New IBD genes?

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New reports of potential novel inflammatory bowel disease (IBD) genes further advance our understanding of the genetic basis of IBD

Crohn's disease (CD) and ulcerative colitis result from an inappropriate response of the mucosal immune system to the normal enteric flora in a genetically susceptible individual. The importance of genetic predisposition was firmly established in 2001 by the identification of the first CD susceptibility gene *NOD2* (*CARD15*) on chromosome 16q12 (*IBD1*).^{1,2} It is now clear that *NOD2* variants contribute only a small part to genetic susceptibility, suggesting the existence of other, as yet unidentified, genes. Although these have remained elusive, linkage studies have provided important clues to their location, implicating at least seven other regions of the human genome.

Two recent studies report the cloning of potential novel inflammatory bowel disease (IBD) genes within linkage areas on chromosomes 5³ and 10.⁴ Linkage between CD and chromosome 5q31-33 (*IBD5*) was first demonstrated in 1999⁵ and further characterisation of this locus refined the region to a highly conserved 250 kb haplotype.⁶ Identifying the *IBD5* causal mutation(s) has been hampered by the degree of linkage disequilibrium and the density of immunoregulatory genes on this haplotype. A Canadian group³ now suggest that the true *IBD5* disease causing mutations occur in *SLC22A4* and *SLC22A5*, encoding OCTN1 and OCTN2 respectively, members of the organic cationic transporter subfamily. These proteins are involved in the elimination of toxins and the uptake of various physiological substrates, including carnitine (required for oxygen burst mediated pathogen killing). OCTN1 and OCTN2 are widely expressed, including in intestinal epithelial cells, macrophages, and T cells. Two putatively functional single nucleotide polymorphisms (SNPs), one in *SLC22A4* and the other in *SLC22A5*, form a risk haplotype enriched in individuals with CD. The *L503F* (leucine to phenylalanine at codon 503) polymorphism maps to a region of *SLC22A4*, important for cellular transport, and functional data presented by the authors suggest the variant allele may affect uptake of carnitine, various xenobiotics, and toxins. The *SLC22A5* SNP is located in the gene promoter where it appears to disrupt a predicted heat shock element required for the binding of heat shock transcription factors. Possession of

Pelteková VD, Wintle RF, Rubin LA, *et al.* Functional variants of OCTN cation transporter genes are associated with Crohn disease. *Nat Genet* 2004;36:471-5.

Crohn disease is a chronic, inflammatory disease of the gastrointestinal tract. A locus of approximately 250 kb at 5q31 (*IBD5*) was previously associated with susceptibility to Crohn disease, as indicated by increased prevalence of a risk haplotype of 11 single-nucleotide polymorphisms among individuals with Crohn disease, but the pathogenic lesion in the region has not yet been identified. We report here that two variants in the organic cation transporter cluster at 5q31 (a missense substitution in *SLC22A4* and a G→C transversion in the *SLC22A5* promoter) form a haplotype associated with susceptibility to Crohn disease. These variants alter transcription and transporter functions of the organic cation transporters and interact with variants in another gene associated with Crohn disease, *CARD15*, to increase risk of Crohn disease. These results suggest that *SLC22A4*, *SLC22A5* and *CARD15* act in a common pathogenic pathway to cause Crohn disease.

Stoll M, Corneliussen B, Costello CM, *et al.* Genetic variation in *DLG5* is associated with inflammatory bowel disease. *Nat Genet* 2004;36:476-80.

Crohn disease and ulcerative colitis are two subphenotypes of inflammatory bowel disease (IBD), a complex disorder resulting from gene-environment interaction. We refined our previously defined linkage region for IBD on chromosome 10q23 and used positional cloning to identify genetic variants in *DLG5* associated with IBD. *DLG5* encodes a scaffolding protein involved in the maintenance of epithelial integrity. We identified two distinct haplotypes with a replicable distortion in transmission ($P=0.000023$ and $P=0.004$ for association with IBD, $P=0.00012$ and $P=0.04$ for association with Crohn disease). One of the risk-associated *DLG5* haplotypes is distinguished from the common haplotype by a nonsynonymous single-nucleotide polymorphism 113G→A, resulting in the amino acid substitution R30Q in the DUF622 domain of *DLG5*. This mutation probably impedes scaffolding of *DLG5*. We stratified the study sample according to the presence of risk-associated *CARD15* variants to study potential gene-gene interaction. We found a significant difference in association of the 113A *DLG5* variant with Crohn disease in affected individuals carrying the risk-associated *CARD15* alleles versus those carrying non-risk-associated *CARD15* alleles. This is suggestive of a complex pattern of gene-gene interaction between *DLG5* and *CARD15*, reflecting the complex nature of polygenic diseases. Further functional studies will evaluate the biological significance of *DLG5* variants.

the risk haplotype is associated with a 3-4-fold risk of disease, similar to that for possession of *CARD15* mutations. Interestingly, the risk was much greater in the presence of both the risk haplotype and CD associated *CARD15* alleles, consistent with an interaction between *IBD5* and *CARD15*. The strong linkage disequilibrium across *IBD5* makes interpretation of association data from individual SNPs difficult. This region is rich in genes, many of which are attractive candidates for disease pathogenesis. It therefore remains possible that other "functional" SNPs, expressed in tissues relevant to IBD, exist on this haplotype.

Precisely how these SNPs might interact with *NOD2* to increase the risk of CD remains unclear. However, tantalising clues are provided by the suggestion that α -defensins are potential substrates of OCTN1 and 2, and by a recent report suggesting that *NOD2* variants may actually increase susceptibility to CD, through a defect in α -defensin production.⁷ The same group has published a study in an independent cohort confirming their findings. Analyses in this cohort have revealed particular association with ileal CD⁸ in contrast to a previous study suggesting an association between *IBD5* and perianal CD.⁹

A second study reports an association between SNPs in *DLG5*, a member of the membrane associated guanylate kinase gene family, and IBD. A German group⁴ performed linkage disequilibrium mapping of a locus on chromosome 10 and demonstrated association with variants in *DLG5*. Four common haplotypes across *DLG5* were identified. Haplotype D (containing the unique *SNP 113A*) conferred a modest risk of CD while haplotype A was protective. The results were replicated in a case control study (but not in an independent family based association study) and once again possible interaction with the CD associated *CARD15* alleles was noted. *DLG5* is expressed in the colon and small intestine where it acts as a multifunctional adapter and scaffold protein involved in the regulation of epithelial cell growth, shape, and polarity. Computer prediction programs suggest that the IBD associated *DLG* variants may impair this scaffolding function, but at present supportive data from functional experiments are not available. One published study has confirmed the association between CD and *SNP113A* (but not with the common haplotype)¹⁰ although two other studies have not been able to confirm CD association with *DLG5*.^{11, 12} Further large adequately powered studies in different populations are now required to assess the probable moderate effect of this gene in CD susceptibility.

The rapid advances in our understanding of the genetic basis of IBD are yet to impact on routine clinical practice although recent discoveries have provided considerable insights for investigators. There is considerable optimism that as IBD genes are identified and their environmental interactions unravelled that we may be able to better define the heterogeneous group of patients with IBD, aid understanding of the molecular mechanisms specific to subgroups of disease, and provide a framework to predict clinical phenotype and response to therapy. These novel findings reported by the Canadian and German make an important contribution towards this goal.

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REFERENCES

- 1 Hugot JP, Chamaillard M, Zouali H, *et al*. Association of NOD2 leucine-rich repeat variants

with susceptibility to Crohn's disease. *Nature* 2001;411:599–603.

- 2 Ogura Y, Bonen DK, Inohara N, *et al*. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 2001;411:603–6.
- 3 Peltekova VD, Wintle RF, Rubin LA, *et al*. Functional variants of OCTN cation transporter genes are associated with Crohn disease. *Nat Genet* 2004;36:471–5.
- 4 Stoll M, Corneliussen B, Costello CM, *et al*. Genetic variation in *DLG5* is associated with inflammatory bowel disease. *Nat Genet* 2004;36:476–80.
- 5 Ma Y, Ohmen JD, Li Z, *et al*. A genome-wide search identifies potential new susceptibility loci for Crohn's disease. *Inflamm Bowel Dis* 1999;5:271–8.
- 6 Rioux JD, Daly MJ, Silverberg MS, *et al*. Genetic variation in the 5q31 cytokine gene cluster confers susceptibility to Crohn disease. *Nat Genet* 2001;29:223–8.
- 7 Wehkamp J, Harder J, Weichenthal M, *et al*. NOD2 (*CARD15*) mutations in Crohn's disease are associated with diminished mucosal alpha-defensin expression. *Gut* 2004;53:1658–64.
- 8 Newman B, Gu X, Wintle R, *et al*. A risk haplotype in the Solute Carrier Family 22A4/22A5 gene cluster influences phenotypic expression of Crohn's disease. *Gastroenterology* 2005;128:260–9.
- 9 Armuzzi A, Ahmad T, Ling KL, *et al*. Genotype-phenotype analysis of the Crohn's disease susceptibility haplotype on chromosome 5q31. *Gut* 2003;52:1133–9.
- 10 Daly MJ, Pearce AV, Farwell L, *et al*. Association of *DLG5* R30Q variant with inflammatory bowel disease. *Eur J Hum Genet* 2005.
- 11 Török HP, Glas J, Tonenchi L, *et al*. Polymorphisms in the *DLG5* and *OCTN* cation transporter genes in Crohn's disease. *Gut* Published Online First: 14 June 2005. doi:10.1136/gut.2005.066340.
- 12 Noble C, Nimmo ER, Drummond H, *et al*. *DLG5* variants do not influence susceptibility to inflammatory bowel disease in the Scottish population. *Gut* Published Online First: 20 April 2005. doi:10.1136/gut.2005.066621.

EDITOR'S QUIZ: GI SNAPSHOT

An unusual cause of rectal bleeding in a patient with rheumatoid arthritis

Robin Spiller, Editor

Clinical presentation

A 75 year old woman presented with rectal bleeding of 10 days' duration. She had a 26 year history of rheumatoid arthritis and duodenal ulcer. Medications included prednisolone 5 mg, famotidine 20 mg, and indomethacin suppositories 100 mg, all daily. On examination, there was no tenderness in the abdomen. Haemoglobin was 10.0 g/dl. Colonoscopy revealed a diaphragm-like stricture with circumferential ulcer in the rectum (fig 1). Biopsies showed mild non-specific inflammation without granulomas and vasculitis. Culture of stool and biopsies were negative. Tuberculin test was negative.

Question

What is the diagnosis?

See page 1071 for answer

This case is submitted by:

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Figure 1 Colonoscopy showing a diaphragm-like stricture with circumferential ulcer in the rectum.