Reduced Metallothionein Expression in Colonic Crohn's Disease: Evidence for a new Disease-modifying Gene

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Background: The identification of genetic determinants of Crohn's disease (CD) remains a challenge. This study aimed at identifying new candidate susceptibility genes for CD by integrating known disease loci with gene expression in unaffected colon biopsies of CD patients. We focused on characterizing one of the candidate genes, metallothionein (MT), which belong to a family of highly conserved stress proteins comprising immunomodulating properties.
Methods: Sixteen CD patients and 11 controls were subjected to microarray analysis using a focus microarray (VIB Crohn 7K2) containing 6,779 expressed sequence tags. The expression of MT was analyzed by quantitative PCR and immunohistochemistry. To model lowered MT expression *in vitro*, a colonic epithelial cell line expressing small interfering RNA against MT was generated. The gene for MRE-binding transcription factor 1 (*MTF1)* was screened for mutations in 96 CD patients and the influence of a polymorphism on transcriptional activity was assessed in a luciferase reporter assay.
Results: Eighteen differentially expressed genes were identified. Metallothionein mRNA expression was reduced in colon biopsies (P=0.008) and in PBMCs (P=0.026) of patients with colonic involvement. This observation was confirmed in ileal biopsies by immunohistochemistry (P=0.046). MT-knockdown HT29 cells showed a reduced IL8 secretion in response to bacterial challenge. Sequence analysis of the main transcriptional regulator of MTs, *MTF1*, revealed two coding mutations: Asp63Glu in 5 patients and Glu385Lys in 2 patients. In addition, a frequent (29%) polymorphism at the splice site junction between exon 8 and 9 (c.1270A>G) was found. An intronic polymorphism (IVS1-128A>T) was significantly associated with colonic disease (Chi-square: 8.297, P=0.004). Moreover, this polymorphism was shown to influence the transcription of a reporter gene, suggesting allele specific transcription of *MTF1* which could be responsible for the reduced MT expression observed in colonic CD.
Conclusions: We showed that deficient basal MT expression in CD patients with colonic involvement is, at least in part, genetically determined. We identified *MTF1* (located within the *IBD7* locus) as a new disease-modifying gene.