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Extraintestinal Manifestations of Inflammatory Bowel Disease (IBD) and Polymorphisms in the TNFα Gene: Further Evidence for Phenotype Determining Genes in the MHC Region on Chromosome 6

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Background - Type 1 and 2 peripheral arthritis (PeA), erythema nodosum (EN) and uveitis are distinct, but overlapping extraintestinal manifestations (EIM's) of inflammatory bowel disease. We have previously described the clinical association of Type 1 PeA with both EN and uveitis, and Type 2 PeA with uveitis only. In addition we have described associations with distinct HLA-B and HLA-DR alleles, which suggest that EIM's may be determined by genes in this region, in linkage disequilibrium with each other. To investigate this further this study was undertaken to examine polymorphisms of the TNFα gene in these EIM's. TNFα is an important cytokine in mediating inflammation, and the gene lies on chromosome 6 in the area of interest between HLA-B and HLA- DR. Methods - EDTA blood was collected from 39 patients with Type 1 PeA, 28 patients with Type 2 PeA, 31 patients with EN and 30 patients with uveitis. DNA was extracted and the presence of four polymorphisms in the TNFα gene, at positions -1031, -380, -308 and -238 was assessed by a pcr based technique using sequence specific primers. The results of the different groups were compared with each other and with 261 healthy controls, using 2x2 contingency tables and Fisher's exact test.A Bonferroni correctio was made for multiple comparisons using a correction factor of 16 (the number of alleles x number of tests). Results - There was a significant association between EN and possession of the -1031C polymorphism - 22/31 (71%) patients vs 96/261 (37%) of controls ($p = 0.0004$, $pc = 0.006$). There was no significant difference in the prevalence of this polymorphism between EN and Type 1 PeA patients (71% vs 46%), but there was a significant difference between EN and Type 2 PeA (71% vs 25%, $p = 0.001$, $pc = 0.016$), and between EN and uveitis (71% vs 34%)$p = 0.016$, although the latter did not withstand correction for multiple comparisons. No associations were seen at positions, -380, -308 or -238 with any EIM's Conclusions - These data demonstrate a strong association between EN and polymorphism -1031 of the TNFα gene. In addition they support the hypothesis that the clinical phenotype of extraintestinal manifestations may be determined by genes in linkage disequilibrium in this region within the MHC. This linkage disequilibrium may determine the distinct yet overlapping clinical syndromes of arthritis, EN and uveitis.

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Transmission Disequilibrium Testing Confirms The Association Of The TNFα 1031C Allele With Crohn's Disease.

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INTRODUCTION: Increased tumour necrosis factor (TNF) expression in the TNFΔARE mouse model results in a Crohn's disease (CD) like phenotype, and anti-TNF therapy is effective in treating CD. Linkage studies from five groups worldwide have confirmed an inflammatory bowel disease (IBD) susceptibility locus on chromosome 6p. We have previously reported a significant association with the TNFα promoter -1031 C allele and CD in both Japanese and European Caucasian case-control populations. In order to overcome potential false positive results from a case control study, IBD families were genotyped and the transmission disequilibrium test (TDT) was used to assess association AIMS: To confirm the association seen in the case-control studies in an independent family based population. METHODS: We obtained complete genotypes for the TNFα 1031 C/T polymorphism using PCR-SSP in 515 parent-child trios from 434 European Caucasian nuclear families (349 simplex, 85 multiply affected). A permutation based probability test was used to calculate association independent of linkage (Aspex). RESULTS: We observed significantly increased transmission of the TNFα 1031 C allele for the CD but not IBD or UC phenotypes. Under a multiplicative model the genotype relative risk of the C allele is 1.5, and the population attributable risk approximately 20%. CONCLUSION: We have confirmed the association of the TNF -1031 polymorphism with Crohn's disease by the TDT. This common allele (22% frequency), or one in linkage disequilibrium with it, influences nearly a fifth of the cases of Crohn's disease in a Caucasian population

Transmission Of the TNFα -1031C

	genotyped trio (n)	Transmit	Nontransmit	Significance
IBD	515	197	179	n.s
CD	273	118	81	$p = 0.01$
UC	233	77	96	n.s

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Genome-wide Scanning in a Belgian IBD Population Reveals Novel Linkages and Validates Previous Linkages

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Introduction: Epidemiological studies addressed the important genetic susceptibility of IBD. Previous genome-wide scans have shown at least nominal evidence for linkage on various chromosomes but only a number of susceptibility regions have been replicated: 16cen (*IBD1*), 12q (*IBD2*) and 6p (*IBD3*). Previously, we found no evidence for linkage on IBD susceptibility regions 3, 7, 12 and 16 in a smaller cohort of Belgian IBD-affected sibling pairs. Therefore, a whole genome search was performed in a larger group of IBD affected relative pairs to see if other IBD susceptibility regions could be identified in this population. Methods: 92 IBD families consisting of 125 affected sibling pairs (100 CD-only - 25 mixed CD-UC)were recruited from the Flemish part of Belgium (all Caucasian) and were genotyped for 323 microsatellite markers spaced over the genome (average spacing 12.6 cM). 42 markers were added to those of the index map to fill in gaps. Markers were run on ABI 373 sequencers, and genotyped using the Genescan 3.1/Genotyper 2.1 software (ABI). Allele frequencies were calculated from the non-related parents in the samples. Two-point and multipoint non-parametric linkage (NPL) analysis was carried out using Genehunter 2.1. Results: Nominal evidence for linkage was obtained on various chromosomes: on chrom 1, two peaks were seen: the first around D1S197 with a multipoint NPL = 2.42 ($p = 0.006$), and another around D1S305 and D1S252 on 1p13 (multipoint NPL = 2.97, $p = 0.001$). On chrom 4 maximal linkage was seen around D4S406 (multipoint NPL = 2.44, $p = 0.007$). Chrom 6 showed nominal linkage on 6q16 around D6S314 (NPL = 2.44, $p = 0.007$). Although weak, linkage was also observed on 10p12 around D10S197 (NPL = 2.05, $p = 0.02$). Chromosome 14q11-12 showed linkage over a broad region, being maximal at D14S80 (multipoint NPL = 2.41, $p = 0.008$). Chrom 20: maximum multipoint NPL around D20S192 (NPL = 2.7, $p = 0.003$). Earlier findings of linkage on Xcen around DXS990 were confirmed (NPL = 1.6, $p = 0.049$). Conclusion: A genome-wide search in a Belgian IBD population showed regions of linkage overlapping with or extending previous genome-wide scans: 1 (overlaps with Cho et al), 4 (overlaps with Hampe et al), 10p12 (partially overlaps and extends with the region found by Hampe et al), and 14q11-12 (overlaps with Ma et al and Duerr et al). We therefore further support the existence of the *IBD4 locus* on 14q11-12. Furthermore, novel linkages were observed on 1p13, 6q16 and 20q.

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Linkage Disequilibrium Mapping in Ashkenazi Jews with Crohn's Disease Localizes the IBD1 Susceptibility Gene to a Region of 600 kb.

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Several genetic studies including an analysis of more than 400 affected sib-pairs by an international consortium have provided compelling evidence for a susceptibility locus for Crohn's disease (CD) on chromosome 16 designated IBD1. More recently, we and others have found increased evidence for linkage to the IBD1 locus among CD patients with an early age of disease onset. In order to more precisely localize the gene responsible for this increased susceptibility to Crohn's disease we have performed linkage disequilibrium (LD) mapping using the TDT test which measures distortion in the expected 50% transmission of genetic markers from parents to the affected child. Methods: Parents and an affected child from 63 Ashkenazi families were genotyped with 21 known microsatellite markers as well as with 18 newly identified markers encompassing the IBD1 region. All the families had two or more affected individuals with Crohn's disease and at least one affected with an age-of-onset ≤21. Results: TDT analysis of the early age-of-onset group with the 21 known microsatellites showed five haplotypes with significant ($p = 0.05$) distortion in transmission to the affected child. Upon further analysis of a chromosome 16 contig only 10 of the 21 microsatellite markers were found to be within the IBD1 region. These 10 markers allowed the identification of only a single haplotype showing significant transmission distortion and two showing suggestive evidence ($p = 0.06$ and 0.07) of transmission distortion. In order to increase the density of markers the chromosome 16 contig was screened for new microsatellite markers leading to the identification of an additional 18 markers distributed at a density of approximately 1 marker/150 kb. When used for LD mapping these markers led to the identification of a single haplotype displaying strong LD ($p = 0.0027$). Conclusion: LD mapping of early age-of-onset affecteds in the genetically homogeneous Ashkenazi population localizes the IBD1 gene to a region of approximately 600 kb.

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Polymorphisms in the ICAM-1 Gene but Not in the CD11 Cluster Are Disease Modifying Factors in Inflammatory Bowel Disease

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Background: Adhesion molecules play an important role in the pathogenesis of intestinal inflammation. Previous genetic linkage studies have identified a susceptibility locus on the pericentromeric region of chromosome 16 (IBD1), which includes the integrin-alpha-L (CD11a) and Integrin-alpha-X (CD11c) genes. Intercellular Adhesion Molecule 1 (ICAM-1) is the ligand of CD11a and has been suggested as a disease modifier gene. Aim: To investigate association of mutations in these adhesion genes with inflammatory bowel disease (IBD). Methods: A cohort of 422 families (523 affected sibling pairs) of German and UK extraction plus 190 German normal controls were examined. Mutation detection by full length sequencing was performed in the CD11a and CD11c genes in 20 unrelated IBD patients. All detected SNPs plus known SNPs (4 in ICAM1, 1 in CD11c) were genotyped using TaqMan technology. Case-control contingency table (χ^2 statistic) analyses were performed, linkage disequilibrium was assessed using the EH program and family association was evaluated using the pedigree disequilibrium test (PDT), AJHG 2000. Results: Significant association was observed in the R allele frequency of the G/R241 polymorphism in the German UC group as compared to controls (UC 17.8%, NC 10.9%, $p = 0.027$) and the E allele frequency of the K/E469 polymorphism was higher in the British CD group (CD 46.1%, NC 40.5%, $p < 0,05$) than in controls. The PDT analysis was negative. Strong linkage disequilibrium (LD) ($\chi^2 = 62.3$, df = 3, $p < 0.001$)) was observed. The two known mutations in the promoter and exon 2 of ICAM1 (as identified previously in an African population) were not present in our cohort. Five novel SNPs in the CD11a gene were identified in intron 10, 14, 20 and exon 12 (Arg791Thr, Asn809Asn), all in pairwise LD ($\chi^2 = 12.8 - 70.4$, df = 3, $p < 0.001$). No significant association

in the case-control or PDT analysis for CD11a and CD11c was observed. Conclusions: The investigated mutations of the CD11a and CD11c genes are not likely to be involved in IBD etiology. As previously suggested in a Japanese and US cohort, the ICAM1 variants (including the G/R241 polymorphism in the MAC-1 binding domain) could act as population dependent disease modifiers in the development of IBD.

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Novel Polymorphisms In The Beta 7 Integrin Gene (ITGB7): Family Based Association Studies In IBD.

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Background: Linkage studies from five groups worldwide have confirmed the presence of an inflammatory bowel disease (IBD) susceptibility locus on chromosome 12q. ITGB7, a positional candidate gene within this region, is involved in lymphocyte homing to the gut and retention of intra-epithelial lymphocytes. Monoclonal antibodies to alpha 4 beta 7 integrin have been shown to ameliorate colitis in animal models. No polymorphisms in ITGB7 had been reported and therefore we screened this gene in order to identify markers to test for association. In order to overcome potential false positive results from a case control study, polymorphisms were genotyped in IBD families and the transmission disequilibrium test (TDT) was used to assess association. Aims: To screen ITGB7 for polymorphisms, and carry out association testing of common or functional polymorphisms in IBD families. Methods: Genomic sequence was obtained for the whole gene and promoter region by direct sequencing of the products of inverse PCR and PCR between exons. PCR fragments covering all 16 exons and 1.7kb of 5′ promoter region were designed and analysed for polymorphisms in 24 individuals by denaturing HPLC (Transgenomic Wave). Samples showing heterozygote traces were sequenced to verify the SNP. PCR-RFLP assays were designed for each SNP and allele frequencies were tested in 90 healthy controls. Common alleles (frequency $\geq$ 10%) and potentially functional polymorphisms were genotyped in 567 trios from 464 IBD families. A permutation based probability test was used to calculate association independent of linkage (Aspex). Results: 14 SNPs were identified and two common intronic and two amino acid changing SNPs were genotyped. Data were available from 102 multiply affected families and 362 simplex families containing 254 ulcerative colitis (UC), 13 indeterminate colitis and 300 Crohn s disease (CD) trios. No significant TDT results were obtained with any SNP for the IBD, UC or CD phenotype. Conclusion: The ITGB7 gene is unlikely to be involved in IBD susceptibility and therefore future studies on chromosome 12 should focus on other positional candidate genes.

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Genetic and Environmental Factors Associated with Diminished Bone Mineral Density (BMD) Differ in Ulcerative Colitis and Crohn's disease

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Background/Aims: Bone loss is a recognized complication of CD and UC. The pathogenesis is unclear but may be influenced by genes regulating bone metabolism (e.g., *VDR, COLIA1*) and/or gut inflammation (e.g., *TNF-α, IL-6*). This study was undertaken to evaluate these genes in relation to the BMD of patients with IBD. Methods: BMD at the lumbar spine (LS) and femoral neck (FN) was determined by DEXA in 106 UC and 203 CD patients randomly recruited from the MSH IBD Centre. Genotypes were determined for the biallelic polymorphisms at position -174 of *IL-6*, positions -238 and -308 of *TNF-α*, the *FokI* site of *VDR* and the Sp1 site of *COLIA1*. Genotype frequencies among groups were compared by χ^2 and means among groups were evaluated by ANOVA. Results: Reduced BMD (T score $\leq$ -1) occurred in 45% of CD patients and was equivalent at the LS and FN. Osteopenia in CD occurred independent of current or prior steroid use. In UC, diminished BMD occurred in 36% at the LS and 28% at the FN. Prior or current steroid use was positively associated with osteopenia at the LS ($p = 0.004$) but not at the FN. Bone loss in both CD and UC was not correlated with gender,disease duration or site of disease. Of UC patients, those with osteopenia at the FN were more likely to carry the G/G genotype (60% vs. 41%, $p = 0.01$) and less likely to carry the C/C genotype (14.3% vs. 0%, $p = 0.01$) at position -174 of *IL-6*. The same associations were not identified for UC patients with reduced BMD at the LS. Mean BMD results were not different when evaluated by genotype grouping. For CD patients, mean BMD (g/cm^2) at the LS was significantly lower in patients carrying G/G (1.13 g/cm^2) at -238 of *TNF-α* than in those with either G/A (1.17 g/cm^2) or A/A (1.53 g/cm^2)($p = 0.046$). Results at the FN demonstrated a similar trend but the results were not statistically signficant. Conclusions: In CD, osteopenia occurs independent of current or prior steroid use. Carriage of the A allele at -238 of *TNF-α* appears to confer resistance to bone loss in CD; as this allele has been associated with reduced levels of LPS-induced TNF production, BMD in CD may be influenced more by disease activity and disease activity-related genetic polymorphisms. In UC, steroid use is an important factor associated with bone loss, particularly at the LS. Genetic factors may play an important role in bone metabolism at the FN in UC; carriage of the C allele at -174 of *IL-6*, which is associated with diminished IL-6 production, may be protective against bone loss.

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A Genome-Wide Screen of Ashkenazi Jews with Crohn's Disease: Evidence for a Unique Susceptibility Locus on Chromosome 14.

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Background: Various epidemiologic and genetic studies support a role for genetic factors in susceptibility to Crohn's disease (CD). Since Ashkenazi Jews have a 10-fold greater risk of developing the disease than their non-Jewish neighbors we sought to determine whether there might be susceptibility genes unique to this population by performing linkage analysis on a population consisting entirely of Ashkenazi Jews. Methods: A genome-wide screen of an Ashkenazi Jewish population of 170 CD patients was initiated. These 170 patients are composed of 105 affected relative pairs consisting of 56 sib-pairs, 31 cousin-cousin pairs, 15 uncle(aunt)-niece(nephew), and 3 grandparent-child pairs. Of these 170 affecteds, 101 have an age-of-onset $\leq$21. For genotyping the ABI medium density linkage mapping panel which consists of 15-20 dinucleotide repeats per chromosome with an average heterozygosity of 0.79 and an average distribution of 9.2 cM was used. Results: We have, to date, screened our patient population for linkage to markers on four chromosomes, 1, 5, 6, and 14. These chromosomes were initially selected because others have obtained suggestive evidence for linkage to these four chromosomes in predominantly non-Jewish populations. The genotyping data was then subjected to non-parametric multipoint linkage analysis using Genehunter 2.0. There was no evidence of linkage on chr 1 and only slight evidence of linkage on chr 5 at D5S424 ($p = 0.06$); this is near the region of suggestive linkage previously observed by others. For chr 6 we observed modest evidence of linkage at D6S1610 ($p = 0.02$) which is near the HLA region where evidence of linkage has also previously been observed. However, the strongest evidence for linkage was observed on chr 14 at D14S65 ($p = 0.007$). Although there have been two other reports of possible linkage to CD on chr 14, the previously reported linkage was observed for markers at 14q11-12 arm whereas the linkage we observe is on the telomeric part of the q arm; we did not observe any evidence for linkage at 14q11-12. Conclusion: These studies suggest that there may be unique susceptibility genes in the Ashkenazi Jewish population that is responsible for their increased risk to CD.

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The 4G/5G Polymorphism of the Type-1 Plasminogen Activator Inhibitor Gene Is a Determinant of Disease Phenotype and Thrombotic Events in Patients with Inflammatory Bowel Disease

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Background & Aims: Thrombosis in intestinal and extraintestinal vasculature is increased in inflammatory bowel disease (IBD). The present study prospectively evaluated the role of the 4G/5G polymorphism of the type-1 plasminogen activator inhibitor (PAI-1) gene, the major inhibitor of fibrinolysis, in IBD phenotype and thrombotic complications. Methods: One hundred and ten consecutive patients with ulcerative colitis, 85 with Crohn's disease (CD), and 350 controls were included. The PAI-1 4G/5G polymorphism, factor V Leiden, and mutation 20210A of the prothrombin gene were assessed by PCR. Plasma PAI-1 antigen was quantified by ELISA. Results: In patients with CD the multivariate analysis showed that the 4G/5G genotype is an independent predictor of penetrating phenotype (p: 0.005, relative risk: 6.30, 95% confidence interval -CI-: 1.7-22.9), along with male sex (p: 0.004, relative risk: 6.15, 95 CI: 1.79-21.16), and age >32 years (p: 0.009, relative risk: 6.32, 95% CI: 1.58-25.0). IBD-related complications were also more frequent in patients with the 4G/4G genotype (43.4%) than in patients with the 4G/5G genotype (22.2%) or the 5G/5G genotype (26.8%) (p: 0.026). Fifteen patients had a history of arterial (n: 6) or venous (n: 9) thrombosis. The 4G/4G genotype was more frequent in IBD patients with arterial thrombosis (83.3%) than in patients with venous thrombosis (0%, p: 0.002), patients without thrombosis (22.7%, p: 0.004), or controls (21.1%, $p<0.001$). The 4G allelic frequency was higher in IBD patients with arterial thrombosis (0.83) than in patients without thrombosis (0.50, p: 0.036) or controls (0.47, p: 0.016). A weak but significant correlation was observed between plasma PAI-1 antigen and disease activity (r: 0.36, $p<0.0001$). Plasma PAI-1 antigen was higher in patients with the 4G/4G genotype and thrombosis (36.2$\pm$3 ng/mL) than in 4G/4G patients without thrombosis (22.4$\pm$1.8 ng/mL, p: 0.013). Conclusions: The 4G/5G polymorphism of the PAI-1 gene plays a role as a disease modifier for CD, with the 4G/4G genotype being an independent risk factor for the development of a penetrating phenotype. This fact may be taken into account in the therapeutic options for patients with CD. In addition, the 4G/4G genotype is associated to the occurrence of arterial thrombosis in patients with IBD. This finding may be useful for identifying a subgroup of IBD patients which may benefit from prophilactic antithrombotic therapy.

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Localization of a Recessive Locus in the SAMP1/Yit Model of Crohn's Disease

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BACKGOUND: The SAMP/Yit (SAMP) mouse offers a unique opportunity to study the genetics of spontaneous ileitis with pathological features of Crohn's disease (CD). We have localized a susceptibility region encoding a dominant locus in a cohort of (B6 X SAMP)F2 mice. The distribution of scores in this cohort suggested that a second recessive locus contributed to the degree of ileitis in these mice, but no additional linkage could be detected. In order to confirm and localize recessive loci relevant to crosses between these two strains, we performed a similar analysis on a test cohort of B6F1 X SAMP backcross mice. METHODS: A colony of SAMP mice was established in a barrier facility at University of Virginia from animals provided by Dr. Satoshi Matsumoto of the Yakult Central Institute for Microbiological Research, Tokyo, Japan. The colony remains free of detectable mouse pathogens, including Helicobacter sp.,