and cancer cell lines (BICR3, BICR56) by immunohistochemistry, real-time polymerase chain reaction (RT-PCR) analysis and western blotting. Scanned images were digitally analysed using ImageJ and the immunomembrane plug-in. ABCB5 expression on protein level was correlated with clinical characteristics and impact on survival. ABCB5 was co-labelled with CD44 in immunohistochemical and

immunofluorescence double labelling experiments. Expression subgroups were identified by receiver operating characteristics (ROC) analysis.

Results: High ABCB5 expression was significantly associated with tumour progression and recurrence of the tumour. Multivariate analysis demonstrated high ABCB5 expression as an independent prognostic factor ($p = 0.0004$). Immunohistochemical and immunofluorescence double labelling experiments revealed ABCB5 expression by CD44+ cancer cells. ABCB5 specificity was confirmed by western blot and RT-PCR analysis.

Conclusions: For the first time, this study provides evidence that ABCB5 expression in OSCC might be associated with tumour formation, metastasis and a putative CSC compartment. One of the principal mechanisms for protecting putative cancer stem cells is through the expression of multifunctional efflux transporters from the ABC gene family, like ABCB5. This provides one mechanism in which putative cancer stem cells could survive and may lead to tumour relapse. Knowledge of expression profiles of ABC transporters and other genes involved in MDR will likely help therapeutic optimisation for cancer patients in clinic. However, this hypothesis requires further in vitro and in vivo studies.

Guariso, G. and M. Gasparetto (2017). "Treating children with inflammatory bowel disease: Current and new perspectives." World J Gastroenterol **23**(30): 5469-5485.

Inflammatory bowel disease (IBD) is a chronic inflammatory condition of the gut characterised by alternating periods of remission and relapse. Whilst the mechanism

underlying this disease is yet to be fully understood, old and newer generation treatments can only target selected pathways of this complex inflammatory process. This narrative review aims to provide an update on the most recent advances in treatment of paediatric IBD. A MEDLINE search was conducted using "paediatric inflammatory bowel disease", "paediatric Crohn's disease", "paediatric ulcerative colitis", "treatment", "therapy", "immunosuppressant", "biologic", "monitoring" and "biomarkers" as key words. Clinical trials, systematic reviews, and meta-analyses published between 2014 and 2016 were selected. Studies referring to earlier periods were also considered in case the data was relevant to our scope. Major advances have been achieved in monitoring the individual metabolism, toxicity and response to relevant medications in IBD including thiopurines and biologics. New biologics acting on novel mechanisms such as selective interference with lymphocyte trafficking are emerging treatment options. Current research is investing in the development of reliable prognostic biomarkers, aiming to move towards personalised treatments targeted to individual patients.

Gunther, C., et al. (2011). "Caspase-8 regulates TNF-alpha-induced epithelial necroptosis and terminal ileitis." Nature **477**(7364): 335-U108.

Dysfunction of the intestinal epithelium is believed to result in the excessive translocation of commensal bacteria into the bowel wall that drives chronic mucosal inflammation in Crohn's disease, an incurable inflammatory bowel disease in humans characterized by inflammation of the terminal ileum(1). In healthy individuals, the

intestinal epithelium maintains a physical barrier, established by the tight contact of cells. Moreover, specialized epithelial cells such as Paneth cells and goblet cells provide innate immune defence functions by secreting mucus and antimicrobial peptides, which hamper access and survival of bacteria adjacent to the epithelium(2). Epithelial cell death is a hallmark of intestinal inflammation and has been discussed as a possible pathogenic mechanism driving Crohn's disease in humans(3). However, the regulation of epithelial cell death and its role in intestinal homeostasis remain poorly understood. Here we demonstrate a critical role for caspase-8 in regulating necroptosis of intestinal epithelial cells (IECs) and terminal ileitis. Mice with a conditional deletion of caspase-8 in the intestinal epithelium (Casp8(Delta IEC)) spontaneously developed inflammatory lesions in the terminal ileum and were highly susceptible to colitis. Casp8(Delta IEC) mice lacked Paneth cells and showed reduced numbers of goblet cells, indicating dysregulated antimicrobial immune cell functions of the intestinal epithelium. Casp8(Delta IEC) mice showed increased cell death in the Paneth cell area of small intestinal crypts. Epithelial cell death was induced by tumour necrosis factor (TNF)-alpha, was associated with increased expression of receptor-interacting protein 3 (Rip3; also known as Ripk3) and could be inhibited on blockade of necroptosis. Lastly, we identified high levels of RIP3 in human Paneth cells and increased necroptosis in the terminal ileum of patients with Crohn's disease, suggesting a potential role of necroptosis in the pathogenesis of this disease. Together, our data demonstrate a critical function of caspase-8 in regulating intestinal homeostasis and in protecting IECs from TNF-alpha-induced necroptotic cell death.

Guo, Q., et al. (2018). "ATP-binding cassette member B5 (ABCB5) promotes tumor cell invasiveness in human colorectal cancer." J Biol Chem **293**(28): 11166-11178.

Gutmann, H., et al. (2005). "Distribution of breast cancer resistance protein (BCRP/ABCG2) mRNA expression along the human GI tract." Biochem Pharmacol **70**(5): 695-699.

Gutmann, H., et al. (2008). "Breast cancer resistance protein and P-glycoprotein expression in patients with newly diagnosed and therapy-refractory ulcerative colitis compared with healthy controls." Digestion **78**(2-3): 154-162.

Haisma, S. M., et al. (2015). "Methotrexate for maintaining remission in paediatric Crohn's patients with prior failure or intolerance to thiopurines: a multicenter cohort study." J Crohns Colitis **9**(4): 305-311.

BACKGROUND AND AIMS: Methotrexate [MTX] is an immunomodulating drug that can be used to maintain remission in patients with Crohn's disease [CD], but data on efficacy and tolerability in children and teenagers are scarce. We evaluated the long-term efficacy and tolerability of MTX monotherapy after thiopurine therapy in paediatric CD patients. METHODS: A multicenter cohort of paediatric MTX users who stopped thiopurines due to ineffectiveness or intolerance between 2002 and 2012 were included and followed for at least 12 months. Relapse-free use was defined as steroid and biologics-free clinical remission after the introduction of MTX, and

included intentional discontinuation of successful therapy before the end of the observation period. RESULTS: A total of 113 patients with CD in remission were followed while on MTX monotherapy, of whom 75 [66%] had failed on thiopurines and 38 [34%] had stopped thiopurines due to side effects. Median age at the introduction of MTX was 14 years [range 7 to 17], and 93% used the subcutaneous route. Kaplan-Meier analysis showed that 52% of the study cohort were still in steroid- and biologics-free remission after 12 months of MTX monotherapy, with a difference that did not reach significance between thiopurine-intolerant and thiopurine-failing patients [p = 0.21, log-rank test]. CONCLUSIONS: The findings of this cohort study suggest that MTX is an effective immunomodulator to maintain remission after stopping thiopurines. MTX maintenance should be considered before stepping up to anti-tumor necrosis factor alpha therapy. MTX is probably somewhat more effective in patients who stopped thiopurines due to side effects than in those who failed on thiopurines.

Halfvarson, J., et al. (2017). "Dynamics of the human gut microbiome in inflammatory bowel disease." Nat Microbiol **2**: 17004.

Inflammatory bowel disease (IBD) is characterized by flares of inflammation with a periodic need for increased medication and sometimes even surgery. The aetiology of IBD is partly attributed to a deregulated immune response to gut microbiome dysbiosis. Cross-sectional studies have revealed microbial signatures for different IBD subtypes, including ulcerative colitis, colonic Crohn's disease and ileal Crohn's

disease. Although IBD is dynamic, microbiome studies have primarily focused on single time points or a few individuals. Here, we dissect the long-term dynamic behaviour of the gut microbiome in IBD and differentiate this from normal variation. Microbiomes of IBD subjects fluctuate more than those of healthy individuals, based on deviation from a newly defined healthy plane (HP). Ileal Crohn's disease subjects deviated most from the HP, especially subjects with surgical resection. Intriguingly, the microbiomes of some IBD subjects periodically visited the HP then deviated away from it. Inflammation was not directly correlated with distance to the healthy plane, but there was some correlation between observed dramatic fluctuations in the gut microbiome and intensified medication due to a flare of the disease. These results will help guide therapies that will redirect the gut microbiome towards a healthy state and maintain remission in IBD.

Han, S. S., et al. (2017). "A PRISMA-compliant meta-analysis of MDR1 polymorphisms and idiopathic nephrotic syndrome: Susceptibility and steroid responsiveness." Medicine (Baltimore) **96**(24): e7191.

BACKGROUND: Studies have investigated rs1128503, rs1045642, and rs2032582 in multidrug resistance protein 1 (MDR1) for association with susceptibility to idiopathic nephrotic syndrome (INS) and steroid resistance. However, because these findings were inconsistent, we performed a meta-analysis to determine whether there was evidence of a role of these MDR1 variants in INS. METHODS: The PubMed, Embase, and Web of Science databases were systematically searched to identify

studies that examined MDR1 polymorphisms with susceptibility to INS and/or to steroid resistance. Pooled odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by a fixed-effects or random-effects model based on heterogeneity. RESULTS: We selected 9 case-control studies that included 928 patients with INS, of which steroid resistance data were available for 724 (236 were steroid resistant and 488 were steroid sensitive), and 879 healthy controls. All subjects were children. No significant relationships between these polymorphisms and INS susceptibility were identified. Significantly increased risk of steroid resistance was observed with rs1128503 allelic (OR = 1.49, 95% CI = 1.20-1.86) and genotypic (OR = 1.97, 95% CI = 1.18-3.30; OR = 2.03, 95% CI = 1.43-2.88) comparisons, and with allelic (OR = 1.56, 95% CI = 1.05-2.31) and genotypic (OR = 2.85, 95% CI = 1.15-7.07; OR = 2.21, 95% CI = 1.01-4.8) comparisons to rs2032582 in Caucasian populations. However, this association between rs2032582 and steroid resistance was not robust enough to withstand corrections for multiple comparisons. Similarly, we found that the rs1128503T-rs2032582G-rs1045642C (T-G-C) haplotype was associated with an increased risk of steroid resistance (OR = 2.02, 95% CI = 1.13-3.59), while the wild-type C-G-C haplotype was associated with a decreased risk (OR = 0.32, 95% CI = 0.12-0.88) in Caucasians; however, these findings were not significant following adjustments for multiple comparisons. CONCLUSIONS: MDR1 rs1128503, rs1045642, and rs2032582 polymorphisms are not associated with INS susceptibility; however, there is evidence of an association between rs1128503 and increased risk of steroid resistance in children with INS, which indicates MDR1 may play a role in steroid resistance found in children with INS.

Hansen, L. F., et al. (2015). "Surgery and postoperative recurrence in children with Crohn disease." J Pediatr Gastroenterol Nutr **60**(3): 347-351.

Haritunians, T., et al. (2010). "Genetic predictors of medically refractory ulcerative colitis." Inflamm Bowel Dis **16**(11): 1830-1840.

BACKGROUND: Acute severe ulcerative colitis (UC) remains a significant clinical challenge and the ability to predict, at an early stage, those individuals at risk of colectomy for medically refractory UC (MR-UC) would be a major clinical advance. The aim of this study was to use a genome-wide association study (GWAS) in a well-characterized cohort of UC patients to identify genetic variation that contributes to MR-UC. METHODS: A GWAS comparing 324 MR-UC patients with 537 non-MR-UC patients was analyzed using logistic regression and Cox proportional hazards methods. In addition, the MR-UC patients were compared with 2601 healthy controls. RESULTS: MR-UC was associated with more extensive disease (P = 2.7 x 10(-6)) and a positive family history of UC (P = 0.004). A risk score based on the combination of 46 single nucleotide polymorphisms (SNPs) associated with MR-UC explained 48% of the variance for colectomy risk in our cohort. Risk scores divided into quarters showed the risk of colectomy to be 0%, 17%, 74%, and 100% in the four groups. Comparison of the MR-UC subjects with healthy controls confirmed the contribution of the major histocompatibility complex to severe UC (peak association: rs17207986, P = 1.4 x 10(-16)) and provided genome-wide suggestive association at

the TNFSF15 (TL1A) locus (peak association: rs11554257, P = 1.4 x 10(-6)). CONCLUSIONS: A SNP-based risk scoring system, identified here by GWAS analyses, may provide a useful adjunct to clinical parameters for predicting the natural history of UC. Furthermore, discovery of genetic processes underlying disease severity may help to identify pathways for novel therapeutic intervention in severe UC.

Harris, R. A., et al. (2016). "Colonic Mucosal Epigenome and Microbiome Development in Children and Adolescents." J Immunol Res **2016**: 9170162.

Epigenetic and microbiome changes during pediatric development have been implicated as important elements in the developmental origins of inflammatory bowel diseases (IBDs) including Crohn's disease (CD) and ulcerative colitis (UC), which are linked to early onset colorectal cancer (CRC). Colonic mucosal samples from 22 control children between 3.5 and 17.5 years of age were studied by Infinium HumanMethylation450 BeadChips and, in 10 cases, by 454 pyrosequencing of the bacterial 16S rRNA gene. Intercalating age-specific DNA methylation and microbiome changes were identified, which may have significant translational relevance in the developmental origins of IBD and CRC.

Harusato, A. and B. Chassaing (2017). "Insights on the impact of diet-mediated microbiota alterations on immunity and diseases." Am J Transplant.

The intestinal tract is inhabited by a large and diverse community of bacteria collectively referred to as the gut microbiota. The intestinal microbiota is composed by 500-1000 distinct species, and alterations in its composition are associated with a variety of diseases including obesity, diabetes, and inflammatory bowel disease (IBD). Importantly, microbiota transplantation from diseased patients or mice (IBD, metabolic syndrome, etc.) to germ-free mice was found to be sufficient to transfer some aspects of disease phenotypes, indicating that altered microbiota is playing a direct role in those particular conditions. Moreover, it is now well admitted that the intestinal microbiota is involved in shaping and maturating the immune system, with for example the observation that germ-free animals harbor a poorly developed intestinal immune system and that some single bacteria species, such as segmented filamentous bacteria (SFB), are sufficient to induce the expansion of Th17 cells (CD4+ T helper cells producing IL-17). We will present herein an overview of the interactions occurring between the intestinal microbiota and the immune system, and we will discuss how a dietary-induced disruption of the intestinal environment may influence transplantation outcomes.

Hawrelak, J. A. and S. P. Myers (2004). "The causes of intestinal dysbiosis: a review." Altern Med Rev **9**(2): 180-197.

Alterations in the bowel flora and its activities are now believed to be contributing factors to many chronic and degenerative diseases. Irritable bowel syndrome,

inflammatory bowel disease, rheumatoid arthritis, and ankylosing spondylitis have all been linked to alterations in the intestinal microflora. The intestinal dysbiosis hypothesis suggests a number of factors associated with modern Western living have a detrimental impact on the microflora of the gastrointestinal tract. Factors such as antibiotics, psychological and physical stress, and certain dietary components have been found to contribute to intestinal dysbiosis. If these causes can be eliminated or at least attenuated then treatments aimed at manipulating the microflora may be more successful

Haycox, A., et al. (2014). "Through a Glass Darkly: Economics and Personalised Medicine." Pharmacoeconomics **32**(11): 1055-1061.

Heap, G. A., et al. (2016). "Clinical Features and HLA Association of 5-Aminosalicylate (5-ASA)-induced Nephrotoxicity in Inflammatory Bowel Disease." J Crohns Colitis **10**(2): 149-158.

BACKGROUND AND AIMS: Nephrotoxicity is a rare idiosyncratic reaction to 5-aminosalicylate (5-ASA) therapies. The aims of this study were to describe the clinical features of this complication and identify clinically useful genetic markers so that these drugs can be avoided or so that monitoring can be intensified in high-risk patients. METHODS: Inflammatory bowel disease patients were recruited from 89 sites around the world. Inclusion criteria included normal renal function prior to commencing 5-ASA, >/=50% rise in creatinine any time after starting 5-ASA, and

physician opinion implicating 5-ASA strong enough to justify drug withdrawal. An adjudication panel identified definite and probable cases from structured case report forms. A genome-wide association study was then undertaken with these cases and 4109 disease controls. RESULTS: After adjudication, 151 cases of 5-ASA-induced nephrotoxicity were identified. Sixty-eight percent of cases were males, with nephrotoxicity occurring at a median age of 39.4 years (range 6-79 years). The median time for development of renal injury after commencing 5-ASA was 3.0 years (95% confidence interval [CI] 2.3-3.7). Only 30% of cases recovered completely after drug withdrawal, with 15 patients requiring permanent renal replacement therapy. A genome-wide association study identified a suggestive association in the HLA region (p = 1x10(-7)) with 5-ASA-induced nephrotoxicity. A sub-group analysis of patients who had a renal biopsy demonstrating interstitial nephritis (n = 55) significantly strengthened this association (p = 4x10(-9), odds ratio 3.1). CONCLUSIONS: This is the largest and most detailed study of 5-ASA-induced nephrotoxicity to date. It highlights the morbidity associated with this condition and identifies for the first time a significant genetic predisposition to drug-induced renal injury.

Heap, G. A., et al. (2014). "HLA-DQA1-HLA-DRB1 variants confer susceptibility to pancreatitis induced by thiopurine immunosuppressants." Nature Genetics **46**(10): 1131-1134.

Pancreatitis occurs in approximately 4% of patients treated with the thiopurines azathioprine or mercaptopurine. Its development is unpredictable and almost always

leads to drug withdrawal. We identified patients with inflammatory bowel disease (IBD) who had developed pancreatitis within 3 months of starting these drugs from 168 sites around the world. After detailed case adjudication, we performed a genome-wide association study on 172 cases and 2,035 controls with IBD. We identified strong evidence of association within the class II HLA region, with the most significant association identified at rs2647087 (odds ratio 2.59, 95% confidence interval 2.07-3.26, P = 2 x 10(-16)). We replicated these findings in an independent set of 78 cases and 472 controls with IBD matched for drug exposure. Fine mapping of the HLA region identified association with the HLA-DQA1*02:01-HLA-DRB1*07:01 haplotype. Patients heterozygous at rs2647087 have a 9% risk of developing pancreatitis after administration of a thiopurine, whereas homozygotes have a 17% risk.

Heazlewood, C. K., et al. (2008). "Aberrant mucin assembly in mice causes endoplasmic reticulum stress and spontaneous inflammation resembling ulcerative colitis." Plos Medicine **5**(3): 440-460.

Background

MUC2 mucin produced by intestinal goblet cells is the major component of the intestinal mucus barrier. The inflammatory bowel disease ulcerative colitis is characterized by depleted goblet cells and a reduced mucus layer, but the aetiology remains obscure. In this study we used random mutagenesis to produce two murine models of

inflammatory bowel disease, characterised the basis and nature of the inflammation in these mice, and compared the pathology with human ulcerative colitis.

Methods and Findings

By murine N-ethyl-N-nitrosourea mutagenesis we identified two distinct noncomplementing missense mutations in Muc2 causing an ulcerative colitis-like phenotype. 100% of mice of both strains developed mild spontaneous distal intestinal inflammation by 6 wk (histological colitis scores versus wild-type mice, $p < 0.01$) and chronic diarrhoea. Monitoring over 300 mice of each strain demonstrated that 25% and 40% of each strain, respectively, developed severe clinical signs of colitis by age 1 y. Mutant mice showed aberrant Muc2 biosynthesis, less stored mucin in goblet cells, a diminished mucus barrier, and increased susceptibility to colitis induced by a luminal toxin. Enhanced local production of IL-1 beta, TNF-alpha, and IFN-gamma was seen in the distal colon, and intestinal permeability increased 2-fold. The number of leukocytes within mesenteric lymph nodes increased 5-fold and leukocytes cultured in vitro produced more Th1 and Th2 cytokines (IFN-gamma, TNF-alpha, and IL- 13). This pathology was accompanied by accumulation of the Muc2 precursor and ultrastructural and biochemical evidence of endoplasmic reticulum (ER) stress in goblet cells, activation of the unfolded protein response, and altered intestinal expression of genes involved in ER stress, inflammation, apoptosis, and wound repair. Expression of mutated Muc2 oligomerisation domains in vitro demonstrated that aberrant Muc2 oligomerisation underlies the ER stress. In human ulcerative colitis we demonstrate similar accumulation of nonglycosylated MUC2 precursor in goblet cells together with ultrastructural and biochemical evidence of ER stress even in

noninflamed intestinal tissue. Although our study demonstrates that mucin misfolding and ER stress initiate colitis in mice, it does not ascertain the genetic or environmental drivers of ER stress in human colitis.

Conclusions

Characterisation of the mouse models we created and comparison with human disease suggest that ER stress-related mucin depletion could be a fundamental component of the pathogenesis of human colitis and that clinical studies combining genetics, ER stress-related pathology and relevant environmental epidemiology are warranted.

Henderson, P. and D. C. Wilson (2012). "The rising incidence of paediatric-onset inflammatory bowel disease." Arch Dis Child **97**(7): 585-586.

Herfarth, H. H., et al. (2014). "Prevalence of a gluten-free diet and improvement of clinical symptoms in patients with inflammatory bowel diseases." Inflamm Bowel Dis **20**(7): 1194-1197.

Herrlinger, K. R., et al. (2011). "ABCB1 single-nucleotide polymorphisms determine tacrolimus response in patients with ulcerative colitis." Clin Pharmacol Ther **89**(3): 422-428.

Tacrolimus (Tac) is effective in the treatment of steroid-refractory ulcerative colitis (UC); however, nonresponse and unpredictable side effects are major limitations.

Because Tac response in patients who have undergone solid-organ transplantation has been associated with the presence of variants in CYP3A and ABCB1, we elucidated the contributions of CYP3A4*1B and CYP3A5*3 and of ABCB1 1236C>T, 2677G>T,A, and 3435C>T polymorphisms to Tac response in 89 patients with UC. Short-term remission and response were achieved in 61 and 14% of the patients, respectively, and were associated with colectomy-free survival. In a linear logistic regression model, patients with homozygous variants for one of the three ABCB1 alleles showed significantly higher short-term remission rates as compared with those of other genotypes. The effects held true after multivariate analysis including multiple comparisons and were more pronounced after correction for dose-adjusted Tac blood trough levels. We suggest that ABCB1, but not CYP3A5, may predict short-term remission of Tac in steroid-refractory UC.

Hirano, T., et al. (2004). "MDR1 mRNA expressions in peripheral blood mononuclear cells of patients with ulcerative colitis in relation to glucocorticoid administration." J Clin Pharmacol **44**(5): 481-486.

Ho, G. T., et al. (2006). "The efficacy of corticosteroid therapy in inflammatory bowel disease: analysis of a 5-year UK inception cohort." Aliment Pharmacol Ther **24**(2): 319-330.

BACKGROUND: Corticosteroids remain the mainstay of first-line therapy in active inflammatory bowel disease. AIMS: To determine the clinical outcome after the first corticosteroid-therapy and to identify factors which predict response/failure.

METHODS: 216 (136 ulcerative colitis and 80 Crohn's disease) patients were identified in this 5-year inception cohort. The outcomes of early (30 days) and late (1 year) responses were used. Multivariate analyses were performed to identify factors associated with outcome. RESULTS: 86 (63%) and 60 (75%) ulcerative colitis and Crohn's disease required corticosteroid therapy, respectively. In ulcerative colitis, at 30 days, 69 (51%), 42 (31%) and 25 (18%) patients demonstrated complete response, partial response and no response, respectively. For Crohn's disease, these outcomes were observed in 32 (40%), 28 (35%) and 20 (25%). After 1 year, 75 (55%), 23 (17%) and 29 (21%) patients with ulcerative colitis demonstrated prolonged response, corticosteroid-dependence or required surgery, respectively. For Crohn's disease, these outcomes were observed in 30 (38%), 19 (24%) and 27 (35%) patients. Extensive ulcerative colitis was a predictor of surgery (P = 0.001, OR: 15.2). In Crohn's disease, inflammatory disease behaviour was negatively associated with surgery (P = 0.02, OR: 0.13). CONCLUSION: Although corticosteroids are effective, dependence/ resistance remains common. Patients with extensive ulcerative colitis and fistulizing/ stricturing Crohn's are most at risk of failing corticosteroid therapy.

Ho, G. T., et al. (2005). "Multidrug resistance (MDR1) gene in inflammatory bowel disease: a key player?" Inflamm Bowel Dis **11**(11): 1013-1019.

Ho, G. T., et al. (2003). "Multidrug resistance 1 gene (P-glycoprotein 170): an important determinant in gastrointestinal disease?" Gut **52**(5): 759-766.

The interface between luminal contents and intestinal epithelium constitutes the largest area of interaction between the host and the environment. There is now strong evidence that the gene product of the multidrug resistant pump (MDR) plays a critical role in host-bacterial interactions in the gastrointestinal tract and maintenance of intestinal homeostasis. This review highlights the efflux mechanism in the intestinal epithelium which is mediated by the multidrug resistant pump, also known as P-glycoprotein 170. Current studies promise to provide further insights into the contribution of the MDR1 gene in the pathogenesis of inflammatory and malignant disorders of the gastrointestinal tract.

Ho, G. T., et al. (2005). "Allelic variations of the multidrug resistance gene determine susceptibility and disease behavior in ulcerative colitis." Gastroenterology **128**(2): 288-296.

BACKGROUND AND AIMS: The MDR1 gene encodes P-glycoprotein 170, an efflux transporter that is highly expressed in intestinal epithelial cells. The MDR1 exonic single nucleotide polymorphisms (SNPs) C3435T and G2677T have been shown to correlate with activity/expression of P-glycoprotein 170. METHODS: This was a case-control analysis of MDR1 C3435T and G2677T SNPs in a large well-characterized Scottish white cohort (335 with ulcerative colitis [UC], 268 with Crohn's disease [CD], and 370 healthy controls). We conducted 2-locus haplotype and detailed univariate and multivariate genotypic-phenotypic analyses. RESULTS: The MDR1 3435 TT genotype (34.6% vs 26.5%; P = .04; odds ratio [OR], 1.60; 95% confidence interval [95% CI], 1.04-2.44) and T-allelic frequencies (58.2% vs 52.8%;

P = .02; OR, 1.28; 95% CI, 1.03-1.58) were significantly higher in patients with UC compared with controls. No association was seen with CD. The association was strongest with extensive UC (TT genotype: 42.4% vs 26.5%; P = .003; OR, 2.64; 95% CI, 1.34-4.99; and T allele: 63.9% vs 52.8%; P = .009; OR, 1.70; 95% CI, 1.24-2.29), and this was also confirmed on multivariate analysis (P = .007). The G2677T SNP was not associated with UC or CD. These 2 SNPs lie in linkage disequilibrium in our population (D', .8-.9; r 2 , .7-.8). Two-locus haplotypes showed both positive (3435T/ G2677 haplotype: P = .03; OR, 1.44) and negative (C3435/2677T haplotype: P = .002; OR, .35) associations with UC. Homozygotes for the haplotype 3435T/G2677 were significantly increased in UC (P = .017; OR, 8.88; 95% CI, 1.10-71.45). CONCLUSIONS: Allelic variations of the MDR1 gene determine disease extent as well as susceptibility to UC in the Scottish population. The present data strongly implicate the C3435T SNP, although the 2-locus haplotype data underline the need for further detailed haplotypic studies.

Ho, G. T., et al. (2006). "ABCB1/MDR1 gene determines susceptibility and phenotype in ulcerative colitis: discrimination of critical variants using a gene-wide haplotype tagging approach." Hum Mol Genet **15**(5): 797-805.

Several lines of evidence suggest a role for the multidrug resistance gene (ABCB1/ MDR1) and its product, P-glycoprotein 170, in the pathogenesis of inflammatory bowel disease (IBD). In addition, P-glycoprotein activity determines bioavailability of many drugs used regularly in many medical specialties, and ABCB/MDR1 variation

appears to be a critical pharmacogenetic determinant. We have utilized a gene-wide haplotype tagging approach to further define the identity of germ-line variations in the ABCB1/MDR1 gene contributing to IBD susceptibility. Six haplotype tagging single nucleotide polymorphisms (tSNPs) representing the haplotypic variations of the ABCB1/MDR1 gene were identified initially following the characterization of the haplotype structure of this gene in 24 Centre d'Etude du Polymorphisme Humain Caucasian trios. Genotyping was performed in 249 ulcerative colitis (UC) and 179 Crohn's disease (CD) patients and 260 healthy controls. Using log-likelihood analysis, we observed a highly significant association between the common haplotypes and UC (P=4.22 x 10(-7)) but not CD (P=0.22). This significant association was critically dependent on one tSNP, intronic variant rs3789243. All haplotypes with this variant retained a highly significant association (P=3.2 x 10(-7)-3.6 x 10(-12)), whereas significance was lost when rs3789243 was dropped in systematic haplotypic analysis. The effect of this tSNP was independent of C3435T SNP, previously suggested to be the critical variant in disease susceptibility and drug transport. The association with UC was shown to be strongest with the phenotype of extensive disease (P=1.7 x 10(-7)). This 'candidate gene' approach provides compelling evidence to support the contribution of the ABCB1/MDR1 gene in determining risk to UC but not to CD and provides new insights into the localization of the critical susceptibility determinants within the gene. In addition, these findings have potentially important implications in the application of pharmacogenetics across a range of common diseases, including HIV, epilepsy and colorectal cancer.

Hoentjen, F., et al. (2007). "CD4(+) T lymphocytes mediate colitis in HLA-B27 transgenic rats monoassociated with nonpathogenic Bacteroides vulgatus." Inflamm Bowel Dis **13**(3): 317-324.

Hoffmeyer, S., et al. (2000). "Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo." Proc Natl Acad Sci U S A **97**(7): 3473-3478.

Horjus Talabur Horje, C. S., et al. (2016). "Prevalence of Upper Gastrointestinal Lesions at Primary Diagnosis in Adults with Inflammatory Bowel Disease." Inflamm Bowel Dis **22**(8): 1896-1901.

BACKGROUND: The prevalence of upper gastrointestinal (GI) involvement in adult inflammatory bowel disease has mostly been studied in patients with long-standing disease. The aim of this study was to prospectively evaluate the prevalence of upper GI involvement in a consecutive series of newly diagnosed, treatment-naive adult patients with inflammatory bowel disease, irrespective of upper GI tract symptoms. METHODS: Consecutive patients with suspected inflammatory bowel disease underwent combined ileocolonoscopy and upper endoscopy with biopsies. Patients diagnosed with either Crohn's disease (CD) or ulcerative colitis (UC), denying use of nonsteroidal anti-inflammatory drug, were included in the study. Helicobacter pylori infection was diagnosed histologically and positive patients were excluded from the analysis. Endoscopic and histologic lesions in the stomach and duodenum were

recorded. Upper GI location (+L4) was defined as a combination of endoscopic and histological lesions. RESULTS: A total of 152 patients (108 CD and 44 UC) were analyzed. Endoscopic lesions were only seen in patients with CD (60 of 108, 55%). Histological lesions were present in both patients with CD and patients with UC: focally enhanced gastritis in 58 CD (54%) and 10 UC (23%), granulomas in 30 CD (28%). Upper GI disease location was diagnosed in 44 patients with CD (41%) and no patients with UC. Upper GI tract symptoms were reported in 14 of 44 patients (32%) with upper GI location. CONCLUSIONS: A high prevalence of upper GI involvement was observed in newly diagnosed patients with CD, with a majority of the patients being asymptomatic. Focally enhanced gastritis was common in both patients with CD and patients with UC, whereas granulomatous inflammation was restricted to patients with CD.

Hou, J. K., et al. (2011). "Dietary intake and risk of developing inflammatory bowel disease: a systematic review of the literature." Am J Gastroenterol **106**(4): 563-573.

OBJECTIVES: The incidence of inflammatory bowel disease (IBD) is increasing. Dietary factors such as the spread of the "Western" diet, high in fat and protein but low in fruits and vegetables, may be associated with the increase. Although many studies have evaluated the association between diet and IBD risk, there has been no systematic review. METHODS: We performed a systematic review using guideline-recommended methodology to evaluate the association between pre-illness intake of nutrients (fats, carbohydrates, protein) and food groups (fruits, vegetables, meats) and

the risk of subsequent IBD diagnosis. Eligible studies were identified via structured keyword searches in PubMed and Google Scholar and manual searches. RESULTS: Nineteen studies were included, encompassing 2,609 IBD patients (1,269 Crohn's disease (CD) and 1,340 ulcerative colitis (UC) patients) and over 4,000 controls. Studies reported a positive association between high intake of saturated fats, monounsaturated fatty acids, total polyunsaturated fatty acids (PUFAs), total omega-3 fatty acids, omega-6 fatty acids, mono- and disaccharides, and meat and increased subsequent CD risk. Studies reported a negative association between dietary fiber and fruits and subsequent CD risk. High intakes of total fats, total PUFAs, omega-6 fatty acids, and meat were associated with an increased risk of UC. High vegetable intake was associated with a decreased risk of UC. CONCLUSIONS: High dietary intakes of total fats, PUFAs, omega-6 fatty acids, and meat were associated with an increased risk of CD and UC. High fiber and fruit intakes were associated with decreased CD risk, and high vegetable intake was associated with decreased UC risk.

Howell, K. J., et al. (2018). "DNA Methylation and Transcription Patterns in Intestinal Epithelial Cells From Pediatric Patients With Inflammatory Bowel Diseases Differentiate Disease Subtypes and Associate With Outcome." Gastroenterology **154**(3): 585-598.

BACKGROUND & AIMS: We analyzed DNA methylation patterns and transcriptomes of primary intestinal epithelial cells (IEC) of children newly diagnosed with inflammatory bowel diseases (IBD) to learn more about pathogenesis. METHODS: We obtained mucosal biopsies (N = 236) collected from terminal ileum

and ascending and sigmoid colons of children (median age 13 years) newly diagnosed with IBD (43 with Crohn's disease [CD], 23 with ulcerative colitis [UC]), and 30 children without IBD (controls). Patients were recruited and managed at a hospital in the United Kingdom from 2013 through 2016. We also obtained biopsies collected at later stages from a subset of patients. IECs were purified and analyzed for genome-wide DNA methylation patterns and gene expression profiles. Adjacent microbiota were isolated from biopsies and analyzed by 16S gene sequencing. We generated intestinal organoid cultures from a subset of samples and genome-wide DNA methylation analysis was performed. RESULTS: We found gut segment-specific differences in DNA methylation and transcription profiles of IECs from children with IBD vs controls; some were independent of mucosal inflammation. Changes in gut microbiota between IBD and control groups were not as large and were difficult to assess because of large amounts of intra-individual variation. Only IECs from patients with CD had changes in DNA methylation and transcription patterns in terminal ileum epithelium, compared with controls. Colon epithelium from patients with CD and from patients with ulcerative colitis had distinct changes in DNA methylation and transcription patterns, compared with controls. In IECs from patients with IBD, changes in DNA methylation, compared with controls, were stable over time and were partially retained in ex-vivo organoid cultures. Statistical analyses of epithelial cell profiles allowed us to distinguish children with CD or UC from controls; profiles correlated with disease outcome parameters, such as the requirement for treatment with biologic agents. CONCLUSIONS: We identified specific changes in DNA methylation and transcriptome patterns in IECs from pediatric patients with IBD compared with controls. These data indicate that IECs undergo changes during IBD

development and could be involved in pathogenesis. Further analyses of primary IECs from patients with IBD could improve our understanding of the large variations in disease progression and outcomes.

Hsu, S. M. and L. Raine (1981). "Protein A, avidin, and biotin in immunohistochemistry." J Histochem Cytochem **29**(11): 1349-1353.

Huang, E. Y., et al. (2015). "Using corticosteroids to reshape the gut microbiome: implications for inflammatory bowel diseases." Inflamm Bowel Dis **21**(5): 963-972.

Huang, S. N., et al. (1976). "Application of immunofluorescent staining on paraffin sections improved by trypsin digestion." Laboratory Investigation **35**(4): 383-390.

Huang, Y., et al. (2004). "Membrane transporters and channels: role of the transportome in cancer chemosensitivity and chemoresistance." Cancer Research **64**(12): 4294-4301.

Huebner, C., et al. (2009). "Genetic analysis of MDR1 and inflammatory bowel disease reveals protective effect of heterozygous variants for ulcerative colitis." Inflamm Bowel Dis **15**(12): 1784-1793.

Hyams, J. S., et al. (2017). "Factors associated with early outcomes following standardised therapy in children with ulcerative colitis (PROTECT): a multicentre inception cohort study." Lancet Gastroenterol Hepatol **2**(12): 855-868.

Icht, O., et al. (2017). "Comparative Study of Two Cohorts of Newly Diagnosed Crohn's Disease Demonstrates Change in Therapeutic Strategies." Digestion **96**(3): 135-141.

BACKGROUND: There has been a paradigm shift in the treatment of Crohn's disease (CD) involving the rapid introduction of biologics and/or immunomodulators after diagnosis. We wished to assess whether this was applied to patients with newly diagnosed CD in a tertiary inflammatory bowel disease referral centre in Israel. METHODS: Newly diagnosed CD patients were stratified into 2 groups: the early group was diagnosed between 2005 and 2007 and the late group was diagnosed between 2010 and 2012. Baseline demographics, medical and surgical treatments, disease course and complications during those 2 periods were analyzed. RESULTS: Each group included 60 patients. Significantly higher rates of immunomodulators and biologics were administered to patients in the late group compared to the early group (81.7 and 36.7% compared to 56.7 and 18.3%, p = 0.004 and p = 0.021, respectively). On the other hand, steroid therapy was less prevalent in the late (36.7%) group compared to that of the early group (56.7%), p = 0.059. Medical and surgical CD outcomes, including exacerbations/hospitalizations and surgeries, were comparable for both groups. CONCLUSIONS: There was a change in treatment strategy between

2005-2007 and 2010-2012, as reflected in higher proportions of biologics/ immunomodulators for patients with newly diagnosed CD. This was associated with a steroid-sparing effect.

Ieiri, I., et al. (2004). "The MDR1 (ABCB1) gene polymorphism and its clinical implications." Clin Pharmacokinet **43**(9): 553-576.

International HapMap, C., et al. (2007). "A second generation human haplotype map of over 3.1 million SNPs." Nature **449**(7164): 851-861.

Ishimoto, O., et al. (2002). "Possible oncogenic potential of DeltaNp73: a newly identified isoform of human p73." Cancer Research **62**(3): 636-641.

p73, a recently identified gene highly homologous to p53, can transactivate p53 target genes and induce apoptosis. Here we report the identification of an NH(2)-terminal truncated isoform of human p73, DeltaNp73, which is capable of suppressing p53- and p73-dependent transactivation. We speculate that this suppression is achieved by competing for the DNA binding site in the case of p53 and by direct association in the case of TAp73. Expression of DeltaNp73 in cancer cell lines also inhibited suppressive activity of p53 and TAp73 in colony formation, implying possible involvement of DeltaNp73 in oncogenesis by inhibiting the tumor-suppressive function of p53 and TAp73. Also reported is the identification of TAp73eta, a new

member of the COOH-terminal truncated isoform of p73 and tissue-specific expression of these isoforms, along with other previously identified p73 isoforms.

Ivell, R., et al. (2014). "Proper application of antibodies for immunohistochemical detection: antibody crimes and how to prevent them." Endocrinology **155**(3): 676-687.

Iwasaki, M., et al. (2015). "Circadian modulation in the intestinal absorption of P-glycoprotein substrates in monkeys." Mol Pharmacol **88**(1): 29-37.

Jin, S. S. and W. J. Song (2017). "Association between MDR1 C3435T polymorphism and colorectal cancer risk: A meta-analysis." Medicine (Baltimore) **96**(51): e9428.

Johnson, J. A., et al. (2017). "Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Pharmacogenetics-Guided Warfarin Dosing: 2017 Update." Clin Pharmacol Ther **102**(3): 397-404.

Johnson, R. L. and J. C. Fleet (2013). "Animal models of colorectal cancer." Cancer Metastasis Rev **32**(1-2): 39-61.

Colorectal cancer is a heterogeneous disease that afflicts a large number of people in the USA. The use of animal models has the potential to increase our understanding of carcinogenesis, tumor biology, and the impact of specific molecular events on colon biology. In addition, animal models with features of specific human colorectal cancers can be used to test strategies for cancer prevention and treatment. In this review, we provide an overview of the mechanisms driving human cancer, we discuss the approaches one can take to model colon cancer in animals, and we describe a number of specific animal models that have been developed for the study of colon cancer. We believe that there are many valuable animal models to study various aspects of human colorectal cancer. However, opportunities for improving upon these models exist.

Jongkhajornpong, P., et al. (2016). "Elevated expression of ABCB5 in ocular surface squamous neoplasia." Sci Rep **6**: 20541.

Jostins, L., et al. (2012). "Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease." Nature **491**(7422): 119-124.

Crohn's disease and ulcerative colitis, the two common forms of inflammatory bowel disease (IBD), affect over 2.5 million people of European ancestry, with rising prevalence in other populations. Genome-wide association studies and subsequent meta-analyses of these two diseases as separate phenotypes have implicated previously unsuspected mechanisms, such as autophagy, in their pathogenesis and showed that some IBD loci are shared with other inflammatory diseases. Here we

expand on the knowledge of relevant pathways by undertaking a meta-analysis of Crohn's disease and ulcerative colitis genome-wide association scans, followed by extensive validation of significant findings, with a combined total of more than 75,000 cases and controls. We identify 71 new associations, for a total of 163 IBD loci, that meet genome-wide significance thresholds. Most loci contribute to both phenotypes, and both directional (consistently favouring one allele over the course of human history) and balancing (favouring the retention of both alleles within populations) selection effects are evident. Many IBD loci are also implicated in other immune-mediated disorders, most notably with ankylosing spondylitis and psoriasis. We also observe considerable overlap between susceptibility loci for IBD and mycobacterial infection. Gene co-expression network analysis emphasizes this relationship, with pathways shared between host responses to mycobacteria and those predisposing to IBD.

Jowett, S. L., et al. (2004). "Influence of dietary factors on the clinical course of ulcerative colitis: a prospective cohort study." Gut **53**(10): 1479-1484.

Juyal, G., et al. (2009). "Associations between common variants in the MDR1 (ABCB1) gene and ulcerative colitis among North Indians." Pharmacogenet Genomics **19**(1): 77-85.

Kammermeier, J., et al. (2014). "Targeted gene panel sequencing in children with very early onset inflammatory bowel disease--evaluation and prospective analysis." J Med Genet **51**(11): 748-755.

Kawahito, Y., et al. (1995). "Corticotropin releasing hormone in colonic mucosa in patients with ulcerative colitis." Gut **37**(4): 544-551.

Corticotropin releasing hormone (CRH) is a key hormone in integrated response to stress, acting as the major regulator of the hypothalamic-pituitary-adrenal axis. Recently, local production of CRH has been detected in normal human colonic enterochromaffin cells. CRH is locally secreted in granulomatous and arthritic tissues in rats and humans, where it seems to act as a local proinflammatory agent. To find out if CRH is present in colonic tissues of patients with ulcerative colitis, this study examined the expression of this peptide in the large bowel of patients with ulcerative colitis. Colonic tissues of patients with ulcerative colitis obtained by endoscopic biopsy were immunostained with anti-CRH antibody. CRH messenger (m) RNA was also examined in biopsy specimens of ulcerative colitis by the reverse transcribed polymerase chain reaction method and by in situ hybridisation. Considerably enhanced expression of immunoreactive CRH was found in mucosal inflammatory cells. Intense staining with anti-CRH antibody was also shown in mucosal macrophages. CRH mRNA was expressed in mucosal epithelial cells. The expression of immunoreactive CRH in colonic mucosal epithelial cells of ulcerative colitis slightly increased, but not significantly, compared with normal colonic mucosal

epithelial cells. These results suggest that CRH may play a part in the modulation of intestinal immune and inflammatory system, and as a modulator in the pathogenesis of ulcerative colitis.

Kawanobe, T., et al. (2012). "Expression of human ABCB5 confers resistance to taxanes and anthracyclines." Biochem Biophys Res Commun **418**(4): 736-741.

Human ABCB5, an ATP-binding cassette (ABC) transporter gene, has two major mRNA species. One transcript encodes an 812 amino acid polypeptide, ABCB5beta, with a transmembrane domain and a nucleotide-binding domain. We isolated the cDNA of another ABCB5 mRNA that encodes a 1257 amino acid polypeptide. The translated ABCB5 protein is a full-sized ABC transporter that has an internally duplicated structure with two transmembrane domains and two nucleotide-binding domains. The 5' and 3' parts of the ABCB5 mRNA were expressed in the prostate and testis. HEK293 cells transfected with the ABCB5 cDNA expressed a 150-160kDa protein. The ABCB5 transfectants showed approximately 1.5-fold higher resistance to doxorubicin, and 2- to 3-fold higher resistance to paclitaxel and docetaxel. Cellular uptake of radiolabeled paclitaxel and docetaxel in the transfectants was lower than that in the parental HEK293 cells. Treatment of the transfectants with ABCB5-targeted siRNA lowered their resistance to docetaxel. Revertant cells that express a reduced amount of ABCB5 also showed a lowered level of docetaxel resistance. These results indicated that the expression of ABCB5 conferred resistance to taxanes and anthracyclines. Membrane vesicles prepared from ABCB5 baculovirus-infected

Sf21 cells showed higher vanadate-sensitive ATPase activity than the Sf21 control vesicles. The k(m) and V(max) values of ATPase activity in the ABCB5 vesicles were 1.8mM and 65nmol/min/mg protein, respectively. ABCB5 ATPase activity was 1.25-fold higher in the presence of 100muM docetaxel than it was in the absence of docetaxel. These results indicates that the full-length ABCB5 protein has ATPase activity that is sensitive to docetaxel.

Kaye, J. B., et al. (2017). "Warfarin Pharmacogenomics in Diverse Populations." Pharmacotherapy **37**(9): 1150-1163.

Kemper, K. E., et al. (2018). "A multi-trait Bayesian method for mapping QTL and genomic prediction." Genetics Selection Evolution **50**.

Keniya, M. V., et al. (2014). "Drug Resistance Is Conferred on the Model Yeast Saccharomyces cerevisiae by Expression of Full-Length Melanoma-Associated Human ATP-Binding Cassette Transporter ABCB5." Molecular Pharmaceutics **11**(10): 3452-3462.

ABCB5, an ATP-binding cassette (ABC) transporter, is highly expressed in melanoma cells, and may contribute to the extreme resistance of melanomas to chemotherapy by efflux of anti-cancer drugs. Our goal was to determine whether we could functionally express human ABCB5 in the model yeast Saccharomyces cerevisiae, in order to demonstrate an efflux function for ABCB5 in the absence of background pump

activity from other human transporters. Heterologous expression would also facilitate drug discovery for this important target. DNAs encoding ABCB5 sequences were cloned into the chromosomal PDR5 locus of a S. cerevisiae strain in which seven endogenous ABC transporters have been deleted. Protein expression in the yeast cells was monitored by immunodetection using both a specific anti-ABCB5 antibody and a cross-reactive anti-ABCB1 antibody. ABCB5 function in recombinant yeast cells was measured by determining whether the cells possessed increased resistance to known pump substrates, compared to the host yeast strain, in assays of yeast growth. Three ABCB5 constructs were made in yeast. One was derived from the ABCB5-beta mRNA, which is highly expressed in human tissues but is a truncation of a canonical full-size ABC transporter. Two constructs contained full-length ABCB5 sequences: either a native sequence from cDNA or a synthetic sequence codon-harmonized for S. cerevisiae. Expression of all three constructs in yeast was confirmed by immunodetection. Expression of the codon-harmonized full-length ABCB5 DNA conferred increased resistance, relative to the host yeast strain, to the putative substrates rhodamine 123, daunorubicin, tetramethylrhodamine, FK506, or clorgyline. We conclude that full-length ABCB5 can be functionally expressed in S. cerevisiae and confers drug resistance.

Kerb, R., et al. (2001). "ABC drug transporters: hereditary polymorphisms and pharmacological impact in MDR1, MRP1 and MRP2." Pharmacogenomics **2**(1): 51-64.

Kerur, B., et al. (2018). "Biologics Delay Progression of Crohn's Disease, but Not Early Surgery, in Children." Clin Gastroenterol Hepatol **16**(9): 1467-1473.

Keshteli, A. H., et al. (2017). "Dietary and metabolomic determinants of relapse in ulcerative colitis patients: A pilot prospective cohort study." World J Gastroenterol **23**(21): 3890-3899.

AIM: To identify demographic, clinical, metabolomic, and lifestyle related predictors of relapse in adult ulcerative colitis (UC) patients. METHODS: In this prospective pilot study, UC patients in clinical remission were recruited and followed-up at 12 mo to assess a clinical relapse, or not. At baseline information on demographic and clinical parameters was collected. Serum and urine samples were collected for analysis of metabolomic assays using a combined direct infusion/liquid chromatography tandem mass spectrometry and nuclear magnetic resolution spectroscopy. Stool samples were also collected to measure fecal calprotectin (FCP). Dietary assessment was performed using a validated self-administered food frequency questionnaire. RESULTS: Twenty patients were included (mean age: 42.7 +/- 14.8 years, females: 55%). Seven patients (35%) experienced a clinical relapse during the follow-up period. While 6 patients (66.7%) with normal body weight developed a clinical relapse, 1 UC patient (9.1%) who was overweight/obese relapsed during the follow-up (P = 0.02). At baseline, poultry intake was significantly higher in patients who were still in remission during follow-up (0.9 oz vs 0.2 oz, P = 0.002). Five patients (71.4%) with FCP > 150 mug/g and 2 patients (15.4%) with normal FCP (</= 150 mug/g) at baseline relapsed during the follow-up (P = 0.02). Interestingly,

baseline urinary and serum metabolomic profiling of UC patients with or without clinical relapse within 12 mo showed a significant difference. The most important metabolites that were responsible for this discrimination were trans-aconitate, cystine and acetamide in urine, and 3-hydroxybutyrate, acetoacetate and acetone in serum. CONCLUSION: A combination of baseline dietary intake, fecal calprotectin, and metabolomic factors are associated with risk of UC clinical relapse within 12 mo.

Khor, B., et al. (2011). "Genetics and pathogenesis of inflammatory bowel disease." Nature **474**(7351): 307-317.

Recent advances have provided substantial insight into the maintenance of mucosal immunity and the pathogenesis of inflammatory bowel disease. Cellular programs responsible for intestinal homeostasis use diverse intracellular and intercellular networks to promote immune tolerance, inflammation or epithelial restitution. Complex interfaces integrate local host and microbial signals to activate appropriate effector programs selectively and even drive plasticity between these programs. In addition, genetic studies and mouse models have emphasized the role of genetic predispositions and how they affect interactions with microbial and environmental factors, leading to pro-colitogenic perturbations of the host-commensal relationship.

Kim, M. S., et al. (2014). "A draft map of the human proteome." Nature **509**(7502): 575-581.

Kiss, K., et al. (2012). "Shifting the paradigm: the putative mitochondrial protein ABCB6 resides in the lysosomes of cells and in the plasma membrane of erythrocytes." PLoS One **7**(5): e37378.

Kiss, K., et al. (2015). "Role of the N-terminal transmembrane domain in the endo-lysosomal targeting and function of the human ABCB6 protein." Biochem J **467**(1): 127-139.

Klaassen, C. D. and L. M. Aleksunes (2010). "Xenobiotic, bile acid, and cholesterol transporters: function and regulation." Pharmacol Rev **62**(1): 1-96.

Kleffel, S., et al. (2014). "ABCB5 inhibition sensitizes Merkel cell carcinoma cells to chemotherapy-induced apoptosis." Journal of Investigative Dermatology **134**: S18-S18.

Kleffel, S., et al. (2014). "ABCB5 inhibition sensitizes Merkel cell carcinoma cells to chemotherapy-induced apoptosis." Journal of Investigative Dermatology **134**: S31-S31.

Kleffel, S., et al. (2016). "ABCB5-Targeted Chemoresistance Reversal Inhibits Merkel Cell Carcinoma Growth." J Invest Dermatol **136**(4): 838-846.

Knops, N., et al. (2013). "From gut to kidney: transporting and metabolizing calcineurin-inhibitors in solid organ transplantation." Int J Pharm **452**(1-2): 14-35.

Since their introduction circa 35 years ago, calcineurin-inhibitors (CNI) have become the cornerstone of immunosuppressive therapy in solid organ transplantation. However, CNI's possess a narrow therapeutic index with potential severe consequences of drug under- or overexposure. This demands a meticulous policy of Therapeutic Drug Monitoring (TDM) to optimize outcome. In clinical practice optimal dosing is difficult to achieve due to important inter- and intraindividual variation in CNI pharmacokinetics. A complex and often interdependent set of factors appears relevant in determining drug exposure. These include recipient characteristics such as age, race, body composition, organ function, and food intake, but also graft-related characteristics such as: size, donor-age, and time after transplantation can be important. Fundamental (in vitro) and clinical studies have pointed out the intrinsic relation between the aforementioned variables and the functional capacity of enzymes and transporters involved in CNI metabolism, primarily located in intestine, liver and kidney. Commonly occurring polymorphisms in genes responsible for CNI metabolism (CYP3A4, CYP3A5, CYP3A7, PXR, POR, ABCB1 (P-gp) and possibly UGT) are able to explain an important part of interindividual variability. In particular, a highly prevalent SNP in CYP3A5 has proven to be an important determinant of CNI dose requirements and drug-dose-interactions. In addition, a discrepancy in genotype between graft and receptor has to be taken into account. Furthermore, common phenomena in solid organ transplantation such as inflammation, ischemia- reperfusion injury, graft function, co-medication, altered food intake and intestinal motility can

have a differential effect on the expression enzymes and transporters involved in CNI metabolism. Notwithstanding the built-up knowledge, predicting individual CNI pharmacokinetics and dose requirements on the basis of current clinical and experimental data remains a challenge.

Kojima, Y., et al. (2018). "Influence of NUDT15 variants on hematological pictures of patients with inflammatory bowel disease treated with thiopurines." World J Gastroenterol **24**(4): 511-518.

Koloyou, P. E., et al. (2010). "Abcb5 Identifies Tumor Initiating Cells in Retinoblastoma." Pediatric Blood & Cancer **55**(5): 897-898.

Konidari, A., et al. (2014). "Thiopurine monitoring in children with inflammatory bowel disease: a systematic review." British Journal of Clinical Pharmacology **78**(3): 467-476.

AIMS

The aim was to systematically review the evidence on the clinical usefulness of thiopurine metabolite and white blood count (WBC) monitoring in the assessment of clinical outcomes in children with inflammatory bowel disease (IBD).

METHODS

Medline, Embase, Cochrane Central Register of controlled trials and http://www.clinicaltrials.gov were screened in adherence to the PRISMA statement by two independent reviewers for identification of eligible studies. Eligible studies were randomized controlled trials (RCTs), cohort studies and large case series of children with inflammatory bowel disease (IBD) (<18 years) who underwent monitoring of thiopurine metabolites and/or WBC.

RESULTS

Fifteen papers were identified (n = 1026). None of the eligible studies were RCTs. High 6-thioguanine nucleotide (6TGN) concentrations were not consistently associated with leucopenia. Leucopenia was not associated with achievement of clinical remission. A positive but not consistent correlation between 6TGN and clinical remission was reported. Haematological toxicity could not be reliably assessed with 6TGN measurements only. A number of studies supported the use of high 6-methylmercaptopurine ribonucleotides (6MMPR) as an indicator of hepatotoxicity. Low thiopurine metabolite concentration may be indicative of non-compliance.

CONCLUSION

Thiopurine metabolite testing does not safely predict clinical outcome, but may facilitate toxicity surveillance and treatment optimization in poor responders. Current evidence favours the combination of thiopurine metabolite/WBC monitoring and clinic follow-up for prompt identification of haematologic/hepatic toxicity safe dose adjustment, and treatment modification in cases of suboptimal clinical outcome or non-

compliance. Well designed RCTs for the identification of robust surrogate markers of thiopurine efficacy and toxicity are required.

Kopp, F. and J. T. Mendell (2018). "Functional Classification and Experimental Dissection of Long Noncoding RNAs." Cell **172**(3): 393-407.

Kozera, B. and M. Rapacz (2013). "Reference genes in real-time PCR." J Appl Genet **54**(4): 391-406.

Kriegsmann, K., et al. (2018). "Combined Immunohistochemistry after Mass Spectrometry Imaging for Superior Spatial Information." Proteomics Clin Appl: e1800035.

Krupoves, A., et al. (2011). "Associations Between Variants in the ABCB1 (MDR1) Gene and Corticosteroid Dependence in Children with Crohn's Disease." Inflammatory Bowel Diseases **17**(11): 2308-2317.

Background: Corticosteroids (CS) effectively induce remission in patients with moderate to severe Crohn's disease (CD). However, CS dependence in children is a significant clinical problem associated with numerous side effects. Identification of molecular markers of CS dependence is of paramount importance. The ABCB1 gene codes for P-glycoprotein, a transporter involved in the metabolism of CS. We

examined whether DNA variation in the ABCB1 gene was associated with CS dependency in children with CD.

Methods: A retrospective study was carried out in two Canadian tertiary pediatric gastroenterology centers. Clinical information was abstracted from medical charts of CD patients (N = 260) diagnosed with CD prior to age 18 and administered a first course of CS during the 1 year since diagnosis. Patients were classified as CS-dependent if they relapsed during drug tapering or after the end of therapy. DNA was extracted from blood or saliva. Thirteen tagging single nucleotide polymorphisms (tag-SNPs) and a synonymous variation (C3435T) in the ABCB1 gene were genotyped. Allelic, genotype, and haplotype associations were examined using logistic regression and Haploview.

Results: Tag-SNP rs2032583 was statistically significantly associated with CS dependency. The rare C allele of this SNP (odds ratio [OR] 0.56, 95% confidence interval [CI]: 0.34-0.95, P = 0.029) and heterozygous genotype TC (OR = 0.52, 95% CI: 0.28-0.95, P = 0.035) conferred protection from CS dependency. A three-marker haplotype was significantly associated with CS dependence (multiple comparison corrected P-value = 0.004).

Conclusions: Our results suggest that the ABCB1 gene may be associated with CS dependence in pediatric CD patients. (Inflamm Bowel Dis 2011;17:2308-2317)

Ksander, B. R., et al. (2014). "ABCB5 is a limbal stem cell gene required for corneal development and repair." Nature **511**(7509): 353-357.

Corneal epithelial homeostasis and regeneration are sustained by limbal stem cells (LSCs), and LSC deficiency is a major cause of blindness worldwide. Transplantation is often the only therapeutic option available to patients with LSC deficiency. However, while transplant success depends foremost on LSC frequency within grafts, a gene allowing for prospective LSC enrichment has not been identified so far. Here we show that ATP-binding cassette, sub-family B, member 5 (ABCB5) marks LSCs and is required for LSC maintenance, corneal development and repair. Furthermore, we demonstrate that prospectively isolated human or murine ABCB5-positive LSCs possess the exclusive capacity to fully restore the cornea upon grafting to LSC-deficient mice in xenogeneic or syngeneic transplantation models. ABCB5 is preferentially expressed on label-retaining LSCs in mice and p63alpha-positive LSCs in humans. Consistent with these findings, ABCB5-positive LSC frequency is reduced in LSC-deficient patients. Abcb5 loss of function in Abcb5 knockout mice causes depletion of quiescent LSCs due to enhanced proliferation and apoptosis, and results in defective corneal differentiation and wound healing. Our results from gene knockout studies, LSC tracing and transplantation models, as well as phenotypic and functional analyses of human biopsy specimens, provide converging lines of evidence that ABCB5 identifies mammalian LSCs. Identification and prospective isolation of molecularly defined LSCs with essential functions in corneal development and repair has important implications for the treatment of corneal disease, particularly corneal blindness due to LSC deficiency.

Ksander, B. R., et al. (2015). "Abcb5-positive retinal pigment epithelial cells are required for normal retinal development and ageing." Investigative Ophthalmology & Visual Science **56**(7).

Kugathasan, S., et al. (2017). "Prediction of complicated disease course for children newly diagnosed with Crohn's disease: a multicentre inception cohort study." Lancet **389**(10080): 1710-1718.

BACKGROUND: Stricturing and penetrating complications account for substantial morbidity and health-care costs in paediatric and adult onset Crohn's disease. Validated models to predict risk for complications are not available, and the effect of treatment on risk is unknown. METHODS: We did a prospective inception cohort study of paediatric patients with newly diagnosed Crohn's disease at 28 sites in the USA and Canada. Genotypes, antimicrobial serologies, ileal gene expression, and ileal, rectal, and faecal microbiota were assessed. A competing-risk model for disease complications was derived and validated in independent groups. Propensity-score matching tested the effect of anti-tumour necrosis factor alpha (TNFalpha) therapy exposure within 90 days of diagnosis on complication risk. FINDINGS: Between Nov 1, 2008, and June 30, 2012, we enrolled 913 patients, 78 (9%) of whom experienced Crohn's disease complications. The validated competing-risk model included age, race, disease location, and antimicrobial serologies and provided a sensitivity of 66% (95% CI 51-82) and specificity of 63% (55-71), with a negative predictive value of 95% (94-97). Patients who received early anti-TNFalpha therapy were less likely to

have penetrating complications (hazard ratio [HR] 0.30, 95% CI 0.10-0.89; p=0.0296) but not stricturing complication (1.13, 0.51-2.51; 0.76) than were those who did not receive early anti-TNFalpha therapy. Ruminococcus was implicated in stricturing complications and Veillonella in penetrating complications. Ileal genes controlling extracellular matrix production were upregulated at diagnosis, and this gene signature was associated with stricturing in the risk model (HR 1.70, 95% CI 1.12-2.57; p=0.0120). When this gene signature was included, the model's specificity improved to 71%. INTERPRETATION: Our findings support the usefulness of risk stratification of paediatric patients with Crohn's disease at diagnosis, and selection of anti-TNFalpha therapy. FUNDING: Crohn's and Colitis Foundation of America, Cincinnati Children's Hospital Research Foundation Digestive Health Center.

Kugimiya, N., et al. (2015). "The c-MYC-ABCB5 axis plays a pivotal role in 5-fluorouracil resistance in human colon cancer cells." J Cell Mol Med **19**(7): 1569-1581.

c-MYC overexpression is frequently observed in various cancers including colon cancer and regulates many biological activities such as aberrant cell proliferation, apoptosis, genomic instability, immortalization and drug resistance. However, the mechanism by which c-MYC confers drug resistance remains to be fully elucidated. In this study, we found that the c-MYC expression level in primary colorectal cancer tissues correlated with the recurrence rate following 5-fluorouracil (5-FU)-based adjuvant chemotherapy. Supporting this finding, overexpression of exogenous c-MYC increased the survival rate following 5-FU treatment in human colon cancer cells, and

knockdown of endogenous c-MYC decreased it. Furthermore, c-MYC knockdown decreased the expression level of ABCB5, which is involved in 5-FU resistance. Using a chromatin immunoprecipitation assay, we found that c-MYC bound to the ABCB5 promoter region. c-MYC inhibitor (10058-F4) treatment inhibited c-MYC binding to the ABCB5 promoter, leading to a decrease in ABCB5 expression level. ABCB5 knockdown decreased the survival rate following 5-FU treatment as expected, and the ABCB5 expression level was increased in 5-FU-resistant human colon cancer cells. Finally, using a human colon cancer xenograft murine model, we found that the combined 5-FU and 10058-F4 treatment significantly decreased tumorigenicity in nude mice compared with 5-FU or 10058-F4 treatment alone. 10058-F4 treatment decreased the ABCB5 expression level in the presence or absence of 5-FU. In contrast, 5-FU treatment alone increased the ABCB5 expression level. Taken together, these results suggest that c-MYC confers resistance to 5-FU through regulating ABCB5 expression in human colon cancer cells.

Kumar, R. and E. B. Thompson (2005). "Gene regulation by the glucocorticoid receptor: structure:function relationship." J Steroid Biochem Mol Biol **94**(5): 383-394.

Lane, E. R., et al. (2017). "The microbiota in inflammatory bowel disease: current and therapeutic insights." J Inflamm Res **10**: 63-73.

Inflammatory bowel disease is a heterogeneous group of chronic disorders that result from the interaction of the intestinal immune system with the gut microbiome. Until

recently, most investigative efforts and therapeutic breakthroughs were centered on understanding and manipulating the altered mucosal immune response that characterizes these diseases. However, more recent studies have highlighted the important role of environmental factors, and in particular the microbiota, in disease onset and disease exacerbation. Advances in genomic sequencing technology and bioinformatics have facilitated an explosion of investigative inquiries into the composition and function of the intestinal microbiome in health and disease and have advanced our understanding of the interplay between the gut microbiota and the host immune system. The gut microbiome is dynamic and changes with age and in response to diet, antibiotics and other environmental factors, and these alterations in the microbiome contribute to disease onset and exacerbation. Strategies to manipulate the microbiome through diet, probiotics, antibiotics or fecal microbiota transplantation may potentially be used therapeutically to influence modulate disease activity. This review will characterize the factors involved in the development of the intestinal microbiome and will describe the typical alterations in the microbiota that are characteristic of inflammatory bowel disease. Additionally, this manuscript will summarize the early but promising literature on the role of the gut microbiota in the pathogenesis of inflammatory bowel disease with implications for utilizing this data for diagnostic or therapeutic application in the clinical management of patients with these diseases.

Langmann, T., et al. (2004). "Loss of detoxification in inflammatory bowel disease: dysregulation of pregnane X receptor target genes." Gastroenterology **127**(1): 26-40.

Lasa, J. and P. Olivera (2017). "Efficacy of Tacrolimus for Induction of Remission in Patients with Moderate-to-Severe Ulcerative Colitis: A Systematic Review and Meta-Analysis." Arq Gastroenterol **54**(2): 167-172.

Lee, W. J., et al. (2016). "Top-down Versus Step-up Prescribing Strategies for Tumor Necrosis Factor Alpha Inhibitors in Children and Young Adults with Inflammatory Bowel Disease." Inflamm Bowel Dis **22**(10): 2410-2417.

BACKGROUND: Early initiation of tumor necrosis factor-alpha inhibitor (TNFI) therapy for children and young adults with inflammatory bowel disease (IBD) is not well described. METHODS: We conducted a retrospective cohort study of children and young adults (</=24 yr) newly diagnosed with IBD using health insurance claims from 2009 to 2013. The conventional "step-up" approach was defined as TNFI initiation >30 days after first IBD medication prescription, whereas the "top-down" approach was defined as new TNFI prescription within 30 days of first IBD medication prescription. Switching rates, time to initiation, discontinuation, and adherence to TNFIs were compared between the 2 strategies. RESULTS: A total of 11,962 IBD patients were identified. Among 3300 TNFI users, 1298 (39.3%) were treated with the top-down approach, whereas 2002 (60.7%) were treated with the step-up approach. Top-down approach use increased from 31.4% to 49.8% during the 5-year period, and under this approach, most patients were treated with TNFIs alone. Time to TNFI initiation was shorter for patients diagnosed in more recent years.

Patients treated with the top-down strategy had lower rates of corticosteroid use (32.5% versus 94.2%) compared with step-up treatment but presented a higher rate of TNFI discontinuation. The 2 strategies both exhibited high adherence (mean proportion of days covered: 83.7%-95.4%). CONCLUSIONS: Early TNFI initiation increased over time for children and young adults with IBD and was related to lower rates of corticosteroid use compared with the conventional approach. However, the higher rate of TNFI discontinuation under the top-down approach requires further examination.

Lennard, L. (1992). "The Clinical-Pharmacology of 6-Mercaptopurine." European Journal of Clinical Pharmacology **43**(4): 329-339.

Lennard, L., et al. (2015). "Thiopurine dose intensity and treatment outcome in childhood lymphoblastic leukaemia: the influence of thiopurine methyltransferase pharmacogenetics." Br J Haematol **169**(2): 228-240.

The impact of thiopurine methyltransferase (TPMT) genotype on thiopurine dose intensity, myelosuppression and treatment outcome was investigated in the United Kingdom childhood acute lymphoblastic leukaemia (ALL) trial ALL97. TPMT heterozygotes had significantly more frequent cytopenias and therefore required dose adjustments below target levels significantly more often than TPMT wild-type patients although the average dose range was similar for both genotypes. Event-free survival (EFS) for patients heterozygous for the more common TPMT*1/*3A variant

allele (n = 99, 5-year EFS 88%) was better than for both wild-type TPMT*1/*1 (n = 1206, EFS 80%, P = 0.05) and TPMT*1/*3C patients (n = 17, EFS 53%, P = 0.002); outcomes supported by a multivariate Cox regression analysis. Poor compliance without subsequent clinician intervention was associated with a worse EFS (P = 0.02) and such non-compliance may have contributed to the poorer outcome for TPMT*1/*3C patients. Patients prescribed escalated doses had a worse EFS (P = 0.04), but there was no difference in EFS by dose intensity or duration of cytopenias. In contrast to reports from some USA and Nordic trials, TPMT heterozygosity was not associated with a higher rate of second cancers. In conclusion, TPMT*1/*3A heterozygotes had a better EFS than TPMT wild-type patients. Thiopurine induced cytopenias were not detrimental to treatment outcome.

Leong, A. S. and J. Wright (1987). "The contribution of immunohistochemical staining in tumour diagnosis." Histopathology **11**(12): 1295-1305.

Leschziner, G., et al. (2006). "Clinical factors and ABCB1 polymorphisms in prediction of antiepileptic drug response: a prospective cohort study." Lancet Neurol **5**(8): 668-676.

Leslie, E. M., et al. (2005). "Multidrug resistance proteins: role of P-glycoprotein, MRP1, MRP2, and BCRP (ABCG2) in tissue defense." Toxicol Appl Pharmacol **204**(3): 216-237.

In tumor cell lines, multidrug resistance is often associated with an ATP-dependent decrease in cellular drug accumulation which is attributed to the overexpression of certain ATP-binding cassette (ABC) transporter proteins. ABC proteins that confer drug resistance include (but are not limited to) P-glycoprotein (gene symbol ABCB1), the multidrug resistance protein 1 (MRP1, gene symbol ABCC1), MRP2 (gene symbol ABCC2), and the breast cancer resistance protein (BCRP, gene symbol ABCG2). In addition to their role in drug resistance, there is substantial evidence that these efflux pumps have overlapping functions in tissue defense. Collectively, these proteins are capable of transporting a vast and chemically diverse array of toxicants including bulky lipophilic cationic, anionic, and neutrally charged drugs and toxins as well as conjugated organic anions that encompass dietary and environmental carcinogens, pesticides, metals, metalloids, and lipid peroxidation products. P-glycoprotein, MRP1, MRP2, and BCRP/ABCG2 are expressed in tissues important for absorption (e.g., lung and gut) and metabolism and elimination (liver and kidney). In addition, these transporters have an important role in maintaining the barrier function of sanctuary site tissues (e.g., blood-brain barrier, blood-cerebral spinal fluid barrier, blood-testis barrier and the maternal-fetal barrier or placenta). Thus, these ABC transporters are increasingly recognized for their ability to modulate the absorption, distribution, metabolism, excretion, and toxicity of xenobiotics. In this review, the role of these four ABC transporter proteins in protecting tissues from a variety of toxicants is discussed. Species variations in substrate specificity and tissue distribution of these transporters are also addressed since these properties have implications for in vivo models of toxicity used for drug discovery and development.

Leung, G. and A. M. Muise (2018). "Monogenic Intestinal Epithelium Defects and the Development of Inflammatory Bowel Disease." Physiology (Bethesda) **33**(5): 360-369.

Levine, A., et al. (2011). "Pediatric modification of the Montreal classification for inflammatory bowel disease: the Paris classification." Inflamm Bowel Dis **17**(6): 1314-1321.

BACKGROUND: Crohn's disease and ulcerative colitis are complex disorders with some shared and many unique predisposing genes. Accurate phenotype classification is essential in determining the utility of genotype-phenotype correlation. The Montreal Classification of IBD has several weaknesses with respect to classification of children. The dynamic features of pediatric disease phenotype (change in disease location and behavior over time, growth failure) are not sufficiently captured by the current Montreal Classification. METHODS: Focusing on facilitating research in pediatric inflammatory bowel disease (IBD), and creating uniform standards for defining IBD phenotypes, an international group of pediatric IBD experts met in Paris, France to develop evidence-based consensus recommendations for a pediatric modification of the Montreal criteria. RESULTS: Important modifications developed include classifying age at diagnosis as A1a (0 to <10 years), A1b (10 to <17 years), A2 (17 to 40 years), and A3 (>40 years), distinguishing disease above the distal ileum as L4a (proximal to ligament of Treitz) and L4b (ligament of Treitz to above distal ileum), allowing both stenosing and penetrating disease to be classified in the same patient (B2B3), denoting the presence of growth failure in the patient at any time as

G(1) versus G(0) (never growth failure), adding E4 to denote extent of ulcerative colitis that is proximal to the hepatic flexure, and denoting ever severe ulcerative colitis during disease course by S1. CONCLUSIONS: These modifications are termed the Paris Classification. By adhering to the Montreal framework, we have not jeopardized or altered the ability to use this classification for adult onset disease or by adult gastroenterologists.

Ley, R. E., et al. (2006). "Ecological and evolutionary forces shaping microbial diversity in the human intestine." Cell **124**(4): 837-848.

The human gut is populated with as many as 100 trillion cells, whose collective genome, the microbiome, is a reflection of evolutionary selection pressures acting at the level of the host and at the level of the microbial cell. The ecological rules that govern the shape of microbial diversity in the gut apply to mutualists and pathogens alike.

Li, Z. G., et al. (2014). "ABCB1 Gene Polymorphisms and Glucocorticoid-Induced Avascular Necrosis of the Femoral Head Susceptibility: A Meta-Analysis." Medical Science Monitor **20**: 2811-2816.

Background: The results of studies on association between ABCB1 gene polymorphisms and glucocorticoid-induced avascular necrosis of the femoral head

(GANFH) are controversial. This study aimed to assess the association of ABCB1 gene polymorphisms with the risk of GANFH by conducting a meta-analysis.

Material/Methods: The PubMed, Cochrane Library, and Embase databases were searched for papers that describe the association between ABCB1 polymorphisms and GANFH risk. Summary odds ratios and 95% confidence intervals (CI) were estimated based on a fixed-effects model or random-effects model, depending on the absence or presence of significant heterogeneity.

Results: A total of 5 studies and 833 patients were included in the final analysis. Significant differences were found for rs1045642 polymorphism in the comparisons of CC vs. CT+TT (OR, 1.462; 95% CI, 1.066-2.007; P=0.019), and rs2032582 polymorphism in the comparisons of GG vs. G(TA)+(TA)(TA) (OR, 1.548; 95% CI, 1.063-2.255; P=0.023).

Conclusions: The study demonstrated that the ABCB1 polymorphisms (rs1045642 and rs2032582) significantly reduced the risk of GANFH.

Liang, J. J., et al. (2013). "TPMT genetic variants are associated with increased rejection with azathioprine use in heart transplantation." Pharmacogenet Genomics **23**(12): 658-665.

OBJECTIVES: Azathioprine (AZA) is an important immunosuppressant drug used in heart transplantation (HTX). Consensus guidelines recommend that patients with thiopurine S-methyltransferase (TPMT) genetic variants be started on lower AZA dose because of higher active metabolite levels and risk of adverse events. However,

in-vitro lymphocyte proliferation assays performed in participants with inactive TPMT alleles have suggested that AZA use may result in decreased immunosuppressant efficacy as compared with wild-type (WT) individuals. The objective of this study was therefore to determine the effect of TPMT genetic variation on AZA efficacy or prevention of rejection in HTX recipients treated with AZA. PARTICIPANTS AND METHODS: We genotyped 93 HTX recipients treated with AZA and measured erythrocyte TPMT enzyme activity. Acute rejection was monitored by routine endomyocardial biopsies. RESULTS: There were 83 WT and 10 heterozygote (HZ) HTX recipients. TPMT activity level was lower in HZ compared with WT (13.1+/-2.8 vs. 21+/-4.5 U/ml red blood cell, P<0.001). Despite similar AZA dose, HZ developed severe rejection earlier (P<0.001), and the total rejection score was higher (P=0.02) than WT. AZA was discontinued more frequently in HZ (P=0.01) because of rejection. The incidence of leukopenia was similar between the groups (40 vs. 43%, P=1.0). CONCLUSION: HTX recipients with TPMT genetic variant alleles who are treated with AZA develop acute rejection earlier, more frequently, and of greater severity. These patients, despite having lower TPMT enzymatic activity, should be monitored carefully for possible increased risk of acute rejection.

Lieberman, J. (2018). "Tapping the RNA world for therapeutics." Nat Struct Mol Biol **25**(5): 357-364.

Liefferinckx, C. and D. Franchimont (2018). "Viewpoint: Toward the Genetic Architecture of Disease Severity in Inflammatory Bowel Diseases." Inflamm Bowel Dis **24**(7): 1428-1439.

Lin, J., et al. (2017). "[Progress in digital PCR technology and application]." Sheng Wu Gong Cheng Xue Bao **33**(2): 170-177.

Lin, J. Y., et al. (2013). "Genetically determined ABCB5 functionality correlates with pigmentation phenotype and melanoma risk." Biochem Biophys Res Commun **436**(3): 536-542.

Liu, J., et al. (2015). "Chronic inflammation up-regulates P-gp in peripheral mononuclear blood cells via the STAT3/Nf-kappab pathway in 2,4,6-trinitrobenzene sulfonic acid-induced colitis mice." Sci Rep **5**: 13558.

Patients with inflammatory bowel diseases, including Crohn's disease and ulcerative colitis, often suffer drug intolerance. This resistance can be divided into intrinsic resistance and acquired resistance. Although there is agreement on acquired resistance, studies regarding intrinsic resistance have demonstrated inconsistencies, especially for Crohn's disease. For this reason, an animal model of Crohn's disease was induced with 2,4,6-trinitrobenzene sulfonic acid solution (TNBS), and intrinsic resistance was analyzed by measuring the function and expression of P-glycoprotein (P-gp) in peripheral mononuclear blood cells (PMBC), followed by mechanistic

studies. The results revealed reduced retention of cyclosporine A in PMBC over-expressing P-gp in a TNBS-treated group and enhanced secretion of the cytokines IL-1beta, IL-6, IL-17, and TNF-alpha as well as LPS in plasma. These cytokines and LPS can induce P-gp expression through the STAT3/Nf-kappab pathway, contributing to a decrease of cyclosporine A retention, which can be reversed by the application of a P-gp inhibitor. Our results demonstrated that the sustained chronic inflammation could induce the intrinsic resistance presented as P-gp over-expression in PBMC in Crohn's disease through STAT3/Nf-kappab pathway and this resistance might be reversed by combinational usage of P-gp inhibitors.

Liu, J. Z., et al. (2015). "Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations." Nature Genetics **47**(9): 979-986.

Ulcerative colitis and Crohn's disease are the two main forms of inflammatory bowel disease (IBD). Here we report the first trans-ancestry association study of IBD, with genome-wide or Immunochip genotype data from an extended cohort of 86,640 European individuals and Immunochip data from 9,846 individuals of East Asian, Indian or Iranian descent. We implicate 38 loci in IBD risk for the first time. For the majority of the IBD risk loci, the direction and magnitude of effect are consistent in European and non-European cohorts. Nevertheless, we observe genetic heterogeneity between divergent populations at several established risk loci driven by differences in allele frequency (NOD2) or effect size (TNFSF15 and ATG16L1) or a combination of

these factors (IL23R and IRGM). Our results provide biological insights into the pathogenesis of IBD and demonstrate the usefulness of trans-ancestry association studies for mapping loci associated with complex diseases and understanding genetic architecture across diverse populations.

Liu, Y. P., et al. (2015). "Association between thiopurine S-methyltransferase polymorphisms and thiopurine-induced adverse drug reactions in patients with inflammatory bowel disease: a meta-analysis." PLoS One **10**(3): e0121745.

PURPOSE: Thiopurine drugs are well established treatments in the management of inflammatory bowel disease (IBD), but their use is limited by significant adverse drug reactions (ADRs). Thiopurine S-methyltransferase (TPMT) is an important enzyme involved in thiopurine metabolism. Several clinical guidelines recommend determining TPMT genotype or phenotype before initiating thiopurine therapy. Although several studies have investigated the association between TPMT polymorphisms and thiopurine-induced ADRs, the results are inconsistent. The purpose of this study is to evaluate whether there is an association between TPMT polymorphisms and thiopurine-induced ADRs using meta-analysis. METHODS: We explored PubMed, Web of Science and Embase for articles on TPMT polymorphisms and thiopurine-induced ADRs. Studies that compared TPMT polymorphisms with-ADRs and without-ADRs in IBD patients were included. Relevant outcome data from all the included articles were extracted and the pooled odds ratio (OR) with corresponding 95% confidence intervals were calculated using Revman 5.3 software.

RESULTS: Fourteen published studies, with a total of 2,206 IBD patients, which investigated associations between TPMT polymorphisms and thiopurine-induced ADRs were included this meta-analysis. Our meta-analysis demonstrated that TPMT polymorphisms were significantly associated with thiopurine-induced overall ADRs and bone marrow toxicity; pooled ORs were 3.36 (95%CI: 1.82-6.19) and 6.67 (95%CI: 3.88-11.47), respectively. TPMT polymorphisms were not associated with the development of other ADRs including hepatotoxicity, pancreatitis, gastric intolerance, flu-like symptoms and skin reactions; the corresponding pooled ORs were 1.27 (95%CI: 0.60-2.71), 0.97 (95%CI: 0.38-2.48), 1.82 (95%CI: 0.93-3.53), 1.28 (95%CI: 0.47-3.46) and 2.32 (95%CI: 0.86-6.25), respectively. CONCLUSIONS: Our meta-analysis demonstrated an association of TPMT polymorphisms with overall thiopurine-induced ADRs and bone marrow toxicity, but not with hepatotoxicity, pancreatitis, flu-like symptoms, gastric intolerance and skin reactions. These findings suggest that pretesting the TPMT genotype could be helpful in clinical practice before initiating thiopurine therapy. However, white blood cell count analysis should be the mainstay for follow-up.

Liu, Y. P., et al. (2015). "Association between Thiopurine S-Methyltransferase Polymorphisms and Azathioprine-Induced Adverse Drug Reactions in Patients with Autoimmune Diseases: A Meta-Analysis." PLoS One **10**(12): e0144234.

PURPOSE: Azathioprine (AZA) is widely used as an immunosuppressive drug in autoimmune diseases, but its use is limited by significant adverse drug reactions

(ADRs). Thiopurine S-methyltransferase (TPMT) is an important enzyme involved in AZA metabolism. Several clinical guidelines recommend determining TPMT genotype or phenotype before initiating AZA therapy. Although several studies have investigated the association between TPMT polymorphisms and AZA-induced ADRs, the results are inconsistent. The purpose of this study is to evaluate whether there is an association between TPMT polymorphisms and AZA-induced ADRs using meta-analysis. METHODS: We explored PubMed, Web of Science and Embase for articles on TPMT polymorphisms and AZA-induced ADRs. Studies that compared TPMT polymorphisms with-ADRs and without-ADRs in patients with autoimmune diseases were included. Relevant outcome data from all the included articles were extracted and the pooled odds ratios (ORs) with corresponding 95% confidence intervals (CIs) were calculated using Revman 5.3 software. RESULTS: Eleven published studies, with a total of 651 patients with autoimmune diseases, investigated associations between TPMT polymorphisms and AZA-induced ADRs, were included in this meta-analysis. Our meta-analysis demonstrated that TPMT polymorphisms were significantly associated with AZA-induced overall ADRs, bone marrow toxicity and gastric intolerance; pooled ORs were 3.12 (1.48-6.56), 3.76 (1.97-7.17) and 6.43 (2.04-20.25), respectively. TPMT polymorphisms were not associated with the development of hepatotoxicity; the corresponding pooled OR was 2.86 (95%CI: 0.32-25.86). However, the association in GI subset could be driven by one single study. After this study was excluded, the OR was 2.11 (95%CI: 0.36-12.42); namely, the association became negative. CONCLUSIONS: Our meta-analysis demonstrated an association of TPMT polymorphisms with overall AZA-induced ADRs, bone marrow toxicity and gastric intolerance, but not with hepatotoxicity. The presence of

the normal TPMT genotypes cannot preclude the development of ADRs during AZA treatment, TPMT genotyping prior to commencing AZA therapy cannot replace, may augment, the current practice of regular monitoring of the white blood cell. Because of small sample sizes, large and extensive exploration was required to validate our findings.

Llopis, M., et al. (2009). "Lactobacillus casei downregulates commensals' inflammatory signals in Crohn's disease mucosa." Inflamm Bowel Dis **15**(2): 275-283.

BACKGROUND: The interaction of commensal bacteria with the intestinal immune system is an essential factor in the development of inflammatory bowel disease (IBD). The study of isolated commensal bacteria's effects on the mucosal immune response might be relevant for a better understanding of pathophysiological mechanisms in IBD. METHODS: We investigated the immune responses to signals from the commensal Escherichia coli ATCC 35345 and the probiotic Lactobacillus casei DN-114 001 in Crohn's disease (CD) mucosa. Ileal specimens were obtained during surgery from CD patients. Mucosal explants were incubated with L. casei or its genomic DNA; TNF-alpha, IFN-gamma, IL-2, IL-6, IL-8, and CXCL1 were measured in the supernatant. Second, tissue expression of key proinflammatory cytokines (IL-6, TGF-beta, IL-23p19, IL-12p35, IL-17F), and chemokines (IL-8, CXCL1, CXCL2) was evaluated after incubation with L. casei or E. coli. Finally, combination experiments were carried out by incubating both strains with mucosal explants at different timepoints. RESULTS: Live L. casei significantly decreased

secretion of TNF-alpha, IFN-gamma, IL-2, IL-6, IL-8, and CXCL1 by CD mucosa, but the effect was not reproduced by L. casei DNA. Second, live L. casei downregulated expression of IL-8, IL-6, and CXCL1 and did not modify expression of IL-23p19, IL-12p35, and IL-17F. In contrast, E. coli significantly upregulated expression of all these cytokines. Interestingly, combination experiments revealed the ability of L. casei to prevent and counteract the proinflammatory effects of E. coli. CONCLUSIONS: Live L. casei can counteract the proinflammatory effects of E. coli on CD inflamed mucosa by specific downregulation of key proinflammatory mediators.

Loftus, E. V., Jr. (2004). "Clinical epidemiology of inflammatory bowel disease: Incidence, prevalence, and environmental influences." Gastroenterology **126**(6): 1504-1517.

Although the incidence and prevalence of ulcerative colitis and Crohn's disease are beginning to stabilize in high-incidence areas such as northern Europe and North America, they continue to rise in low-incidence areas such as southern Europe, Asia, and much of the developing world. As many as 1.4 million persons in the United States and 2.2 million persons in Europe suffer from these diseases. Previously noted racial and ethnic differences seem to be narrowing. Differences in incidence across age, time, and geographic region suggest that environmental factors significantly modify the expression of Crohn's disease and ulcerative colitis. The strongest environmental factors identified are cigarette smoking and appendectomy. Whether other factors such as diet, oral contraceptives, perinatal/childhood infections, or

atypical mycobacterial infections play a role in expression of inflammatory bowel disease remains unclear. Additional epidemiologic studies to define better the burden of illness, explore the mechanism of association with environmental factors, and identify new risk factors are needed.

Lopes, C. V., et al. (2018). "Differential diagnosis of mesenchymal neoplasms of the digestive tract by cell block and immunohistochemistry." Cytopathology.

Lutgendorff, F., et al. (2008). "The role of microbiota and probiotics in stress-induced gastro-intestinal damage." Curr Mol Med **8**(4): 282-298.

Lutz, N. W., et al. (2016). "Expression of Cell-Surface Marker ABCB5 Causes Characteristic Modifications of Glucose, Amino Acid and Phospholipid Metabolism in the G3361 Melanoma-Initiating Cell Line." PLoS One **11**(8): e0161803.

We present a pilot study aimed at determining the effects of expression of ATP-binding cassette member B5 (ABCB5), a previously described marker for melanoma-initiating cells, on cellular metabolism. Metabolic profiles for two groups of human G3361 melanoma cells were compared, i.e. wildtype melanoma cells with intact ABCB5 expression (ABCB5-WT) and corresponding melanoma cell variants with inhibited ABCB5 expression, through shRNA-mediated gene knockdown (ABCB5-KD). A comprehensive metabolomic analysis was performed by using proton and

phosphorus NMR spectroscopy of cell extracts to examine water-soluble metabolites and lipids. Parametric and non-parametric statistical analysis of absolute and relative metabolite levels yielded significant differences for compounds involved in glucose, amino acid and phospholipid (PL) metabolism. By contrast, energy metabolism was virtually unaffected by ABCB5 expression. The sum of water-soluble metabolites per total protein was 17% higher in ABCB5-WT vs. ABCB5-KD G3361 variants, but no difference was found for the sum of PLs. Enhanced abundance was particularly pronounced for lactate (+ 23%) and alanine (+ 26%), suggesting an increase in glycolysis and potentially glutaminolysis. Increases in PL degradation products, glycerophosphocholine and glycerophosphoethanolamine (+ 85 and 123%, respectively), and redistributions within the PL pool suggested enhanced membrane PL turnover as a consequence of ABCB5 expression. The possibility of glycolysis modulation by an ABCB5-dependent IL1beta-mediated mechanism was supported by functional studies employing monoclonal antibody (mAb)-dependent ABCB5 protein inhibition in wildtype G3361 melanoma cells. Our metabolomic results suggest that the underlying biochemical pathways may offer targets for melanoma therapy, potentially in combination with other treatment forms.

Mahfouz, M., et al. (2019). "Hepatic Complications of Inflammatory Bowel Disease." Clin Liver Dis **23**(2): 191-208.

Hepatobiliary disorders are commonly encountered in patients with inflammatory bowel disease (IBD). Although primary sclerosing cholangitis is the stereotypical

hepatobiliary disorder associated with IBD, other diseases, including autoimmune hepatitis and nonalcoholic fatty liver disease, also are encountered in this population. Several agents used for treatment of IBD may cause drug-induced liver injury, although severe hepatotoxicity occurs infrequently. Furthermore, reactivation of hepatitis B virus infection may occur in patients with IBD treated with systemic corticosteroids and biologic agents.

Mahid, S. S., et al. (2006). "Smoking and inflammatory bowel disease: a meta-analysis." Mayo Clin Proc **81**(11): 1462-1471.

Mahid, S. S., et al. (2007). "The role of smoking in Crohn's disease as defined by clinical variables." Dig Dis Sci **52**(11): 2897-2903.

Malik, N. J. and M. E. Daymon (1982). "Improved double immunoenzyme labeling using alkaline phosphatase and horseradish peroxidase." J Clin Pathol **35**(10): 1092-1094.

Manceau, S., et al. (2012). "Expression and induction by dexamethasone of ABC transporters and nuclear receptors in a human T-lymphocyte cell line." J Chemother **24**(1): 48-55.

Mansoori, M., et al. (2015). "Genetic Variation in the ABCB1 Gene May Lead to mRNA Level Chabge: Application to Gastric Cancer Cases." Asian Pac J Cancer Prev **16**(18): 8467-8471.

BACKGROUND: One of the major mechanisms for drug resistance is associated with altered anticancer drug transport, mediated by the human-adenosine triphosphate binding cassette (ABC) transporter superfamily proteins. The overexpression of adenosine triphosphate binding cassette, sub-family B, member 1 (ABCB1) by multidrug-resistant cancer cells is a serious impediment to chemotherapy. In our study we have studied the possibility that structural single-nucleotide polymorphisms (SNP) are the mechanism of ABCB1 overexpression. MATERIALS AND METHODS: A total of 101 gastric cancer multidrug resistant cases and 100 controls were genotyped with sequence-specific primed PCR (SSP-PCR). Gene expression was evaluated for 70 multidrug resistant cases and 54 controls by real time PCR. The correlation between the two groups was based on secondary structures of RNA predicted by bioinformatics tool. RESULTS: The results of genotyping showed that among 3 studied SNPs, rs28381943 and rs2032586 had significant differences between patient and control groups but there were no differences in the two groups for C3435T. The results of real time PCR showed over-expression of ABCB1 when we compared our data with each of the genotypes in average mode. Prediction of secondary structures in the existence of 2 related SNPs (rs28381943 and rs2032586) showed that the amount of DeltaG for original mRNA is higher than the amount of DeltaG for the two mentioned SNPs. CONCLUSIONS: We have observed that 2 of our studied SNPs

(rs283821943 and rs2032586) may elevate the expression of ABCB1 gene, through increase in mRNA stability, while this was not the case for C3435T.

Martini, E., et al. (2017). "Mend Your Fences: The Epithelial Barrier and its Relationship With Mucosal Immunity in Inflammatory Bowel Disease." Cell Mol Gastroenterol Hepatol **4**(1): 33-46.

The intestinal epithelium can be easily disrupted during gut inflammation as seen in inflammatory bowel disease (IBD), such as ulcerative colitis or Crohn's disease. For a long time, research into the pathophysiology of IBD has been focused on immune cell-mediated mechanisms. Recent evidence, however, suggests that the intestinal epithelium might play a major role in the development and perpetuation of IBD. It is now clear that IBD can be triggered by disturbances in epithelial barrier integrity via dysfunctions in intestinal epithelial cell-intrinsic molecular circuits that control the homeostasis, renewal, and repair of intestinal epithelial cells. The intestinal epithelium in the healthy individual represents a semi-permeable physical barrier shielding the interior of the body from invasions of pathogens on the one hand and allowing selective passage of nutrients on the other hand. However, the intestinal epithelium must be considered much more than a simple physical barrier. Instead, the epithelium is a highly dynamic tissue that responds to a plenitude of signals including the intestinal microbiota and signals from the immune system. This epithelial response to these signals regulates barrier function, the composition of the microbiota, and mucosal immune homeostasis within the lamina propria. The epithelium can thus

be regarded as a translator between the microbiota and the immune system and aberrant signal transduction between the epithelium and adjacent immune cells might promote immune dysregulation in IBD. This review summarizes the important cellular and molecular barrier components of the intestinal epithelium and emphasizes the mechanisms leading to barrier dysfunction during intestinal inflammation.

Martino, J. V., et al. (2017). "The Role of Carrageenan and Carboxymethylcellulose in the Development of Intestinal Inflammation." Front Pediatr **5**: 96.

Although the exact pathophysiology remains unknown, the development of inflammatory bowel disease (IBD) is influenced by the interplay between genetics, the immune system, and environmental factors such as diet. The commonly used food additives, carrageenan and carboxymethylcellulose (CMC), are used to develop intestinal inflammation in animal models. These food additives are excluded from current dietary approaches to induce disease remission in Crohn's disease such as exclusive enteral nutrition (EEN) using a polymeric formula. By reviewing the existing scientific literature, this review aims to discuss the role that carrageenan and CMC may play in the development of IBD. Animal studies consistently report that carrageenan and CMC induce histopathological features that are typical of IBD while altering the microbiome, disrupting the intestinal epithelial barrier, inhibiting proteins that provide protection against microorganisms, and stimulating the elaboration of pro-inflammatory cytokines. Similar trials directly assessing the influence of carrageenan and CMC in humans are of course unethical to conduct, but recent

studies of human epithelial cells and the human microbiome support the findings from animal studies. Carrageenan and CMC may trigger or magnify an inflammatory response in the human intestine but are unlikely to be identified as the sole environmental factor involved in the development of IBD or in disease recurrence after treatment. However, the widespread use of carrageenan and CMC in foods consumed by the pediatric population in a "Western" diet is on the rise alongside a corresponding increase in IBD incidence, and questions are being raised about the safety of frequent usage of these food additives. Therefore, further research is warranted to elucidate the role of carrageenan and CMC in intestinal inflammation, which may help identify novel nutritional strategies that hinder the development of the disease or prevent disease relapse post-EEN treatment.

McGilligan, V. E., et al. (2007). "The effect of nicotine in vitro on the integrity of tight junctions in Caco-2 cell monolayers." Food and Chemical Toxicology **45**(9): 1593-1598.

Mijac, D., et al. (2018). "MDR1 gene polymorphisms are associated with ulcerative colitis in a cohort of Serbian patients with inflammatory bowel disease." PLoS One **13**(3): e0194536.

Misaka, S., et al. (2013). "Clinical relevance of drug efflux pumps in the gut." Curr Opin Pharmacol **13**(6): 847-852.

Important export pumps expressed in the apical membrane of enterocytes are P-glycoprotein (P-gp), breast cancer resistance protein (BCRP) and multidrug resistance protein 2 (MRP2). They are believed to be a crucial part of the bodies' defense mechanisms against potentially toxic, orally administered xenobiotics. In particular P-gp and BCRP also limit the bioavailability of drugs. Inhibition of these intestinal export pumps by concomitantly administered drugs leads to increased plasma concentrations, whereas induction can reduce absorption of the substrate drugs and decrease plasma concentrations. The role of polymorphisms in genes encoding for these transporters will also be discussed. Taken together this review will focus on the role of intestinal export pumps using selected examples from clinical studies in humans.

Moitra, K. (2015). "Overcoming Multidrug Resistance in Cancer Stem Cells." Biomed Res Int **2015**: 635745.

The principle mechanism of protection of stem cells is through the expression of ATP-binding cassette (ABC) transporters. These transporters serve as the guardians of the stem cell population in the body. Unfortunately these very same ABC efflux pumps afford protection to cancer stem cells in tumors, shielding them from the adverse effects of chemotherapy. A number of strategies to circumvent the function of these transporters in cancer stem cells are currently under investigation. These strategies include the development of competitive and allosteric modulators, nanoparticle mediated delivery of inhibitors, targeted transcriptional regulation of ABC

transporters, miRNA mediated inhibition, and targeting of signaling pathways that modulate ABC transporters. The role of ABC transporters in cancer stem cells will be explored in this paper and strategies aimed at overcoming drug resistance caused by these particular transporters will also be discussed.

Moitra, K. and M. Dean (2011). "Evolution of ABC transporters by gene duplication and their role in human disease." Biol Chem **392**(1-2): 29-37.

The ATP-binding cassette (ABC) transporter genes represent the largest family of transporters and these genes are abundant in the genome of all vertebrates. Through analysis of the genome sequence databases we have characterized the full complement of ABC genes from several mammals and other vertebrates. Multiple gene duplication and deletion events were identified in ABC genes in different lineages indicating that the process of gene evolution is still ongoing. Gene duplication resulting in either gene birth or gene death plays a major role in the evolution of the vertebrate ABC genes. The understanding of this mechanism is important in the context of human health because these ABC genes are associated with human disease, involving nearly all organ systems of the body. In addition, ABC genes play an important role in the development of drug resistance in cancer cells. Future genetic, functional, and evolutionary studies of ABC transporters will provide important insight into human and animal biology.

Moitra, K., et al. (2011). "Molecular evolutionary analysis of ABCB5: the ancestral gene is a full transporter with potentially deleterious single nucleotide polymorphisms." PLoS One **6**(1): e16318.

BACKGROUND: ABCB5 is a member of the ABC protein superfamily, which includes the transporters ABCB1, ABCC1 and ABCG2 responsible for causing drug resistance in cancer patients and also several other transporters that have been linked to human disease. The ABCB5 full transporter (ABCB5.ts) is expressed in human testis and its functional significance is presently unknown. Another variant of this transporter, ABCB5 beta possess a "half-transporter-like" structure and is expressed in melanoma stem cells, normal melanocytes, and other types of pigment cells. ABCB5 beta has important clinical implications, as it may be involved with multidrug resistance in melanoma stem cells, allowing these stem cells to survive chemotherapeutic regimes. METHODOLOGY/PRINCIPAL FINDINGS: We constructed and examined in detail topological structures of the human ABCB5 protein and determined in-silico the cSNPs (coding single nucleotide polymorphisms) that may affect its function. Evolutionary analysis of ABCB5 indicated that ABCB5, ABCB1, ABCB4, and ABCB11 share a common ancestor, which began duplicating early in the evolutionary history of chordates. This suggests that ABCB5 has evolved as a full transporter throughout its evolutionary history. CONCLUSIONS/ SIGNIFICANCE: From our in-silco analysis of cSNPs we found that a large number of non-synonymous cSNPs map to important functional regions of the protein suggesting that these SNPs if present in human populations may play a role in diseases associated with ABCB5. From phylogenetic analyses, we have shown that

ABCB5 evolved as a full transporter throughout its evolutionary history with an absence of any major shifts in selection between the various lineages suggesting that the function of ABCB5 has been maintained during mammalian evolution. This finding would suggest that ABCB5 beta may have evolved to play a specific role in human pigment cells and/or melanoma cells where it is predominantly expressed.

Molodecky, N. A., et al. (2012). "Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review." Gastroenterology **142**(1): 46-54 e42; quiz e30.

BACKGROUND & AIMS: We conducted a systematic review to determine changes in the worldwide incidence and prevalence of ulcerative colitis (UC) and Crohn's disease (CD) in different regions and with time. METHODS: We performed a systematic literature search of MEDLINE (1950-2010; 8103 citations) and EMBASE (1980-2010; 4975 citations) to identify studies that were population based, included data that could be used to calculate incidence and prevalence, and reported separate data on UC and/or CD in full manuscripts (n = 260). We evaluated data from 167 studies from Europe (1930-2008), 52 studies from Asia and the Middle East (1950-2008), and 27 studies from North America (1920-2004). Maps were used to present worldwide differences in the incidence and prevalence of inflammatory bowel diseases (IBDs); time trends were determined using joinpoint regression. RESULTS: The highest annual incidence of UC was 24.3 per 100,000 person-years in Europe, 6.3 per 100,000 person-years in Asia and the Middle East, and 19.2 per 100,000 person-

years in North America. The highest annual incidence of CD was 12.7 per 100,000 person-years in Europe, 5.0 person-years in Asia and the Middle East, and 20.2 per 100,000 person-years in North America. The highest reported prevalence values for IBD were in Europe (UC, 505 per 100,000 persons; CD, 322 per 100,000 persons) and North America (UC, 249 per 100,000 persons; CD, 319 per 100,000 persons). In time-trend analyses, 75% of CD studies and 60% of UC studies had an increasing incidence of statistical significance ($P < .05$). CONCLUSIONS: Although there are few epidemiologic data from developing countries, the incidence and prevalence of IBD are increasing with time and in different regions around the world, indicating its emergence as a global disease.

Moran, C. J., et al. (2013). "IL-10R Polymorphisms Are Associated with Very-early-onset Ulcerative Colitis." Inflammatory Bowel Diseases **19**(1): 115-123.

Background: Interleukin-10 (IL-10) signaling genes are attractive inflammatory bowel disease (IBD) candidate genes as IL-10 restricts intestinal inflammation, IL-10 polymorphisms have been associated with IBD in genome-wide association studies, and mutations in IL-10 and IL-10 receptor (IL-10R) genes have been reported in immunodeficient children with severe infantile-onset IBD. Our objective was to determine if IL-10R polymorphisms were associated with early-onset IBD (EO-IBD) and very-early-onset IBD (VEO-IBD).

Methods: Candidate-gene analysis of IL10RA and IL10RB was performed after initial sequencing of an infantile onset-IBD patient identified a novel homozygous mutation.

The discovery cohort included 188 EO-IBD subjects and 188 healthy subjects. Polymorphisms associated with IBD in the discovery cohort were genotyped in an independent validation cohort of 422 EO-IBD subjects and 480 healthy subjects.

Results: We identified a homozygous, splice-site point mutation in IL10RA in an infantile-onset IBD patient causing a premature stop codon (P206X) and IL-10 insensitivity. IL10RA and IL10RB sequencing in the discovery cohort identified five IL10RA polymorphisms associated with ulcerative colitis (UC) and two IL10RB polymorphisms associated with Crohn's disease (CD). Of these polymorphisms, two IL10RA single nucleotide polymorphisms, rs2228054 and rs2228055, were associated with VEO-UC in the discovery cohort and replicated in an independent validation cohort (odds ratio [OR] 3.08, combined P = 2 x 10(-4); and OR = 2.93, P = 6 x 10(-4), respectively).

Conclusions: We identified IL10RA polymorphisms that confer risk for developing VEO-UC. Additionally, we identified the first splice site mutation in IL10RA resulting in infantile-onset IBD. This study expands the phenotype of IL10RA polymorphisms to include both severe arthritis and VEO-UC. (Inflamm Bowel Dis 2013;19:115-123)

Morgan, X. C., et al. (2012). "Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment." Genome Biology **13**(9).

Background: The inflammatory bowel diseases (IBD) Crohn's disease and ulcerative colitis result from alterations in intestinal microbes and the immune system. However, the precise dysfunctions of microbial metabolism in the gastrointestinal microbiome

during IBD remain unclear. We analyzed the microbiota of intestinal biopsies and stool samples from 231 IBD and healthy subjects by 16S gene pyrosequencing and followed up a subset using shotgun metagenomics. Gene and pathway composition were assessed, based on 16S data from phylogenetically-related reference genomes, and associated using sparse multivariate linear modeling with medications, environmental factors, and IBD status.

Results: Firmicutes and Enterobacteriaceae abundances were associated with disease status as expected, but also with treatment and subject characteristics. Microbial function, though, was more consistently perturbed than composition, with 12% of analyzed pathways changed compared with 2% of genera. We identified major shifts in oxidative stress pathways, as well as decreased carbohydrate metabolism and amino acid biosynthesis in favor of nutrient transport and uptake. The microbiome of ileal Crohn's disease was notable for increases in virulence and secretion pathways.

Conclusions: This inferred functional metagenomic information provides the first insights into community-wide microbial processes and pathways that underpin IBD pathogenesis.

Mrowicka, M., et al. (2017). "Association between SOD1, CAT, GSHPX1 polymorphisms and the risk of inflammatory bowel disease in the Polish population." Oncotarget **8**(65): 109332-109339.

Mukkavilli, R., et al. (2017). "Evaluation of Drug Transport in MDCKII-Wild Type, MDCKII-MDR1, MDCKII-BCRP and Caco-2 Cell Lines." Curr Pharm Biotechnol **18**(14): 1151-1158.

BACKGROUND: Drug transporters function as gatekeepers and modulate drug access into body and various tissues. Thus, a thorough and precise understanding of transporter liability for compound uptake and efflux is critical during drug development. METHODS: In the present study, we assessed the apparent permeability (Papp) and compared efflux ratio of various compounds in stably transfected Madin-Darby Canine Kidney (MDCKII) cells overexpressing human P-gp (MDCKII-MDR1), human BCRP (MDCKII-BCRP), wild-type (MDCKII-WT), and Caco-2 cell monolayers. RESULTS: We observed that quinidine, a substrate for MDR1 transporter, showed efflux ratio (Papp B-A/ Papp A-B) of 838 in MDCKII-MDR1 cells which plummeted to 14 in presence of verapamil, a known inhibitor of MDR1. With MDCKII-WT cells, Papp of quinidine dropped from 2 to 1, in the presence of verapamil. Caco-2 cells showed a diminutive decrease in efflux ratio of quinidine from 2.5 to 1.6 by verapamil. Prazosin and dantrolene were evaluated in MDCKII-BCRP cells and were found to have 80-fold higher efflux ratio compared to MDCKII-WT cells. In Caco-2 cells, prazosin and dantrolene showed efflux ratio of 4 and 2, respectively. Rhodamine-123, a fluorogenic probe substrate of MDR1 showed an efflux ratio of 4 in Caco-2 cells and BCRP substrate estrone-3-sulphate showed an efflux ratio of 7. In presence of BCRP inhibitor fumitremorgin-c, the efflux ratio of estrone-3-sulfate dropped to 1 in Caco-2 cells. CONCLUSION: The very high efflux ratios of MDR1 and BCRP substrates in transfected MDCKII cells clearly

demonstrate the potential usefulness of these models to provide more definitive data to evaluate the transporter involvement compared to Caco-2 or MDCKII-WT cells.

Munkholm, P., et al. (1994). "Frequency of glucocorticoid resistance and dependency in Crohn's disease." Gut **35**(3): 360-362.

The outcome of the first steroid treatment course was prospectively studied in a regional cohort of 196 patients with Crohn's disease diagnosed 1979-1987. The immediate outcome after 30 days, and the prolonged outcome 30 days after treatment had stopped, are described. In all 109 patients treatment was analysed. Complete remission was obtained in 48%, partial remission in 32%, and no response in 20% within 30 days of treatment. Among primary responders (complete and partial remission), 55% remained in prolonged response after treatment had finished, while 45% relapsed or could not be withdrawn from treatment within one year. Localisation of disease, age, sex or clinical symptoms did not significantly correlate with outcome, which can be summarised as prolonged steroid response in 44%, steroid dependency in 36%, and steroid resistant in 20% of the patients.

Nami, Y., et al. (2014). "Assessment of probiotic potential and anticancer activity of newly isolated vaginal bacterium Lactobacillus plantarum 5BL." Microbiol Immunol **58**(9): 492-502.

Numerous bacteria in and on its external parts protect the human body from harmful threats. This study aimed to investigate the potential beneficial effects of the vaginal ecosystem microbiota. A type of bacteria was isolated from vaginal secretions of adolescent and young adult women, cultured on an appropriate specific culture medium, and then molecularly identified through 16S rDNA gene sequencing. Results of 16S rDNA sequencing revealed that the isolate belongs to the Lactobacillus plantarum species. The isolated strain exhibited probiotic properties such as low pH and high bile salt concentration tolerance, antibiotic susceptibility and antimicrobial activity against some pathogenic bacteria. The anticancer effects of the strain on human cancer cell lines (cervical, HeLa; gastric, AGS; colon, HT-29; breast, MCF-7) and on a human normal cell line (human umbilical vein endothelial cells [HUVEC]) were investigated. Toxic side effects were assessed by studying apoptosis in the treated cells. The strain exhibited desirable probiotic properties and remarkable anticancer activity against the tested human cancer cell lines (P </= 0.05) with no significant cytotoxic effects on HUVEC normal cells (P </= 0.05). Overall, the isolated strain showed favorable potential as a bioactive therapeutic agent. Therefore, this strain should be subjected to the other required tests to prove its suitability for clinical therapeutic application.

Narula, N., et al. (2017). "Systematic Review and Meta-analysis: Fecal Microbiota Transplantation for Treatment of Active Ulcerative Colitis." Inflamm Bowel Dis.

BACKGROUND: Changes in the colonic microbiota may play a role in the pathogenesis of ulcerative colitis (UC) and restoration of healthy gut microbiota may ameliorate disease. A systematic review and meta-analysis was conducted to assess fecal microbiota transplantation (FMT) as a treatment for active UC. METHODS: A literature search was conducted to identify high-quality studies of FMT as a treatment for patients with UC. The primary outcome was combined clinical remission and endoscopic remission or response. Secondary outcomes included clinical remission, endoscopic remission, and serious adverse events. Odds ratios with 95% confidence intervals (CIs) are reported. RESULTS: Overall, 4 studies with 277 participants were eligible for inclusion. Among 4 randomized controlled trials, FMT was associated with higher combined clinical and endoscopic remission compared with placebo (risk ratio UC not in remission was 0.80; 95% CI: 0.71-0.89) with a number needed to treat of 5 (95% CI: 4-10). There was no statistically significant increase in serious adverse events with FMT compared with controls (risk ratio adverse event was 1.4; 95% CI: 0.55-3.58). CONCLUSIONS: Among randomized controlled trials, short-term use of FMT shows promise as a treatment to induce remission in active UC based on the efficacy and safety observed. However, there remain many unanswered questions that require further research before FMT can be considered for use in clinical practice.

Nettleship, J. E., et al. (2015). "Transient expression in HEK 293 cells: an alternative to E. coli for the production of secreted and intracellular mammalian proteins." Methods Mol Biol **1258**: 209-222.

Newby, E. A., et al. (2008). "Natural history of paediatric inflammatory bowel diseases over a 5-year follow-up: a retrospective review of data from the register of paediatric inflammatory bowel diseases." J Pediatr Gastroenterol Nutr **46**(5): 539-545.

OBJECTIVES: The natural history of paediatric inflammatory bowel diseases (IBDs) is poorly understood. We aim to describe the disease course in this cohort and generate prognostic information for patients and clinicians. MATERIALS AND METHODS: Patient records from 6 tertiary paediatric gastroenterology centres were reviewed to generate data concerning original diagnosis, change in diagnosis, family history, surgical interventions, growth, and presence of extragastrointestinal manifestations. RESULTS: Data were collected on 116 children with Crohn disease (CD), 74 with ulcerative colitis (UC), and 20 with indeterminate colitis (IC), followed for a mean period of 3.42, 3.3, and 2.9 years from date of diagnosis, respectively. A male predominance is demonstrated in CD. Revision of diagnosis in patients with IC is mainly to UC, with most children receiving a definitive diagnosis within 2 years of initial presentation. Of the children with UC, 17.6% underwent 1 or more major operations with a median time to surgery of 1.92 years. Of children with CD, 11.6% underwent 1 or more major intraabdominal procedures with a median time to surgery of 1.83 years. We recorded a positive family history in 2.7%, 8.2%, and 10% of cases for CD, UC, and IC, respectively. For both boys and girls with CD, but only for boys with UC, height standard deviation score became more negative over time. CONCLUSIONS: This retrospective study quantifies certain distinctions between IBDs diagnosed in paediatric and adult populations. We document a trend toward

male predominance in children with CD. We also note impaired linear growth in children with CD, whereas it appears maintained in girls with UC. We also have recorded a low incidence of IBDs in the families of this cohort and suggest that environmental influences may be of greater importance. We document that major intraabdominal surgery may be required in about 15% of patients with either UC or CD within 2 years of diagnosis, and that the majority of those diagnosed initially with IC will be reclassified as either UC or CD within 2 years.

Nicol, M. R., et al. (2014). "Expression of six drug transporters in vaginal, cervical, and colorectal tissues: Implications for drug disposition in HIV prevention." J Clin Pharmacol **54**(5): 574-583.

Nishida, A., et al. (2018). "Gut microbiota in the pathogenesis of inflammatory bowel disease." Clin J Gastroenterol **11**(1): 1-10.

Inflammatory bowel disease (IBD), including ulcerative colitis and Crohn's disease, is a chronic and relapsing inflammatory disorder of the intestine. Although its incidence is increasing globally, the precise etiology remains unclear and a cure for IBD has yet to be discovered. The most accepted hypothesis of IBD pathogenesis is that complex interactions between genetics, environmental factors, and the host immune system lead to aberrant immune responses and chronic intestinal inflammation. The human gut harbors a complex and abundant aggregation of microbes, collectively referred to as the gut microbiota. The gut microbiota has physiological functions associated with

nutrition, the immune system, and defense of the host. Recent advances in next-generation sequencing technology have identified alteration of the composition and function of the gut microbiota, which is referred to as dysbiosis, in IBD. Clinical and experimental data suggest dysbiosis may play a pivotal role in the pathogenesis of IBD. This review is focused on the physiological function of the gut microbiota and the association between the gut microbiota and pathogenesis in IBD. In addition, we review the therapeutic options for manipulating the altered gut microbiota, such as probiotics and fecal microbiota transplantation.

Noble, C. L., et al. (2010). "Characterization of intestinal gene expression profiles in Crohn's disease by genome-wide microarray analysis." Inflamm Bowel Dis **16**(10): 1717-1728.

Okada, Y., et al. (2014). "Genetics of rheumatoid arthritis contributes to biology and drug discovery." Nature **506**(7488): 376-381.

A major challenge in human genetics is to devise a systematic strategy to integrate disease-associated variants with diverse genomic and biological data sets to provide insight into disease pathogenesis and guide drug discovery for complex traits such as rheumatoid arthritis (RA). Here we performed a genome-wide association study meta-analysis in a total of >100,000 subjects of European and Asian ancestries (29,880 RA cases and 73,758 controls), by evaluating approximately 10 million single-nucleotide polymorphisms. We discovered 42 novel RA risk loci at a genome-wide level of significance, bringing the total to 101 (refs 2 - 4). We devised an in silico pipeline

using established bioinformatics methods based on functional annotation, cis-acting expression quantitative trait loci and pathway analyses--as well as novel methods based on genetic overlap with human primary immunodeficiency, haematological cancer somatic mutations and knockout mouse phenotypes--to identify 98 biological candidate genes at these 101 risk loci. We demonstrate that these genes are the targets of approved therapies for RA, and further suggest that drugs approved for other indications may be repurposed for the treatment of RA. Together, this comprehensive genetic study sheds light on fundamental genes, pathways and cell types that contribute to RA pathogenesis, and provides empirical evidence that the genetics of RA can provide important information for drug discovery.

Olbjorn, C., et al. (2017). "Serological markers in diagnosis of pediatric inflammatory bowel disease and as predictors for early tumor necrosis factor blocker therapy." Scand J Gastroenterol **52**(4): 414-419.

OBJECTIVE: To describe the prevalence of serological markers in newly diagnosed treatment-naive pediatric inflammatory bowel disease (IBD), their utility in differentiating Crohn's disease (CD), ulcerative colitis (UC) and symptomatic non-IBD patients and whether serological markers are associated with early TNF blocker treatment. MATERIAL AND METHODS: Ninety-six children and adolescents <18 years, 58 with IBD and 38 symptomatic non-IBD controls were included. At diagnosis and after 1-2 years, serological antibodies (anti-Saccharomyces cerevisiae antibodies (ASCA), perinuclear anti-neutrophil cytoplasmic antibody (pANCA),

flagellin expressed by Clostridial phylum (anti-CBir1), outer membrane porin of Escherichia coli (anti-OmpC), Pseudomonas fluorescens-associated sequence (anti-I2), CRP, ESR and fecal calprotectin were analyzed. The choice of treatment was made at the discretion of the treating pediatrician. RESULTS: Of the IBD patients, 20 (36%) and 26 (47%) were positive for ASCA and pANCA compared to 3(8%), $p < .01$ and 10 (27%), $p = .04$ of the controls. Thirteen (72%) of UC patients were pANCA positive, versus 13 (35%) of CD patients ($p < .01$). None of the UC patients was ASCA positive versus 20 (54%) of CD patients ($p < .0001$). Compared to conventionally treated patients, the 18 (49%) TNF blocker treated CD patients had higher presence of ASCA ($p < .01$), lower presence of pANCA ($p = .02$) and higher levels of fecal calprotectin, CRP and ESR at diagnosis. In multivariate analyses ASCA and pANCA status, but not CRP, ESR or calprotectin, were independently associated with early TNF blocker treatment. CONCLUSIONS: ASCA and pANCA status were associated with having IBD and with early TNF blocker treatment in CD.

Olen, O., et al. (2017). "Childhood onset inflammatory bowel disease and risk of cancer: a Swedish nationwide cohort study 1964-2014." BMJ **358**: j3951.

Objective To assess risk of cancer in patients with childhood onset inflammatory bowel disease in childhood and adulthood.Design Cohort study with matched general population reference individuals using multivariable Cox regression to estimate hazard ratios.Setting Swedish national patient register (both inpatient and non-primary outpatient care) 1964-2014.Participants Incident cases of childhood onset

(<18 years) inflammatory bowel disease (n=9405: ulcerative colitis, n=4648; Crohn's disease, n=3768; unclassified, n=989) compared with 92 870 comparators from the general population matched for sex, age, birth year, and county.Main outcome measures Any cancer and cancer types according to the Swedish Cancer Register.Results During follow-up through adulthood (median age at end of follow-up 27 years), 497 (3.3 per 1000 person years) people with childhood onset inflammatory bowel disease had first cancers, compared with 2256 (1.5 per 1000 person years) in the general population comparators (hazard ratio 2.2, 95% confidence interval 2.0 to 2.5). Hazard ratios for any cancer were 2.6 in ulcerative colitis (2.3 to 3.0) and 1.7 in Crohn's disease (1.5 to 2.1). Patients also had an increased risk of cancer before their 18th birthday (2.7, 1.6 to 4.4; 20 cancers in 9405 patients, 0.6 per1000 person years). Gastrointestinal cancers had the highest relative risks, with a hazard ratio of 18.0 (14.4 to 22.7) corresponding to 202 cancers in patients with inflammatory bowel disease. The increased risk of cancer (before 25th birthday) was similar over time (1964-1989: 1.6, 1.0 to 2.4; 1990-2001: 2.3, 1.5 to 3.3); 2002-06: 2.9, 1.9 to 4.2; 2007-14: 2.2, 1.1 to 4.2).Conclusion Childhood onset inflammatory bowel disease is associated with an increased risk of any cancer, especially gastrointestinal cancers, both in childhood and later in life. The higher risk of cancer has not fallen over time.

Ong, H. S. and H. C. H. Yim (2017). "Microbial Factors in Inflammatory Diseases and Cancers." Adv Exp Med Biol **1024**: 153-174.

The intestinal microbes form a symbiotic relationship with their human host to harvest energy for themselves and their host and to shape the immune system of their host. However, alteration of this relationship, which is named as a dysbiosis, has been associated with the development of different inflammatory diseases and cancers. It is found that metabolites, cellular components, and virulence factors derived from the gut microbiota interact with the host locally or systemically to modulate the dysbiosis and the development of these diseases. In this book chapter, we discuss the role of these microbial factors in regulating the host signaling pathways, the composition and load of the gut microbiota, the co-metabolism of the host and the microbiota, the host immune system, and physiology. In particular, we highlight how each microbial factor can contribute in the manifestation of many diseases such as cancers, Inflammatory Bowel Diseases, obesity, type-2 diabetes, non-alcoholic fatty liver diseases, nonalcoholic steatohepatitis, and cardiovascular diseases.

Oster, S. K., et al. (2002). "The myc oncogene: MarvelouslY Complex." Adv Cancer Res **84**: 81-154.

The activated product of the myc oncogene deregulates both cell growth and death check points and, in a permissive environment, rapidly accelerates the affected clone through the carcinogenic process. Advances in understanding the molecular mechanism of Myc action are highlighted in this review. With the revolutionary developments in molecular diagnostic technology, we have witnessed an unprecedented advance in detecting activated myc in its deregulated, oncogenic form

in primary human cancers. These improvements provide new opportunities to appreciate the tumor subtypes harboring deregulated Myc expression, to identify the essential cooperating lesions, and to realize the therapeutic potential of targeting Myc. Knowledge of both the breadth and depth of the numerous biological activities controlled by Myc has also been an area of progress. Myc is a multifunctional protein that can regulate cell cycle, cell growth, differentiation, apoptosis, transformation, genomic instability, and angiogenesis. New insights into Myc's role in regulating these diverse activities are discussed. In addition, breakthroughs in understanding Myc as a regulator of gene transcription have revealed multiple mechanisms of Myc activation and repression of target genes. Moreover, the number of reported Myc regulated genes has expanded in the past few years, inspiring a need to focus on classifying and segregating bona fide targets. Finally, the identity of Myc-binding proteins has been difficult, yet has exploded in the past few years with a plethora of novel interactors. Their characterization and potential impact on Myc function are discussed. The rapidity and magnitude of recent progress in the Myc field strongly suggests that this marvelously complex molecule will soon be unmasked.

Ouahed, J., et al. (2018). "Mucosal Gene Expression in Pediatric and Adult Patients With Ulcerative Colitis Permits Modeling of Ideal Biopsy Collection Strategy for Transcriptomic Analysis." Inflamm Bowel Dis.

Palmieri, O., et al. (2015). "Systematic analysis of circadian genes using genome-wide cDNA microarrays in the inflammatory bowel disease transcriptome." Chronobiol Int **32**(7): 903-916.

Pan, J., et al. (2001). "Dexamethasone inhibits the antigen presentation of dendritic cells in MHC class II pathway." Immunol Lett **76**(3): 153-161.

Panwala, C. M., et al. (1998). "A novel model of inflammatory bowel disease: mice deficient for the multiple drug resistance gene, mdr1a, spontaneously develop colitis." J Immunol **161**(10): 5733-5744.

The murine multiple drug resistance (mdr) gene, mdr1a, encodes a 170-kDa transmembrane protein that is expressed in many tissues including intestinal epithelial cells, a subset of lymphoid cells and hematopoietic cells. We report that mdr1a knockout (mdr1a-/-) mice are susceptible to developing a severe, spontaneous intestinal inflammation when maintained under specific pathogen-free animal facility conditions. The intestinal inflammation seen in mdr1a-/- mice has a pathology similar to that of human inflammatory bowel disease (IBD) and is defined by dysregulated epithelial cell growth and leukocytic infiltration into the lamina propria of the large intestine. Treating mdr1a-/- mice with oral antibiotics can both prevent the development of disease and resolve active inflammation. Lymphoid cells isolated from mice with active colitis are functionally reactive to intestinal bacterial Ags, providing evidence that there is enhanced immunologic responsiveness to the normal

bacterial flora during IBD. This study is the first description of spontaneous colitis in a gene knockout mouse with an apparently intact immune system. This novel model of spontaneous colitis may provide new insight into the pathogenesis of IBD, the nature of dysregulated immune reactivity to intestinal bacterial Ags, and the potential functional role of mdr genes expressed in the cells and tissues of the colonic microenvironment.

Papamichael, K. and A. S. Cheifetz (2016). "Use of anti-TNF drug levels to optimise patient management." Frontline Gastroenterology **7**(4): 289-300.

Anti-tumour necrosis factor (TNF) therapies, such as infliximab, adalimumab, certolizumab pegol and golimumab, have been proven to be effective for the treatment of patients with Crohn's disease and ulcerative colitis. However, 10%-30% of patients with inflammatory bowel disease (IBD) show no initial clinical benefit to anti-TNF therapy (primary non-response), and over 50% after an initial favourable outcome will lose response over time (secondary loss of response (SLR)). Numerous recent studies in IBD have revealed an exposure-response relationship suggesting a positive correlation between high serum anti-TNF concentrations and favourable therapeutic outcomes including clinical, biomarker and endoscopic remission, whereas antidrug antibodies have been associated with SLR and infusion reactions. Currently, therapeutic drug monitoring (TDM) is typically performed when treatment failure occurs either for SLR, drug intolerance (potential immune-mediated reaction) or infusion reaction (reactive TDM). Nevertheless, recent data demonstrate that

proactive TDM and a treat-to-target (trough) therapeutic approach may more effectively optimise anti-TNF therapy efficacy, safety and cost. However, implementing TDM in real-life clinical practice is currently limited by the diversity in study design, therapeutic outcomes and assays used, which have hindered the identification of robust clinically relevant concentration thresholds. This review will focus mainly on the pharmacodynamic properties of anti-TNF therapy and the role of TDM in guiding therapeutic decisions in IBD.

Parkes, G. C., et al. (2014). "Smoking in inflammatory bowel disease: impact on disease course and insights into the aetiology of its effect." J Crohns Colitis **8**(8): 717-725.

Pedersen, J. M., et al. (2017). "Substrate and method dependent inhibition of three ABC-transporters (MDR1, BCRP, and MRP2)." European Journal of Pharmaceutical Sciences **103**: 70-76.

Peng, R., et al. (2016). "Impacts of ABCB1 (G1199A) polymorphism on resistance, uptake, and efflux to steroid drugs." Xenobiotica **46**(10): 948-952.

1. P-glycoprotein (P-gp) substrates, including steroid drugs, involve in the inter-individual differences in resistant phenotype. This study was performed to evaluate whether G1199A polymorphism in ABCB1 gene can alter the sensitivity, accumulation, and transepithelial efflux to steroids in LLC-PK1 cells. 2. The stable

recombinant LLC-PK1 cell lines transfected with ABCB1 1199G and ABCB1 1199A were used to assess the sensitivity, accumulation, and transepithelial permeability to steroids. 3. The cells transfected with 1199A allele displayed stronger resistance to aldosterone, dexamethasone, and cortisol (2.5-, 2.0-, and 1.6-fold, respectively) than cells overexpressing 1199G allele, while the two types of recombinant cells showed a similar resistance to corticosterone. The accumulation of aldosterone, dexamethasone, and cortisol in recombinant 1199A cells were significantly decreased when compared to 1199G cells (2.9-, 4.4-, and 3.9-fold, respectively). The net efflux ratios of P-gp-mediated aldosterone, dexamethasone, and cortisol in cells expressing 1199A allele were apparently greater than cells transfected with 1199G allele (3.3-, 3.5-, and 4.0-fold, respectively). 4. The impacts of ABCB1 (G1199A) single nucleotide polymorphism on the efflux of P-gp substrates presented as drug-specific. Overall, the transport ability of P-gp-dependent steroid drugs in recombinant model overexpressing variant 1199A allele is stronger in comparison to cells overexpressing wild-type 1199G allele. Therefore, the ABCB1 (G1199A) polymorphism may affect effective steroids concentration in target cells by regulating the drug transport and distribution.

Perezgonzalez, J. F. and J. R. Rojas (1976). "Inhibition of Baroreceptor Input to Cardiac Vagal Preganglionic Neurons by Stimulation of Medullary Reticular-Formation." Journal of Physiology-London **263**(1): P152-P154.

Petersen, B. S., et al. (2017). "Targeted Gene Panel Sequencing for Early-onset Inflammatory Bowel Disease and Chronic Diarrhea." Inflamm Bowel Dis **23**(12): 2109-2120.

Peterson, D. A., et al. (2008). "Metagenomic approaches for defining the pathogenesis of inflammatory bowel diseases." Cell Host Microbe **3**(6): 417-427.

The human gastrointestinal tract is home to immense and complex populations of microorganisms. Using recent technical innovations, the diversity present in this human body habitat is now being analyzed in detail. This review focuses on the microbial ecology of the gut in inflammatory bowel diseases and on how recent studies provide an impetus for using carefully designed, comparative metagenomic approaches to delve into the structure and activities of the gut microbial community and its interrelationship with the immune system.

Petryszyn, P. W. and A. Wiela-Hojenska (2018). "The importance of the polymorphisms of the ABCB1 gene in disease susceptibility, behavior and response to treatment in inflammatory bowel disease: A literature review." Adv Clin Exp Med **27**(10): 1459-1463.

Pfaffl, M. W. (2001). "A new mathematical model for relative quantification in real-time RT-PCR." Nucleic Acids Res **29**(9): e45.

Plevy, S., et al. (2013). "Combined serological, genetic, and inflammatory markers differentiate non-IBD, Crohn's disease, and ulcerative colitis patients." Inflamm Bowel Dis **19**(6): 1139-1148.

BACKGROUND: Previous studies have demonstrated that serological markers can assist in diagnosing inflammatory bowel disease (IBD). In this study, we aim to build a diagnostic tool incorporating serological markers, genetic variants, and markers of inflammation into a computational algorithm to examine patterns of combinations of markers to (1) identify patients with IBD and (2) differentiate patients with Crohn's disease (CD) from ulcerative colitis (UC). METHODS: In this cross-sectional study, patient blood samples from 572 CD, 328 UC, 437 non-IBD controls, and 183 healthy controls from academic and community centers were analyzed for 17 markers: 8 serological markers (ASCA-IgA, ASCA-IgG, ANCA, pANCA, OmpC, CBir1, A4-Fla2, and FlaX), 4 genetic markers (ATG16L1, NKX2-3, ECM1, and STAT3), and 5 inflammatory markers (CRP, SAA, ICAM-1, VCAM-1, and VEGF). A diagnostic Random Forest algorithm was constructed to classify IBD, CD, and UC. RESULTS: Receiver operating characteristic analysis compared the diagnostic accuracy of using a panel of serological markers only (ASCA-IgA, ASCA-IgG, ANCA, pANCA, OmpC, and CBir1) versus using a marker panel that in addition to the serological markers mentioned above also included gene variants, inflammatory markers, and 2 additional serological markers (A4-Fla2 and FlaX). The extended marker panel increased the IBD versus non-IBD discrimination area under the curve from 0.80 (95% confidence interval [CI], +/-0.05) to 0.87 (95% CI, +/-0.04; $P < 0.001$). The CD versus UC discrimination increased from 0.78 (95% CI, +/-0.06) to 0.93 (95% CI,

+/-0.04; P < 0.001). CONCLUSIONS: Incorporating a combination of serological, genetic, and inflammation markers into a diagnostic algorithm improved the accuracy of identifying IBD and differentiating CD from UC versus using serological markers alone.

Press, M. F., et al. (1993). "Her-2/neu expression in node-negative breast cancer: direct tissue quantitation by computerized image analysis and association of overexpression with increased risk of recurrent disease." Cancer Research **53**(20): 4960-4970.

Qosa, H., et al. (2016). "Astrocytes drive upregulation of the multidrug resistance transporter ABCB1 (P-Glycoprotein) in endothelial cells of the blood-brain barrier in mutant superoxide dismutase 1-linked amyotrophic lateral sclerosis." Glia **64**(8): 1298-1313.

The efficacy of drugs targeting the CNS is influenced by their limited brain access, which can lead to complete pharmacoresistance. Recently a tissue-specific and selective upregulation of the multidrug efflux transporter ABCB1 or P-glycoprotein (P-gp) in the spinal cord of both patients and the mutant SOD1-G93A mouse model of amyotrophic lateral sclerosis (ALS), a fatal neurodegenerative disease that prevalently kills motor neurons has been reported. Here, we extended the analysis of P-gp expression in the SOD1-G93A ALS mouse model and found that P-gp upregulation was restricted to endothelial cells of the capillaries, while P-gp expression was not detected in other cells of the spinal cord parenchyma such as astrocytes, oligodendrocytes, and neurons. Using both in vitro human and mouse models of the

blood-brain barrier (BBB), we found that mutant SOD1 astrocytes were driving P-gp upregulation in endothelial cells. In addition, a significant increase in reactive oxygen species production, Nrf2 and NFkappaB activation in endothelial cells exposed to mutant SOD1 astrocytes in both human and murine BBB models were observed. Most interestingly, astrocytes expressing FUS-H517Q, a different familial ALS-linked mutated gene, also drove NFkappaB-dependent upregulation of P-gp. However, the pathway was not dependent on oxidative stress but rather involved TNF-alpha release. Overall, these findings indicated that nuclear translocation of NFkappaB was a converging mechanism used by endothelial cells of the BBB to upregulate P-gp expression in mutant SOD1-linked ALS and possibly other forms of familial ALS. GLIA 2016 GLIA 2016;64:1298-1313.

Ramamoorthy, S. and J. A. Cidlowski (2016). "Corticosteroids: Mechanisms of Action in Health and Disease." Rheum Dis Clin North Am **42**(1): 15-31, vii.

Ramesh, R., et al. (2014). "Pro-inflammatory human Th17 cells selectively express P-glycoprotein and are refractory to glucocorticoids." J Exp Med **211**(1): 89-104.

IL-17A-expressing CD4(+) T cells (Th17 cells) are generally regarded as key effectors of autoimmune inflammation. However, not all Th17 cells are pro-inflammatory. Pathogenic Th17 cells that induce autoimmunity in mice are distinguished from nonpathogenic Th17 cells by a unique transcriptional signature, including high Il23r expression, and these cells require Il23r for their inflammatory

function. In contrast, defining features of human pro-inflammatory Th17 cells are unknown. We show that pro-inflammatory human Th17 cells are restricted to a subset of CCR6(+)CXCR3(hi)CCR4(lo)CCR10(-)CD161(+) cells that transiently express c-Kit and stably express P-glycoprotein (P-gp)/multi-drug resistance type 1 (MDR1). In contrast to MDR1(-) Th1 or Th17 cells, MDR1(+) Th17 cells produce both Th17 (IL-17A, IL-17F, and IL-22) and Th1 (IFN-gamma) cytokines upon TCR stimulation and do not express IL-10 or other anti-inflammatory molecules. These cells also display a transcriptional signature akin to pathogenic mouse Th17 cells and show heightened functional responses to IL-23 stimulation. In vivo, MDR1(+) Th17 cells are enriched and activated in the gut of Crohn's disease patients. Furthermore, MDR1(+) Th17 cells are refractory to several glucocorticoids used to treat clinical autoimmune disease. Thus, MDR1(+) Th17 cells may be important mediators of chronic inflammation, particularly in clinical settings of steroid resistant inflammatory disease.

Rapozo, D. C., et al. (2017). "Diet and microbiota in inflammatory bowel disease: The gut in disharmony." World J Gastroenterol **23**(12): 2124-2140.

Bacterial colonization of the gut shapes both the local and the systemic immune response and is implicated in the modulation of immunity in both healthy and disease states. Recently, quantitative and qualitative changes in the composition of the gut microbiota have been detected in Crohn's disease and ulcerative colitis, reinforcing the hypothesis of dysbiosis as a relevant mechanism underlying inflammatory bowel

disease (IBD) pathogenesis. Humans and microbes have co-existed and co-evolved for a long time in a mutually beneficial symbiotic association essential for maintaining homeostasis. However, the microbiome is dynamic, changing with age and in response to environmental modifications. Among such environmental factors, food and alimentary habits, progressively altered in modern societies, appear to be critical modulators of the microbiota, contributing to or co-participating in dysbiosis. In addition, food constituents such as micronutrients are important regulators of mucosal immunity, with direct or indirect effects on the gut microbiota. Moreover, food constituents have recently been shown to modulate epigenetic mechanisms, which can result in increased risk for the development and progression of IBD. Therefore, it is likely that a better understanding of the role of different food components in intestinal homeostasis and the resident microbiota will be essential for unravelling the complex molecular basis of the epigenetic, genetic and environment interactions underlying IBD pathogenesis as well as for offering dietary interventions with minimal side effects.

Relling, M. V. and W. E. Evans (2015). "Pharmacogenomics in the clinic." Nature **526**(7573): 343-350.

Relling, M. V., et al. (2011). "Clinical Pharmacogenetics Implementation Consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing." Clin Pharmacol Ther **89**(3): 387-391.

Thiopurine methyltransferase (TPMT) activity exhibits monogenic co-dominant inheritance, with ethnic differences in the frequency of occurrence of variant alleles. With conventional thiopurine doses, homozygous TPMT-deficient patients (~1 in 178 to 1 in 3,736 individuals with two nonfunctional TPMT alleles) experience severe myelosuppression, 30-60% of individuals who are heterozygotes (~3-14% of the population) show moderate toxicity, and homozygous wild-type individuals (~86-97% of the population) show lower active thioguanine nucleolides and less myelosuppression. We provide dosing recommendations (updates at http://www.pharmgkb.org) for azathioprine, mercaptopurine (MP), and thioguanine based on TPMT genotype.

Relling, M. V., et al. (2019). "Clinical Pharmacogenetics Implementation Consortium Guideline for Thiopurine Dosing Based on TPMT and NUDT15 Genotypes: 2018 Update." Clin Pharmacol Ther **105**(5): 1095-1105.

Thiopurine methyltransferase (TPMT) activity exhibits a monogenic codominant inheritance and catabolizes thiopurines. TPMT variant alleles are associated with low enzyme activity and pronounced pharmacologic effects of thiopurines. Loss-of-function alleles in the NUDT15 gene are common in Asians and Hispanics and reduce the degradation of active thiopurine nucleotide metabolites, also predisposing to myelosuppression. We provide recommendations for adjusting starting doses of azathioprine, mercaptopurine, and thioguanine based on TPMT and NUDT15 genotypes (updates on www.cpicpgx.org).

Replication, D. I. G., et al. (2014). "Genome-wide trans-ancestry meta-analysis provides insight into the genetic architecture of type 2 diabetes susceptibility." Nature Genetics **46**(3): 234-244.

To further understanding of the genetic basis of type 2 diabetes (T2D) susceptibility, we aggregated published meta-analyses of genome-wide association studies (GWAS), including 26,488 cases and 83,964 controls of European, east Asian, south Asian and Mexican and Mexican American ancestry. We observed a significant excess in the directional consistency of T2D risk alleles across ancestry groups, even at SNPs demonstrating only weak evidence of association. By following up the strongest signals of association from the trans-ethnic meta-analysis in an additional 21,491 cases and 55,647 controls of European ancestry, we identified seven new T2D susceptibility loci. Furthermore, we observed considerable improvements in the fine-mapping resolution of common variant association signals at several T2D susceptibility loci. These observations highlight the benefits of trans-ethnic GWAS for the discovery and characterization of complex trait loci and emphasize an exciting opportunity to extend insight into the genetic architecture and pathogenesis of human diseases across populations of diverse ancestry.

Ricciuto, A., et al. (2018). "Clinical outcomes with therapeutic drug monitoring in inflammatory bowel disease: A systematic review with meta-analysis." J Crohns Colitis.

Richard, M. L., et al. (2017). "Mucosa-associated microbiota dysbiosis in colitis associated cancer." Gut Microbes: 0.

Gut microbiota dysbiosis has been associated with inflammatory bowel diseases (IBD). In colorectal cancer, the gut microbiota has also been recognized as potentially involved in aggravating or favoring the tumor development. However, very little is known on the structure and role of the microbiota in colitis associated cancer (CAC), an important complication of IBD in human. Here we analyzed the bacterial and fungal composition of the mucosa associated microbiota of patients suffering CAC, sporadic cancer (SC) and of healthy subjects (HS) by barcode sequences analysis on the following cohort: 7 CAC patients, 10 SC patients and 10 HS using 16S (MiSeq) and ITS2 (pyrosequencing) sequencing, for bacteria and fungi respectively. Mucosa-associated bacterial microbiota in CAC was significantly different from the ones in SC or in HS, while the fungal showed no differences. Comparison between mucosa-associated microbiota on the tumor site or in normal mucosa near the tumor showed very similar patterns. The global mucosa-associated bacterial microbiota in cancer patients was characterized by a restriction in biodiversity but no change for the fungal community. Compared to SC, CAC was characterized by an increase of Enterobacteriacae family and Sphingomonas genus and a decrease of Fusobacterium and Ruminococcus genus. Our study confirms the alteration of the mucosa-associated bacterial microbiota in IBD and SC. Although the cohort is limited in number, this is the first evidence of the existence of an altered bacterial microbiota in CAC clearly different from the one in SC patients.

Riches, Z., et al. (2016). "ATP-binding cassette proteins BCRP, MRP1 and P-gp expression and localization in the human umbilical cord." Xenobiotica **46**(6): 548-556.

Riedel, C. U., et al. (2006). "Anti-inflammatory effects of bifidobacteria by inhibition of LPS-induced NF-kappaB activation." World J Gastroenterol **12**(23): 3729-3735.

Rizzello, F., et al. (2018). "The safety of beclomethasone dipropionate in the treatment of ulcerative colitis." Expert Opin Drug Saf **17**(9): 963-969.

Rocco, A., et al. (2012). "MDR1-P-glycoprotein behaves as an oncofetal protein that promotes cell survival in gastric cancer cells." Laboratory Investigation **92**(10): 1407-1418.

Roda, G., et al. (2016). "Loss of Response to Anti-TNFs: Definition, Epidemiology, and Management." Clinical and Translational Gastroenterology **7**.

Tumor necrosis factor-alpha (TNF alpha) antagonists have advanced the management of inflammatory bowel diseases patients leading to an improvement of patient's quality of life with the reduction of number of surgeries and hospitalizations. Despite these advances, many patients do not respond to the induction therapy (primary non-response-PNR) or lose response during the treatment (secondary loss of response-LOR). In this paper we will provide an overview of the definition, epidemiology and

risk factors for PNR and LOR, as well as discuss the therapeutic options for managing LOR.

Rojas, J. R., et al. (2005). "Formation, distribution, and elimination of infliximab and anti-infliximab immune complexes in cynomolgus monkeys." Journal of Pharmacology and Experimental Therapeutics **313**(2): 578-585.

Infliximab (IFX) is a chimeric IgG1 monoclonal antibody specific for human tumor necrosis factor-alpha that is approved in the United States and Europe for the treatment of rheumatoid arthritis (RA) and Crohn's disease (CD). Approximately 10% of RA and CD patients receiving maintenance treatment with IFX will develop antibodies to IFX. The objective of this study was to develop a model to assess the in vivo formation, distribution, and elimination of immune complexes resulting from a low-level immune response in the presence of the excess concentration of a therapeutic antigen. In this model, cynomolgus monkeys were treated with a single intravenous injection of IFX, followed by injection of either radiolabeled, purified monkey anti-IFX IgG antibody (n = 3, test group) or radiolabeled monkey, nonimmune IgG (n = 3, control group). High-performance liquid chromatography analysis of collected sera revealed a rapid formation of immune complexes comprised of IFX and radiolabeled anti-IFX IgG antibody immune complexes. The terminal half-life of the anti-IFX IgG antibody immune complex was approximately 38 h compared with 86 h for the nonimmune antibody. However, the pharmacokinetic profile of IFX, although slightly lower in concentration over time for the test group,

was not notably different relative to the control group. There were no macroscopic or microscopic histological findings in either treatment group. These data confirm that immune complexes between IFX and anti-IFX IgG antibodies can form in vivo and that these immune complexes are eliminated more rapidly than nonimmune antibodies in the presence of excess IFX.

Roussomoustakaki, M., et al. (1997). "Genetic markers may predict disease behavior in patients with ulcerative colitis." Gastroenterology **112**(6): 1845-1853.

BACKGROUND & AIMS: Recent studies have suggested that HLA DRB1*0103 and allele 2 of the interleukin 1 receptor antagonist (IL-1RA) gene predict severe and extensive ulcerative colitis, respectively. The aim of this study was to test these hypotheses in patients undergoing surgery for their colitis. METHODS: HLA DRB1 and DQB1 genotyping was performed in 99 patients and 472 controls. Genotyping for polymorphisms of genes encoding tumor necrosis factor alpha and IL-1RA was performed in 107 patients and 89 controls. Measurement of antineutrophil cytoplasmic antibody (ANCA) was performed in 72 patients and 58 healthy subjects by fixed neutrophil enzyme-linked immunosorbent assay and indirect immunofluorescence. RESULTS: The DRB1*0103 allele was increased in patients (14.1% vs. 3.2% in controls; P < 1 x 10[-5]). This association was greatest in patients with extensive disease (15.8%; P < 0.0001) or extraintestinal manifestations (22.8%; P < 0.0001): mouth ulcers (25.8%; P < 0.0001), arthritis (27.2%; P < 0.0001), and uveitis (35.7%; P < 0.0001). The DRB1*04 alleles were reduced in patients (P =

0.005). Differences were noted between extensive and distal disease in the frequency of allele 2 of IL-1RA (10.9% in distal vs. 28.6% in extensive; P = 0.01) and allele 2 homozygosity. ANCA was detected in 76.4% of patients. Carriage of IL-1RA allele 2 and tumor necrosis factor 2 allele was increased in ANCA-positive patients. CONCLUSIONS: Genetic markers may predict disease behavior in ulcerative colitis.

Ruemmele, F. M. and D. Turner (2014). "Differences in the management of pediatric and adult onset ulcerative colitis--lessons from the joint ECCO and ESPGHAN consensus guidelines for the management of pediatric ulcerative colitis." J Crohns Colitis **8**(1): 1-4.

Ruemmele, F. M., et al. (2014). "Consensus guidelines of ECCO/ESPGHAN on the medical management of pediatric Crohn's disease." J Crohns Colitis **8**(10): 1179-1207.

Russell, R. K., et al. (2011). "A British Society of Paediatric Gastroenterology, Hepatology and Nutrition survey of the effectiveness and safety of adalimumab in children with inflammatory bowel disease." Aliment Pharmacol Ther **33**(8): 946-953.

BACKGROUND: Adalimumab is efficacious therapy for adults with Crohn's disease (CD). AIM: To summarise the United Kingdom and Republic of Ireland paediatric adalimumab experience. METHODS: British Society of Paediatric Gastroenterology, Hepatology and Nutrition (BSPGHAN) members with Inflammatory Bowel Disease (IBD) patients <18 years old commencing adalimumab with at least 4 weeks follow-

up. Patient demographics and details of treatment were then collected. Response and remission was assessed using the Paediatric Crohn's Disease Activity Index (PCDAI)/ Physicians Global Assessment (PGA). RESULTS: Seventy-two patients [70 CD, 1 ulcerative colitis (UC), 1 IBD unclassified (IBDU)] from 19 paediatric-centres received adalimumab at a median age of 14.8 (IQR 3.1, range 6.1-17.8) years; 66/70 CD (94%) had previously received infliximab. A dose of 80 mg then 40 mg was used for induction in 41(59%) and 40 mg fortnightly for maintenance in 61 (90%). Remission rates were 24%, 58% and 41% at 1, 6 and 12 months, respectively. Overall 43 (61%) went into remission at some point, with 24 (35%) requiring escalation of therapy. Remission rates were higher in those on concomitant immunosuppression cf. those not on immunosuppression [34/46 (74%) vs. 9/24 (37%), respectively, (chi(2) 8.8, P=0.003)]. There were 15 adverse events (21%) including four (6%) serious adverse events with two sepsis related deaths in patients who were also on immunosuppression and home parenteral nutrition (3% mortality rate). CONCLUSIONS: Adalimumab is useful in treatment of refractory paediatric patients with a remission rate of 61%. This treatment benefit should be balanced against side effects, including in this study a 3% mortality rate.

Russo, E., et al. (2017). "Crohn's Colitis: Development of a multiplex gene expression assay comparing mRNA levels of susceptibility genes." Clin Res Hepatol Gastroenterol **41**(4): 435-444.

Sakil, H. A. M., et al. (2017). "DeltaNp73 regulates the expression of the multidrug-resistance genes ABCB1 and ABCB5 in breast cancer and melanoma cells - a short report." Cell Oncol (Dordr).

PURPOSE: Multidrug resistance (MDR) is a major cause of treatment failure. In cancer cells, MDR is often caused by an increased efflux of therapeutic drugs mediated by an up-regulation of ATP binding cassette (ABC) transporters. It has previously been shown that oncogenic DeltaNp73 plays an important role in chemo-resistance. Here we aimed at unraveling the role of DeltaNp73 in regulating multidrug resistance in breast cancer and melanoma cells. METHODS: KEGG pathway analysis was used to identify pathways enriched in breast cancer samples with a high DeltaNp73 expression. We found that the ABC transporter pathway was most enriched. The expression of selected ABC transporters was analyzed using qRT-PCR upon siRNA/shRNA-mediated knockdown or exogenous overexpression of DeltaNp73 in the breast cancer-derived cell lines MCF7 and MDA-MB-231, as well as in primary melanoma samples and in the melanoma-derived cell line SK-MEL-28. The ability to efflux doxorubicin and the concomitant effects on cell proliferation were assessed using flow cytometry and WST-1 assays. RESULTS: We found that high DeltaNp73 levels correlate with a general up-regulation of ABC transporters in breast cancer samples. In addition, we found that exogenous expression of DeltaNp73 led to an increase in the expression of ABCB1 and ABCB5 in the breast cancer-derived cell lines tested, while knocking down of DeltaNp73 resulted in a reduction in ABCB1 and ABCB5 expression. In addition, we found that DeltaNp73 reduction leads to an intracellular retention of doxorubicin in MDA-MB-231 and MCF7 cells

and a concomitant decrease in cell proliferation. The effect of DeltaNp73 on ABCB5 expression was further confirmed in metastases from melanoma patients and in the melanoma-derived cell line SK-MEL-28. CONCLUSIONS: Our data support a role for DeltaNp73 in the multidrug-resistance of breast cancer and melanoma cells.

Saleh, M. and C. O. Elson (2011). "Experimental Inflammatory Bowel Disease: Insights into the Host-Microbiota Dialog." Immunity **34**(3): 293-302.

Inflammatory bowel disease appears to result from an abnormal host immune response to the intestinal microbiota. Experimental models have allowed the dissection of the complex dialog between the host and its microbiota. Through genetic manipulation of the host genome the immune compartments, cells, molecules, and genes that are critical for maintenance of intestinal homeostasis are being identified. Genetic association studies in humans have identified over 100 susceptibility loci. Although there is remarkable coherence between the experimental model and the human genetic data, a full understanding of the mechanisms involved in genetic susceptibility to IBD and of gene-gene and gene-environmental interactions will require a "next generation" of experimental models.

Samaan, M. A., et al. (2014). "A systematic review of the measurement of endoscopic healing in ulcerative colitis clinical trials: recommendations and implications for future research." Inflamm Bowel Dis **20**(8): 1465-1471.

BACKGROUND: Assessment of endoscopic disease activity, as measured by various endoscopic evaluative instruments, is an essential part of quantifying disease activity in clinical trials in patients with ulcerative colitis (UC). Evaluative instruments have specific definitions and operating properties that influence the interpretation of clinical trial results. Our objective was to systematically review all endoscopic evaluative instruments that measure endoscopic disease activity in UC and to describe their definitions and operating characteristics (reliability, responsiveness, and predictive validity). METHODS: We performed a systematic review of evaluative instruments assessing endoscopic disease activity in UC. MEDLINE (Ovid), EMBASE (Ovid), PubMed, the Cochrane Library (CENTRAL), and Digestive Disease Week abstracts of clinical trials were searched from inception to January 2013. RESULTS: In total, 5885 studies were identified and screened for inclusion criteria. Four hundred twenty-two studies involving 31 evaluative instruments were identified. Two types of indices were found, numerical scoring systems and stepwise grading scales. CONCLUSIONS: Both the endoscopic evaluative instrument selected and the definition chosen for mucosal healing affect the validity of assessing endoscopic disease activity during a clinical trial for UC. Currently, the sigmoidoscopic component of the Mayo Score and the ulcerative colitis endoscopic index of severity show the most promise as reliable evaluative instruments of endoscopic disease activity. However, further validation is required.

Samaan, M. A., et al. (2017). "Vedolizumab: early experience and medium-term outcomes from two UK tertiary IBD centres." Frontline Gastroenterol **8**(3): 196-202.

OBJECTIVE: To gain an understanding of the efficacy of vedolizumab in a 'real-world' setting. DESIGN: Retrospective cohort study using prospectively maintained clinical records. SETTING: Two UK tertiary inflammatory bowel disease (IBD) centres. PATIENTS: Patients with IBD commenced on vedolizumab at Guy's & St Thomas' and King's College Hospitals during November 2014-November 2015. INTERVENTION: Vedolizumab, a monoclonal antibody to alpha-4 beta-7 integrins that selectively inhibit leucocyte migration into the gut. MAIN OUTCOME MEASURES: Clinical disease activity was assessed at baseline, weeks 14 and 30 using Harvey-Bradshaw Index (HBI) for Crohn's disease (CD) and Simple Clinical Colitis Activity Index (SCCAI) for ulcerative colitis (UC). Response was defined as HBI or SCCAI reduction >/=3. Remission was defined as HBI <5 or SCCAI <3. Continuous data are summarised as medians, followed by range. RESULTS: Fifty patients were included: 27 CD, 20 UC and 3 IBD-U (included in the UC group for analysis). At baseline visit, the median HBI was 8 (1-16) and SCCAI was 6 (0-15). At week 14, these values had fallen to 5 (0-15) (p=0.117) and 4 (0-10) (p=0.005), respectively. Additionally, week 30 data were available for 19 patients (9 CD, 10 UC). The clinical disease activity scores at that point were HBI 2 (0-7) (p=0.039) and SCCAI 2 (0-10) (p=0.023). At baseline, 37 (74%) of the 50 patients had clinically active disease. Of the patients with active disease, 22 (59%) responded and 14 (38%) achieved remission at week 14. CONCLUSIONS: Our early experience with vedolizumab demonstrates a clear benefit in terms of disease control as well as a steroid-sparing effect in a cohort, which included patients with complex and previously refractory disease.

Sandborn, W. J., et al. (2013). "Vedolizumab as Induction and Maintenance Therapy for Crohn's Disease." New England Journal of Medicine **369**(8): 711-721.

BACKGROUND

The efficacy of vedolizumab, an alpha(4)beta(7) integrin antibody, in Crohn's disease is unknown.

METHODS

In an integrated study with separate induction and maintenance trials, we assessed intravenous vedolizumab therapy (300 mg) in adults with active Crohn's disease. In the induction trial, 368 patients were randomly assigned to receive vedolizumab or placebo at weeks 0 and 2 (cohort 1), and 747 patients received open-label vedolizumab at weeks 0 and 2 (cohort 2); disease status was assessed at week 6. In the maintenance trial, 461 patients who had had a response to vedolizumab were randomly assigned to receive placebo or vedolizumab every 8 or 4 weeks until week 52.

RESULTS

At week 6, a total of 14.5% of the patients in cohort 1 who received vedolizumab and 6.8% who received placebo were in clinical remission (i.e., had a score on the Crohn's Disease Activity Index [CDAI] of <= 150, with scores ranging from 0 to approximately 600 and higher scores indicating greater disease activity) (P = 0.02); a total of 31.4% and 25.7% of the patients, respectively, had a CDAI-100 response (>=

100-point decrease in the CDAI score) (P = 0.23). Among patients in cohorts 1 and 2 who had a response to induction therapy, 39.0% and 36.4% of those assigned to vedolizumab every 8 weeks and every 4 weeks, respectively, were in clinical remission at week 52, as compared with 21.6% assigned to placebo (P<0.001 and P = 0.004 for the two vedolizumab groups, respectively, vs. placebo). Antibodies against vedolizumab developed in 4.0% of the patients. Nasopharyngitis occurred more frequently, and headache and abdominal pain less frequently, in patients receiving vedolizumab than in patients receiving placebo. Vedolizumab, as compared with placebo, was associated with a higher rate of serious adverse events (24.4% vs. 15.3%), infections (44.1% vs. 40.2%), and serious infections (5.5% vs. 3.0%).

CONCLUSIONS

Vedolizumab-treated patients with active Crohn's disease were more likely than patients receiving placebo to have a remission, but not a CDAI-100 response, at week 6; patients with a response to induction therapy who continued to receive vedolizumab (rather than switching to placebo) were more likely to be in remission at week 52. Adverse events were more common with vedolizumab.

Sarrabayrouse, G., et al. (2014). "CD4CD8alphaalpha lymphocytes, a novel human regulatory T cell subset induced by colonic bacteria and deficient in patients with inflammatory bowel disease." PLoS Biol **12**(4): e1001833.

Satsangi, J., et al. (2006). "The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications." Gut **55**(6): 749-753.

> In recent years, investigators have readdressed the complex issues involved in the classification of inflammatory bowel diseases. In 2003, a Working Party of investigators with an interest in the issues involved in disease subclassification was formed with the aim of summarising recent developments in disease classification and establishing an integrated clinical, molecular, and serological classification of inflammatory bowel disease. The results of the Working Party were reported at the 2005 Montreal World Congress of Gastroenterology. Here we highlight the key issues that have emerged from discussions of the Montreal Working Party and the relevance to clinical practice and research activities.

Sawczenko, A., et al. (2001). "Prospective survey of childhood inflammatory bowel disease in the British Isles." Lancet **357**(9262): 1093-1094.

> The incidence of inflammatory bowel disease in children in western countries may be rising. Since there is no prospective national data on the incidence of inflammatory bowel disease in the UK and Republic of Ireland (ROI), we undertook a prospective survey to determine this incidence. The incidence during 1998 and 1999 was 5.2/100,000 per year in children aged younger than 16 years. Those from an Asian background were over-represented and more likely to have ulcerative colitis.

Sazonovs, A. and J. C. Barrett (2018). "Rare-Variant Studies to Complement Genome-Wide Association Studies." Annu Rev Genomics Hum Genet **19**: 97-112.

Genome-wide association studies (GWASs) have revolutionized human disease genetics by discovering tens of thousands of associations between common variants and complex diseases. In parallel, huge technological advances in DNA sequencing have made it possible to measure and analyze rare variation in populations. This review considers these two stories and how they have come together. We first review the history of GWASs and sequencing. We then consider how to understand the biological mechanisms that drive signals of strong association in the absence of rare-variant studies. We describe how rare-variant studies complement these approaches and highlight both data generation and statistical challenges in their interpretation. Finally, we consider how certain special study designs, such as those for families and isolated populations, fit in this paradigm.

Scaldaferri, F., et al. (2017). "Body mass index influences infliximab post-infusion levels and correlates with prospective loss of response to the drug in a cohort of inflammatory bowel disease patients under maintenance therapy with Infliximab." PLoS One **12**(10): e0186575.

Scarpa, M. and E. Stylianou (2012). "Epigenetics: Concepts and relevance to IBD pathogenesis." Inflamm Bowel Dis **18**(10): 1982-1996.

Schaedler, T. A., et al. (2015). "Structures and functions of mitochondrial ABC transporters." Biochem Soc Trans **43**(5): 943-951.

Schatton, T., et al. (2015). "ABCB5 Identifies Immunoregulatory Dermal Cells." Cell Reports **12**(10): 1564-1574.

Cell-based strategies represent a new frontier in the treatment of immune-mediated disorders. However, the paucity of markers for isolation of molecularly defined immunomodulatory cell populations poses a barrier to this field. Here, we show that ATP-binding cassette member B5 (ABCB5) identifies dermal immunoregulatory cells (DIRCs) capable of exerting therapeutic immunoregulatory functions through engagement of programmed cell death 1 (PD-1). Purified Abcb5(+) DIRCs suppressed T cell proliferation, evaded immune rejection, homed to recipient immune tissues, and induced Tregs in vivo. In fully major-histocompatibility-complex-mismatched cardiac allotransplantation models, allogeneic DIRCs significantly prolonged allograft survival. Blockade of DIRC-expressed PD-1 reversed the inhibitory effects of DIRCs on T cell activation, inhibited DIRC-dependent Treg induction, and attenuated DIRC-induced prolongation of cardiac allograft survival, indicating that DIRC immunoregulatory function is mediated, at least in part, through PD-1. Our results identify ABCB5(+) DIRCs as a distinct immunoregulatory cell population and suggest promising roles of this expandable cell subset in cellular immunotherapy.

Schechter, A., et al. (2015). "Early endoscopic, laboratory and clinical predictors of poor disease course in paediatric ulcerative colitis." Gut **64**(4): 580-588.

OBJECTIVE: Data to support treatment algorithms in ambulatory paediatric UC are scarce. We aimed to explore the 1 year outcome in an inception cohort of paediatric UC patients and to identify early predictors of good outcome that might serve as short term treatment targets. DESIGN: A chart review of 115 children with new onset UC was performed (age 11 +/- 4.1 years; 58 (50%) males; 86 (75%) extensive colitis; 70 (61%) moderate-severe disease; 63 (55%) received steroids at baseline). We assessed the Paediatric Ulcerative Colitis Activity Index (PUCAI) and laboratory variables at the time of diagnosis and at 3 months, and endoscopy at diagnosis. RESULTS: The 3 month PUCAI was the strongest predictor of 1 year sustained steroid free remission (SSFR) (area under the receiver operating characteristic curve (AUROC)=0.7 (95% CI 0.6 to 0.8) and colectomy by 2 years (AUROC=0.75 (0.6 to 0.89)). SSFR was achieved in 9/54 (17%) children who had active disease (PUCAI >/= 10) at 3 months (negative predictive value (NPV)=83%) and by 4/46 (8.6%) of those with a PUCAI score >10; (NPV=91%, positive predictive value=52%; p<0.001), implying that PUCAI >10 at 3 months has a probability of 9% for achieving SSFR versus 48% with a PUCAI value of </=10. None of the variables at baseline was predictive of SSFR or colectomy (endoscopic severity, disease extent, age, PUCAI or C reactive protein/ erythrocyte sedimentation rate/albumin/haemoglobin; all AUROC<0.6, p>0.05) but baseline PUCAI predicted subsequent acute severe colitis and the need for salvage medical therapy. CONCLUSIONS: Completeness of the early response appears more important than baseline UC severity for predicting outcome in children, and supports

using PUCAI<10 as a feasible treatment goal. Our data suggest that treatment escalation should be considered with a PUCAI value of >/= 10 at 3 months.

Scherf, U., et al. (2000). "A gene expression database for the molecular pharmacology of cancer." Nat Genet **24**(3): 236-244.

Schicho, R., et al. (2010). "Quantitative Metabolomic Profiling of Serum and Urine in DSS-Induced Ulcerative Colitis of Mice by H-1 NMR Spectroscopy." Journal of Proteome Research **9**(12): 6265-6273.

Quantitative profiling of a large number of metabolic compounds is a promising method to detect biomarkers in inflammatory bowel diseases (IBD) such as ulcerative colitis (UC) We induced an experimental form of UC in mice by treatment with dextran sulfate sodium (DSS) and characterized 53 serum and 69 urine metabolites by use of H-1 NMR spectroscopy and quantitative ("targeted) analysis to distinguish between diseased and healthy animals Hierarchical multivariate orthogonal partial least-squares (OPLS) models were developed to detect and predict separation of control and DSS treated mice DSS treatment resulted in weight loss, colonic inflammation, and increase in myeloperoxidase activity Metabolomic patterns generated from the OPLS data clearly separated DSS-treated from control mice with a slightly higher predictive power (Q(2)) for serum (0 73) than urine (0 71) During DSS colitis creatine, carnitine, and methylamines increased in urine while in serum, maximal increases were observed for ketone bodies, hypoxanthine, and tryptophan

Antioxidant metabolites decreased in urine whereas in serum, glucose and Krebs cycle intermediates decreased strongly Quantitative metabolic profiling of serum and urine thus discriminates between healthy and DSS-treated mice Analysis of serum or urine seems to be equally powerful for detecting experimental colitis, and a combined analysis offers only a minor improvement

Schicho, R., et al. (2012). "Quantitative Metabolomic Profiling of Serum, Plasma, and Urine by H-1 NMR Spectroscopy Discriminates between Patients with Inflammatory Bowel Disease and Healthy Individuals." Journal of Proteome Research **11**(6): 3344-3357.

Serologic biomarkers for inflammatory bowel disease (IBD) have yielded variable differentiating ability. Quantitative analysis of a large number of metabolites is a promising method to detect IBD biomarkers. Human subjects with active Crohn's disease (CD) and active ulcerative colitis (UC) were identified, and serum, plasma, and urine specimens were obtained. We characterized 44 serum, 37 plasma, and 71 urine metabolites by use of H-1 NMR spectroscopy and "targeted analysis" to differentiate between diseased and non-diseased individuals, as well as between the CD and UC cohorts. We used multiblock principal component analysis and hierarchical OPLS-DA for comparing several blocks derived from the same "objects" (e.g., subject) to examine differences in metabolites. In serum and plasma of IBD patients, methanol, mannose, formate, 3-methyl-2-oxovalerate, and amino acids such as isoleucine were the metabolites most prominently increased, whereas in urine, maximal increases were observed for mannitol, allantoin, xylose, and carnitine. Both

serum and plasma of UC and CD patients showed significant decreases in urea and citrate, whereas in urine, decreases were observed, among others, for betaine and hippurate. Quantitative metabolomic profiling of serum, plasma, and urine discriminates between healthy and IBD subjects. However, our results show that the metabolic differences between the CD and UC cohorts are less pronounced.

Schoepfer, A., et al. (2019). "Systematic Analysis of the Impact of Diagnostic Delay on Bowel Damage in Paediatric Versus Adult Onset Crohn's Disease." J Crohns Colitis.

BACKGROUND AND AIMS: Length of diagnostic delay is associated with bowel strictures and intestinal surgery in adult patients with Crohn's disease [CD]. Here we assessed whether diagnostic delay similarly impacts on the natural history of paediatric CD patients. METHODS: Data from the Swiss IBD Cohort Study were analysed. Frequency of CD-related complications [bowel stenosis, perianal fistula, internal fistula, any fistula, resection surgery, fistula/abscess surgery, any complication] at diagnosis and in the long term [up to 30 years after CD diagnosis] was compared between paediatric patients [diagnosed <18 years] and adult patients [diagnosed >/=18 years] using multivariate Cox proportional hazard regression modelling. RESULTS: From 2006 to 2016, 387 paediatric and 1163 adult CD patients were included. Median [interquartile range: IQR] diagnostic delay was 3 [1-9] for the paediatric and 6 [1-24] months for the adult group, respectively. Adult onset CD patients presented at diagnosis more frequently with bowel stenosis [p <0.001] and bowel surgery [p <0.001] compared with paediatric CD patients. In the long term,

length of diagnostic delay was significantly associated with bowel stenosis [p = 0.001], internal fistula [p = 0.038], and any complication [p = 0.024] in the adult onset CD population. No significant association between length of diagnostic delay and CD-related outcomes in the long term was observed in the paediatric population. CONCLUSIONS: Adult CD patients have longer diagnostic delay compared with paediatric CD patients and present at diagnosis more often with bowel stenosis and surgery. Length of diagnostic delay was found to be predictive for CD-related complications only in the adult but not in the paediatric CD population.

Schreiber, S., et al. (1998). "Activation of nuclear factor kappa B inflammatory bowel disease." Gut **42**(4): 477-484.

Schroder, M. and R. J. Kaufman (2005). "ER stress and the unfolded protein response." Mutat Res **569**(1-2): 29-63.

Schwab, M., et al. (2003). "Association between the C3435T MDR1 gene polymorphism and susceptibility for ulcerative colitis." Gastroenterology **124**(1): 26-33.

Selinger, C. P., et al. (2017). "A multi-centre audit of excess steroid use in 1176 patients with inflammatory bowel disease." Aliment Pharmacol Ther.

BACKGROUND: Corticosteroids are central to inducing remission in inflammatory bowel disease (IBD) but are ineffective maintenance agents. AIM: To benchmark steroid usage in British outpatients and assess factors associated with excess exposure. METHODS: We recorded steroid use in unselected IBD outpatients. Cases meeting criteria for steroid dependency or excess were blind peer reviewed to determine whether steroid prescriptions were avoidable. Associations between steroid use and patient/institutional factors were analysed. RESULTS: Of 1176 patients, 30% received steroids in the prior 12 months. 14.9% had steroid dependency or excess, which was more common in moderate/severe ulcerative colitis (UC) than Crohn's disease (CD) (42.6% vs 28.1%; P = .027). Steroid dependency or excess was deemed avoidable in 49.1%. The annual incidence of inappropriate steroid excess was 7.1%. Mixed-effects logistic regression analysis revealed independent predictors of inappropriate steroid excess. The odds ratio (OR, 95%CI) for moderate/severe compared to mild/quiescent disease activity was 4.59 (1.53-20.64) for UC and 4.60 (2.21-12.00) for CD. In CD, lower rates of inappropriate steroid excess were found in centres with an IBD multi-disciplinary team (OR 0.62 [0.46-0.91]), whilst dedicated IBD clinics protected against inappropriate steroid excess in UC (OR 0.64, 95% CI 0.21-0.94). The total number of GI trainees was associated with rates of inappropriate steroid excess. CONCLUSION: Steroid dependency or excess occurred in 14.9% of British IBD patients (in 7.1% potentially avoidable). We demonstrated positive effects of service configurations (IBD multi-disciplinary team, dedicated IBD clinics). Routine recording of steroid dependency or excess is feasible and should be considered a quality metric.

Senhaji, N., et al. (2015). "Genetic Polymorphisms of Multidrug Resistance Gene-1 (MDR1/ ABCB1) and Glutathione S-Transferase Gene and the Risk of Inflammatory Bowel Disease among Moroccan Patients." Mediators Inflamm **2015**: 248060.

Inflammatory bowel diseases (IBD) are multifactorial disorders resulting from environmental and genetic factors. Polymorphisms in MDR1 and GSTs genes might explain individual differences in susceptibility to IBD. We carried out a case-control study to examine the association of MDR1 (C1236T and C3435T), GSTT1, and GSTM1 polymorphisms with the risk of IBD. Subjects were genotyped using PCR-RFLP for MDR1 gene and multiplex PCR for GSTT1 and GSTM1. Meta-analysis was performed to test the association of variant allele carriage with IBD risk. We report that GSTT1 null genotype is significantly associated with the risk of CD (OR: 2.5, CI: 1.2-5, P = 0.013) and UC (OR: 3.5, CI: 1.5-8.5, P = 0.004) and can influence Crohn's disease behavior. The interaction between GSTT1 and GSTM1 genes showed that the combined null genotypes were associated with the risk of UC (OR: 3.1, CI: 1.1-9, P = 0.049). Furthermore, when compared to combined 1236CC/CT genotypes, the 1236TT genotype of MDR1 gene was associated with the risk of UC (OR: 3.7, CI: 1.3-10.7, P = 0.03). Meta-analysis demonstrated significantly higher frequencies of 3435T carriage in IBD patients. Our results show that GSTT1 null and MDR1 polymorphisms could play a role in susceptibility to IBD.

Senhaji, N., et al. (2017). "Polymorphisms in oxidative pathway related genes and susceptibility to inflammatory bowel disease." World J Gastroenterol **23**(47): 8300-8307.

Shanahan, F. (2010). "Probiotics in perspective." Gastroenterology **139**(6): 1808-1812.

Shanahan, F. (2012). "A commentary on the safety of probiotics." Gastroenterol Clin North Am **41**(4): 869-876.

Probiotics have a long record of safety, which relates primarily to lactobacilli and bifidobacteria. Experience with other forms of probiotic is more limited. There is no such thing as zero risk, particularly in the context of certain forms of host susceptibility. There is poor public understanding of the concept of risk, in general, and risk/benefit analysis, in particular. Uncertainty persists regarding the potential for transfer of antibiotic resistance with probiotics, but the risk seems to be low with currently available probiotic products. As with other forms of therapeutics, the safety of probiotics should be considered on a strain-by-strain basis.

Shaul, O. (2017). "How introns enhance gene expression." International Journal of Biochemistry & Cell Biology **91**: 145-155.

Shaw, K. A., et al. (2019). "Genetic variants and pathways implicated in a pediatric inflammatory bowel disease cohort." Genes Immun **20**(2): 131-142.

In the United States, approximately 5% of individuals with inflammatory bowel disease (IBD) are younger than 20 years old. Studies of pediatric cohorts can provide unique insights into genetic architecture of IBD, which includes Crohn's disease (CD) and ulcerative colitis (UC). Large genome-wide association studies have found more than 200 IBD-associated loci but explain a minority of disease variance for CD and UC. We sought to characterize the contribution of rare variants to disease development, comparing exome sequencing of 368 pediatric IBD patients to publicly available exome sequencing (dbGaP) and aggregate frequency data (ExAC). Using dbGaP data, we performed logistic regression for common variants and optimal unified association tests (SKAT-O) for rare, likely-deleterious variants. We further compared rare variants to ExAC counts with Fisher's exact tests. We did pathway enrichment analysis on the most significant genes from each comparison. Many variants overlapped with known IBD-associated genes (e.g. NOD2). Rare variants were enriched in CD-associated loci ($p = 0.009$) and showed suggestive enrichment in neutrophil function genes ($p = 0.05$). Pathway enrichment implicated immune-related pathways, especially cell killing and apoptosis. Variants in extracellular matrix genes also emerged as an important theme in our analysis.

Sheehan, D. and F. Shanahan (2017). "The Gut Microbiota in Inflammatory Bowel Disease." Gastroenterol Clin North Am **46**(1): 143-154.

Genes, bacteria, and immunity contribute to the pathogenesis of inflammatory bowel disease. Most genetic risk relates to defective sensing of microbes and their metabolites or defective regulation of the host response to the microbiota. Because the composition of the microbiota shapes the developing immune system and is determined in early life, the prospect of therapeutic manipulation of the microbiota in adulthood after the onset of disease is questionable. However, the microbiota may be a marker of risk and a modifier of disease activity and a contributor to extraintestinal manifestations and associations in some patients with inflammatory bowel disease.

Shouval, D. S., et al. (2018). "The Treatment of Inflammatory Bowel Disease in Patients with Selected Primary Immunodeficiencies." J Clin Immunol **38**(5): 579-588.

Sigall Boneh, R., et al. (2017). "Dietary Therapy with the Crohn's Disease Exclusion Diet is a Successful Strategy for Induction of Remission in Children and Adults Failing Biological Therapy." J Crohns Colitis.

Background: Loss of response (LoR) to biologics in Crohn's disease (CD) is a significant clinical problem. Dietary therapy as a treatment strategy in this setting has not been previously reported. We report the use of dietary strategies using enteral nutrition coupled with the Crohn's Disease Exclusion Diet (CDED) for LoR to infliximab or adalimumab as a single center experience. Methods: Patients with LoR to a biologic despite dose escalation or combination therapy were treated with partial enteral nutrition (PEN) by a polymeric formula and the CDED for 12 weeks.

Paediatric patients with severe flares received 14 days of exclusive enteral nutrition followed by PEN+CDED as above. All patients were seen at week 6 & 12 for follow up. Current and prior treatment, Harvey Bradshaw Index (HBI), CRP and albumin were recorded. Remission was defined as HBI <5 at week 6. Results: Twenty one patients, mean age 22.1+/- 8.9 years. (11 adults and 10 children) met study criteria. Seventeen patients (81%) had used combination therapy, 10/21 (47.6%) had failed a second biologic. Seven patients had a prior intestinal resection. Dose escalation had failed in 13/21 (62%) patients. Clinical remission by PGA & HBI after 6 weeks was obtained in 13/21 (61.9%). Mean HBI decreased from 9.4 to 2.6+/-3.8 ($p<0.001$), mean CRP decreased from 2.8+/- 3.4 to 0.7+/-0.5 ($p=0.005$) and mean albumin increased from 3.5+/-0.6 to 3.8+/-0.5 ($p=0.06$). Conclusion: Dietary treatment combining partial enteral nutrition with the CDED may be a useful salvage regimen for patients failing biological therapy despite dose escalation.

Silverberg, M. S., et al. (2005). "Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a Working Party of the 2005 Montreal World Congress of Gastroenterology." Can J Gastroenterol **19 Suppl A**: 5A-36A.

The discovery of a series of genetic and serological markers associated with disease susceptibility and phenotype in inflammatory bowel disease has led to the prospect of an integrated classification system involving clinical, serological and genetic parameters. The Working Party has reviewed current clinical classification systems in Crohn's disease, ulcerative colitis and indeterminate colitis, and provided

recommendations for clinical classification in practice. Progress with respect to integrating serological and genetic markers has been examined in detail, and the implications are discussed. While an integrated system is not proposed for clinical use at present, the introduction of a widely acceptable clinical subclassification is strongly advocated, which would allow detailed correlations among serotype, genotype and clinical phenotype to be examined and confirmed in independent cohorts of patients and, thereby, provide a vital foundation for future work.

Simpson, H. L., et al. (2014). "IBD: microbiota manipulation through diet and modified bacteria." Dig Dis **32 Suppl 1**: 18-25.

BACKGROUND/AIMS: Crohn's disease (CD) and ulcerative colitis (UC) are both typified by an altered intestinal microbiota, and gene associations imply various defects in the mucosal barrier and in the innate immune response to bacteria. This review aims to assess how alterations in diet or use of modified bacteria could have therapeutic effects in CD or UC. METHODS: A MEDLINE search using the terms 'prebiotic', 'genetically modified bacteria', 'mucosal barrier in association with ulcerative colitis', 'Crohn's disease' or 'microbiota'. RESULTS: A large body of data from in vitro and animal studies shows promise for therapeutic approaches that target the microbiota. Approaches include dietary supplementation with fermentable fibres (prebiotics) and soluble fibres that block bacterial-epithelial adherence (contrabiotics), enhancement of the mucosal barrier with phosphatidylcholine, and use of genetically modified bacteria that express IL-10 or protease inhibitors. Vitamin D

supplementation also shows promise, acting via enhancement of innate immunity. Clinical trials have shown benefit with enterically delivered phosphatidylcholine supplementation in UC and near-significant benefit with vitamin D supplementation in CD. CONCLUSION: Strategies that target the microbiota or the host defence against it appear to be good prospects for therapy and deserve greater investment.

Slager, S. L., et al. (2003). "Candidate-gene association studies with pedigree data: controlling for environmental covariates." Genet Epidemiol **24**(4): 273-283.

Smids, C., et al. (2017). "The value of serum antibodies in differentiating inflammatory bowel disease, predicting disease activity and disease course in the newly diagnosed patient." Scand J Gastroenterol **52**(10): 1104-1112.

BACKGROUND: Data on serum antibodies in untreated adult inflammatory bowel disease (IBD) patients at diagnosis are scarcely available, and results on the stability of antibody presence over time are inconsistent. Our aim was to investigate antibodies in newly diagnosed, untreated IBD patients in relation to disease phenotype and course. Furthermore, we analyzed antibody presence over time. METHODS: Baseline anti-Saccharomyces cerevisiae antibodies (ASCA), anti-chitobioside carbohydrate antibodies (ACCA), anti-laminaribioside carbohydrate antibodies (ALCA) and anti-mannobioside carbohydrate antibodies (AMCA) were measured with enzyme-linked immunosorbent assays and perinuclear anti-neutrophilic cytoplasmic antibodies (pANCA) was measured by indirect immunofluorescence in serum of 120 untreated

IBD patients at diagnosis and 19 healthy controls. Antibodies were related to disease outcomes. Serial measurements were available in 71 patients. RESULTS: The combination of pANCA and ASCA enabled good discrimination between UC and CD (p = .004). Antibody presence was relatively stable over time, even though there were significant changes in concentrations. There was a trend towards larger fluctuations in concentration with immunosuppressive medication. Baseline pANCA in UC patients correlated with calprotectin values (rho = .545, p = .019) and change in pANCA status over time was associated with disease activity at that moment. No associations were found with antibodies at diagnosis and disease outcomes. CONCLUSION: Antibody profiles at diagnosis support the distinction between CD and UC. Anti-glycan antibodies are reasonably stable over time, but may fluctuate under the influence of immunosuppressive treatment which may explain the inconsistency in findings hitherto. The appearance or disappearance of pANCA antibodies during follow-up correlated with disease activity in UC and may be used in disease monitoring.

Smith, M. M., Marinaki A., Sanderson J. (2010). "Genetic polymorphisms in thr multi-drug resistance 5 gene is associated with non-response to azathioprine in inflammatory bowel disease." United European Week Journal.

Sokol, H., et al. (2008). "Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients." Proc Natl Acad Sci U S A **105**(43): 16731-16736.

A decrease in the abundance and biodiversity of intestinal bacteria within the dominant phylum Firmicutes has been observed repeatedly in Crohn disease (CD) patients. In this study, we determined the composition of the mucosa-associated microbiota of CD patients at the time of surgical resection and 6 months later using FISH analysis. We found that a reduction of a major member of Firmicutes, Faecalibacterium prausnitzii, is associated with a higher risk of postoperative recurrence of ileal CD. A lower proportion of F. prausnitzii on resected ileal Crohn mucosa also was associated with endoscopic recurrence at 6 months. To evaluate the immunomodulatory properties of F. prausnitzii we analyzed the anti-inflammatory effects of F. prausnitzii in both in vitro (cellular models) and in vivo [2,4,6-trinitrobenzenesulphonic acid (TNBS)-induced] colitis in mice. In Caco-2 cells transfected with a reporter gene for NF-kappaB activity, F. prausnitzii had no effect on IL-1beta-induced NF-kappaB activity, whereas the supernatant abolished it. In vitro peripheral blood mononuclear cell stimulation by F. prausnitzii led to significantly lower IL-12 and IFN-gamma production levels and higher secretion of IL-10. Oral administration of either live F. prausnitzii or its supernatant markedly reduced the severity of TNBS colitis and tended to correct the dysbiosis associated with TNBS colitis, as demonstrated by real-time quantitative PCR (qPCR) analysis. F. prausnitzii exhibits anti-inflammatory effects on cellular and TNBS colitis models, partly due to secreted metabolites able to block NF-kappaB activation and IL-8 production. These results suggest that counterbalancing dysbiosis using F. prausnitzii as a probiotic is a promising strategy in CD treatment.

Song, R., et al. (2018). "Identification and analysis of key genes associated with ulcerative colitis based on DNA microarray data." Medicine (Baltimore) **97**(21): e10658.

Soranzo, N., et al. (2004). "Identifying candidate causal variants responsible for altered activity of the ABCB1 multidrug resistance gene." Genome Research **14**(7): 1333-1344.

Stein, U., et al. (2012). "Impact of mutant beta-catenin on ABCB1 expression and therapy response in colon cancer cells." Br J Cancer **106**(8): 1395-1405.

Steinhart, A. H., et al. (2003). "Corticosteroids for maintenance of remission in Crohn's disease." Cochrane Database Syst Rev(4): CD000301.

Sullivan, K. J., et al. (2017). "Value of upper endoscopic biopsies in predicting medical refractoriness in pediatric patients with ulcerative colitis." Hum Pathol **66**: 167-176.

Refractory ulcerative colitis (UC) occurs in patients who experience a severe disease manifestation that is unresponsive to medical therapy. The assessment of upper endoscopic microscopic findings and its correlation with refractory UC has not been fully studied in pediatric patients and is the focus of this study. Medical records of UC patients treated at a tertiary pediatric center between 2000 and 2014 were reviewed. Endoscopic biopsies of the upper gastrointestinal (GI) tract of patients meeting a

priori inclusion criteria were compared between refractory UC patients and nonrefractory UC patients for active inflammation. Statistically significant differences were determined between groups, and tissues shown to have significant differences were further evaluated for their diagnostic performance. A total of 52 patients were included, 26 in each group. Significant differences were observed in intraepithelial neutrophil infiltration and percentage involvement of crypts/glands for the antrum, body, and duodenal bulb (P </= .001, .005, and .01 [intraepithelial neutrophil infiltration] and P = .001, .009, and .015 [% involvement], respectively). Microabscesses of mucosal glands/crypts were also experienced in a greater number of refractory UC patients in the stomach (ie, antrum and/or body of stomach; P = . 005) and duodenum (ie, duodenum and/or duodenal bulb; P = .023). The sensitivity and specificity of upper GI tissues to predict refractory UC were moderate, with sensitivities ranging from 38% to 67% and specificities ranging from 81% to 100%. Our results suggest that children with refractory UC are more likely to have active inflammation in the upper GI tract, and thus, this may represent a predictor of responsiveness to current medical therapy.

Sutiman, N., et al. (2018). "Predictive role of NUDT15 variants on thiopurine-induced myelotoxicity in Asian inflammatory bowel disease patients." Pharmacogenomics **19**(1): 31-43.

Taipalensuu, J., et al. (2001). "Correlation of gene expression of ten drug efflux proteins of the ATP-binding cassette transporter family in normal human jejunum and in human intestinal epithelial Caco-2 cell monolayers." Journal of Pharmacology and Experimental Therapeutics **299**(1): 164-170.

This investigation describes the expression and interindividual variability in transcript levels of multiple drug efflux systems in the human jejunum and compares the expression profiles in these cells with that of the commonly used Caco-2 cell drug absorption model. Transcript levels of ten-drug efflux proteins of the ATP-binding cassette (ABC) transporter family [MDR1, MDR3, ABCB5, MRP1-6, and breast cancer resistance protein (BCRP)], lung resistance-related protein (LRP), and CYP3A4 were determined using quantitative polymerase chain reaction in jejunal biopsies from 13 healthy human subjects and in Caco-2 cells. All genes except ABCB5 were expressed, and transcript levels varied between individuals only by a factor of 2 to 3. Surprisingly, BCRP and MRP2 transcripts were more abundant in jejunum than MDR1 transcripts. Jejunal transcript levels of the different ABC transporters spanned a range of three log units with the rank order: BCRP approximately MRP2 > MDR1 approximately MRP3 approximately MRP6 approximately MRP5 approximately MRP1 > MRP4 > MDR3. Furthermore, transcript levels of 9 of 10 ABC transporters correlated well between jejunum and Caco-2 cells (r2 = 0.90). However, BCRP exhibited a 100-fold lower transcript level in Caco-2 cells compared with jejunum. Thus, the expression of a number of efflux protein transcripts in jejunum are equal to, or even higher than, that of MDR1, suggesting that the roles of these proteins (in particular BCRP and MRP2) in

intestinal drug efflux have been underestimated. Also, we tentatively conclude that the Caco-2 cell line is a useful model of jejunal drug efflux, if the low expression of BCRP is taken into account.

Tamboli, C. P., et al. (2003). "Probiotics in inflammatory bowel disease: a critical review." Best Pract Res Clin Gastroenterol **17**(5): 805-820.

Intestinal bacteria play a key role in inflammatory bowel disease. Probiotics attempt to modify disease by favourably altering bacterial composition, immune status, and inflammation. Until recently, probiotic therapy was considered 'folk' medicine, but there now is emerging interest on the part of the general public and scientific communities in the use of probiotics in human disease. This practical, evidence-based review examines probiotics as therapy for inflammatory bowel disease in humans. There are very few such published randomized clinical trials, but some data exist that possibly show an efficacy of probiotics as maintenance therapy in chronic relapsing pouchitis. Obstacles to providing probiotic therapy include selection of appropriate strains, poorly regulated probiotic quality standardization, processing and human biologic factors which impair probiotic viability, difficulty in maintaining new bacterial populations in the gut, and local product unavailability. Studies have focused on specific inflammatory bowel disease subgroups, limiting general applicability for the practitioner. Basic research highlights the importance of bacteria in these conditions, and the possibility that probiotics will modify physiological parameters.

Well-designed, randomized clinical studies are still required to define the role of probiotics as therapeutic agents in inflammatory bowel disease.

Tang, K. H., et al. (2012). "CD133(+) liver tumor-initiating cells promote tumor angiogenesis, growth, and self-renewal through neurotensin/interleukin-8/CXCL1 signaling." Hepatology **55**(3): 807-820.

UNLABELLED: A novel theory in the field of tumor biology postulates that cancer growth is driven by a population of stem-like cells, called tumor-initiating cells (TICs). We previously identified a TIC population derived from hepatocellular carcinoma (HCC) that is characterized by membrane expression of CD133. Here, we describe a novel mechanism by which these cells mediate tumor growth and angiogenesis by systematic comparison of the gene expression profiles between sorted CD133 liver subpopulations through genome-wide microarray analysis. A significantly dysregulated interleukin-8 (IL-8) signaling network was identified in CD133(+) liver TICs obtained from HCC clinical samples and cell lines. IL-8 was found to be overexpressed at both the genomic and proteomic levels in CD133(+) cells isolated from HCC cell lines or clinical samples. Functional studies found enhanced IL-8 secretion in CD133(+) liver TICs to exhibit a greater ability to self-renew, induce tumor angiogenesis, and initiate tumors. In further support of these observations, IL-8 repression in CD133(+) liver TICs by knockdown or neutralizing antibody abolished these effects. Subsequent studies of the IL-8 functional network identified neurotensin (NTS) and CXCL1 to be preferentially expressed in CD133(+)

liver TICs. Addition of exogenous NTS resulted in concomitant up-regulation of IL-8 and CXCL1 with simultaneous activation of p-ERK1/2 and RAF-1, both key components of the mitogen-activated protein kinase (MAPK) pathway. Enhanced IL-8 secretion by CD133(+) liver TICs can in turn activate an IL-8-dependent feedback loop that signals through the MAPK pathway. Further, in its role as a liver TIC marker CD133 also plays a functional part in regulating tumorigenesis of liver TICs by way of regulating NTS, IL-8, CXCL1, and MAPK signaling. CONCLUSION: CD133(+) liver TICs promote angiogenesis, tumorigenesis, and self-renewal through NTS-induced activation of the IL-8 signaling cascade.

Taylor, C. R. and R. M. Levenson (2006). "Quantification of immunohistochemistry--issues concerning methods, utility and semiquantitative assessment II." Histopathology **49**(4): 411-424.

Tennessen, J. A., et al. (2011). "The promise and limitations of population exomics for human evolution studies." Genome Biology **12**(9): 127.

Exome sequencing is poised to yield substantial insights into human genetic variation and evolutionary history, but there are significant challenges to overcome before this becomes a reality.

The UniProt, C. (2017). "UniProt: the universal protein knowledgebase." Nucleic Acids Res **45**(D1): D158-D169.

Thibault, R., et al. (2010). "Butyrate utilization by the colonic mucosa in inflammatory bowel diseases: a transport deficiency." Inflamm Bowel Dis **16**(4): 684-695.

The short-chain fatty acid butyrate, which is mainly produced in the lumen of the large intestine by the fermentation of dietary fibers, plays a major role in the physiology of the colonic mucosa. It is also the major energy source for the colonocyte. Numerous studies have reported that butyrate metabolism is impaired in intestinal inflamed mucosa of patients with inflammatory bowel disease (IBD). The data of butyrate oxidation in normal and inflamed colonic tissues depend on several factors, such as the methodology or the models used or the intensity of inflammation. The putative mechanisms involved in butyrate oxidation impairment may include a defect in beta oxidation, luminal compounds interfering with butyrate metabolism, changes in luminal butyrate concentrations or pH, and a defect in butyrate transport. Recent data show that butyrate deficiency results from the reduction of butyrate uptake by the inflamed mucosa through downregulation of the monocarboxylate transporter MCT1. The concomitant induction of the glucose transporter GLUT1 suggests that inflammation could induce a metabolic switch from butyrate to glucose oxidation. Butyrate transport deficiency is expected to have clinical consequences. Particularly, the reduction of the intracellular availability of butyrate in colonocytes may decrease its protective effects toward cancer in IBD patients.

Thul, P. J., et al. (2017). "A subcellular map of the human proteome." Science **356**(6340).

Tighe, D., et al. (2017). "One-Year Clinical Outcomes in an IBD Cohort Who Have Previously Had Anti-TNFa Trough and Antibody Levels Assessed." Inflamm Bowel Dis **23**(7): 1154-1159.

BACKGROUND: Loss of response (LOR) is a big concern for anti-TNFa therapies in inflammatory bowel disease. Immunomonitoring may be useful to optimize response rates and overcome secondary LOR. METHODS: This was an observational retrospective cohort study of a group of patients with inflammatory bowel disease on infliximab (IFX) and adalimumab (ADA) who had anti-TNFa trough and antibody levels measured, during maintenance phase of treatment. Anti-TNFa trough and antibody levels were measured using standard enzyme-linked immunosorbent assay techniques. Baseline patient characteristics were determined and patients were reviewed 1 year later. Clinical assessment took place with partial Mayo scores for ulcerative colitis and Harvey-Bradshaw index for Crohn's disease. C-reactive protein (CRP) and albumin were also measured. Poor outcomes were defined as the following: need for rescue steroids, dose intensification, surgery, or treatment discontinuation. RESULTS: Seventy-four patients were included in the study, 37 (50%) were female, mean age 41 years, 61 (82%) had Crohn's disease, and 42 (57%) ulcerative colitis. Forty-two (57%) patients received IFX and 32 (43%) ADA. Mean IFX trough was 3.6 mug/mL and mean ADA troughs were 3.78 mug/mL. Twenty-

seven percent of patients (n = 20) overall had a poor outcome, with a similar proportion in each group 24% (n = 10) IFX and 31% (n = 10) ADA (P value 0.24). Of the cohort, 14.2% (6/42) treated with IFX had subtherapeutic trough levels, 6.2% (2/32) of ADA patients had a trough level <1 mug/mL (P value = 0.273) There was no difference in mean trough according to outcome (4.9 mug/mL poor versus 5.4 mug/mL good, P value 0.14). Low IFX trough levels did correlate with high CRP, low albumin and response rates, mean CRP 6.66 mug/mL (n = 3), mean albumin 37 g/L for patients with low trough levels and poor response versus CRP 2.0 mug/mL (n = 24), mean albumin 43 g/L for patients with high trough levels and good response (P = 0.009, 95% confidence interval, -0.78 to -0.12). CONCLUSIONS: LOR is still a big concern with anti-TNFa therapies. Stand-alone anti-TNFa trough and antibody levels are not useful at predicting LOR/disease progression at 1 year, but low trough levels do correlate well with elevated CRP, hypoalbuminaemia, and poor response rates.

Timmer, A., et al. (2017). "Current health status and medical therapy of patients with pediatric-onset inflammatory bowel disease: a survey-based analysis on 1280 patients aged 10-25 years focusing on differences by age of onset." Eur J Gastroenterol Hepatol.

OBJECTIVE: There are inconsistent reports on age-related differences in inflammatory bowel disease (IBD). On the basis of patient information, we describe the clinical presentation and therapy in relation to age at diagnosis in longstanding pediatric IBD. PATIENTS AND METHODS: Two surveys were conducted in children and young adults (age: 10-25 years) by pretested postal questionnaires. The

main analyses are descriptive, showing proportions and distributions per grouped age of diagnosis. Exploratory logistic regression was used to identify sociodemographic and disease-related factors associated with prognosis. Recent disease course, use of biological therapy, and resecting surgery were chosen as indicators of disease severity. Patients with a diagnosis in infancy (<2 years of age) are presented as a case series. RESULTS: Information of 1280 cases was available [804 Crohn's disease (CD), 382 ulcerative colitis (UC), 94 IBD not specified] (response: 44.6 and 49.6%). Stable remission during the preceding year was reported by 675 (56.7%) patients; 825 (60.9%) patients reported feeling currenty well. Anti-tumor necrosis factor therapy was reported by 33% of CD patients and 9.3% of UC patients, immunomodulation in 82.1 and 63.2%, and corticosteroids by 78.4 and 76.1%, respectively (ever use). Age at diagnosis was not associated with indicators of severe disease. Diagnosis in infancy was reported by 37 patients. CONCLUSION: Our data do not support age at diagnosis-related differences in prognosis in pediatric-onset IBD.

Tomiyasu, H., et al. (2013). "Regulation of expression of ABCB1 and LRP genes by mitogen-activated protein kinase/extracellular signal-regulated kinase pathway and its role in generation of side population cells in canine lymphoma cell lines." Leuk Lymphoma **54**(6): 1309-1315.

The concept of the cancer stem cell (CSC) has been recognized as key for elucidation of the mechanisms that confer the multidrug resistance (MDR) phenotype to tumor cells, and the side population (SP) fraction has been shown to be enriched by cells

with the CSC phenotype. The purpose of the present study was to identify the mechanism that induces a difference of phenotype between the SP and the remaining major population (MP) using two canine lymphoma cell lines. Expression levels of ABCB1 and LRP genes, which encode efflux pumps, were significantly higher in the SP than in the MP. Microarray analysis revealed up-regulation of the expression of transforming growth factor-beta (TGF-beta) type II receptor in SP compared with MP, and the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathway was more up-regulated in the SP than in the MP. Stimulation of the MAPK/ERK pathway significantly increased the mRNA expression of both ABCB1 and LRP genes. These results indicate increased expression of the efflux pumps through the MAPK/ERK pathway in SP cells.

Torok, H. P., et al. (2017). "Functional Toll-Like Receptor (TLR)2 polymorphisms in the susceptibility to inflammatory bowel disease." PLoS One **12**(4): e0175180.

Toyonaga, T., et al. (2017). "Usefulness of fecal calprotectin for the early prediction of short-term outcomes of remission-induction treatments in ulcerative colitis in comparison with two-item patient-reported outcome." PLoS One **12**(9): e0185131.

BACKGROUND: Fecal calprotectin (FC) is well accepted as a non-invasive biomarker which objectively reflects colonic inflammation in ulcerative colitis (UC). However, its value as a marker of response during the early phase of remission induction treatment has not been well studied. The aim of this study is to evaluate the

significance of FC for predicting the short-term outcomes of remission induction treatment in patients with UC. METHODS: A prospective observational study was conducted among 31 patients with active UC. FC was monitored with two-item patient-reported outcome (PRO2), partial Mayo score (PMS), and Lichtiger clinical activity index (CAI) during the first 4 weeks of remission induction treatment. Clinical response was defined as a decrease in CAI of 3 or more points below baseline. Mucosal healing (MH) was defined as Mayo endoscopic subscore 0 or 1. Within-day and within-stool variability of FC were assessed during the first week of treatment. RESULTS: In week 4-clinical responders, PRO2, PMS, and CAI significantly decreased from day 3, however, FC did not show significant reduction until week 2. Among all markers, the decrease in PRO2 at week 4 most accurately predicted MH at week 12. Within-day variability of FC was remarkably wide even at the first week in clinical responders. Within-stool variability was extremely small. CONCLUSIONS: PRO2 predicted the short-term outcomes of remission induction treatment earlier than FC possibly because of the wide within-day variability of FC in active UC.

Triadafilopoulos, G. (2014). "Glucocorticoid therapy for gastrointestinal diseases." Expert Opin Drug Saf **13**(5): 563-572.

Truelove, S. C. and D. P. Jewell (1974). "Intensive Intravenous Regimen for Severe Attacks of Ulcerative-Colitis." Lancet **1**(7866): 1067-1070.

Trumpi, K., et al. (2015). "ABC-Transporter Expression Does Not Correlate with Response to Irinotecan in Patients with Metastatic Colorectal Cancer." J Cancer **6**(11): 1079-1086.

Tung, J., et al. (2006). "A population-based study of the frequency of corticosteroid resistance and dependence in pediatric patients with Crohn's disease and ulcerative colitis." Inflamm Bowel Dis **12**(12): 1093-1100.

BACKGROUND: The goal of this study was to examine the 1-year outcome after the first course of systemic corticosteroids in an inception cohort of pediatric patients with inflammatory bowel disease. METHODS: All Olmsted County (Minnesota) residents diagnosed with Crohn's disease (n = 50) or ulcerative colitis (n = 36) before 19 years of age from 1940 to 2001 were identified. Outcomes at 30 days and 1 year after the initial course of corticosteroids were recorded. RESULTS: Twenty-six patients with Crohn's disease (65%) and 14 with ulcerative colitis (44%) were treated with corticosteroids before age 19. Thirty-day outcomes for corticosteroid-treated Crohn's disease were complete remission in 16 (62%), partial remission in 7 (27%), and no response in 3 (12%), with 2 of these patients requiring surgery. Thirty-day outcomes for treated ulcerative colitis were complete remission in 7 (50%), partial remission in 4 (29%), and no response in 3 (21%). One-year outcomes for Crohn's disease were prolonged response in 11 (42%) and corticosteroid dependence in 8 (31%), whereas 7 (27%) were postsurgical. One-year outcomes for ulcerative colitis were prolonged response in 8 (57%) and corticosteroid dependence in 2 (14%),

whereas 4 (29%) were postsurgical. CONCLUSIONS: Most pediatric patients with inflammatory bowel disease initially responded to corticosteroids. However, after 1 year, 58% of pediatric patients with Crohn's disease and 43% of pediatric patients with ulcerative colitis either were steroid dependent or required surgery. This finding emphasizes the need for early steroid-sparing medications in pediatric inflammatory bowel disease.

Turner, D., et al. (2012). "Management of pediatric ulcerative colitis: joint ECCO and ESPGHAN evidence-based consensus guidelines." J Pediatr Gastroenterol Nutr **55**(3): 340-361.

Turner, D., et al. (2007). "Development, validation, and evaluation of a pediatric ulcerative colitis activity index: a prospective multicenter study." Gastroenterology **133**(2): 423-432.

Turner, D., et al. (2018). "Management of Paediatric Ulcerative Colitis, Part 1: Ambulatory Care- an Evidence-Based Guideline from ECCO and ESPGHAN." J Pediatr Gastroenterol Nutr.

Tusnady, G. E., et al. (2006). "Membrane topology of human ABC proteins." FEBS Lett **580**(4): 1017-1022.

In this review, we summarize the currently available information on the membrane topology of some key members of the human ABC protein subfamilies, and present the predicted domain arrangements. In the lack of high-resolution structures for eukaryotic ABC transporters this topology is based only on prediction algorithms and biochemical data for the location of various segments of the polypeptide chain, relative to the membrane. We suggest that topology models generated by the available prediction methods should only be used as guidelines to provide a basis of experimental strategies for the elucidation of the membrane topology.

Ufer, M., et al. (2008). "Influence of CYP3A4, CYP3A5, and ABCB1 genotype and expression on budesonide pharmacokinetics: a possible role of intestinal CYP3A4 expression." Clin Pharmacol Ther **84**(1): 43-46.

Ufer, M., et al. (2009). "Decreased sigmoidal ABCB1 (P-glycoprotein) expression in ulcerative colitis is associated with disease activity." Pharmacogenomics **10**(12): 1941-1953.

AIMS: The modulation of the intestinal expression of detoxifying proteins by relevant transcription factors, intracellular receptors and cytokines in ulcerative colitis (UC) is poorly understood. Here, we compared the intestinal expression of drug transporters, metabolizing enzymes and putative regulatory genes between inflamed and noninflamed tissue and studied their modulation by disease activity. MATERIALS & METHODS: Sigmoidal biopsies of 18 UC patients and 18 healthy volunteers matched for age, gender and ABCB1 3435C>T genotype were investigated for mRNA

expression levels of 43 systematically selected candidate genes by low-density array real-time PCR. Additionally, the ABCB1 gene product P-glycoprotein was visualized by immunohistochemistry and quantified by western blotting. Disease phenotype was categorized by clinical, endoscopic and histopathological examination. Disease activity was quantified by clinical activity index. RESULTS: In inflamed sigmoidal tissue from UC patients, 11 genes (NAT1, NR2B1, CEBPB, IFG, IL8, IL10, S100A12, SPP1, DEFA5, DEFA6 and HAMP) were overexpressed. By contrast, only the major human efflux transporter ABCB1 showed significantly lower expression levels, that were inversely correlated with those of certain antimicrobial peptides (DEFA5/6) and cytokines (IL1beta and IL8). Cell culture experiments revealed a time-dependent decrease of ABCB1 expression upon IL8 exposure. Disease activity profoundly modified ABCB1 expression, indicated by an inverse correlation of clinical activity index values with ABCB1 mRNA expression ($r = -0.603$; $p = 0.017$) and markedly reduced protein expression in UC patients with moderate and severe symptomology ($p = 0.011$). CONCLUSION: Cytokine-mediated downregulation of the major human efflux transporter ABCB1 in inflamed intestine of UC patients is presumably dependent on disease activity, with a possible contribution from IL8.

Uhlig, H. H., et al. (2014). "The diagnostic approach to monogenic very early onset inflammatory bowel disease." Gastroenterology **147**(5): 990-1007 e1003.

Uno, T., et al. (2018). "Metabolism of steroids by cytochrome P450 2C9 variants." Biopharm Drug Dispos.

Vancamelbeke, M., et al. (2017). "Genetic and Transcriptomic Bases of Intestinal Epithelial Barrier Dysfunction in Inflammatory Bowel Disease." Inflamm Bowel Dis **23**(10): 1718-1729.

BACKGROUND: Intestinal barrier defects are common in patients with inflammatory bowel disease (IBD). To identify which components could underlie these changes, we performed an in-depth analysis of epithelial barrier genes in IBD. METHODS: A set of 128 intestinal barrier genes was selected. Polygenic risk scores were generated based on selected barrier gene variants that were associated with Crohn's disease (CD) or ulcerative colitis (UC) in our study. Gene expression was analyzed using microarray and quantitative reverse transcription polymerase chain reaction. Influence of barrier gene variants on expression was studied by cis-expression quantitative trait loci mapping and comparing patients with low- and high-risk scores. RESULTS: Barrier risk scores were significantly higher in patients with IBD than controls. At single-gene level, the associated barrier single-nucleotide polymorphisms were most significantly enriched in PTGER4 for CD and HNF4A for UC. As a group, the regulating proteins were most enriched for CD and UC. Expression analysis showed that many epithelial barrier genes were significantly dysregulated in active CD and UC, with overrepresentation of mucus layer genes. In uninflamed CD ileum and IBD colon, most barrier gene levels restored to normal,

except for MUC1 and MUC4 that remained persistently increased compared with controls. Expression levels did not depend on cis-regulatory variants nor combined genetic risk. CONCLUSIONS: We found genetic and transcriptomic dysregulations of key epithelial barrier genes and components in IBD. Of these, we believe that mucus genes, in particular MUC1 and MUC4, play an essential role in the pathogenesis of IBD and could represent interesting targets for treatment.

Vandevyver, S., et al. (2013). "New Insights into the Anti-inflammatory Mechanisms of Glucocorticoids: An Emerging Role for Glucocorticoid-Receptor-Mediated Transactivation." Endocrinology **154**(3): 993-1007.

Vasquez-Moctezuma, I., et al. (2010). "ATP-binding cassette transporter ABCB5 gene is expressed with variability in malignant melanoma." Actas Dermosifiliogr **101**(4): 341-348.

Venkateswaran, S., et al. (2018). "Enhanced Contribution of HLA in Pediatric Onset Ulcerative Colitis." Inflamm Bowel Dis **24**(4): 829-838.

Volpicelli, E. R., et al. (2014). "The multidrug-resistance transporter ABCB5 is expressed in human placenta." Int J Gynecol Pathol **33**(1): 45-51.

ATP-binding cassette (ABC) transporters in placenta protectively transport drugs and xenobiotics. ABCB5 [subfamily B (MDR/TAP)] is a novel ABC multidrug-resistance transporter that also mediates cell fusion, stem cell function, and vasculogenic plasticity. Immunohistochemistry and double-labeling immunofluorescence staining for ABCB5 and ABCB5/CD200, respectively, was performed on formalin-fixed, paraffin-embedded placental tissue from 5 first trimester, 5 second trimester, and 5 term pregnancies as well as 5 partial moles, and 5 complete moles. In addition, tumor cells from 5 choriocarcinoma and 5 placental site trophoblastic tumor cases were examined. ABCB5 staining was observed in villous trophoblasts in 100% (5/5) of first trimester placentas (with progressive decrease in term placentas); 100% of partial moles (5/5); and 100% of complete moles (5/5). Notably, reactivity was discretely restricted to the inner trophoblast layer, with no staining of overlying syncytiotrophoblast. Antibody specificity and localization was confirmed further by in situ hybridization. ABCB5 expression was retained in 20% of choriocarcinomas (1/5) and 40% of placental site trophoblastic tumors (2/5). Prior studies have localized expression of multidrug-resistance-1, also known as ABCB1, within the syncytiotrophoblast of early placentas, where it serves a protective function as an efflux transporter. Our results show that ABCB5 is preferentially expressed in the cytotrophoblast layer of placental villi. The expression of this novel biomarker at the maternal-fetal interface raises questions on its role in placental structure and function as well as on its potential contribution to the protective efflux provided by other P-glycoprotein transporters.

Voskuil, M. D., et al. (2019). "Predicting (side) effects for patients with inflammatory bowel disease: The promise of pharmacogenetics." World J Gastroenterol **25**(21): 2539-2548.

Inflammatory bowel disease (IBD) is a chronic and heterogeneous intestinal inflammatory disorder. The medical management of IBD aims for long-lasting disease remission to prevent complications and disease progression. Early introduction of immunosuppression forms the mainstay of medical IBD management. Large inter-individual variability in drug responses, in terms of both efficacy and toxicity, leads to high rates of therapeutic failure in the management of IBD. Better patient stratification is needed to maximize patient benefit and minimize the harm caused by adverse events. Pre-treatment pharmacogenetic testing has the potential to optimize drug selection and dose, and to minimize harm caused by adverse drug reactions. In addition, optimizing the use of cheap conventional drugs, and avoiding expensive ineffective drugs, will lead to a significant reduction in costs. Genetic variation in both TPMT and NUDT15, genes involved in thiopurine metabolism, is associated to an increased risk of thiopurine-induced myelosuppression. Moreover, specific HLA haplotypes confer risk to thiopurine-induced pancreatitis and to immunogenicity to tumor necrosis factor-antagonists, respectively. Falling costs and increased availability of genetic tests allow for the incorporation of pre-treatment genetic tests into clinical IBD management guidelines. In this paper, we review clinically useful pharmacogenetic associations for individualized treatment of patients with IBD and discuss the path from identification of a predictive pharmacogenetic marker to implementation into IBD clinical care.

Walters, T. D., et al. (2014). "Increased effectiveness of early therapy with anti-tumor necrosis factor-alpha vs an immunomodulator in children with Crohn's disease." Gastroenterology **146**(2): 383-391.

BACKGROUND & AIMS: Standard therapy for children newly diagnosed with Crohn's disease (CD) includes early administration of immunomodulators after initial treatment with corticosteroids. We compared the effectiveness of early (</=3 mo after diagnosis) treatment with an anti-tumor necrosis factor (TNF)alpha with that of an immunomodulator in attaining clinical remission and facilitating growth of pediatric patients. METHODS: We analyzed data from the RISK study, an observational research program that enrolled patients younger than age 17 diagnosed with inflammatory (nonpenetrating, nonstricturing) CD from 2008 through 2012 at 28 pediatric gastroenterology centers in North America. Patients were managed by physician dictate. From 552 children (median age, 11.8 y; 61% male; 63% with pediatric CD activity index scores >30; and median C-reactive protein level 5.6-fold the upper limit of normal), we used propensity score methodology to identify 68 triads of patients matched for baseline characteristics who were treated with early anti-TNFalpha therapy, early immunomodulator, or no early immunotherapy. We evaluated relationships among therapies, corticosteroid and surgery-free remission (pediatric CD activity index scores, </=10), and growth at 1 year for 204 children. Treatment after 3 months was a covariate. RESULTS: Early treatment with anti-TNFalpha was superior to early treatment with an immunomodulator (85.3% vs 60.3% in remission; relative risk, 1.41; 95% confidence interval [CI], 1.14-1.75; P = . 0017), whereas early immunomodulator therapy was no different than no early

immunotherapy (60.3% vs 54.4% in remission; relative risk, 1.11; 95% CI, 0.83-1.48; P = .49) in achieving remission at 1 year. Accounting for therapy after 3 months, early treatment with anti-TNFalpha remained superior to early treatment with an immunomodulator (relative risk, 1.51; 95% CI, 1.20-1.89; P = .0004), whereas early immunomodulator therapy was no different than no early immunotherapy (relative risk, 1.00; 95% CI, 0.75-1.34; P = .99). The mean height z-score increased compared with baseline only in the early anti-TNFalpha group. CONCLUSIONS: In children newly diagnosed with comparably severe CD, early monotherapy with anti-TNFalpha produced better overall clinical and growth outcomes at 1 year than early monotherapy with an immunomodulator. Further data will be required to best identify children most likely to benefit from early treatment with anti-TNFalpha therapy.

Wang, H., et al. (2017). "Hypomethylation agent decitabine restores drug sensitivity by depressing P-glycoprotein activity through MAPK signaling pathway." Mol Cell Biochem **433**(1-2): 141-148.

The multidrug resistance (MDR) continues to be an obstacle in the treatment of both hematological and solid tumors. Hypomethylation agent, decitabine (5-Aza-dC), is an experimental agent in MDR therapy, while the mechanism is not very clear. In the present study, we demonstrated 5-Aza-dC may reverse MDR induced by P-glycoprotein (P-gp) coded by mdr1 gene in both hematologic K562/ADR cells and solid tumor MCF-7/ADR cells with time and dose-dependent manner. 5-Aza-dC significantly increased drug sensitivity in patients' leukemic cells which had higher

expression of mdr1 gene. Both total protein and membrane P-gp expression were up-regulated with 5-Aza-dC treatment in K562/ADR and MCF-7/ADR cells. However, accumulation of adriamycin and rhodamine 123 were increased which suggested the depression of P-gp activity. Gene expression profiling was performed and activation of MAPK signaling pathway was identified as the most significant change affected by 5-Aza-dC. Inhibition of MAPK pathway could increase P-gp activity. Our data suggested that hypomethylation agent decitabine restores drug sensitivity in the P-gp-induced MDR phenotype by depressing of P-gp activity as drug pump partly through MAPK signaling pathway.

Wang, H., et al. (2003). "Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation." Nature **421**(6921): 384-388.

Wang, J., et al. (2014). "MDR1 C3435T polymorphism and inflammatory bowel disease risk: a meta-analysis." Mol Biol Rep **41**(4): 2679-2685.

Wang, Z. C., et al. (2015). "Genetic polymorphisms of the multidrug resistance 1 gene MDR1 and the risk of hepatocellular carcinoma." Tumor Biology **36**(9): 7007-7015.

A possible association between multiple drug resistance 1 gene (MDR1) polymorphisms and the risk of developing hepatocellular carcinoma (HCC) is currently under debate, and evidence from various epidemiological studies has

yielded controversial results. To derive a more precise estimation of the association between MDR1 polymorphisms and HCC risk, the present meta-analysis was performed. A total of 8 studies containing 11 cohorts with 4407 cases and 4436 controls were included by systematic literature search of EMBASE, PubMed, Web of Science, and CNKI. All polymorphisms were classified as mutant/wild-type alleles. In particular, the variation type, functional impact, and protein domain location of the polymorphisms were assessed and used as stratified indicators. The pooled odds ratio (OR) with 95 % confidence interval (CI) was calculated to evaluate the association. Overall, our results suggested that the mutant alleles of the MDR1 gene were associated with a significantly increased risk for HCC under all genetic models (allelic model: OR = 1.28, 95 % CI = 1.20-1.36, P < 0.001; dominant model: OR = 1.27, 95 % CI = 1.16-1.38, P < 0.001; recessive model: OR = 1.59, 95 % CI = 1.36-1.85, P < 0.001). Furthermore, increased risks for HCC were also revealed in stratified analyses by ethnicity, sample size, and quality scores of cohorts as well as variation type, functional impact, and protein domain location of polymorphisms. In conclusion, the present meta-analysis suggested that the presence of MDR1 mutant alleles might be a risk factor for HCC.

Wei, H., et al. (2018). "Overexpression of long non coding RNA CA3-AS1 suppresses proliferation, invasion and promotes apoptosis via miRNA-93/PTEN axis in colorectal cancer." Gene.

Wilhelm, M., et al. (2014). "Mass-spectrometry-based draft of the human proteome." Nature **509**(7502): 582-587.

Wilson, B. J., et al. (2011). "ABCB5 Identifies a Therapy-Refractory Tumor Cell Population in Colorectal Cancer Patients." Cancer Research **71**(15): 5307-5316.

Identification and reversal of treatment resistance mechanisms of clinically refractory tumor cells is critical for successful cancer therapy. Here we show that ATP-binding cassette member B5 (ABCB5) identifies therapy-refractory tumor cells in colorectal cancer patients following fluorouracil (5-FU)-based chemoradiation therapy and provide evidence for a functional role of ABCB5 in colorectal cancer 5-FU resistance. Examination of human colon and colorectal cancer specimens revealed ABCB5 to be expressed only on rare cells within healthy intestinal tissue, whereas clinical colorectal cancers exhibited substantially increased levels of ABCB5 expression. Analysis of successive, patient-matched biopsy specimens obtained prior to and following neoadjuvant 5-FU-based chemoradiation therapy in a series of colorectal cancer patients revealed markedly enhanced abundance of ABCB5-positive tumor cells when residual disease was detected. Consistent with this finding, the ABCB5-expressing tumor cell population was also treatment refractory and exhibited resistance to 5-FU-induced apoptosis in a colorectal cancer xenograft model of 5-FU monotherapy. Mechanistically, short hairpin RNA-mediated ABCB5 knockdown significantly inhibited tumorigenic xenograft growth and sensitized colorectal cancer cells to 5-FU-induced cell killing. Our results identify ABCB5 as a novel molecular

marker of therapy-refractory tumor cells in colorectal cancer patients and point to a need for consistent eradication of ABCB5-positive resistant tumor cell populations for more effective colorectal cancer therapy. Cancer Res; 71(15); 5307-16. (C) 2011 AACR.

Wilson, B. J., et al. (2011). "ABCB5 identifies a therapy-refractory tumor cell population in colorectal cancer patients." Cancer Research **71**(15): 5307-5316.

Identification and reversal of treatment resistance mechanisms of clinically refractory tumor cells is critical for successful cancer therapy. Here we show that ATP-binding cassette member B5 (ABCB5) identifies therapy-refractory tumor cells in colorectal cancer patients following fluorouracil (5-FU)-based chemoradiation therapy and provide evidence for a functional role of ABCB5 in colorectal cancer 5-FU resistance. Examination of human colon and colorectal cancer specimens revealed ABCB5 to be expressed only on rare cells within healthy intestinal tissue, whereas clinical colorectal cancers exhibited substantially increased levels of ABCB5 expression. Analysis of successive, patient-matched biopsy specimens obtained prior to and following neoadjuvant 5-FU-based chemoradiation therapy in a series of colorectal cancer patients revealed markedly enhanced abundance of ABCB5-positive tumor cells when residual disease was detected. Consistent with this finding, the ABCB5-expressing tumor cell population was also treatment refractory and exhibited resistance to 5-FU-induced apoptosis in a colorectal cancer xenograft model of 5-FU monotherapy. Mechanistically, short hairpin RNA-mediated ABCB5 knockdown

significantly inhibited tumorigenic xenograft growth and sensitized colorectal cancer cells to 5-FU-induced cell killing. Our results identify ABCB5 as a novel molecular marker of therapy-refractory tumor cells in colorectal cancer patients and point to a need for consistent eradication of ABCB5-positive resistant tumor cell populations for more effective colorectal cancer therapy.

Wong, G. K., et al. (2003). "A population threshold for functional polymorphisms." Genome Res **13**(8): 1873-1879.

Wu, C. P., et al. (2018). "SIS3, a specific inhibitor of Smad3 reverses ABCB1- and ABCG2-mediated multidrug resistance in cancer cell lines." Cancer Lett **433**: 259-272.

One of the major challenges in cancer chemotherapy is the development of multidrug resistance phenomenon attributed to the overexpression of ATP-binding cassette (ABC) transporter ABCB1 or ABCG2 in cancer cells. Therefore, re-sensitizing MDR cancer cells to chemotherapy by directly inhibiting the activity of ABC transporters has clinical relevance. Unfortunately, previous attempts of developing clinically applicable synthetic inhibitors have failed, mostly due to problems associated with toxicity and unforeseen drug-drug interactions. An alternative approach is by repositioning drugs with known pharmacological properties as modulators of ABCB1 and ABCG2. In this study, we discovered that the transport function of ABCB1 and ABCG2 is strongly inhibited by SIS3, a specific inhibitor of Smad3. More importantly, SIS3 enhances drug-induced apoptosis and resensitizes ABCB1- and

ABCG2-overexpressing cancer cells to chemotherapeutic drugs at non-toxic concentrations. These findings are further supported by ATPase assays and by a docking analysis of SIS3 in the drug-binding pockets of ABCB1 and ABCG2. In summary, we revealed an additional action of SIS3 that re-sensitizes MDR cancer cells and a combination therapy with this drug and other chemotherapeutic agents may be beneficial for patients with MDR tumors.

Wu, G. D., et al. (2011). "Linking long-term dietary patterns with gut microbial enterotypes." Science **334**(6052): 105-108.

Diet strongly affects human health, partly by modulating gut microbiome composition. We used diet inventories and 16S rDNA sequencing to characterize fecal samples from 98 individuals. Fecal communities clustered into enterotypes distinguished primarily by levels of Bacteroides and Prevotella. Enterotypes were strongly associated with long-term diets, particularly protein and animal fat (Bacteroides) versus carbohydrates (Prevotella). A controlled-feeding study of 10 subjects showed that microbiome composition changed detectably within 24 hours of initiating a high-fat/low-fiber or low-fat/high-fiber diet, but that enterotype identity remained stable during the 10-day study. Thus, alternative enterotype states are associated with long-term diet.

Wu, Q., et al. (2014). "Multi-drug resistance in cancer chemotherapeutics: mechanisms and lab approaches." Cancer Lett **347**(2): 159-166.

Multi-drug resistance (MDR) has become the largest obstacle to the success of cancer chemotherapies. The mechanisms of MDR and the approaches to test MDR have been discovered, yet not fully understood. This review covers the in vivo and in vitro approaches for the detection of MDR in the laboratory and the mechanisms of MDR in cancers. This study also envisages the future developments toward the clinical and therapeutic applications of MDR in cancer treatment. Future therapeutics for cancer treatment will likely combine the existing therapies with drugs originated from MDR mechanisms such as anti-cancer stem cell drugs, anti-miRNA drugs or anti-epigenetic drugs. The challenges for the clinical detection of MDR will be to find new biomarkers and to determine new evaluation systems before the drug resistance emerges.

Wurm, P., et al. (2017). "Antibiotic-Associated Apoptotic Enterocolitis in the Absence of a Defined Pathogen: The Role of Intestinal Microbiota Depletion." Crit Care Med **45**(6): e600-e606.

OBJECTIVE: Antibiotic therapy is a major risk factor for the development of diarrhea and colitis with varying severity. Often the origin of antibiotic-associated gastrointestinal deterioration remains elusive and no specific infectious agents could be discerned. PATIENTS: We represent three cases of intractable high-volume diarrhea associated with combined antibiotic and steroid therapy in critically ill patients not fitting into established disease entities. Cases presented with severe apoptotic enterocolitis resembling acute intestinal graft-versus-host-disease.

Microbiologic workup precluded known enteropathogens, but microbiota analysis revealed a severely depleted gut microbiota with concomitant opportunistic pathogen overgrowth. INTERVENTIONS: Fecal microbiota transplantation, performed in one patient, was associated with correction of dysbiosis, rapid clinical improvement, and healing of enterocolitis. CONCLUSIONS: Our series represents a severe form of antibiotic-associated colitis in critically ill patients signified by microbiota depletion, and reestablishment of a physiologic gastrointestinal microbiota might be beneficial for this condition.

Xiao, J., et al. (2018). "Differential expression of ABCB5 in BRAF inhibitor-resistant melanoma cell lines." BMC Cancer **18**(1): 675.

Xu, K., et al. (2017). "[Analysis on the novel compound heterozygous mutation F of a patient with hereditary factor deficiency]." Zhonghua Yi Xue Za Zhi **97**(48): 3774-3778.

Yang, G., et al. (2015). "MicroRNA-522 reverses drug resistance of doxorubicin-induced HT29 colon cancer cell by targeting ABCB5." Mol Med Rep **12**(3): 3930-3936.

MicroRNAs (miRNAs) are small non-coding RNAs, which are important in the development of multidrug resistance in cancer by regulating gene expression at the posttranscriptional level. The present study investigated the functional effects of miR522 in chemoresistant colon cancer cells. The results demonstrated that miR522

was significantly downregulated in doxorubicin (DOX) resistant colon cell line, HT29/DOX, compared with the parental HT29 colon cancer cell line. Overexpression of miR522 in the HT29/DOX cells partially restored DOX sensitivity. miRNA target prediction algorithms suggested that ABCB5 was a target gene for miR522. A fluorescent reporter assay confirmed that miR522 was able to specifically bind to the predicted site of the ABCB5 mRNA 3'untranslated region. When miR522 was overexpressed in the HT29/DOX cells, the protein expression levels of ABCB5 were downregulated. Furthermore, knockdown of ABCB5 significantly increased the growth inhibition rate of the HT29/DOX cells, compared with the control group. These results suggested that miR522 may affect the sensitivity of colon cancer cell lines to DOX treatment by targeting ABCB5.

Yang, J. Y., et al. (2010). "p-Glycoprotein ABCB5 and YB-1 expression plays a role in increased heterogeneity of breast cancer cells: correlations with cell fusion and doxorubicin resistance." BMC Cancer **10**: 388.

Yang, M., et al. (2012). "Expression of ABCB5 gene in hematological malignances and its significance." Leukemia & Lymphoma **53**(6): 1211-1215.

We examined ABCB5 gene expression using real-time polymerase chain reaction (PCR) in leukemia cells from 29 patients with acute lymphoblastic leukemia (ALL), 24 patients with chronic lymphocytic leukemia (CLL), 42 with acute myeloid leukemia (AML), 22 with chronic myeloid leukemia (CML), 17 with lymphoma and

10 with multiple myeloma (MM). It was confirmed that expression of the ABCB5 gene is highly increased in B-precursor ALL and French-American-British (FAB) M1 and M2 types of AML and lymphoma. The ABCB5 gene is expressed more highly in patients with relapsed or refractory disease than in patients with drug sensitive acute leukemia. Furthermore, there was an evident positive correlation between ABCB5 mRNA expression and MDR1 mRNA expression, but no correlation with MRP mRNA expression or BCRP mRNA expression. Quantification of the ABCB5 gene by real-time PCR offers particular promise as a prognostic marker and a marker for drug resistance in acute leukemia. Our findings raise the possibility that ABCB5 may be responsible for both the progression and chemotherapeutic refractoriness of advanced acute leukemia, and that ABCB5-targeted approaches might therefore represent novel and translationally relevant therapeutic strategies for drug resistance in leukemia.

Yang, Q. F., et al. (2015). "Contribution of MDR1 gene polymorphisms on IBD predisposition and response to glucocorticoids in IBD in a Chinese population." J Dig Dis **16**(1): 22-30.

OBJECTIVE: The cornerstone of conventional treatment for inflammatory bowel disease (IBD) is glucocorticoid (GC). Single nucleotide polymorphisms (SNPs) of genes such as multidrug resistance 1 (MDR1) are related to patient's response to GC, and MDR1 polymorphisms are associated with susceptibility to IBD in Caucasians. We aimed to investigate whether the polymorphisms of five genes including MDR1 influence the response to GC in Chinese patients and the relationship between MDR1

and IBD susceptibility. METHODS: SNPs were selected and genotyped in 156 IBD patients treated with GC and 223 healthy controls. Patients were evaluated and classified as GC-dependent, GC-resistant or responsive to GC after treatment. RESULTS: The CC genotypes of rs1128503 and rs1045642 in MDR1 gene were more frequent in Crohn's disease (CD) patients who were GC-dependent than in those responsive to GC (odds ratio [OR] 6.583, 95% confidence interval [CI] 1.760-24.628, P = 0.019 and OR 3.873, 95% CI 1.578-9.506, P = 0.009, respectively). The G allele of MDR1 rs2032582 was less frequent among CD patients than in controls (OR 0.668, 95% CI 0.484-0.921, P = 0.014). G allele carriers were also less likely to develop non-stricturing and non-penetrating CD (OR 0.661, 95% CI 0.462-0.946, P = 0.023) and ileocolonic CD (OR 0.669, 95% CI 0.472-0.948, P = 0.024). CONCLUSIONS: Polymorphisms of MDR1 are associated with patient's GC response and a predisposition to CD in Chinese population. Further studies are needed to elucidate the role of MDR1 polymorphisms in IBD and that as genetic markers for GC response.

Yang, S., et al. (2018). "Long noncoding RNA ROR as a novel biomarker for progress and prognosis outcome in human cancer: a meta-analysis in the Asian population." Cancer Manag Res **10**: 4641-4652.

Yang, S. Y., et al. (2018). "Transcriptome-wide identification of transient RNA G-quadruplexes in human cells." Nat Commun **9**(1): 4730.

Yang, Y. and C. Jobin (2017). "Novel insights into microbiome in colitis and colorectal cancer." Curr Opin Gastroenterol.

PURPOSE OF REVIEW: Microbiota is a major player in the pathogenesis of inflammatory bowel diseases (IBD) and colorectal cancer (CRC). Here, we summarize the key advances achieved in the past 18 months (ending June 2017) toward a better understanding of the role of microbiota in colitis and CRC development. RECENT FINDINGS: Accumulating evidence shows the essential role of intestinal barrier function (e.g. mucus, IgA, LCN2, LYPD8) in protecting against bacteria-induced inflammation and tumor development. Numerous signaling pathways (e.g. TLRs and NLRs), metabolites (e.g. indole, bile acids, retinoic acid) and small noncoding RNAs (e.g. miRNA) have been identified as key mediators regulating host-microbe interactions in the intestine. Novel microbial drivers of colitis and tumorigenesis (e.g. Alistipes finegoldii, Atopobium parvalum, Peptostreptococcus anaerobius) have been identified and their disease-promoting activities have been described. SUMMARY: IBD-associated colorectal cancer results from a complex breakdown of communication between the host and its microbiota, involving barrier function, immune signaling and metabolites.

Yao, J. T., et al. (2017). "ABCB5-ZEB1 Axis Promotes Invasion and Metastasis in Breast Cancer Cells." Oncology Research **25**(3): 305-316.

ABCB5 belongs to the ATP-binding cassette (ABC) superfamily, which is recognized for playing a role in the failure of chemotherapy. ABCB5 has also been found to be overexpressed at the transcriptional level in a number of cancer subtypes, including breast cancer. However, the exact mechanism ABCB5 uses on cancer cell metastasis is still unclear. In the present study, we demonstrate that ABCB5 expression was increased in metastatic tissues when compared with nonmetastatic tissues. ABCB5 can significantly enhance metastasis and epithelial-mesenchymal transition (EMT), while knockdown of ABCB5 inhibited these processes. Microarray analysis indicated that ZEB1 may function as a downstream factor of ABCB5. Furthermore, the expression of ZEB1 in tissues is positively relevant to ABCB5 in breast cancer. Knocking down ZEB1 inhibits ABCB5 ectopic expression-induced migration and invasion, as well as EMT. Taken together, these results helped to realize the oncogene functions of ABCB5 in breast cancer cells and provided a new direction in treating breast cancer.

Yu, A. M., et al. (2016). "MicroRNA Pharmacoepigenetics: Posttranscriptional Regulation Mechanisms behind Variable Drug Disposition and Strategy to Develop More Effective Therapy." Drug Metabolism and Disposition **44**(3): 308-319.

Yue, Q., et al. (2015). "MDR1 C3435T polymorphism and childhood acute lymphoblastic leukemia susceptibility: an updated meta-analysis." Biomed Pharmacother **69**: 76-81.

BACKGROUND: Acute lymphoblastic leukemia (ALL) is the most common malignancy in children, though the etiology of the leukemia is poorly understood, both genetic and environmental factors appear to be involved. Previous studies investigating the association between MDR1 C3435T polymorphisms and risk of children with ALL reported controversial results. METHODS: We performed a comprehensive meta-analysis to clarify the effect of MDR1 C3435T polymorphisms on risk of childhood ALL. The strength of the association was measured by odds ratio (OR) with 95% confidence interval (CI). RESULTS: Nine studies were finally included, involving a total of 1462 cases and 1522 controls. There was no association between MDR1 C3435T polymorphism and children ALL risk in all of four models in overall populations (CT vs. CC: OR=0.86, 95% CI=0.65-1.15, P=0.310; TT vs. CC: OR=1.50, 95% CI=0.96-2.35, P=0.076; TT/CT vs. CC: OR=1.12, 95% CI=0.95-1.33, P=0.166; TT vs. CC/CT: OR=1.58, 95% CI=0.97-2.56, P=0.067). Subgroup analysis by race suggested that this association existed in Asians under the under the homozygote model and recessive model (TT vs. CC: OR=2.45, 95% CI=1.24-4.86, P=0.010; TT vs. CT/CC: OR=2.65, 95% CI=1.22-5.75, P=0.014). CONCLUSIONS: This meta-analysis suggests there was no association between MDR1 C3435T polymorphism and children ALL risk in overall populations, but significant association with an increased risk in Asians.

Zhang, Y. J., et al. (2014). "Multidrug resistance gene and its relationship to ulcerative colitis and immune status of ulcerative colitis." Genet Mol Res **13**(4): 10837-10851.

Zhao, J. J., et al. (2015). "CTLA-4 and MDR1 polymorphisms increase the risk for ulcerative colitis: A meta-analysis." World Journal of Gastroenterology **21**(34): 10025-10040.

AIM: To evaluate the correlations between cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) and multidrug resistance 1 (MDR1) genes polymorphisms with ulcerative colitis (UC) risk.

METHODS: PubMed, EMBASE, Web of Science, Cochrane Library, CBM databases, Springerlink, Wiley, EBSCO, Ovid, Wanfang database, VIP database, China National Knowledge Infrastructure, and Weipu Journal databases were exhaustively searched using combinations of keywords relating to CTLA-4, MDR1 and UC. The published studies were filtered using our stringent inclusion and exclusion criteria, the quality assessment for each eligible study was conducted using Critical Appraisal Skill Program and the resultant high-quality data from final selected studies were analyzed using Comprehensive Meta-analysis 2.0 (CMA 2.0) software. The correlations between SNPs of CTLA-4 gene, MDR1 gene and the risk of UC were evaluated by OR at 95% CI. Z test was carried out to evaluate the significance of overall effect values. Cochran's Q -statistic and I-2 tests were applied to quantify heterogeneity among studies. Funnel plots, classic fail-safe N and Egger's linear regression test were inspected for indication of publication bias.

RESULTS: A total of 107 studies were initially retrieved and 12 studies were eventually selected for meta-analysis. These 12 case-control studies involved 1860 UC patients and 2663 healthy controls. Our major result revealed that single nucleotide

polymorphisms (SNPs) of CTLA-4 gene rs3087243 G > A and rs231775 G > A may increase the risk of UC (rs3087243 G > A: allele model: OR = 1.365, 95% CI: 1.023-1.822, P = 0.035; dominant model: OR = 1.569, 95% CI: 1.269-1.940, P < 0.001; rs231775 G > A: allele model: OR = 1.583, 95% CI: = 1.306-1.918, P < 0.001; dominant model: OR = 1.805, 95% CI: 1.393-2.340, P < 0.001). In addition, based on our result, SNPs of MDR1 gene rs1045642 C > T might also confer a significant increases for the risk of UC (allele model: OR = 1.389, 95% CI: 1.214-1.590, P < 0.001; dominant model: OR = 1.518, 95% CI: 1.222-1.886, P < 0.001).

CONCLUSION: CTLA-4 gene rs3087243 G > A and rs231775 G > A, and MDR1 gene rs1045642 C > T might confer an increase for UC risk.

Zhao, L., et al. (2018). "Expression profile analysis identifies the long non-coding RNA landscape and the potential carcinogenic functions of LINC00668 in laryngeal squamous cell carcinoma." Gene.

Zheng, C., et al. (2018). "Infantile Onset Intractable Inflammatory Bowel Disease Due to Novel Heterozygous Mutations in TNFAIP3 (A20)." Inflamm Bowel Dis.

Zheng, M., et al. (2015). "The role of Abcb5 alleles in susceptibility to haloperidol-induced toxicity in mice and humans." Plos Medicine **12**(2): e1001782.

Zhong, X. B. and J. S. Leeder (2013). "Epigenetic regulation of ADME-related genes: focus on drug metabolism and transport." Drug Metabolism and Disposition **41**(10): 1721-1724.

Zijlmans, J. M., et al. (1995). "Modification of rhodamine staining allows identification of hematopoietic stem cells with preferential short-term or long-term bone marrow-repopulating ability." Proc Natl Acad Sci U S A **92**(19): 8901-8905.

Zittan, E., et al. (2016). "Development of the Harvey-Bradshaw Index-pro (HBI-PRO) Score to Assess Endoscopic Disease Activity in Crohn's Disease." J Crohns Colitis.

BACKGROUND: There is a need for better, less-invasive disease activity indices that provide a representative assessment of endoscopic disease activity. We developed a new clinical score that incorporates the Harvey-Bradshaw index [HBI] with modified patient-reported outcomes [PROp] and physician [clinician]-reported outcomes [PROc] and assessed its ability to measure endosopic disease activity in ileocolonic Crohn's disease [CD]. METHODS: A cohort of 88 CD patients undergoing colonoscopy was accrued in a prospective fashion. In total, 48 of the subjects were CD cases and 40 had already undergone a post-operative ileocolonic resection [post-op CD]. Each patient underwent multiple, endoscopist-blinded assessments including: HBI score, a PROp question asking for patient perception of disease activity status, a PROc question for clinician perception of disease activity status and C-reactive

protein [CRP]. Active endoscopic disease was defined as Simple Endoscopic Score for CD [SES-CD] >/= 3 for CD subjects and Rutgeerts score > i1 for post-op CD subjects. RESULTS: Clinical remission as defined by the HBI did not accurately reflect endoscopic remission as defined by the SES-CD (area under the curve [AUC] = 0.54). Combining the HBI with PROp and PROc scores and then further adding CRP significantly improved the correlation with SES-CD [AUC = 0.78 and AUC = 0.88, respectively, p < 0.00001]. In post-op CD, HBI-defined remission also performed poorly against endoscopic remission defined by the Rutgeerts score [AUC = 0.52]. Combining HBI with PROp and the PROc scores and then further adding CRP did not significantly improve the model [AUC = 0.65 and AUC = 0.61, respectively, p = NS]. CONCLUSION: In CD, the HBI correlates poorly with endoscopic disease activity. However, the HBI-PRO score, which incorporated PROp, PROc, CRP and HBI, significantly improved its ability to predict endoscopic activity in ileocolonic CD without prior surgery.

Ziv-Baran, T., et al. (2018). "Response to treatment is more important than disease severity at diagnosis for prediction of early relapse in new-onset paediatric Crohn's disease." Aliment Pharmacol Ther **48**(11-12): 1242-1250.

BACKGROUND: Paediatric Crohn's disease is characteried by frequently relapsing disease which may lead to hospitalisations and complications. AIM: To develop predictive models for early relapse following first remission. METHODS: The GROWTH CD prospective inception cohort was designed to predict risk for early

disease relapse and poor outcomes. Newly diagnosed children underwent endoscopies and imaging. They were phenotyped and followed at scheduled visits through 78 weeks for relapses. Twenty-eight dichotomous and continuous variables were assessed at baseline and week 12, including phenotype, inflammatory markers, disease activity (PCDAI) and other markers. Clinical relapses defined as PCDAI >10 after remission were recorded using a relapse form. Logistic regression & risk modelling was performed. RESULTS: We enrolled 282 eligible patients of whom 178 (63.6%) patients achieved steroid free remission by week 12. Disease complications developed in 22/76(29%) of patients with relapse compared to 20/206 (9.7%) without relapse (P = 0.01). Multivariable analysis demonstrated that while variables from age/ gender at diagnosis were not predictive, week 12 variables including PCDAI >5 (P = 0.02), CRP >20 mg/L (P = 0.02), and faecal calprotectin >400 microg/g (P = 0.03) as optimal cut-offs were associated with increased risk of relapse. A prediction model for patients in remission including gender, age, week 12 PCDAI, calprotectin and CRP had sensitivity 43%, specificity 92%, PPV 78%, NPV 71% for relapse. CONCLUSIONS: Early relapses were associated with a higher risk for disease complications at followup. Relapse prediction based on week 12 disease activity or inflammation is superior to prediction using data from diagnosis.

Zundler, S., et al. (2017). "Anti-Adhesion Therapies in Inflammatory Bowel Disease-Molecular and Clinical Aspects." Frontiers in Immunology **8**: 891.

The number of biologicals for the therapy of immunologically mediated diseases is constantly growing. In contrast to other agents that were previously introduced in rheumatologic or dermatologic diseases and only later adopted for the treatment of inflammatory bowel diseases (IBDs), the field of IBD was ground breaking for the concept of anti-adhesion blockade. Anti-adhesion antibodies selectively target integrins controlling cell homing to the intestine, which leads to reduction of inflammatory infiltration to the gut in chronic intestinal inflammation. Currently, the anti-alpha4beta7-antibody vedolizumab is successfully used for both Crohn's disease and ulcerative colitis worldwide. In this mini-review, we will summarize the fundamental basis of intestinal T cell homing and explain the molecular groundwork underlying current and potential future anti-adhesion therapies. Finally, we will comment on noteworthy clinical aspects of anti-adhesion therapy and give an outlook to the future of anti-integrin antibodies and inhibitors.