M26

Genetic risk factors for severe Ulcerative Colitis requiring surgery – the role of innate immunogenetics

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Background: The identification of NOD2(CARD15) as a susceptibility gene for Crohn’s disease (CD) has emphasised the role of the innate immune system in the pathogenesis of IBD.

Aims: To identify genetic variants within innate immunity genes and to test for association with IBD and IBD phenotypes.

Methods: Genetic variants were identified from public databases and direct sequencing. Genotyping was performed by RFLP or TETRA-ARMS PCR. Testing for association performed by case-control analysis (191 CD, 267 UC, and 245 controls) or by the transmission disequilibrium test (TDT)(556 IBD (294 CD and 252 UC) trios). All results stratified by phenotype and NOD2/IBD5 status.

Results: No association seen with SNPs in PPARγ, TLR (toll like receptor3), TLR4, TLR6, TLR9, TIRAP, TREM-1 and 2, P2X7, CD14 and ECSIT. There was a trend towards increased allele frequency of a promoter SNP in Myd88 (p=0.059) in mild colitics. The NOD2 702Trp is associated with UC (p=0.047), particularly in IBD+ cases (p=0.0045) confirming a NOD2/IBD5 interaction in the susceptibility to UC. A MEKK1 SNP (Asp643Asn) is associated with the need for colectomy (p=0.0069). A TLR2 SNP (Arg753Gln) is associated with mild colitics (p=0.0027). We combined these results with previous genetic associations with colectomy (MDR-1 SNP and HLA-DRB1*0103) to produce a ‘panel’ to predict colectomy in UC. Carriage of 2 or more at risk genotypes: colectomy 61.1%, mild UC 42.2%, RR 2.16 (1.26-3.61), p=0.0044. Carriage of 3 or more at risk alleles: colectomy 34.8%, mild UC 15.4%, RR 2.89 (1.53-5.45); p=0.0007.

Conclusions: Polymorphisms within these genes confirm the role of the innate immune system in UC pathogenesis and may additionally help to identify individuals at risk of severe disease requiring surgery. These data may help elucidate the underlying biological mechanisms of disease, permit the individualisation of appropriate therapy and aid the development of future novel therapies.

M27

Expression of the homeobox protein, Cdx2, during malignant progression in Barrett’s oesophagus and in vitro modulation by components of the refluxate

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Background: The molecular basis for intestinal metaplasia is poorly understood. The intestinal transcription factor, cdx2, is a key regulator of intestinal development and homeostasis. Cdx2 expression is normally confined to the epithelium of small and large intestine. Cdx2 is neo-expressed in gastric intestinal metaplasia, and ectopic gastric expression of Cdx2 leads to an intestinalized mucosa in transgenic mice. We aimed to examine Cdx2 expression in intestinal metaplasia of the oesophagus (Barrett’s metaplasia, BM) during malignant progression. The effects of acid and bile on Cdx2 expression was also studied in an in vitro model of BM.

Methods: Cdx2 expression was examined by immunohistochemistry (IHC) in oesophageal biopsies (n=107), including cases of dysplasia and Barrett’s adenocarcinoma. Mucin staining (Gomori’s) and IHC for E-cadherin (a cdx2 target gene) were performed in serial sections. Cdx2 expression in an oesophageal adenocarcinoma cell line (TE7) was examined. We studied the effect of a 5-minute pulse of acid (pH=5) and/or bile salt ( mimicning a gastro-oesophageal reflux event) on Cdx2 expression in LS174T cells cultured for 24hrs, using immunoblotting and immunofluorescence microscopy.

Results: Cdx2 is invariably expressed in specialised BM, but is absent from squamous oesophagus, native oesophageal glands and gastric-type metaplasia variants. E-cadherin expression correlated with that of Cdx2. There was a significant trend towards down-regulation of Cdx2 in the progression from metaplasia to dysplasia/cancer (semi-quantitative IHC scores, p<0.01). Cdx2 expression was absent from TE7 cells. A 5-minute pulse of acid or acidified bile salt (deoxycholic acid, 100mM) resulted in down-regulation of Cdx2 expression and reduced nuclear fluorescence in LS174T cells without cytotoxicity.

Conclusions: Cdx2 neo-expression of Cdx2 is almost invariably in specialised BM. Down-regulation of Cdx2 may occur during oesophageal malignant progression, consistent with a tumour suppressor function. Short term modulation of cdx2 expression by acid/bile may lead to pro-proliferative, de-differentiating and anti-apoptotic effects along the vertical crypt-villus axis in BM.

M28

Polymorphisms in the MEPlA gene: a role in inflammatory bowel disease (IBD)?

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Background: Meprin alpha is an endopeptidase, produced in the epithelial cells of the intestine. It cleaves and has the potential to modify a wide range of luminal gut proteins. MEPlA is located on chromosome 6 in proximity to the HLA region, which has been widely replicated as a susceptibility locus for both Crohn’s disease (CD) and ulcerative colitis (UC).

Aim: Identify MEPlA polymorphisms (SNPs) and test for association with IBD.

Methods: We sequenced samples from 12 coeliac, 6 NOD2 negative CD, and 6 UC patients looking for variants. We genotyped by direct sequencing due to the presence of highly homologous pseudogenes. Association was tested by case-control studies in 380 CD, 379 UC and 372 healthy controls.

Results: Eleven exonic (8 novel) and 2 intronic variants were identified. Seven exonic variants (6 common) were then sequence genotyped. This sample size had the power to analyse the 6 common exonic SNPs found. SNP1 (exon 8 synonymous) allele frequencies: HC 35.1% vs UC 43% (p=0.0025), HC 35.1% vs CD 36% (p=0.73), SNP3 (exon12 synonymous): HC 57.6% vs UC 67.3% (p=0.0067), HC 57.6% vs CD 61% (p=0.24), SNP7 (exon 14 noncoding): HC 61% vs UC 75.2% (p=0.00001), HC 61% vs CD 71.3% (p=0.00033); the remaining variants showed no significant association.

Conclusions: Significant associations were found with SNPs1, 3 and UC, and SNP7 with both CD and UC. Further work is required to identify the haplotype pattern across this gene and to identify the functional importance of these significant SNPs.