promoter region, and intronic boundaries were resequenced in 16 unrelated Centre d'Etude du Polymorphisme Humain (CEPH) individuals using 10 randomly spaced amplicons across the PXR/NRII2 gene. Haplotypes and their respective frequencies were constructed by further genotyping in 32 CEPH trios.

No association was seen between rs1523127/-24381 ($r^2 = 0.96$ with rs3814055/-23585) SNP and UC, CD, or IBD (A-allelic frequency: 60.5% UC, 59.5% CD, 60.0% IBD, and 60.6% HC; $p = 0.96, 0.69$, and 0.82 , respectively) (table 1). The significant rs6785049 (-7635) variant used in Dring's study was in strong linkage with one of the tSNPs used in this study (rs2472682, $r^2 = 0.86$). In the Scottish dataset, this SNP (rs2472682) was not associated with IBD (T-allele: $p = 0.97$, OR 1.00 (95% CI 0.82-1.22); TT genotype: $p = 0.82$, OR 0.96 (95% CI 0.62-1.47)). Log likelihood analyses comparing overall haplotypic distribution of the five tSNPs demonstrated no associations with UC, CD, or IBD ($p = 0.90, 0.90$, and 0.79 , respectively). There were no associations observed between each of the five tSNPs and the common constructed haplotypes with UC, CD, and IBD, respectively. Genotype-phenotype analyses using the Montreal classification did not show any associations with the studied variants or haplotypes (full data available on request).

These negative data, based on a true candidate gene approach, combine the use of the single marker and multimarker haplotype tagging approach and set a statistical limit to the importance of the contribution of this gene to disease susceptibility in our population. Discordance between the findings of this study and those data presented in Dring's study require clarification and explanation. When the allelic frequencies and homozygosity rates of the rs1523127/-24381 SNP in these two studies are compared, it is of interest to note that differences in the conclusions between these studies may be driven primarily by differences in allelic and genotypic frequencies in healthy controls studied in these datasets (Scottish v Irish controls: A-allele frequencies: 60.6% v 54.8%, respectively, $p = 0.03$, OR 1.27 (95% CI 1.02-1.58); AA-genotype frequencies: 35.6% v 32.2%, respectively, $p = 0.01$, odds ratio 1.75 (95% CI 1.12-2.72)). Differences in frequencies in the Scottish and Irish patient populations were less marked (Scottish v Irish cases: A-allele frequencies: 60.0% v 64.3%, $p = 0.05$, OR 1.20 (95% CI 1.00-1.44); AA-genotype frequencies: 33.7% v 41.1%, $p = 0.17$, odds ratio 1.33 (95% CI 0.90-1.98)). Thus potentially a number of explanations exist, including cryptic population stratification, genetic heterogeneity, or a type 1 error in the initial dataset, each of which may explain the divergent findings in these two populations, in whom previous data had suggested consistent founder effects.⁹ Further studies in these and other Northern European populations may help to resolve this issue formally.

G-T Ho

Molecular Medicine Centre, Gastrointestinal Unit,
University of Edinburgh, Western General Hospital,
Edinburgh, UK

N Soranzo, S K Tate

Department of Clinical and Experimental Epilepsy,
Institute of Neurology, London, UK

H Drummond, E R Nimmo

Molecular Medicine Centre, Gastrointestinal Unit,
University of Edinburgh, Western General Hospital,
Edinburgh, UK

A Tenesa

MRC Human Genetics Unit, Edinburgh, UK

I D Arnott, J Satsangi

Molecular Medicine Centre, Gastrointestinal Unit,
University of Edinburgh, Western General Hospital,
Edinburgh, UK

Correspondence to: Dr G-T Ho, Molecular Medicine
Unit, Gastrointestinal Unit, University of Edinburgh,
Western General Hospital, Edinburgh EH4 2XU, UK;
gwotzerho@aol.com

doi: 10.1136/gut.2006.105106

Dr Gwo-Tzer Ho was supported by the Chief
Scientist's Office, Scottish Executive, UK (2001-04).
Dr Albert Tenesa is funded by Cancer Research UK
grant C348/A3758. Dr Elaine Nimmo and Mrs Hazel
Drummond are supported by a program grant from
the Wellcome Trust, C72789/Z/03/Z.

Conflict of interest: None declared.

References

- Willson TM, Kiewer SA. PXR, CAR and drug metabolism. *Nat Rev Drug Discov* 2002;1:259-66.
- Kiewer SA, Goodwin B, Willson TM. The nuclear pregnane X receptor: a key regulator of xenobiotic metabolism. *Endocr Rev* 2002;23:687-702.
- Langmann T, Moehle C, Maurer R, et al. Loss of detoxification in inflammatory bowel disease: Dysregulation of pregnane X receptor target genes. *Gastroenterology* 2004;127:26-40.
- Dring MM, Goulding CA, Trimble VI, et al. The Pregnane X receptor locus is associated with susceptibility to inflammatory bowel disease. *Gastroenterology* 2006;130:341-8.
- Goldstein DB, Ahmadi KR, Weale ME, et al. Genome scans and candidate gene approaches in the study of common diseases and variable drug responses. *Trends Genet* 2003;19:615-22.
- Weale ME, Depondt C, Macdonald SJ, et al. Selection and evaluation of tagging SNPs in the neuronal-sodium-channel gene SCN1A: implications for linkage-disequilibrium gene mapping. *Am J Hum Genet* 2003;73:551-65.
- Ho GT, Soranzo N, Nimmo ER, et al. ABCB1/MDR1 gene determines susceptibility and phenotype in ulcerative colitis: discrimination of critical variants using a gene-wide haplotype

tagging approach. *Hum Mol Genet* 2006;15:797-805.

- Ahmadi KR, Weale ME, Xue ZY, et al. A single-nucleotide polymorphism tagging set for human drug metabolism and transport. *Nat Genet* 2005;37:84-9.
- Arnott ID, Nimmo ER, Drummond HE, et al. NOD2/CARD15, TLR4 and CD14 mutations in Scottish and Irish Crohn's disease patients: evidence for genetic heterogeneity within Europe? *Genes Immun* 2004;5:417-25.

Genetic association between factor V Leiden and coeliac disease

We identified a family in which a previously unknown genetic association between coeliac disease (CD) and mutation of factor V (factor V Leiden) occurred. The index case was a young woman who abruptly presented with severe ascites due to Budd-Chiari syndrome. At endoscopy, she presented with a scalloped duodenal mucosa suggestive of CD, further confirmed by histology and serological tests. Haematological assessment unveiled the mutation of factor V due to the Arg⁵⁰⁶→Gln mutation at position 506.¹ As shown in fig 1, five siblings were also found to be affected by CD (histology performed in all cases) and were heterozygous carriers of factor V Leiden for the same mutation. The two diseases segregated as one in all cases while no sibling was affected by only one of the two diseases. This pattern of inheritance strongly suggests that the genetic mutation responsible for the onset of CD in this family occurs in a gene which is in high linkage disequilibrium with factor V gene, which is sited on the long arm of chromosome 1. The penetrance of such a gene in our family was found to be 100% as all of the carriers demonstrated coeliac disease at endoscopy.

Although susceptibility to CD is strongly determined by environmental gluten, it is clearly a common genetic disorder. HLA-DQ2 and HLA-DQ8 located in the MHC region on chromosome 6 have been related to a genetic predisposition to CD, although these genes are also present in those not affected by CD.²

Figure 1 Genealogical tree of the family in which an association between factor V Leiden and coeliac disease was unveiled.

The association between coeliac disease and factor V Leiden in the present family is strongly suggestive of the presence of a gene in linkage disequilibrium with the long arm of chromosome 1 which is able to trigger the development of CD in all carriers. We feel that further studies are needed to identify such a gene in order to unravel the pathogenesis of CD. Moreover, further assessment of the degree of association between CD and factor V Leiden may be clinically important to avoid severe episodes of thromboembolism as that which occurred in our patient.

T Mari, A Zullo, C Hassan

Gastroenterology and Digestive Endoscopy Unit,
"Nuovo Regina Margherita" and "S Giacomo"
Hospitals, Rome, Italy

L Di Giulio

Department of Vascular Surgery, University Tor
Vergata, Rome, Italy

S Morini

Gastroenterology and Digestive Endoscopy Unit,
"Nuovo Regina Margherita" and "S Giacomo"
Hospitals, Rome, Italy

Correspondence to: Professor S Morini, Ospedale
Nuovo Regina Margherita, Gastroenterologia ed
Endoscopia Digestiva, Via Morosini 30, 00153,
Roma, Italia; gastroroma@virgilio.it

doi: 10.1136/gut.2006.104786

Conflict of interest: None declared.

References

- 1 Simioni P, Prandoni P, Lensing AW, *et al.* The risk of recurrent venous thromboembolism in patients with an Arg506→Gln mutation in the gene for factor V (factor V Leiden). *N Engl J Med* 1997;**336**:399–403.
- 2 Schuppan D, Esslinger B, Dieterich W. Innate immunity and coeliac disease. *Lancet* 2003;**362**:3–4.

Urease induced calcium precipitation by *Helicobacter* species may initiate gallstone formation

Helicobacter species can colonise the mammalian gastrointestinal and hepatobiliary tract which usually results in a chronic infection coupled to an inflammatory host response. It is therefore not surprising that colonisation with *Helicobacter* species is linked with a range of inflammation associated gastrointestinal and hepatobiliary diseases.¹ Recently, this range has been expanded, with an association of infection with enterohepatic *Helicobacter* species and the formation of cholesterol gallstones.²

In their study, Maurer and colleagues² demonstrated that murine infection with the enterohepatic *Helicobacter* species *H bilis* and *H hepaticus* accelerated the formation of cholesterol gallstones in mice fed a lithogenic diet. Although the gallbladder mucosa in mice with gallstones displayed signs of inflammation, *Helicobacter* species were not cultured from the inflamed gallbladder or bile. Therefore, Maurer *et al* hypothesised that the chronic immune stimulation caused by *Helicobacter* species, rather than a direct bacterial factor, led to the production of nucleating agents, thus indirectly linking *Helicobacter* species and cholesterol gallstone formation.² Although a role for inflammation cannot be excluded, we believe that identification of *Helicobacter* DNA in gallstones^{3,4} is

an indication that *Helicobacter* species may also play a direct role in gallstone formation.

Gallstones are crystalline bodies formed by accretion or concretion of bile components. Approximately 80% of gallstones are cholesterol gallstones, and 20% are pigment stones consisting of bilirubin and calcium, two components present in bile. There is a connection between the two types of gallstones as calcium-bilirubin salts form surfaces that are highly attractive for

cholesterols to adhere to.⁵ In addition, almost all types of gallstones contain a nidus of calcium.^{6,7} Bacteria have been shown to be able to initiate calcium precipitation and subsequent stone formation,^{8,9} similar to *Proteus mirabilis* and kidney stones.¹⁰ An important factor in bacteria induced stone formation is the enzyme urease which hydrolyses urea into ammonia and bicarbonate. Ammonia increases the pH and thereby favours generation of an insoluble form of

Figure 1 Urease activity is required for calcium precipitation by *Helicobacter* species. Top panel: Comparison of *H hepaticus* strain ATCC51449, *H pylori* strain NCTC12198 (left) and their isogenic urease negative mutants (right). Bottom panel: Comparison of wild-type *H bilis*, *H pullorum*, and *H cinaedi* isolates. All *Helicobacter* species were grown on precipitation agar (Columbia agar supplemented with Dent selective supplement (Oxoid), 0.2% β -cyclodextrins, 333 mM urea, 100 μ M NiCl_2 , 20 mM NaHCO_3 , 187 mM NH_4Cl , 30 mM CaCl_2 , and 0.04% triphenyl tetrazolium chloride; all concentrations given are final concentrations). After overnight growth under microaerobic conditions (5% O_2 , 10% CO_2 , 85% N_2), calcium crystals were visible with urease positive *Helicobacter* species but not with urease negative *Helicobacter* species or with the isogenic urease negative mutants. Magnification 3 $\times$, photos taken with three megapixel digital camera. The figure represents a representative example of three independent experiments.