contribute to CD susceptibility of the twins but are shared by affected as well as healthy individual. Comparative pairwise analyses between twin samples yielded several hundred potentially differing SNVs and CNVs. Manual inspection of alignments as well as validations by Sanger were not able to confirm any genetic differences between samples so far. Yet, our data provide an exceptionally thorough genetic characterization of the examined twin pairs and are the first example of whole genome sequencing applied to monozygotic twins discordant for CD.

Su1750

Transethnic Fine-Mapping of the *IL12B* Locus Identifies Two Independent Signals Associated With IBD Susceptibility and Disease Behaviors

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Background: Recent GWA studies have confirmed the *IL12B* locus as an IBD susceptibility locus and there has been increasing interest in this locus as a consequence of the efficacy of IL12B monoclonal antibodies in the treatment of Crohn's disease (CD). The aim of this study was to refine the IBD signal from the *IL12B* locus and to test for genotype-phenotype associations. Methods: Genotyping was performed using the 200K SNP ImmunoChip genotyping array. 240 SNPs covering *IL12B* were tested for association in non-Jewish Caucasian (NJ; 779 CD, 462 UC, 4182 controls) and Ashkenazi Jewish (AJ; 492 CD, 305 UC, 395 controls) populations. An additional 300 CD, 194 UC and 235 controls from Puerto Rico (PR) and 729 CD and 469 controls from South Korea (SK) were used for replication and transferability. Population substructure was corrected for using principal components analysis. Disease and clinical phenotypic associations were analyzed using logistic regression and Cox proportional hazards models. Results: In NJ IBD, 36 SNPs were significantly associated with CD. The majority of these SNPs are located 6.7-77kb upstream of *IL12B*. The most associated CD SNP was rs6897260 (p=4.07E-9, OR=0.69). This SNP is associated with CD independent of rs6871626 (p=8.75E-5, OR=1.25), where rs6871626 was recently reported by Jostins et al. (Nature 2012). Thus, at least 2 SNPs from *IL12B* are associated with CD. These 2 SNPs are also associated with NJ UC (rs6897260: p=3.34E-3, OR=0.79; rs6871626: p=9.67E-3, OR=1.22). No association was seen with these 2 SNPs in AJ IBD. The analyses in PR and SK demonstrated a modest association of rs6987260 with PR CD (p=0.02, OR=0.70) but not SK (p=0.82). The other SNP, rs6871626 was associated with SK CD (p=2.05E-3, OR=1.32), but not PR (p=0.68). These trans-ethnic results support the assertion there are two independent *IL12B* associations in NJ CD. These 2 SNPs were not associated with CD phenotypes (disease location, behavior) in NJ, but were both associated with extensive UC in NJ (rs6897260: p=7.23E-3, OR=0.68; rs6871626: p=9.40E-4, OR=1.52). Three other SNPs (including the intronic SNP rs2853694) were associated with time to surgery in NJ CD (p=1.66E-4, HR=2.11) but not with CD susceptibility (p=0.457), thus suggesting a marker of disease severity. Conclusion: We identified 2 independent SNP associations at the *IL12B* locus in NJ CD and those findings were confirmed by differential associations in the SK and PR populations. These 2 SNPs were also associated with extensive UC in NJ. In keeping with previous findings, a SNP association at this locus was associated with disease severity in CD. These data support the value of trans-ethnic mapping and suggest that both susceptibility and severity signals are likely to 'co-exist' in loci although these effects, as seen here, may be independent.

Su1751

Genetic Markers of Medically-Refractory Ulcerative Colitis and Proximal Disease Extension on Long-Term Follow-up - A Genome-Wide Association Study

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Background: More than 50% of patients with ulcerative colitis (UC) have poorly controlled disease and about 20% of patients require a colectomy. Previous genetic studies identified several loci associated with medically refractory (MR) disease leading to colectomy and with extensive disease. Aim: To identify genetic markers that can predict severe disease course in UC Methods: A retrospective study was performed by reviewing the medical records of UC patients with at least 5 years of follow-up (F/U) and with DNA available for genotyping. Genotyping was performed using Illumina OmniExpress (Cedars-Sinai Medical Center, CA). Clinical and epidemiological data, disease location at initial endoscopy, medication requirement and response, endoscopic extension and indication for colectomy were recorded. A genome-wide association study (GWAS) was performed comparing UC patients requiring colectomy for severe disease or for non-response to medications (MR-UC) vs. those who had non-MR-UC. We also compared UC patients who had limited colitis (Montreal E1/E2) at diagnosis whose disease extended proximally (Montreal E3) on F/U vs. those who remained with limited colitis. Comparisons among the clinical characteristics, endoscopic extent at diagnosis, were made between the 2 groups using Chi-square tests. Single marker association analysis of MR-UC versus non-MR-UC was performed using a logistic regression model correcting for population stratification using 3 principal components as covariates (PLINK v.1.06). Results: 603 UC patients were classified into MR-UC (n=355) and non-MR-UC (n=248). There were no differences in gender, smoking and appendectomy rates, age at diagnosis and family history of IBD. MR-UC was associated with Montreal E3 at diagnosis (p=0.0001). GWA data for analysis was available in 252/603 (42%). No association with MR-UC reached genome-wide significance, but 19 SNPs were associated with MR-UC with p-values $<$1x10E-4: 3 were protective (RGS7, POU6F1) and 16 increased the risk (KIAA1239, MST4, SCIN, FAS, PTPN5, SMARCC2, TPP2, GPR65, RNF24). Overall, 286 patients had limited colitis at initial endoscopy and 174 (61%) had proximal disease extension during F/U. There were no clinical associations with proximal disease extension. GWA data were available on 122/286 (43%), but no SNP reached genome-wide association with proximal extension. 20 SNPs had p- value$<$1x10E-4 and were located in the following genes CYP4Z1, DOCK2, PLS3 and OR2H2. Conclusions: Caucasian ethnicity and extensive disease at initial endoscopy are associated with MR-UC leading to colectomy. GWA study did not find significant associations with MR-UC or proximal disease extension. Larger studies are required to explore the genetics of severe UC requiring colectomy.

Su1752

Identification of New Genetic Variants Related to Thiopurine-Induced Myelotoxicity in Inflammatory Bowel Disease (IBD) Patients With Normal Thiopurines-Methyltransferase (TPMT): A Genome-Wide Association Study

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Background: Patients with low TPMT activity are at increased risk of developing thiopurine-induced myelotoxicity. However, only a minority of patients with myelotoxicity are carriers of a mutant TPMT allele. **Aim:** To identify genetic variants associated with thiopurine-induced myelotoxicity in IBD patients with TPMT wild-type alleles and normal activity of this enzyme. **Methods:** 93 IBD patients with normal TPMT activity and wild-type genotype treated with thiopurines were included. Case group: patients with thiopurine-induced myelotoxicity and without other thiopurine-induced adverse effect. Control group: patients without thiopurine-induced side effects consecutively included. TPMT activity over 13.7 UI/mL was considered normal. DNA was extracted from peripheral blood nucleated cells. TPMT genotype was determined by sequencing (TPMT*2, *3A, *3B, *3C, *3D, *4, *5, *6, *7, *9, *10, *15, *16, *19 and *22 alleles). Myelotoxicity was defined as $<$3,000/ml leucocytes, $<$1,500/ml neutrophils, or $<$100,000/ml platelets. Genomic DNA was analysed using Illumina OmniExpress Exome BeadChip genotyping array. This array interrogates a total of 951,117 SNPs (660K common and 200k rare coding variants). Genotype calls were generated using Illumina GenomeStudio. After standard QC control, associations between SNPs and myelotoxicity were assessed using logistic regression analysis. **Results:** 93 patients were included (37 cases and 56 controls). The distribution of gender, type of IBD, mean age, mean TPMT activity and mean dose of mercaptopurine (1.3 vs. 1.4 mg/kg) were similar between cases and controls. The percentage of patients with mercaptopurine was higher among cases (32.4 vs. 12.5%, p=0.02), while mean azathioprine dose was slightly higher among controls (2.2 vs. 2.5 mg/kg, p=0.03). A total of four SNPs showed significant association: SNP1: p=6.5 x 10-5, OR=10.4 (2.5-21); SNP2: p=6.6 x 10-5, OR=6.2 (2.4-12); SNP3: p=8.5 x 10-5, OR=0.1 (0.07-0.5); and SNP4: p=8.8 x 10-5, OR=0.2 (0.1-0,4).The SNP1 is located close to a gene that encodes a relevant enzyme of the thiopurine metabolic pathway. **Conclusion:** An exome-wide association study identified four new SNPs that could explain thiopurine-induced toxicity not related to TPMT deficiency. The SNP with the strongest association could regulate the expression of an enzyme of the thiopurine metabolic pathway. Although further validation is required, these variants could be promising genetic predictors of thiopurine-induced myelotoxicity.

Su1753

Primary Response to Infliximab in Crohn's Disease Is Associated With the *Tnfrsf1a* Rs1800693 Gene Polymorphism

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Background: The introduction of anti-TNF alpha antibodies has had a major impact on the treatment of patients with chronic inflammatory diseases. Remarkably the response to infliximab differs depending on the underlying disease. Whereas inflammatory bowel disease (IBD), rheumatoid arthritis and psoriasis all show good efficacy, there is no efficacy or even worsening of disease observed in multiple sclerosis patients. The *TNFRSF1A* gene has been implicated in susceptibility to multiple sclerosis but not to Crohn's disease (CD), rheumatoid arthritis or psoriasis. Both genetic and functional evidence suggested that rs1800693 is the causal variant in this gene(1). This variant leads to expression of a novel soluble form of TNFR1 which blocks TNF and therefore mimics the effect of anti-TNF agents. We hypothesized that the G risk allele of rs1800693 is associated with primary nonresponse to infliximab in IBD patients. **Methods**: A single-center cohort of 863 IBD patients (616 CD and 247 ulcerative colitis (UC)) were evaluated for primary clinical response to infliximab at weeks 4-10 following initiation of infliximab and were genotyped for rs1800693. A control group of 885 healthy controls were genotyped as well. Patients who had no clinical/CRP benefit after two or three infusions were considered as primary non-responders. Statistical analyses were conducted using PLINK. **Results**: In our population of 885 healthy controls the G allele frequency (44%) was similar to the frequency observed in the control population in the multiple sclerosis study (40%). A statistically significant association between the G risk-allele and primary nonresponse to infliximab was found in the CD cohort. In the CD non-responders group 27% of patients had GG genotype compared to 14% in the responders group (p=6.09e-03 for the recessive model, OR=2.34 [1.26 - 4.37]). The risk for non response increased from 8% in heterozygous carriers of the risk allele to 17% in homozygous mutant patients. However, in the (smaller) UC cohort, this association could not be confirmed (P=0.53 for the recessive model, OR=0.69 [0.33 - 1.45]). **Discussion**: We found that the TNFRSF1A rs1800693 GG genotype was associated with a 2.3 fold increased chance for primary non-response to infliximab in CD patients. The absence of effect in UC can be explained by low sample size, or could indicate different mechanisms driving response in CD or UC. The exact mechanisms how this variant leads to more resistance to anti-TNF agents in CD is unclear. Possibly, the anti-TNF effects exerted by the new soluble form of TNFR1 in the GG homozygous patients result in less TNFalpha-driven inflammation. It will be important to study mucosal expression profiles in these patients. (1) Gregory, A.P. et al. TNF receptor 1 genetic risk mirrors outcome of anti-TNF therapy in multiple sclerosis. Nature 488, 508-511 (2012)

	CD				UC			
	Allele	Genotypes			Allele	Genotypes		
	G	AA	AG	GG	G	AA	AG	GG
Responders	38%	211 (38%)	269 (48%)	78 (14%)	43%	55 (32%)	88 (50%)	32 (18%)
Non-responders	47%	19 (33%)	23 (40%)	16 (27%)	42%	23 (32%)	38 (53%)	11 (15%)

Su1754

The Crohn's Disease Associated DMBT1 Gene Variant Rs2981804 Influences IL-22-Induced DMBT1 Gene Expression by Modifying the DNA Binding of the Transcription Factors CREB1 and ATF2

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INTRODUCTION: Deleted in malignant brain tumors 1 (DMBT1) is an antibacterial scavenger receptor with increased expression observed in intestinal inflammation. A strong inducer of DMBT1 is the Th17 cytokine IL-22. We recently demonstrated associations of several single nucleotide polymorphisms (SNPs) in the DMBT1 gene with the susceptibility to Crohn's disease (CD) and ulcerative colitis (UC) [1]. AIMS&METHODS: The most strongly CD-associated SNP in our previous study was the non-coding DMBT1 variant rs2981804. Here, we aimed to analyse the functional role of this SNP. Potential transcription factor binding sites were identified in silico with the program TFsearch [2]. Electrophoretic mobility shift assays (EMSA) and siRNA transfections were performed to analyse binding of transcription factors to DNA encoding the CD risk and protective alleles of rs2981804. Expression of DMBT1 in intestinal biopsies and regulation of IL-22-induced DMBT1 gene expression was analysed by quantitative PCR. RESULTS: DMBT1 expression in intestinal biopsies was significantly increased in inflamed tissue of CD patients ($p < 0.01$). Several transcription factors were predicted to bind differentially to the genomic sequence containing the CD risk or CD protective allele of DMBT1 SNP rs2981804. Initial screening by EMSA analysis confirmed that a larger amount of nuclear protein binds to a DNA probe with the CD risk allele of rs2981804 in comparison to a probe with the protective allele. siRNA-mediated silencing of CREB1 or ATF2 protein expression in intestinal epithelial cells resulted in a nearly complete loss of protein binding to the DMBT1 probe with the risk allele, proving that CREB1 and ATF2 either directly bind to this genomic region or are an indispensable part of a larger DNA-binding protein complex. Stimulation of intestinal epithelial cells with the Th17 cytokine IL-22 induced DMBT1 expression. In cells with knocked-down CREB1 or ATF2 expression, IL-22-stimulated DMBT1 expression was significantly reduced, confirming that CREB1 and ATF2 are involved in the transcriptional regulation of the DMBT1 gene. CONCLUSION: We discovered a novel functional role of the non-coding DMBT1 SNP rs2981804 as a modulator of CREB1 and ATF2 binding, thereby regulating DMBT1 transcription, providing a possible explanation for the increased DMBT1 expression observed in CD patients. REFERENCES: [1] Diegelmann J et al. Identification of Novel DMBT1 Gene Variants As Susceptibility Variants for Crohn's Disease and Ulcerative Colitis, Gastroenterology Vol. 140, Issue 5, Supplement 1, Page S-272 (abstract presented at DDW 2011) [2] http://www.cbrc.jp/research/db/TFSEARCH.html

Su1755

Integration of Expression and Other Genomic Data to Further Study and Prioritize Genes Associated With Inflammatory Bowel Disease

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Presently, 163 loci containing 1645 genes have been associated with IBD. It is likely, however, that only a subset of these genes is truly associated with IBD. Further refinement of this gene list will allow us to better understand disease-causing mechanisms, potentially identify pathways, and identify targets for novel drug design related to IBD by eliminating erroneous signals and allowing for more focused analysis of related genes. Here, we study features that are enriched in the 163 loci and propose a statistical model to find the combination of features that gives additional evidence to a gene's association with IBD. More specifically, our main focus is to inform our gene list based on precise gene expression levels, for which we have generated RNA-seq data. We find that genes in IBD loci have higher expression levels in the intestine than in other tissues (Figure 1), adding evidence that these loci contain genes truly associated with IBD. Furthermore, we find that in the intestine, genes within IBD loci have significantly higher expression level than those outside of IBD loci (p-value= 1.3EXP-5), i.e., in full biopsy intestine samples, 78% of genes within IBD loci have RNA expression level greater than 0.1 in at least one sample while only 66% of genes outside of IBD loci have an expression level greater than 0.1. Additionally, genes within our identified IBD loci are 1.2 fold more likely to be differentially expressed between cases and controls than non-associated genes, indicating that differences in protein levels may be linked to disease state. Besides expression data, we also look into ENCODE transcription factor binding site data and expression quantitative trait loci (eQTL) data. We find that 1). Most transcription factors located in IBD loci have regulatory targets enriched in IBD loci (Table 1); 2). Variants significantly associated with CD/ UC are 2.7/ 3.4 times more likely to be in eQTL than randomly chosen SNPs (p-value<2.3EXP-13 for both CD and UC significant SNPs). To make our analysis more complete, we also collect data on genes' association with other immune diseases and SNP- protein coding variant data, since previous studies suggest that diseases with similar etiology share risk variants and that protein-coding variants are likely to be disease associated. We then build a statistical model to integrate the data and find the combination of features that gives additive evidence to a gene's association with IBD. Our analysis gives systematic insight into the features of IBD genes. Moreover, we can use the combined gene feature information to prioritize IBD genes and identify potential IBD genes whose GWAS signal fall below the significance threshold.

Table 1. 13 transcription factors in IBD loci have ChIP-Seq data. According to ChIP-Seq, 9 of these transcription factors have targets enriched in IBD loci (p-value < 0.01). Enrichment test uses total IBD genes in human genome as comparison: 1645 (6.2%) out of 26375 autosome genes are in IBD loci.

Transcription factor	Number of regulatory targets located in IBD loci	Total number of regulatory targets	Proportion of targets in IBD loci (%)	Enrichment p-value
NFKB1	682	8696	7.84	2EXP-6
FOSL2	228	2563	8.90	3EXP-6
USF1	601	7933	7.58	0.00010
STAT1	108	1142	9.46	0.00012
IRF1	490	6391	7.67	0.00015
HNF4A	241	2930	8.23	0.00018
FOSL1	90	928	9.70	0.00020
FOS	385	5013	7.68	0.00049
CEBPB	546	7662	7.13	0.00988
STAT3	402	5758	6.98	0.05389
ESRRA	8	114	7.02	0.69828
POU5F1	5	101	4.95	0.83503
PRDM1	19	304	6.25	1

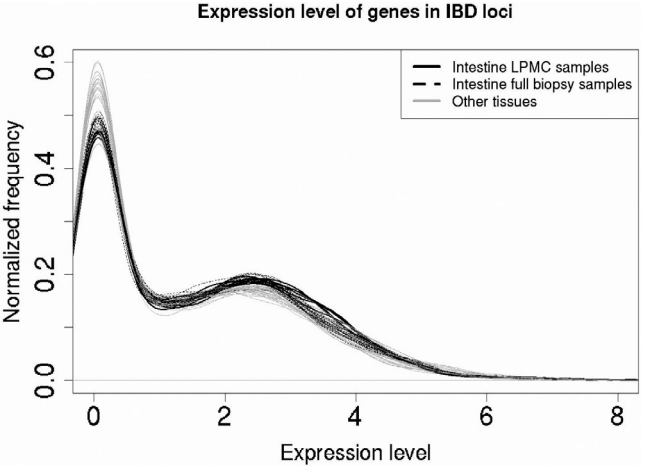

Figure 1. Expression level (FPKM, Fragments Per Kilobase of transcript per Million mapped fragments) of genes in IBD loci in different tissues. Black curves represent intestine lamina propria mononuclear (LPMC) samples and full biopsy samples; grey curves represent samples from 16 non-intestinal human tissues.

Su1756

Overexpression of Mknk2 and Thr-α in Dysplastic Inflamed Colonic Mucosa Correlates to the Risk of Colorectal Cancer in Pancolitis

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Background: Patients with extensive, severe, and early onset ulcerative colitis (UC) have an increased risk of colorectal cancer (CRC). Why extensive colitis (pancolitis) predisposes to CRC is unknown. Aim: This study used DNA microarray-based gene expression profiles from colonic pinch biopsies from patients with left-sided colitis, pancolitis, UC-associated dysplasia, and controls in order to identify possible predisposing molecular signatures. Methods: Mucosal pinch biopsies were obtained from the descending colon of forty-seven patients with active UC, Mayo score ≥ 2 (7 UC-associated dysplasia, 20 pancolitis, and 20 left-sided colitis) and 15 controls. Genome-wide gene expression analyses were performed using Affymetrix GeneChip Human Genome U133 Plus 2.0. Real time RT-PCR and immunohistochemistry was applied to validate selected microarray data. The microarray data were analyzed by principal component analysis, Hotelling T2 tests, and overrepresentation analysis for Gene Ontology terms. The RT-PCR data were analyzed with the Wilcoxons' rank sum test. Results: Based on the microarray data the inflammation was found molecular distinct within the three groups (dysplasia, pancolitis, and left-sided colitis), but annotation analysis suggested similarities in transcripts involved in peptide hormone signaling in dysplasia and pancolitis. Based on these findings and their known involvement in neoplasia, MAP kinase interacting serine/threonine kinase 2 (MKNK2) and the thyroid receptor-α (THR-α) were selected for further validation in terms of RT-PCR and immunohistochemistry. Both MKNK2 and THR-α were found significantly ($p < 0.05$) increased in all inflamed samples irrespective of group belonging when compared to controls. As suggested by the microarray data MKNK2 and THR-α were also found increased in both dysplastic and pancolitis samples when compared to left-sided colitis, although MKNK2 did not quite reach significance. These results were reproduced with immunohistochemistry. Conclusions: The gene expression profiles of the colonic mucosa from patients with left-sided colitis, pancolitis, and UC-associated dysplasia are distinct, but transcripts in peptide hormone signaling pathways, in this case MKNK2 and THR-α, seem to be common features in both pancolitis and UC-associated dysplasia. Consequently, MKNK2 and THR-α might be important for the inflammation-driven neoplastic development observed in UC-associated dysplasia.