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Genome Wide Association (GWA) Predictors of Anti-TNFα Therapeutic Responsiveness in Pediatric Inflammatory Bowel Disease (IBD)

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Background: Natural history observations in early onset IBD have prompted the increasing use of anti-TNFα therapy. Inter-individual variation, however, is seen in efficacy outcomes. These differences may be explained in part by genetic variability as it relates to disease pathogenesis or directed to the mechanism of action of these therapies. Recent GWA studies in IBD have increased our understanding of the genetic susceptibility to IBD and provide insight regarding the various mechanisms of inflammation. We hypothesize that interindividual differences in therapeutic outcomes to anti-TNFα may be associated with IBD susceptibility genes. Aim: Test associations of GWA identified IBD susceptibility loci (34 SNPs) with infliximab (IFX) responsiveness in pediatric IBD patients. Methods: Complete follow up and GWAS data was available on 63 children receiving IFX. Harvey Bradshaw index (HBI) was used to calculate disease activity. Outcomes were primary non- response: no change or increase in HBI at week 10; and secondary loss of response: drop in HBI by >3 points at week 10 (response) and then increase to or above baseline HBI week 14 or later. Chi square testing examined the association between 34 SNPs and IFX responsiveness. Time to loss of response was calculated using Kaplan Meier analysis. Results: Six SNPs (Table 1) were associated with primary non-response ($p < 0.05$). There were 5 different SNPs (Chromosome) associated with loss of response; rs11174631 (12q12); OR =10.6, p= 0.03; rs8049439 (16p11); OR = 3.8, p= 0.03, rs2456449 (8q24); OR= 4.8, p = 0.02, rs10044354 (5q15); OR = 3.8, p = 0.03, rs6908425 (6p22); OR = 4.1, p = 0.03. Of these 5 SNPs, 4 were also associated with the time to loss of response (median 9 months): rs11174631 (p= 0.03), rs2456449 (p = 0.03), rs10044354 (p = 0.04), rs6908425 (p = 0.02). Conclusion: These findings suggest that there may be different genetic predictors and perhaps biological explanations for primary non response vs. secondary loss of response. Replication studies are currently underway. Defining predictors of response to anti-TNFα will allow the identification of patients with a high probability of response before initiating therapy so to negate exposure to ineffective therapies and protect patients from treatment related serious adverse events.

Primary Non Response

SNP	Chromosome	P value	OR	Gene of Interest
rs2241880	2q37	0.04	0.1	ATG16L1
rs2188962	5q23	0.03	6.7	unknown
rs3764147	13q14	0.004	20.1	Orf13
rs762421	21q22	0.03	10.2	ICOSLG
rs9271568	6p21.32	0.004	21.0	HLADQA1
rs2836878	21q22.2	0.01	17.4	BRWD1

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Human Alpha Defensin 5 mRNA Levels Are Decreased in Children with Untreated, Newly Diagnosed Crohn Disease

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Introduction: Paneth cell defensins, including human alpha-defensins 5 (HD5) and 6 (HD6), are key effectors of the intestinal innate immune system. Prior studies have demonstrated decreased HD5 and HD6 levels in adult patients with established ileal Crohn's disease (CD), suggesting that decreased defensin levels may initiate and perpetuate inflammation in susceptible individuals. To circumvent the potentially confounding factors of longstanding disease duration and anti-inflammatory treatment effects in these studies, we measured HD5 and HD6 mRNA levels in the ileum of untreated children with newly diagnosed CD. Methods: Patients ages 7 to 18 years undergoing first-time colonoscopy for any reason were approached for study participation. Mucosal pinch biopsies from the terminal ileum (TI) were obtained in addition to standard clinical TI and colonic biopsies. RT-PCR analysis of ileal tissue samples was performed to quantify Paneth cell HD5 and HD6 as well as sPLA2 and lysozyme, two antimicrobial peptides also produced by intestinal Paneth cells. Levels of the neutrophil chemoattractant IL-8 were assayed to assess inflammation. GAPDH, a constitutively expressed glycolytic enzyme, was used as an internal control. Each of the biomarkers was standardized to GAPDH levels. Ileal defensin levels from patients with normal ileal histology and no other pathologic diagnosis (controls) were compared to patients with CD ileitis using standard t-tests. Diagnosis of CD was made according to standard diagnostic criteria. Results: Terminal ileal biopsies from 8 patients with CD (5 female) and 17 patients with no pathologic diagnosis (10 female) were analyzed. Average age of CD patients was 13.6 years (range 9-18 years). Average age of control patients was 14.8 years (range 8-18 years). In children with ileal CD, ileal HD5 mRNA levels were 65% of control values (p=0.04). Ileal HD6 mRNA levels in patients with CD ileitis were 89% of control values (p=0.68). SPLA2 and lysozyme levels were 1.6 and 2 times higher in CD ileitis than in the control group (p=0.01 and p= 0.004 respectively). IL-8 levels were increased 42-fold in the CD ileitis group (p=0.002). Conclusions: This is the first study to compare terminal ileal defensin levels in children with ileal CD at the time of diagnosis. HD5 mRNA levels are lower in children with newly diagnosed, untreated ileal Crohn's disease than in normal control patients, perhaps contributing to the immunopathogenesis of pediatric CD. Given an increase in SPLA2 and lysozyme mRNA levels in these CD patients, the decrease in HD5 is likely not explained by decreased Paneth cell mass.

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Rate of Recurrence of *Clostridium difficile* in Pediatric Patients with Inflammatory Bowel Disease

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BACKGROUND: The incidence and associated morbidity of *Clostridium difficile* (CD) infection has been increasing at an alarming rate in North America. *Clostridium difficile* associated diarrhea (CDAD) is the leading cause of nosocomial diarrhea in the USA. Patients with CDAD have longer average hospital admissions and additional hospital costs. Evidence has demonstrated that patients with inflammatory bowel disease (IBD) have a higher incidence of CD in comparison to the general population. The aim of this study was to compare the rate of recurrence of CD in hospitalized pediatric patients with IBD compared to hospitalized controls. The secondary aim was to evaluate whether infection with CD resulted in a more severe disease course of IBD. METHODS: This was a nested case control retrospective study of hospitalized pediatric patients. Diagnosis of CD was confirmed with stool Toxin A and B analysis. The following data was obtained from the medical records: demographic information, classification of IBD including location of disease, IBD therapy, and prior surgeries. In addition prior hospital admissions within 1 year and antibiotic exposure were recorded. The same information was recorded following CD infection. RESULTS: A total of 138 patients with IBD and 80 control patients were included. The rate of recurrence of CD in the IBD population was 43% compared to 7.5% in the control population ($p < 0.0001$). In evaluating the effect on IBD disease severity, 57% of patients were readmitted with an exacerbation of disease within 6 months of infection with CD and 67% required escalation of therapy following CD infection. Of the patients with IBD, 44% of the cases were new onset IBD, 63% were on immunosuppression therapy and 33% were on gastric acid suppression prior to infection. In comparing the two populations, there was no significant difference in antibiotic exposure, 33% of IBD patients and 26% of control patients were on antibiotics, ($p < 0.2$). In regards to prior hospitalization, 10% of patients with IBD patients were hospitalized in the 30 days prior to infection in comparison to 27% of the control patients ($p < 0.002$). CONCLUSION: CD infection in patients with IBD results in higher rate of recurrence and is associated with higher morbidity than the general population. Patients with IBD often required hospitalization and escalation of therapy following infection with CD, indicating that CD resulted in increased severity of disease. In addition, IBD patients were more likely develop community acquired CD, while the control patients developed noscomial infections, indicating a higher susceptibility to CD infection in patients with IBD.

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Ipilimumab-Related Colitis in Patients with Advanced Melanoma Receiving Ipilimumab Therapy: Histopathologic and Serologic Characterization

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BACKGROUND: Ipilimumab is a fully human monoclonal antibody that is directed against human cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) that overcomes CTLA-4-mediated T-cell suppression to enhance the immune response against tumors. Clinical trials have demonstrated its activity in the treatment of advanced melanoma. However, Ipilimumab therapy is associated with immune-related adverse events including diarrhea, which can be severe and life-threatening. The histopathologic alterations associated with Ipilimumab-induced gastrointestinal toxicity have not been well characterized. DESIGN: A randomized and double-blind phase II trial of Ipilimumab administered with or without prophylactic budesonide enrolled 115 patients with unresectable stage III or IV melanoma. Endoscopic colonic biopsies were performed during the first 2 weeks after the first dose and after the onset of grade ≥2 diarrhea. Hematoxylin and eosin stained tissue sections were evaluated by a gastrointestinal pathologist. Serologic markers of inflammatory bowel disease (pANCA, ASCA, I2, CBir1 and OmpC) were measured by enzyme-linked immunosorbent assay. RESULTS: Grade ≥2 diarrhea developed in 39 of 115 (34%) patients treated with Ipilimumab therapy. There was no significant difference in the frequency of GI adverse events between the group that received prophylactic budesonide (33%) and the group that received concomitant placebo (35%). Histopathologic examination of colonic biopsies obtained from patients after the onset of grade ≥2 diarrhea showed active colitis characterized by marked lamina propria mixed inflammatory cell infiltrates consisting of neutrophils, lymphocytes, plasma cells and eosinophils. Foci of neutrophilic cryptitis, crypt abscesses, glandular destruction and erosions of the mucosal surface were evident. Ulceration was noted occasionally. The inflammatory changes were diffuse in 75% of the biopsies and patchy in the remaining cases. There was no meaningful increase in the number of intraepithelial lymphocytes or apoptotic activity in colonocytes. Histologic evidence of chronicity, such as crypt architectural distortion, basal plasmacytosis, granuloma, and Paneth cell or pyloric metaplasia, was not appreciated. All the serologic markers showed fluctuated increase in their titers after Ipilimumab administration.. CONCLUSIONS: Ipilimumab therapy may induce active colitis that is histopathologically distinct from inflammatory bowel disease or graft-versus-host disease. Prophylactic budesonide does not appear to be helpful in preventing Ipilimumab-related gastrointestinal toxicity.

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Increased Risk of Inflammatory Bowel Disease After Salmonella or Campylobacter Gastroenteritis: A Population-Based Cohort Study

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Genetic as well as environmental factors, including infections, are believed to be involved in the pathogenesis of inflammatory bowel diseases (IBD). Various commensal enteric and potentially pathogenic bacteria have been studied. Most studies, however, have been cross-sectional and not population-based. We compared the risk of IBD between a cohort of patients with documented Salmonella or Campylobacter gastroenteritis and an age- and gender matched control group from the same population in Denmark. We identified 13,324 patients with Salmonella/Campylobacter gastroenteritis from laboratory registries for the

period 1991-2003 from North Jutland and Aarhus counties, Denmark and 26,648 unexposed controls from the same counties. Of these, 175 exposed patients with IBD before the infection, their 350 unexposed controls, and 80 unexposed individuals with IBD before the Salmonella/Campylobacter infection were excluded. The final study cohort of 13,149 exposed and 26,218 unexposed individuals were followed for up to 15 years (mean 7.5 years). A first-time diagnosis of IBD was reported in 107 exposed individuals (1.2%) and 74 unexposed individuals (0.5%) during follow-up. By Cox's proportional-hazards regression analysis the hazard rate ratio for IBD was 2.9 [95% CI 2.1-3.9] for the whole period and 1.9 [1.3-2.6] if the first year after Salmonella/Campylobacter infection was excluded. The increased risk in exposed subjects was observed throughout the observation period of 15 years. The increased risk was similar for Salmonella (n=6,464) and Campylobacter (n=6,685) and independent of age and gender of the patient. The increase in risk for a first-time diagnosis of Crohn's disease (n=47) and Ulcerative Colitis (n=134) was not significantly different. We conclude, that from a large, population-based cohort study with complete follow-up an increased risk of IBD was demonstrated in individuals notified in laboratory registries with an episode of Salmonella/Campylobacter gastroenteritis. This may have implications for the understanding of the pathogenesis of inflammatory bowel diseases, and it emphasizes the importance of food safety for disease prevention.

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Stool Coliform Testing Can Predict Effective Treatment Regimes for Patients with Antibiotic Resistant Pouchitis

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Background: Empirical antibiotic therapy remains the mainstay of treatment for pouchitis. Combination regimes of ciprofloxacin and metronidaxole can be effective for patients resistant to a single antibiotic agent but failure to enter remission and relapse on withdrawal or during maintenance treatment can occur. A novel method using stool coliform antibiotic sensitivity testing was used to select optimal treatment for pouchitis resistant to standard therapy. Method: Stool samples from patients with active pouchitis (pouch disease activity index (PDAI) ≥7) who failed standard antibiotic treatment were inoculated onto Iso-sensitest agar (Oxoid) using a sterile swab and rotary spreader. Antibiotic discs containing ciprofloxacin, trimethoprim, cefalexin, co-amoxiclav, nitrofurantoin, cefpodoxime, cefuroxime, and cefixime were added. The plates were incubated at 370 C for 18 - 24 hours and the sensitivity patterns recorded. Following 4 weeks treatment with an antibiotic selected on its sensitivity, the clinical component of the PDAI was remeasured. Results: 15 patients with endoscopic and histologically proven chronic pouchitis were studied. 13 had failed to enter remission with a combination of ciprofloxacin and metronidazole, two had relapsed on maintenance ciprofloxacin. Antibiotic coliform sensitivity testing showed: ciprofloxacin resistance in all samples; co-amoxiclav resistance in four samples; trimethoprim resistance in 11 samples, cefixime resistance in eight samples. Four samples contained extended spectrum beta lactamase (ESBL) producing organisms. All 15 patients were treated with an antibiotic to which their stool coliforms were sensitive. Twelve (80%) entered clinical remission with a PDAI symptom score of 0. Conclusion: Stool coliform testing and targeted antibiotic therapy is a novel investigation and treatment strategy which is effective in the majority of patients with antibiotic resistant pouchitis. Stool coliform sensitivity testing and targeted antibiotic therapy should be used in all patients who fail to respond to empirical antibiotic treatment or relapse on long term antibiotic therapy.

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Surgical Rates in Ulcerative Colitis: Have We Made a Difference?

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Introduction: The management of Ulcerative Colitis (UC) has changed considerably over the last 20 years. Immunomodulators and biologic therapies now play a role in treating patients with UC. If these therapies are effective a reduction in surgical rates should be expected. Aim: To review the colectomy rates for UC in an Irish tertiary referral centre over a 20-year period and to determine if newer therapies had impacted on surgical rates Method: We performed a retrospective review of the St Vincent's University Hospital Inflammatory Bowel Disease (IBD) database of all patients newly diagnosed with UC over a 20-year period. Surgery rates were determined. Emergency surgery was defined as all patients admitted with fulminant colitis or who failed standard in-hospital rescue medical treatment. Elective surgery was defined as all patients with pre-planned surgery Results: We studied 989 patients with newly diagnosed UC over four periods (1986-90, 1991-95, 1996-2000, 2001-05). Three-year colectomy rates were 9.5% (14), 14.5% (39), 15.7% (44) and 16.8% (50) respectively. No statistical difference was noted between the 4 quartiles (p=0.0568). However, a significant decrease in rates of emergency surgery was observed; 78%, 69%, and 38% to 20% (p<0.0001). This reduction was noted despite a corresponding increase in tertiary referrals from 25.6% to 33.9%. Azathioprine use in patients undergoing colectomy over the same period was 28.5%, 43.8%, 65.9% and 34% for the 4 quartiles. Ciclosporin use rose from 0%, 23%, and 38.6% to 52% in the last quartile. No patients received Infliximab in the first three quartiles. From 2001-2005, 18% of patients undergoing colectomy received Infliximab. Three of these patients (18%) received both Infliximab and Ciclosporin. Conclusion: Surgical rates, 3 years from diagnosis, have remained broadly similar in the last 20 years. This is despite increased usage of potent immunomodulors, including ciclosporin. However rates of emergency surgery have decreased dramatically over the same period. Surgery in UC is now predominantly employed in an elective setting.

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Efficacy and Tolerability of a Once Daily Treatment with Budesonide Capsules Versus Mesalamine Granules for the Treatment of Active Ulcerative Colitis: A Randomized, Double-Blind, Double-Dummy, Multicenter Study

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BACKGROUND: Recently, it has been shown in a small pilot trial that budesonide capsules (9mg budesonide once daily [OD]) might be effective for the treatment of active, distal ulcerative colitis.[1] **AIM:** Therefore, this pivotal study aimed to evaluate the efficacy and tolerability of capsules, containing gastric juice-resistant budesonide granules with a pH>6.4 dependent release profile (9mg budesonide OD), versus gastric juice-resistant mesalamine granules with a pH≥6.0 dependent, prolonged release profile (3g mesalamine OD) in patients with active ulcerative colitis. **METHODS:** Patients with active ulcerative colitis (Clinical Activity Index [CAI] ≥6, Endoscopic Index [EI] ≥4) were eligible for this double-blind, double-dummy, randomized, multicenter, phase III study. Patients received 9mg budesonide OD (n=177) or 3g mesalamine OD (n=166) for 8 weeks. Primary endpoint was clinical remission (CAI≤4, with stool frequency and rectal bleeding subscores of '0') at the final/withdrawal visit (per protocol (PP) population). **RESULTS:** 343 patients were randomized, treated and evaluable for safety and intention-to-treat (ITT), 302 for PP analysis. Primary efficacy endpoint: In the PP analysis, 67/153 patients (44%) in the budesonide group and 89/149 (60%) in the mesalamine group achieved clinical remission (p=0.5657 for non-inferiority). In the ITT analysis, 70/177 patients (40%) in the budesonide group and 91/166 (55%) in the mesalamine group achieved clinical remission (p=0.5203). Both treatment regimens were safe and no drug-related serious adverse events were observed. 3g mesalamine OD showed better efficacy rates in all efficacy endpoints. **CONCLUSION:** In active ulcerative colitis, treatment with 3g mesalamine OD is superior to a 9mg budesonide OD treatment. Both treatments were safe. **REFERENCES:** 1.) Kolkman JJ, Möllmann HW, Möllmann AC, Pena AS, Greinwald R, Tauschel HD, et al. Evaluation of oral budesonide in the treatment of active distal ulcerative colitis. Drugs Today. 2004;40(7):589-601. Supported by Dr. Falk Pharma GmbH, Freiburg, Germany

Secondary efficacy endpoints

	Number (%) of patients at week 8 (LOCF)	
	9mg budesonide OD	3g mesalamine OD
Mucosal healing ($DAI_{mucosal} \leq 1$)*	83/148 (56%)**	101/137 (74%)**
Histological remission	83/177 (47%)	97/166 (58%)
Therapeutic success (PGA)***	91/177 (51%)	114/166 (69%)
Therapeutic benefit (PGA)****	136/177 (77%)	142/166 (86%)

* $DAI_{mucosal} \leq 1$: no friability ** Patients with baseline $DAI_{mucosal} \geq 2$ only *** At least marked improvement **** At least slight improvement LOCF, Last observation carried forward; PGA, Physicians Global Assessment (acc. to Hanauer)

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The Effect of Inflammatory Bowel Disease (IBD) During Pregnancy On Long-Term Health and Illness in Children of IBD Patients-a Multicenter Israeli Study

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Background: Several studies describe the effect of IBD during pregnancy on perinatal outcomes. However, limited data exists regarding the long-term effect of maternal IBD on the childs' health and development. Aim: to investigate long-term morbidity or developmental defects in children of IBD mothers. Methods: Questionnaires containing IBD mothers and child medical details and children's developmental data were filled and compared to matched controls. Results: A hundred and forty six IBD mothers (93 Crohn's disease, 53 ulcerative colitis) were compared to 70 controls. IBD mothers had 385 children (age 16± 10.8 years, 52.8% born after IBD diagnosis) that were compared to 144 children (age 13±9 years) of the control group. Mean mother age: 43+ 10 vs. 39+8.3 years in IBD vs. controls. Disease duration: 10.8±7.5 years. A Third of the patients had IBD exacerbation, mostly (45%) during the first trimester. IBD patients had more spontaneous abortions (0.68±1.2 vs. 0.33±0.6 in controls, p=0.01). Birth weights were significantly lower in IBD mothers vs. controls' offspring: 3.13±0.6 vs. 3.27±0.45 kg, p=0.05. Importantly, this trend was found also in adolescence where IBD mothers offspring were significantly shorter and lighter compared to controls' offspring (1.28±0.41 vs. 1.47±0.33m, 29.3±21 vs. 39±24 Kg, p<0.01). IBD in study group offspring was diagnosed in 3% (p<0.05 vs. controls) whereas atopic dermatitis was more frequent in controls' offspring (11 vs. 5%, p<0.05). First-year intercurrent infections were more frequent in study group offspring (p=0.001 vs. controls). In contrast, wheezy bronchitis was more common in offspring controls (p<0.05). More learning difficulties and attention deficit disorders (5 vs. 0.8% and 4.6 vs. 0.8%, respectively,p<0.05), and gross neurologic abnormalities (4.4 vs. 0.7%), but not autism, dyslexia or abdominal pain were detected in the study group offspring. Conclusions: IBD has significant and diverse short and long-term effects on the health and development of IBD mothers' offspring.