selected. To ensure reliability of results, histology was confirmed by a single specialist GI pathologist. All subjects had a similar disease severity. No subject was on immunosuppressive treatment, to eliminate influence on gene expression. After scaling, absolute, fold- change and comparative clustering analyses were performed using Affymetrix® Microarray Suite 4.0 and Data Mining Tool to detect genes with consistent under- or over- expression between disease groups and controls.
Results:
Our results identify a number of novel genes not previously associated with IBD. 183 genes were identified with a fold- change >2 that discriminate colonic CD from UC; many are involved in adhesion, inflammatory response, proliferation, proteolysis and signaling. In addition to inflammatory response genes, certain cancer- related genes were differentially expressed only in UC with down- regulation or absence of tumour suppressor genes including CEACAM and WAF1, highlighting the increased malignant potential in this disorder. In CD, a number of inflammatory response genes were differentially expressed, including IL18. Importantly, only 40 common genes were consistently differentially expressed in both UC and CD compared to controls.
Conclusion:
Novel candidate IBD genes have been identified using gene expression analysis and these may lead to the development of a gene-based classification system which assists in differentiating UC from colonic CD.

M1543

Toll-like receptor (TLR)-4 Asp299Gly polymorphism is associated with ulcerative colitis (UC)

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Background and aim: Studies on genetically engineered animals suggest the critical role of colonic bacterial micro flora in the pathogenesis of ulcerative colitis. Toll-like receptors are pattern-recognition receptors (PRRs) involved in the early phase of the innate immune response to bacterial products. Toll-like receptor (TLR)-4 specifically binds lipopolysaccharides (LPS) and transduces its signal trough the NF-kB pathway. The recently described Asp299Gly polymorphism in the TLR4 gene is associated with impaired bacterial recognition (e.g. decreased sensitivity to LPS) and increased susceptibility to gram negative infections. We therefore hypothesised that TLR4 could be a good candidate gene in UC. Patients and Methods: A cohort of 163 patients with a well established diagnosis of UC and 136 healthy hospital workers were genotyped after informed consent for the TLR4 variant Asp299Gly and for the three CARD15 variants (Arg702Trp, Gly908Arg and Leu1007InsC) using Taqman PCR and PCR-RFLP's, respectively. Clinical charts were reviewed for the following phenotypes: localisation (rectosigmoiditis, left colitis and pancolitis), surgery, extra-intestinal manifestations, familial disease, pANCA and smoking at diagnosis. Groups were compared using Chi-square test. Results: There were 31/163 (19.02%) UC patients carrying the TLR4 variant compared to only 13/136 (9.5%) healthy controls ($p = 0.021$). The mutated allele frequency was significantly higher in UC than in the control population (10.1% vs. 5.2%, $p=0.015$) . There were 2 UC patients homozygous for the mutant allele, compared to 1 control. Univariate analysis failed to show any significant association between TLR4 variant Asp299Gly and UC phenotypes. 4.9% of the patients were CARD15+/TLR4+, 12.3% were CARD15+/TLR4-, 14.1% CARD15-/TLR4+ and 68.7% CARD15-/TLR4-. Among the different combinations, the CARD15-/TLR4+ was the only one significantly more frequent in UC than in controls ($p=0.037$). Conclusion: A positive association is observed between the TLR4 Asp299Gly polymorphism and UC. The frequency of this mutant allele was twice as high in UC patients compared to healthy controls. This association may further help to understand the role of the bacterial flora in the development of ulcerative colitis.

M1544

Candidate Genes for Experimental IBD: Microarray Analysis in Combination with Quantitative Trait Locus (QTL) Mapping Data in IL-10-Deficient Mice

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Background & Aim: IL10-deficient (*Il10*^{tm1Cgn}) mice serve as a model for inflammatory bowel disease (IBD). The severity of colitis depends on the genetic background strain carrying the disrupted *Il10* gene: C3H/HeJBir-*Il10*^{tm1Cgn} mice develop severe colitis, while C57BL/6J-*Il10*^{tm1Cgn} mice are colitis-resistant. Two previous studies reported 10 QTLs associated with colitis susceptibility in these strains. The aim of this study was to identify candidate genes within the QTL intervals, which likely contribute to colitis susceptibility. Therefore, we analyzed gene expression patterns in colonic tissue of both strains and combined the results with previous QTL mapping data. Methods: Microarray analyses were performed by using Affymetrix GeneChip technology. Total RNA was isolated from colonic tissue of 4-week-old C3H/HeJBir- and C57BL/6J-*Il10*^{tm1Cgn} mice (before onset of colitis) as well as C3H/HeJBir and C57BL/6J wildtype mice. In each of the four groups, RNA extracted from five colons was pooled. Expression was filtered using the following threshold values: signal difference >40, change fold >2.0, change p-values >0.99 and <0.01 respectively; for some genes change fold was >1.5, signal difference >200 and change p values >0.99 and <0.01. Results: 651 genes were differentially expressed between colitis-susceptible C3H/HeJBir-*Il10*^{tm1Cgn} and colitis-resistant C57BL/6J-*Il10*^{tm1Cgn} mice, 290 genes between C3H/HeJBir and C57BL/6J wildtype mice. 124 of them showed similar expression differences in both subsets and were therefore disregarded for further analysis. Of the remaining 527 differentially expressed genes (C3H/HeJBir- vs C57BL/6J-*Il10*^{tm1Cgn}), 134 genes were located within the 10 QTL intervals. 29 of these 134 genes represent attractive candidate genes because of their known role in immune and defense responses, such as *Nfkbia*, *Stat1*, *Il1r2*, *Cd14*, genes of the major histocompatibility complex and related genes like *Tap1* and *Tap2*. Other attractive candidates are the genes encoding vitamin D receptor, gap junction membrane channel protein, and endomucin. Conclusion: Using a combination of QTL analysis with microarrays, we were able to identify likely candidate genes, which may contribute to colitis susceptibility in the *Il10*^{tm1Cgn} model. Expression differences of the candidate genes have to be confirmed by realtime PCR before conducting sequence analyses and functional assays to determine their relevance to colitis susceptibility.

M1545

Haplotype Structure Analysis in the HLA Region and Implications for Association Mapping in Inflammatory Bowel Disease

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Background: Linkage to chromosome 6p ("IBD3") has been established for inflammatory bowel disease and in several autoimmune/inflammatory diseases. The region comprises the HLA genes but also a host of important immunoregulatory genes. The extent and structure of linkage disequilibrium (LD) in the region is unclear. The existence of long range LD would have significant impact on association mapping strategies. Methods: A linkage disequilibrium (LD) map was constructed with a set of 320 SNP marker covering 3.53 Mb of the HLA region. SNPs were supplied by Applied Biosystems (https://store.appliedbiosystems.com) and are typed by dye based allelic discrimination (TaqMan) technology in 548 German controls, 90 controls from Norway, 73 controls from England, 45 self-described Caucasian Americans and 45 self-described African Americans. Pairwise linkage disequilibrium (D-prime) is computed for all markers followed by clustering (Unweighted Pair Group Method with Arithmetic mean). Pearson's correlation coefficient was calculated as a measure of similarity between the populations and the genetic distance between markers was estimated on LD units using the LDMAP program. Results: Haplotype block structure is conserved to a different degree in the 3.53 Mb region analysed. Areas with well conserved block structures intermingle with areas in which less defined LD structures can be observed. For all populations a decrease in LD could be observed from the telomeric to the centromeric part of the region. A similar picture was seen for all European populations and (on a lower level) for African Americans. Similarities between populations are generally higher where LD is high. Conclusion: LD results support the establishment of SNP marker-sets for the more structured regions for use in further association mapping of inflammatory bowel disease or different chromosome 6p linked diseases. The size of the population has a crucial impact on the detection level of LD pattern. Regions with block structures could be represented by fewer SNP markers or by a smaller population while in regions without block structure more markers need to be analysed.

M1546

The TNF-857C/T Polymorphism Is Associated with Early Onset, Smoking and Arthritic Complications In Inflammatory Bowel Disease and Acts Independently Of CARD15

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Background & Aims: van Heel et al. (Hum Mol Genet. 11:1281-9, 2002) recently reported association of the TNF-857C/T polymorphism with inflammatory bowel disease (IBD). They reported strong association with ulcerative colitis (UC) but with Crohn's disease (CD) only after removal of individuals carrying the CARD15, CD predisposing, mutations. This association is examined in detail here with important consequences for association studies. Methods: The association of the TNF-857C/T polymorphism with IBD, CD and UC was tested in four independent cohorts using family-based and case-control association tests. Multivariate logistic regression procedures examined the correlation of the genotype with clinical traits and with CARD15 genotype. Results: The TNF-857C allele was over represented relative to controls in both German trios and German families for both IBD ($P=0.024$) and CD ($P=0.002$). Allele frequencies were identical between cases and controls in a chronic acute CD cohort lacking certain complications (e.g. fistulae, stenosis, arthritis). No significance for UC was observed ($P=0.666$). Identical results were obtained using the TDT test, although the TNF-857C allele was over-transmitted in all cases. Logistic regression revealed that the TNF-857C/T polymorphism was correlated with early age at onset, arthritis, smoking and right colonic disease in both CD and UC. There was no correlation with CARD15 genotype. Conclusions: The TNF-857C/T polymorphism is associated with both CD and UC in a similar manner. This association is independent of CARD15. Unless association is examined at the level of detailed clinical traits then studies may be conflicting because patient collections vary at this level. The TNF-857T allele may be protective against early onset, arthritis and smoking related IBD and may protect against persistant inflammatory conditions in general.

M1547

Card15/Nod2 Mutations in Ulcerative Colitis (Uc) and Crohn's Disease (Cd) Patients Belonging To Mixed Ibd-Families: A Distinct Entity?

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Background and Aims: The Inflammatory bowel diseases (IBD) CD and UC are complex polygenic diseases. Ten to 15% of patients report a positive familial history of the disease, with a high concordance in disease type and location. CARD15/NOD2 has been identified as the first gene associated with CD. We previously showed that the serologic response to ASCA (Anti-Saccharomyces cerevisiae antibodies) and pANCA (antineutrophil cytoplasmic antibodies) is different in UC and CD patients belonging to so-called mixed IBD families compared to sporadic CD and UC patients and patients from pure CD or UC families. We therefore studied the CARD15 mutation frequency in IBD patients belonging to mixed families. Methods: From our IBD database, we identified 31 families where both CD ($n=31$) and UC ($n=26$) were present. Clinical files were reviewed by three physicians. ASCA and pANCA were available for all patients. 344 patients with sporadic or pure familial CD, 114 sporadic or pure familial UC patients and 141 healthy volunteers served as controls. All patients were genotyped for Arg702Trp, Leu3020insC and Gly908Arg in CARD15 using PCR-RFLP's. Statistical analysis was done with Chi-squared test or Fisher's exact test when appropriate. Results: (tabel 1). UC patients belonging to mixed families had significantly more mutations in CARD15 and more ASCA in comparison with sporadic and pure familial UC patients ($p = 0.0126$ and $p = 0.0014$ respectively) and more CARD15 mutations compared to healthy controls (42.3% compared to 21.3%) ($p=0.0221$). There were significantly less ASCA positive CD patients belonging to mixed families than there were in the sporadic and pure familial CD group ($p = 0.0472$). The observed pANCA prevalence