GENOTYPES AND PHENOTYPES IN CROHN'S DISEASE: DO THEY HELP IN CLINICAL MANAGEMENT?

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Rather than a unique disorder, Crohn's disease is regarded as a common denominator for various immune mediated inflammatory diseases of the gastrointestinal tract. These various subforms of Crohn's disease are characterised by T cell mediated tissue destruction in the gut. In contrast with autoimmune diseases where autoantibodies react with self antigens, inflammation in Crohn's disease is directed against antigens that are mainly derived from the environment.¹ Although it may sound trivial, this adjustment in the understanding of the pathogenetic mechanisms has a vast number of consequences for the search of the disease causing factors and identification of therapeutic targets.

CROHN'S PHENOTYPES

The various subforms of Crohn's disease have not been recognised until recently. Historically, colonic Crohn's disease was not distinguished from ulcerative colitis. Approximately 30 years ago, the first studies highlighted the diverse natural history of colonic and ileal Crohn's disease.² Inherent differences in disease behaviour (obstructing versus perforating) were later recognised by a group of physicians at Mount Sinai Hospital in New York.³ Biological discrimination between various subgroups of Crohn's patients led to a proposal for a phenotypic classification,⁴ which was taken up and further developed and evaluated by an international working group, leading to the Vienna classification.⁵ Accordingly, Crohn's disease is separated into three categories: age at diagnosis, location, and behaviour (table 1). Several teams have now tested the Vienna classification in their databases⁶⁻⁸ and found that it is feasible and reproducible. The behaviour and age categories earned some criticism which will be discussed later. The location category was found to be very useful and was also stable over time, with only one of seven patients having changes over a 10 year observation period.⁷

CLASSIFICATION BY AETIOLOGY

The best classification would be by cause of disease, but unfortunately this is not known. However, there is overwhelming indirect evidence for the presence of environmental triggers, although the specific antigen(s) that drives the immune reaction in Crohn's disease is still obscure. Cigarette smoking is the only confirmed external factor involved in the pathogenesis of Crohn's disease. It has been linked to disease progression and unfavourable disease outcome across the world. The underlying mechanism is incompletely understood.⁹ Smoking seems to be a disease aggravating rather than a disease causing factor. In fact, smokers are more likely to develop strictures or fistulae.¹⁰ No other external trigger has been generally recognised.

CROHN'S GENOTYPES

Apart from the disease causing environment, variations in the human genome contribute to disease susceptibility and shape the clinical phenotype. The higher frequency of Crohn's disease in certain ethnic groups (particularly in Caucasians), familial aggregation, and a greater disease concordance in monozygotic twins are strong indicators of the impact of genetic factors in disease pathogenesis. However, the genetic trait of Crohn's disease is complex, with genetic heterogeneity and incomplete phenotype penetrance. To date, seven susceptibility loci have been identified cosegregating preferentially with disease phenotype¹¹ but no dominant inflammatory bowel disease gene has been detected. Genetic variations are loading the gun but the environmental factor still pulls the trigger.

This paradigm was further supported by identification of caspase activation and recruitment domain 15 (CARD15) variants that confer susceptibility to Crohn's disease.¹² What have we learned about the function of CARD15?

CARD15/NOD2

In 2001, Nod2 was identified as a Nod1/Apaf-1 homologous gene which mapped to chromosome 16q12.¹³ Later the gene was renamed CARD15 by the Human Genome Organisation (HUGO)

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Table 1 Vienna classification of Crohn’s disease

Age at diagnosis Age when diagnosis of Crohn’s disease was first definitively established by radiology, endoscopy, pathology, or surgery.	
▶ A1—<40 y	
▶ A2—≥40 y	
Location Maximum extent of disease involvement at any time before the first resection. Minimum involvement for a location is defined as any aphthous lesion or ulceration. Mucosal erythema and oedema are insufficient. For classification, at least both a small bowel and a large bowel examination are required.	
▶ L1—Terminal ileum	- Disease limited to the terminal ileum (the lower third of the small bowel) with or without spill over into the caecum.
▶ L2—Colon	- Any colonic location between the caecum and rectum with no small bowel or upper gastrointestinal (GI) involvement.
▶ L3—Ileocolon	- Disease of the terminal ileum with or without spill over into the caecum and any location between the ascending colon and rectum.
▶ L4—Upper GI	- Any disease location proximal to the terminal ileum (excluding the mouth) regardless of additional involvement of the terminal ileum or colon.
Behaviour	
▶ B1—Non-stricturing non-penetrating	- Inflammatory disease which never has been complicated at any time in the course of disease.
▶ B2—Stricturing:	- Stricturing disease is defined as the occurrence of constant luminal narrowing demonstrated by radiological, endoscopic, or surgical-pathological methods, with prestenotic dilatation or obstructive signs/symptoms without the presence of penetrating disease at any time in the course of disease.
▶ B3—Penetrating:	- Penetrating disease is defined as the occurrence of intra-abdominal or perianal fistulas, inflammatory masses, and/or abscesses at any time in the course of disease. Perianal ulcers are also included. Excluded are postoperative intra-abdominal complications and perianal skin tags.

Adapted from Gasche and colleagues.⁵

nomenclature committee.^{12 14 15} CARD15 consists of 11 constant exons and an alternative in the 5’ region. Its 1040 amino acids are structured into four distinct domains: two N terminal CARDs, a central nucleotide binding domain (NBD), and a C terminal leucine rich repeat domain (LRR). CARDs are responsible for interaction with CARDs from other proteins, such as RIP-like interacting CLARP kinase (RICK), and potentially with proteins of the apoptosis pathway. NBD is responsible for self oligomerisation. The LRR domain is required for peptidoglycan (PGN) induced nuclear factor κB (NFκB) activation.

Table 2 Diversity of caspase activation and recruitment domain 15 (CARD15) allele frequencies in Europe

Population	Controls/Crohn’s disease (%)		
	SNP8 (R702W)	SNP12 (G908R)	SNP13 (L1007fs)
Finland ⁴⁷	1.8/3.3	0.0/0.6	1.7/4.8
Norway ⁴⁸	2.8/3.5	1.2/0.9	1.2/2.6
Scotland ³⁴	—/8.0	—/2.8	—/4.2
Ireland ⁴⁹	4.0/7.0	1.0/3.0	1.0/4.0
Great Britain ³³	5.2/12.5	1.4/3.3	1.6/9.4
Germany ⁵⁰	—/—	—/—	3.3/23.7
Germany ⁴⁸	4.8/9.9	0.7/4.0	4.1/13.7
Poland ⁵¹	—/—	—/—	3.6/—
Netherlands ⁵²	—/—	3.0/4.3	1.0/8.5
Spain ⁵³	2.2/7.1	1.1/4.2	2.3/7.4
Italy ⁵⁴	—/—	—/—	1.3/11.7
Italy ⁵⁵	5.9/9.0	1.4/4.3	2.3/6.3
Crete ⁵⁶	—/—	—/—	1.5/2.7
European mix ¹⁶	4.0/11.0	1.0/6.0	1.9/11.0
European mix ⁵⁷	4.2/11.4	0.2/4.2	3.4/14.6

SNP, single nucleotide polymorphism; —, not investigated.

Three variants (SNP8 [R702W], SNP12 [G908R], and SNP13 [L1007fs]) within the LRR were initially described to be associated with Crohn’s disease.¹² Screening of the entire coding region of CARD15 identified another 30 non-conservative missense mutations.¹⁶ The three common mutations are present in up to 50% of Crohn’s patients but also in up to 20% of healthy individuals, and they account for 82% of mutated alleles.¹⁶ CARD15 variants seem to be rather disease specific because they do not confer risk to other chronic inflammatory or autoimmune diseases.^{17 18} Variants in the NBD were found to be associated with Blau syndrome, a rare autosomal dominant disorder characterised by early onset granulomatous arthritis, uveitis, and skin rash with camptodactyly.¹⁷

One important observation is that CARD15 mutations are thought to have a dose dependent effect as mutated homozygotes and compound heterozygotes are found more frequently in Crohn’s patients than expected. The three common CARD15 mutations are neither necessary nor sufficient for expression of the disease phenotype. In Caucasians, they do not explain more than 20% of the genetic predisposition to Crohn’s disease. This also varies between different European countries as a high diversity exists in different European populations (table 2). The absence of CARD15 variants in Asian and sub-Saharan African populations precludes any role for CARD15 variants in Crohn’s disease in these populations.¹⁹

The CARD15 pathway

CARD15 is one of the mammalian bacteria sensing proteins that is part of innate immunity leading to activation of the NFκB pathway (fig 1). In the intestine, CARD15 is

Figure 1 Caspase activation and recruitment domain 15 (CARD15) is mainly expressed in the granulocyte-macrophage lineage (including dendritic cells) and also in Paneth cells of the intestinal tract.^{13 25 36 37} CARD15 is activated by binding of naturally occurring muropeptides, enzymatic degradation products of peptidoglycans (PGNs) derived from bacterial cells walls.²² Their presence triggers CARD15 oligomerisation (as indicated by three overlapping CARD15 molecules) and recruitment of RIP-like interacting CLARP kinase (RICK) via CARD-CARD interaction. RICK then activates the nuclear factor κ B inhibitor ($I\kappa$ B) kinase complex (IKK) via phosphorylation of $IKK\gamma$. The IKK complex next phosphorylates $I\kappa$ B resulting in nuclear factor κ B (NF κ B) translocation to the nucleus and transcriptional activation of NF κ B responsive genes such as proinflammatory cytokines or defensins.⁶⁰

preferentially expressed in Paneth cells and macrophages. Because of the overwhelming presence of bacteria in proximity to the mucosal lining, toll-like receptor function is downregulated in intestinal epithelial cells thereby preventing undesired permanent immune activation.²⁰ Therefore, prevention of bacterial invasion in the gut relies mainly on CARD15 and Nod1. CARD15 and Nod1 have an overlapping recognition pattern for bacterial PGNs, with CARD15 being broader than Nod1. Thus CARD15 has a distinct protective role in the intestinal tract because loss of function mutations are only partially compensated for by Nod1.^{21 22}

Apart from activation of NF κ B, Nod1 and CARD15 are capable of activating caspase-1 and caspase-9.^{23 24} However, the mechanism responsible for excess activity of NF κ B in Crohn's lesions is not fully understood and may theoretically be related to insufficient induction of apoptosis by CARD15 mutants. In return, the CARD15 promoter contains NF κ B response elements mediating its transcriptional activation after tumour necrosis factor α (TNF- α) stimulation.^{25 26} TNF- α dependent expression of CARD15 can be enhanced but not independently induced by interferon γ (IFN- γ). In contrast, Nod1 responds to IFN- γ ²⁷ but not to TNF- α . This positive feedback loop of CARD15 was found in infected intestinal

epithelial cells and may explain increased CARD15 expression in epithelial cells surrounding colonic Crohn's lesions.^{28 29}

GENOTYPE-PHENOTYPE CORRELATIONS IN CROHN'S DISEASE

Several groups have independently studied the association between CARD15 variants and Crohn's disease phenotype (summarised in table 3). Appreciable differences were detected in double mutation carriers. They showed unanimously that CARD15 variants only confer susceptibility to ileal (L1 and L3 in the Vienna classification) but not colonic (L2 in the Vienna classification) Crohn's disease.^{30 31} Also, CARD15 polymorphisms are not associated with ulcerative colitis or indeterminate colitis, two distinct colonic forms of inflammatory bowel disease.^{12 31 32} Carriers are further characterised by a younger age at onset (A1 in the Vienna classification).^{16 33} This fits with a general observation that the relative importance of genetic (inherited) and environmental (acquired) factors is shifted towards genes in younger patients and towards environment in the elderly. Some studies found a relation between CARD15 and complicating (stricturing or perforating) disease. Multivariate analyses however identified smoking habits and anti-saccharomyces antibody profiles as the underlying factor.^{10 16 34 35} Intestinal biopsies from double mutant carriers are reported to display more granulomas,¹⁶ underlining the link between CARD15 and granuloma formation, as also observed in Blau syndrome.

The relationship between CARD15 mutations and a certain Crohn's disease phenotype may serve as proof of principle: Crohn's disease is not a single disease but a common denominator for various immune mediated inflammatory diseases of the gastrointestinal tract. We may ask how CARD15 mutations can determine the ileal location of Crohn's disease. The reason for this may be related to the predominant expression of CARD15 in Paneth cells.^{36 37} Paneth cells, on the other hand, are most numerous in the terminal ileum and are critically important in enteric antibacterial defence (fig 2). These findings suggest a role

Table 3 Genotype-phenotype correlations in Crohn's disease

	L1 (ileal)	L2 (colonic)	L3 (ileocolonic)
Cuthbert ³²	+	-	+
Lesage ¹⁶	+	-	-
Murillo ⁵²	+/-	-	+/-
Ahmad ³³	+	-	+
Vermeire ³⁸	+	-	+
Hampe ⁴⁸	+	+/-	+
Abreu ⁵⁹	+	-	+
Mendoza ⁵³	+	-	-
Helio ⁴⁷	+	-	-
Bairead ⁴⁹	+	-	-
Giachino ⁵⁵	+	-	-

+/-, trend but not statistically significant.

Figure 2 Three caspase activation and recruitment domain 15 (CARD15) variants (R702W, G908R, and L1007fs) within the leucine rich repeat (LRR) were initially described to genetically predispose to the development of Crohn's disease.¹² These variants are disease specific because they do not confer risk to other chronic inflammatory or autoimmune diseases.^{17 18} In the intestinal tract, CARD15 is primarily expressed in Paneth cells, which are critical in enteric antibacterial defence.²⁸ In CARD15 mutant individuals, nuclear factor κ B (NF κ B) activation is reduced and thus the necessary antibacterial response, such as expression of defensins, does not occur.^{61 62} Chronic NF κ B activation through alternative pathways is regarded as secondary to the lack of an appropriate antibacterial response.²¹ Paneth cells are most numerous in the terminal ileum and seem to be of local importance. A CARD15 deficit in Paneth cells, as present in homozygous or compound heterozygous mutants, is phenotypically related to Crohn's ileitis. NBD, nucleotide binding domain.

for CARD15 in the regulation of the Paneth cell mediated response against intestinal bacteria and a plausible mechanism explaining the selective association of CARD15 mutations with ileal Crohn's disease. The impaired capacity of Crohn's disease associated CARD15 mutations to sense the intracellular presence of bacterial cell wall components (that is, PGN) may result in overgrowth and invasion of certain gut microbes, perhaps through impaired production of human defensins leading to chronic inflammation.³⁸

DOES PHENOTYPING AND GENOTYPING HAVE RELEVANCE IN CLINICAL PRACTICE?

Assessment of disease phenotype

There is little controversy that the phenotypic discrimination of Crohn's subgroups is a key to the clinical management of patients. Information regarding the patient's age and sex, disease location, and disease progression has been collected long before the Vienna classification was established because

it is needed for appropriate patient care. For example, information on disease location is crucial for prescribing topical therapy³⁹; information on disease progression (mild versus aggressive) is pertinent when considering immuno-suppressive therapy. The Vienna classification did not introduce new disease variables but rather provided a structured format to collect a minimal amount of information on the most important variables.

The Vienna classification has however drawn legitimate criticism for some shortcomings. Notable among these is the observation that the behavioural classification progresses with disease duration.^{7 8} Definitions for the aggressive behaviour phenotype need to be adjusted in the future.⁴⁰ Suggestions include implementing a certain point in time after diagnosis (for example, five or 10 years) to finally establish a complicating (B2 or B3) disease phenotype. When setting this point at five years post diagnosis, one could expect approximately 48% of cases to be B1, 12% B2, and 40% B3, and at 10 years approximately 30% B1, 14% B2, and 66% B3.⁸ Another suggestion relates to the separation of perianal and intra-abdominal fistulising disease which are currently grouped within the B3 category.⁴¹ Thereby, perianal disease supersedes any internal disease characteristic and forces a case to be classified as B3. Perforating disease behaviour was primarily recognised as an indicator of aggressive disease that requires repeated operations.³ This feature needs further attention, particularly in relation to perianal disease which is often the most debilitating aspect of Crohn's disease.⁴² However, the behaviour category aims to depict the degree, speed, and quality of tissue destruction, a clinically obvious and relevant biological distinction between individual patients. The age category has been criticised for its arbitrary cut off at age 40 years. It is difficult however to identify solid arguments for other cut off points. The higher proportion of patients with colonic disease (L2) within the A2 group is a good indicator that this separation has biological meaning.⁵ For now, the Vienna classification remains a simple tool to minimally describe patient populations in clinical trials and eases communication between physicians in clinical practice.

Assessment of disease genotype

Although laboratory assays for detection of CARD15 variants are trivial and robust, its widespread application in clinical practice should be strongly reconsidered because of jurisdictional, ethical, and economical limitations. The central question is: What is the benefit for the individual who undergoes genetic testing?

Identifying individuals at risk

One of the advantages of genetic testing in general, is the possibility of identifying individuals at risk of developing a certain disease, thereby facilitating appropriate action for disease prevention. With regard to Crohn's disease, we have to question the general risk of developing this phenotype in the first instance? Such risk depends on the prevalence of Crohn's disease in the population studied. In most Western European or North American countries the incidence now plateaus at 6–7/100 000/year, with a disease prevalence of approximately 150–180/100 000.⁴³ Canada, exceptionally and without any obvious explanation, has a significantly higher incidence of 15/100 000/year and a prevalence of 200/100 000.⁴⁴ In accordance, the lifetime risk for development of Crohn's disease was estimated to be between 0.15% and 0.3%.

What is the risk of developing Crohn's disease in individuals with a disease associated CARD15 variant? Compared with individuals carrying two wild-type alleles, the odds ratio of disease development was estimated to be 2–3 for heterozygous carriers and 20–40 for homozygotes or compound heterozygotes.^{12 14 15 32} Therefore, the lifetime risk for heterozygote individuals increases to 0.45–0.9%, and for homozygote or compound heterozygote individuals to 3.0–12.0%. However, even in the highest incidence areas (such as Canada) and using the highest risk estimates (3 and 40), only 1 of 111 heterozygous and 1 of 8 homozygous or compound heterozygous individuals will develop the disease phenotype. In addition, in the average Caucasian population (assuming a combined allele frequency (sum of the three major CARD15 SNPs) of approximately 7%), one has to screen 200 individuals to identify a single homozygous or compound heterozygous individual. Genetic variations on different genes may add some risk to phenotype penetrance. Most likely however the clue to this remains undiscovered within the environment of Western countries. Because of these shortcomings, screening for CARD15 variants in Western (and also any other) populations is currently not useful.

Is there a role for genetic testing in Crohn's family members? Genetic testing in gastroenterology has become a precious tool in several familial diseases, including hereditary cancer syndromes such as familial adenomatous polyposis and hereditary non-polyposis colorectal cancer. On the one hand, genetic testing allows for exclusion of transmission of a certain mutation and thereby relieves the individual at risk from the burden of early cancer development. On the other hand, preventive actions such as drug therapy, surveillance endoscopy, or proctocolectomy can be offered to mutation carriers. As CARD15 variants cover only 20% of genetic susceptibility to Crohn's disease and because of the low phenotype penetrance, it seems currently unreasonable to perform genetic testing in Crohn's families. In other words, neither a negative (double wild-type) nor a positive test result (double mutant) carries a certainty in predicting phenotype penetrance. Most importantly, no measures are available to prevent Crohn's disease in individuals at risk. All that physicians can do is recommend that the patient not smoke. This however does not require genetic testing.

Differential diagnosis

Can CARD15 variants help to confirm or exclude the diagnosis of Crohn's disease, specifically in the case of indeterminate colitis? Again, negative tests do not rule out a diagnosis of Crohn's disease and a positive test does not prove its presence. Moreover, the ulcerative colitis-like Crohn's phenotype (L2 in the Vienna classification) is very uncommon in patients carrying two mutations.³¹ This low frequency of CARD15 mutations in Crohn's colitis suggests that CARD15 genotyping has a limited role in this situation.

Pharmacogenetics

Genetic variations are considered important variables which may define a response or adverse event of drug therapy. Our wish is to tailor drug therapy to the genetic background of individual patients. For example, some centres perform thiopurine methyltransferase genotyping for improving 6-mercaptopurine and azathioprine safety in Crohn's disease. So far, CARD15 variants were studied in relation to infliximab therapy^{45 46} without detecting any association with treatment response or side effects. Drug development

programmes intend to interfere with the specific genetic defect in Crohn's disease but no drug has been marketed yet that interacts with the CARD15 pathway.

Genotyping within clinical trials

The fact that Crohn's disease is not a single entity is readily appreciated when studying genetic susceptibility. CARD15 double mutants are a distinct genetically defined Crohn's subgroup. As clinical trials generally report on the composition of the study cohort, future trials could implement the frequency of CARD15 mutations. This could improve transferring the results of a certain study into other patient groups. A different response of CARD15 carriers to certain therapies may be observed in the future and will then allow stratification of patients on the basis of genotyping. Apart from the use of the Vienna classification, we therefore endorse subgroup analysis on the basis of CARD15 genetics in clinical trials

FINAL REMARKS

Identification of CARD15 variants as inheritable susceptibility factors in Crohn's disease defines a milestone in the understanding of disease pathogenesis. CARD15 has provided a logical explanation for some of the phenomenon observed within families or twins. It has opened a door towards unravelling the network of Crohn's pathogenesis. So far we have learned that it relates to ileal disease location, possibly through its selective expression in Paneth cells and regulation of antibacterial host defence. The pathogenetic understanding of CARD15 functions may open new avenues to specific preventive measures and therapies that interfere with the underlying disease mechanism and that are tailored to the patient's genotype. Until then, physicians have to rely on clinical phenotype and their proper judgement of the patient's symptoms.

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PostScript

LETTERS

Fatigue in primary biliary cirrhosis

We would like to take the opportunity to clarify some of the points in response to the recent leading article (*Gut* 2004;53:475-7) which accompanied our report¹ of reduced globus pallidus (GP) magnetisation ratios (MTRs) in patients with fatigue and primary biliary cirrhosis (PBC).

As we stated in the paper, fatigue in PBC is a subjective multidimensional symptom with many potential determinants, including sleep disturbance, depression, and personality, in addition to a potential central neurological cause.¹ We therefore wholeheartedly concur with Drs Milkiewicz and Heathcote when they state that brain manganese (Mn) deposition is certainly not the cause of fatigue in all patients with PBC. We certainly do not believe that we drew this conclusion. However, we do believe that our findings of reduced GP MTRs in patients with stage I-II disease, which were associated with hypermanganaemia and measured fatigue, do open up a novel avenue of research into a poorly understood symptom in patients with PBC.

In order to control for inter-examination system variability, it is necessary to normalise the raw MTRs against an internal region of interest (ROI). Although it might initially appear easier to analyse the raw MTR data, normalisation to an internal standard allows external sources of variation, unrelated to the patient, to be removed. We followed previously published protocols to calculate GP indices, normalised to the putamen and to the frontal white matter,^{2,3} and these were used to test associations with fatigue and blood Mn levels. The raw MTR data were used for the primary comparison between PBC patients and healthy volunteers. We chose two rather than one internal control ROI because, contrary to the assertion in the editorial, there is evidence for Mn accumulation in brain structures, other than the GP, in patients with cirrhosis.^{4,5} Rose *et al* reported significantly elevated Mn concentrations in the frontal and occipital cortex, pallidum, putamen, and caudate⁴ while Maeda *et al* showed elevated Mn concentrations in the GP, putamen, and frontal white matter.⁵ In both series, the highest Mn concentration was in the GP. Our choice of two standard ROIs was made to maximise the interpretation of the raw data although we accept that the a priori assumption that pathology is absent from these regions in this and all relevant magnetic resonance studies to date, which have used internal controls, may be false. This may explain the unexpected trend towards a positive association between blood Mn and the putamen index normalised to white matter.

Drs Milkiewicz and Heathcote have expressed concern about an apparent autocorrelation in our data that did not equal 1. Table 2 in our paper¹ shows the correlation coefficients between individual MTR indices and blood Mn level. We did not compare the normalised putamen index against the normalised putamen index.

We are grateful to the two commentators for extending our interpretations and naturally agree that bile duct loss, rather than liver fibrosis, governs the severity of cholestasis and that there may be dissociation between these features in PBC. For the purposes of this study, we chose to examine patients with stage I-II disease to remove the possibility of hepatic encephalopathy or cirrhosis as a cause for the MTR findings. We believe that both this patient selection and the demonstration of normal cerebral magnetic resonance spectroscopy (MRS) in these patients, compared with healthy volunteers, does indeed achieve this. We found reduced GP MTRs in patients with stage I-II disease, which were associated with hypermanganaemia and measured fatigue, but we also studied four patients with stage III-IV disease and, as a group, there were no significant differences in GP MTR indices compared with stage I-II patients. Although this may be due to the small number of individuals studied, the lack of clear distinction between stage I-II and stage III-IV disease may also reflect a process that adversely affects the brain long before the development of cirrhosis, owing to early bile duct loss.

The commentators point out that the value of liver biopsy staging of PBC is limited owing to sampling error and that there may not have been a true distinction between the stage I-II and III-IV groups. We accept the possibility of sampling error but, in our view, liver biopsy still remains the gold standard for diagnosing cirrhosis. We disagree with the suggestion that cerebral MRS would have been useful in supporting the histological diagnoses as cerebral MRS abnormalities are only seen in a minority of patients with Child-Pugh A cirrhosis.^{6,7} We did not assume that MRS would be abnormal in stage III-IV patients; in fact, there were no significant differences between these patients and stage I-II patients.

Fatigue in PBC merits further research. We hope that we will be able to take further "steps in the right direction".

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Acute ulcerative colitis during successful interferon/ribavirin treatment for chronic hepatitis

A 54 year old man was treated with pegylated interferon alpha 2a 180 µg weekly and ribavirin 1000 mg daily for chronic hepatitis C genotype 3a ($>5 \times 10^5$ IU/ml). There was no history of gastrointestinal disease or morbidity.

At week 12, hepatitis C virus-polymerase chain reaction (HCV-PCR) was negative and alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels remained elevated at 2-3 times above the upper limit. Continuation of this well tolerated therapy was planned until week 24.

However, at week 14, the patient reported a sudden onset of watery and sometimes bloody diarrhoea. Colonoscopy showed continuous pancolitis, macroscopically suggestive of inflammatory bowel disease (IBD). Histology revealed a severe highly active pancolitis with basal plasmacytosis, crypt abscesses, and crypt distortion, as seen in ulcerative colitis.

The antiviral treatment was stopped and treatment with prednisone and mesalazine (5-ASA) was initiated. Steroids were tapered over four weeks, which had been ongoing with clinical remission. 5-ASA was continued at a dose of 3 g daily for eight weeks followed by 2 g daily.

Three months later (receiving 5-ASA 2 g daily) there was complete clinical and endoscopic remission. Histology showed a mild residual increase in mononuclear inflammatory cells. PCR revealed a virological relapse of HCV (high viraemia $>6 \times 10^5$ IU/ml) and an unchanged twofold elevation in ALT and AST.

We suspect that the ulcerative colitis-like severe pancolitis in this patient with no

history of IBD was probably an adverse effect of the antiviral treatment with interferon/ribavirin rather than a concomitant disease. Similar observations have been made by others.¹⁻³ To our knowledge, the present case is the fourth reported in the literature. Interferon has immune stimulating properties⁴ and may trigger autoimmune diseases and transplant rejections.⁵

Hence, in light of this, the report on interferon treatment in active ulcerative colitis (*Gut* 2003;**52**:1728-33) seems interesting and warrants further research.

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Author's reply

As interferon alpha (IFN alpha) suppresses the synthesis of proinflammatory cytokines and induces various anti-inflammatory cytokines, it may show efficacy in chronic inflammatory disorders of the gut. In Crohn's disease, lamina propria cells manifest increased secretion of IFN- γ whereas in ulcerative colitis lamina propria cells and natural killer T cells demonstrate increased secretion of the Th2 cytokines interleukin 5 (IL-5) and IL-13.^{1,2}

IFN alpha has been demonstrated to potently suppress synthesis of both IL-5 and IL-13 in human leucocytes, making it an attractive agent for the treatment of ulcerative colitis. IFN alpha therapy showed no benefit in patients with Crohn's disease.³ This may be explained by the fact that Crohn's disease is thought to be a Th1 linked disease. IFN alpha therapy seems to be more successful in chronic active ulcerative colitis, a more Th2 linked disorder. Sumer and Palabiyikoglu reported that more than 80% of patients with active ulcerative colitis responded to high dose IFN alpha therapy within two weeks of treatment and were in complete clinical and endoscopic remission after six months of therapy.⁴ Madsen et al recently presented a study comparing systemic IFN alpha therapy and prednisolone enemas in the treatment of left sided ulcerative colitis.⁵ Ulcerative colitis is accompanied by high levels of IL-5 in colonic tissue and IFN alpha effectively suppresses IL-5 synthesis in leucocytes. IFN

beta has been used in a pilot study investigating its role in patients with steroid refractory active UC.⁶ In this study, a high responder rate was observed with a mean time to response of three weeks.

Another IFN beta study in ulcerative colitis has been presented recently.⁷ In this small, placebo controlled, randomised, dose escalating study, clinical improvement was observed in 50% of IFN beta treated patients compared with 14% in the placebo group. We recently presented data on the first placebo controlled use of IFN alpha in the treatment of active UC in patients with or without corticosteroid and/or immunosuppressive treatment.⁸ We observed no significant advantage of any IFN group over placebo but did not observe worsening of disease in any IFN treated patient.

The mechanisms of action of IFN alpha are probably multiple but the possible interactions of IFN alpha with the cytokine cascade and immune system are usually not considered. Favouring Th1 responses and suppressing Th2 type immune responses could imply that type I IFNs may be therapeutic in diseases such as ulcerative colitis or allergic disorders. We agree with the authors that IFN alpha might have the potential to enhance inflammatory reactions and alloreactivity in certain situations but are also convinced that it has strong immunomodulatory and anti-inflammatory properties. Larger controlled trials with IFN alpha in ulcerative colitis are eagerly awaited.

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The toll-like receptor 4 (TLR4) Asp299Gly polymorphism is associated with colonic localisation of Crohn's disease without a major role for the *Saccharomyces cerevisiae* mannan-LBP-CD14-TLR4 pathway

It is with great interest that we read the paper by Frachimont and colleagues (*Gut* 2004;**53**:987-92) in which they describe a novel association of the toll-like receptor 4 (TLR4) +896 A>G polymorphism with both Crohn's disease (CD) and ulcerative colitis (UC), supporting the genetic influence of pattern recognition receptors (PRRs) in triggering inflammatory bowel disease (IBD). PRRs are sensors of pattern associated molecular patterns of microorganisms in the intestinal flora. Independently, we performed a similar study. However, special attention to the presence of anti-*Saccharomyces cerevisiae* antibody (ASCA) was taken, as Tada and colleagues¹ have recently reported that the *S. cerevisiae* mannan-LBP complex is recognised by CD14 on monocytes and signalling through TLR4 leads to the production of proinflammatory cytokines in a manner similar to that induced by lipopolysaccharide (LPS).

Patients and controls were recruited from the Outpatient Department of Gastroenterology, VU University Medical Centre, Amsterdam, the Netherlands. The group consisted of 112 CD patients and 170 unrelated Dutch Caucasian controls. Diagnosis of disease was based on clinical, histopathological, and endoscopic findings. CD patients were categorised using the Vienna classification (general patient characteristics are described elsewhere²). ASCA IgA and IgG ELISAs were performed as described previously.³ Genotyping for the *CD14*-260 C>T and *TLR4*+896 A>G single nucleotide polymorphisms (SNPs) was performed as described previously by our group.⁴ The *CD14*-260 and *TLR4*+896 genotypes, allele, and carrier frequencies were compared between the different clinical patient groups and controls. In addition, synergism between *CD14* and *TLR4* genotypes and alleles (carrier trait analyses) was studied. Vienna classification and ASCA status were included in the statistical modelling.

The results are shown in table 1. The frequency of the G allele of *TLR4*+896 was significantly increased in CD patients compared with controls (19% v 10%; p=0.049; odds ratio (OR) 2.1 (95% confidence interval (CI) 1.0-4.1)). Disease phenotype was assessed in patients using the Vienna classification. Carriage of *TLR4* +896*G significantly increased the risk of colonic localisation of CD compared with non-colonic localisation (43% v 12%; p=0.0017; OR 5.5 (95% CI 1.9-15.4)). There was a clear trend (test for trend: χ^2 : 16, p<0.0001) when we compared the increasing frequency of the G allele of *TLR4* +896 in controls (10%) to CD patients (19%) and to CD patients with colonic localisation (43%).

We also assessed if ASCA status was correlated with carriage of the *TLR4* G allele. However, there was no difference between

Table 1 CD14-260 and TLR4+896 genotype distribution in Crohn's disease (CD) patients and healthy controls (HC)

Group	Vienna classification	n _{total}	CD14-260 C>T			TLR4+896 A>G		
			1.1 (CC)	1.2 (CT)	2.2 (TT)	1.1 (AA)	1.2 (AG)	2.2 (GG)
HC		170	48 (28)	82 (48)	40 (24)	153 (90)	16 (9)	1 (1)
CD		112	35 (31)	54 (48)	23 (21)	91 (81)	19 (17)	2 (2)*
	A1	97	29 (30)	49 (51)	19 (20)	77 (79)	19 (20)	1 (0)
	A2	15	6 (40)	5 (33)	4 (27)	14 (93)	0 (0)	1 (6)
	B1	43	14 (33)	20 (47)	9 (21)	36 (84)	6 (14)	1 (2)
	B2	45	15 (33)	21 (47)	9 (20)	37 (82)	7 (16)	1 (2)
	B3	24	6 (25)	13 (54)	5 (21)	18 (75)	6 (25)	0 (0)
	L1	41	14 (34)	17 (41)	10 (24)	36 (88)	4 (10)	1 (2)
	L2	23	6 (26)	13 (57)	4 (17)	13 (57)	9 (39)	1 (4)†
	L3	47	15 (32)	23 (49)	9 (19)	41 (87)	6 (13)	0 (0)
	L4	1	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	0 (0)

*TLR4*G was more frequent in CD patients compared with HC (19% v 10%; p=0.0489; odds ratio (OR) 2.076 (95% confidence interval (CI) 1.041–4.142)).

†TLR4*G was significantly associated with colonic localisation compared with non-colonic localisation (43% v 12%, p=0.0017; OR 5.455 (95% CI 1.931–15.410)).

Vienna Classification: (age) A1: age <40 years; A2: age >40 years; (behaviour) B1: non-stricturing, non-penetrating; B2: stricturing; B3: penetrating; (localisation) L1: ileal; L2: colonic; L3: ileocolonic; L4: upper gastrointestinal tract.

TLR4 G allele carriage in ASCA positive and ASCA negative patients (23% v 14%; p=0.33) (data not shown) and there was no difference between TLR4 G allele carriage in ASCA positive and negative CD patients with colonic localisation (40% v 46%; p=1.00) while the frequency of G allele carriage was identical to that of CD patients with colonic localisation (43%) without correcting for ASCA status.

Several studies have described both TLR4+896 A>G and CD14-260 C>T in CD. Klein *et al* have described a German population and found an increased incidence of CD14-260 heterozygous and homozygous mutants in CD patients compared with healthy controls.⁵ This association could not be confirmed in our population. Preliminary data by Braat *et al* demonstrated an increased risk of suffering from CD in a Dutch population carrying the TLR4+896 SNP,⁶ confirming our results. Franchimont and colleagues (*Gut* 2004;**53**:987–92) corroborated the results of Braat *et al*. In contrast with Franchimont *et al*, we found a clear association between the G allele of TLR4+896 and disease phenotype (colonic localisation). In contrast with the aforementioned studies and results, Arnott *et al* were unable to demonstrate an association between susceptibility to CD and the TLR4 and CD14 SNPs in a Scottish and Irish population.⁷

The association between TLR4 and CD underscores the role of impaired innate immunity in CD. TLR4 signalling is based on both exogenous (for example, LPS) and endogenous (for example, human HSPs) agonists, and as heterozygous carriership of the TLR4+896 A>G does not seem to impair LPS signalling,^{8,9} further agonist identification to elucidate the microorganisms involved in CD and especially in colonic localisation is essential to obtain insight into both the pathophysiological and immunogenetic aspects of CD. This insight may be helpful in developing strategies for the prevention and treatment of CD.

The association we demonstrated between TLR4 and CD is most likely not strongly based on the *S cerevisiae* mannan-LBP-CD14-TLR4 pathway but, as we have demonstrated, on

the ASCA data in our group. It would be interesting to know whether Franchimont *et al* tested for ASCA in their CD patients and whether or not an association between ASCA and TLR4 was observed.

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Conflict of interest: None declared.

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Reoperative chemoradiotherapy for oesophageal cancer: a systematic review and meta-analysis

Just as the weakest link in a chain determines how much weight the chain will hold, the weakest link in the data used by Fiorica *et al* will determine how much weight we as readers should give to their findings and conclusions regarding neoadjuvant chemoradiotherapy for oesophageal adenocarcinoma (*Gut* 2004;**53**:925–30). Clearly, the weakest link in their data is the material by Walsh *et al*, and prior to placing any confidence in the conclusions by Fiorica *et al*, a careful assessment of the reliability of the Walsh data is imperative.¹ Well known criticisms of the Walsh trial include the lack of routine staging with computed tomography scanning that led to five patients undergoing surgery alone for stage 4 disease, the exclusion of a number of patients in the neoadjuvant arm for "protocol violations" when in fact several had evidence of progressive disease and should have been considered treatment failures, and the lack of a uniform surgical technique that led to five different types of operations being performed and what are arguably the worst surgical results for oesophageal adenocarcinoma reported in the literature. However, these concerns are overshadowed by an even greater problem in the Walsh trial related to internal inconsistencies in the survival data. Careful review of the Walsh manuscript reveals that the survival data in the text of the report does not match the data in the Kaplan-Meier survival curves, but interestingly the discrepancy is only for the neoadjuvant arm.¹ In all cases the survival data for the surgery alone arm matches up precisely. For example, in the text of the manuscript, survival by intention to treat at three years in the neoadjuvant arm is reported as 32%, yet on the Kaplan-Meier graph survival by intention to treat in the neoadjuvant arm is approximately 48%.¹ Similar discrepancies occur at essentially every data point for both the intention to treat and the treatment actually received graphs, but only for the neoadjuvant arm, with survival on the Kaplan-Meier graphs much improved over the data in the text. Importantly, the statistics for survival are calculated from the Kaplan-Meier curves, raising concern that the difference in survival between groups is in fact not significant. This alarming discrepancy has never been adequately addressed despite a letter to the *New England Journal of Medicine* and a subsequent reply by Dr Walsh.²

The response by Walsh was that the graphs were mislabelled, but even with a different label the data points continue to be incongruent.

In light of this, I would like to know how Fiorica *et al* handled the data from the Walsh trial. Did they use data from the Kaplan-Meier survival curves or from the text and tables in the manuscript? Were they aware of the discrepancy and if so why did they not comment on it in their manuscript and specify how they dealt with it in their meta-analysis? In light of these concerns, as well as other issues regarding this trial, is it appropriate to even include it in a meta-analysis unless the raw data are independently reviewed and the statistics validated? This is an especially important issue as the Walsh study is the only trial that included just patients with adenocarcinoma, and as stated in the manuscript by Fiorica *et al*, robust analysis showed that exclusion of the Walsh trial would lead to loss of statistical significance for overall mortality (*Gut* 2004;**53**:925–30). This would leave us where we started, lacking any significant evidence that neoadjuvant therapy improves survival for patients with oesophageal adenocarcinoma.

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Systemic lidocaine and mexiletine for the treatment of a patient with total ulcerative colitis

In basic research, neural modulation in ulcerative colitis has been shown.¹ In clinical settings, local anaesthetics such as lidocaine and ropivacaine were used, administered per rectum, for the treatment of distal ulcerative colitis with a response rate of 83% after long treatment periods (6–34 weeks) for proctosigmoiditis (n = 49).² We report a patient with total ulcerative colitis that was ameliorated by continuous intravenous administration of lidocaine followed by oral administration of mexiletine (a congener of lidocaine).

A 24 year old man suffering from exacerbation of ulcerative colitis was admitted to our hospital. Total ulcerative colitis had been initially diagnosed one year previously. Disease extent was re-examined by barium study to reveal a total type of colitis. Conventional medical therapies, including steroids (60 mg prednisolone and steroid pulse therapy), 5-aminosalicylate, and leucocytapheresis were not effective. Abdominal pain with bleeding per rectum was very severe at night. We administered continuous systemic lidocaine (1 mg/min on the first day and 1.3 mg/min on subsequent days) only at night, resulting in complete disappearance of abdominal pain and bloody diarrhoea on the

first day of treatment. This therapy was given for one week followed by oral mexiletine (300 mg/day on the first two days and 200 mg/day thereafter) administration. Prednisolone was tapered without exacerbation of colitis during this treatment, and the patient left our hospital.

Clinical reports by Kemler and colleagues,³ who reported on a patient with ulcerative colitis exacerbated by spinal cord stimulation, and by Peck and Wood,⁴ who obtained complete remission of a patient with ulcerative colitis after spinal cord injury, support the involvement of neural control in ulcerative colitis. Systemic lidocaine, which has been shown to suppress only spontaneous ectopic discharges without blocking nerve conduction,⁵ and mexiletine may modulate central and/or peripheral nerve function. Thus, in this case, the effectiveness of these drugs could be attributed to modulation of nerve function. Björck *et al* found that when using a 2% gel (400 mg lidocaine), maximum plasma levels were 0.5–1.9 mg/l in patients with proctitis two hours after application of the gel.² In experimental models, plasma concentrations of 1.2–2.1 mg/l of lidocaine has been shown to be effective for neuropathic pain.^{6,7} Therefore, it is possible that in ulcerative colitis, lidocaine administered per rectum could exert its pharmacological effects after being absorbed into blood and has an effect on central and/or peripheral nerves. Another possibility is direct anti-inflammatory effects of these drugs on immune cells.⁸ However, it is not known whether systemic administration of lidocaine can achieve adequate concentrations in colonic tissue to have a direct anti-inflammatory effect on immunocytes.

A prominent feature of this case was the close association between pain and other symptoms such as bloody diarrhoea. Systemic lidocaine caused prompt symptomatic relief followed by amelioration of ulcerative colitis which was assessed by sigmoidoscopy and blood inflammatory parameters (data not shown), suggesting that pain or pain inducing substances could be a cause of exacerbation of ulcerative colitis as well as a result of the disease.

Lidocaine and mexiletine therapy could be useful for the treatment of the subgroup of patients with ulcerative colitis that are refractory to conventional medical treatments. While we do not know how to select responders to this treatment, pain could be one of the indicators.

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New treatment for bile salt malabsorption

Currently available binding resins used for symptomatic bile salt malabsorption are generally poorly tolerated because of unpalatability and associated gastrointestinal side effects. We suggest that there is now a viable alternative, colessevelam hydrochloride (WelChol, Sankyo Pharmaceuticals Inc., Japan).

A 30 year old man presented with steatorrhoea, progressive weight loss, marked abdominal bloating, and lethargy after a right hemicolectomy following a road traffic accident in 1966.

Physical examination, relevant blood tests, barium follow through, colonoscopy, and microscopic examination of colonic biopsies were normal. A trial of cholestyramine in preference to a SeCHAT scan caused cessation of diarrhoea on one sachet per day. However, his abdominal bloating continued unabated and he found the treatment unpalatable. Cholestyramine was therefore changed to colessevelam 2.5 g/3.75 g on alternate days. This was well tolerated, with complete cessation of his steatorrhoea and lethargy, and no side effects. In addition, he rapidly gained weight.

A further four patients with markedly symptomatic bile salt malabsorption resistant to antidiarrhoeal agents and intolerant of cholestyramine were subsequently commenced on colessevelam (table 1). In all of these cases colessevelam was well tolerated with no side effects.

Colessevelam is a non-absorbed water insoluble polymer which sequesters bile.¹ It has been approved for usage by the US FDA, and has been received as a valuable alternative for lowering cholesterol.² Colessevelam has high affinity for dihydroxy and trihydroxy bile acids in the intestine which causes increased faecal bile acid secretion, reducing the enterohepatic circulation of bile acids.² This allows 7-hydroxylase, the rate limiting enzyme in bile acid synthesis, to increase the conversion of hepatic cholesterol to bile acids.³ It has not yet been approved for use in the UK. One abstract suggests that colessevelam may be beneficial for patients with diarrhoea who have undergone small bowel resection for Crohn's disease.³ There are no other published data to support its role in bile salt induced diarrhoea. Colessevelam is reported to be 4–6 times as potent as traditional bile salt sequestrants, possibly due to its greater binding affinity for glycocholic acid.⁴ It is administered in tablet form, and in one study the rate of compliance with colessevelam was 93%.⁴ The unique hydrogel polymeric

Table 1 Characteristics of four patients with markedly symptomatic bile salt malabsorption resistant to antidiarrhoeal agents and intolerant of cholestyramine given colessevelam

Age (y)	Sex	Reason for bile salt malabsorption	Outcome with cholestyramine	Outcome with colessevelam	Duration of colessevelam treatment (months)	Current dose of colessevelam
37	M	Idiopathic	Diarrhoea improved but not tolerated because it induced severe dyspepsia	Diarrhoea resolved, no side effects	7	3.75 g/day
59	F	Right hemicolectomy	Diarrhoea improved but not tolerated due to unpalatability	Diarrhoea resolved, no side effects	3	3.75 g/day
68	F	Radiation enteritis and right hemicolectomy	Diarrhoea improved although suffered intractable vomiting	Diarrhoea resolved, no side effects	2	2.5 g/day
40	F	Radiation enteritis	Diarrhoea improved although suffered intractable nausea	Diarrhoea resolved, no side effects	2	1.25 g/day

structure enables greater tolerability with less potential drug interactions than with resins.¹

Reported adverse events from the largest clinical trial to date include flatulence, dyspepsia, and diarrhoea although the incidence of adverse events does differ significantly from that observed with placebo, and is lower than with cholestyramine.² It is rarely associated with constipation, unlike cholestyramine.⁴ Colessevelam is non-absorbed and is excreted entirely via the gastrointestinal tract, preventing systemic side effects.⁵ Furthermore, there is little evidence for clinically significant interactions involving colessevelam.⁴ Pharmacokinetic studies with colessevelam have not shown clinically significant effects of absorption of six other coadministered drugs.⁶

There is a theoretical risk of fat soluble vitamin deficiency following such efficient bile acid sequestration. None of our patients developed any significant change in fasting triglycerides or fat soluble vitamin levels to date.

Each film coated tablet contains colessevelam 625 mg (active ingredient).² The recommended starting dose for monotherapy for hypercholesterolaemia is 3.75 g once a day or 1.875 g twice per day, although the optimal dose is 4.375 g in adults.² The optimal dose for bile salt malabsorption is not clear but an effective dose has varied between two and six tablets/day in our series. Colessevelam was obtained from IDIS Ltd.

Thus colessevelam is a novel bile acid binding resin in tablet form that maintains the benefits of cholestyramine, yet is palatable, associated with decreased adverse effects, and has greater potency. It provides a very attractive alternative therapy for patients with bile salt malabsorption and further study is warranted.

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Any researchers interested in applying for access to information held within the national register should contact Dr Helen Harris (Register Co-ordinator) or Ms Shirley Cole (Research Assistant), Immunisation Department, CDSC, Centre for Infections, Health Protection Agency, 61 Colindale Avenue, London NW9 6EQ, UK (tel: +44 (0)20 8200 6868 ext. 7676 (Wednesday to Friday) or ext. 7906 (Monday to Friday); fax: +44 (0)20 8200 7868; email: helen.harris@hpa.org.uk or shirley.cole@hpa.org.uk).

No data will be released that could identify individual patients directly or via linkage to other data. Any study proposals should then be submitted to the register co-ordinator for consideration by the steering group by Thursday 31 March 2005 (deadline).

NOTICES

The national register of hepatitis C infections with a known date of acquisition

A new call for study proposals.

In 1998, a national register of hepatitis C virus (HCV) infections with a known date of acquisition was established. The register was set up to help inform the natural history of HCV related disease in the UK and now contains anonymous data for one of the largest cohorts of individuals with known date HCV infections, with over 1120 registered patients. The majority of infections in the register are those that were acquired following transfusion of HCV infected blood that was issued before the introduction of routine screening of the blood supply for HCV, but other routes of acquisition are represented.

In order to get maximum benefit from this national resource, the register steering group would like to invite clinical and epidemiological researchers to submit proposals to access data held in the register. It is envisaged that a variety of studies might benefit from linkage with or access to the register, and proposals from all specialties and institutions are welcomed. Such studies are urgently needed to help determine the current and future burden of HCV related disease on healthcare services, and to assess the impact of currently available treatments as well as those that may become available in the future.

6th International Symposium on Functional Gastrointestinal Disorders

This symposium is co-sponsored by the Office of Continuing Medical Education, University of Wisconsin Medical School, and the International Foundation for Functional Gastrointestinal Disorders (IFFGD). It will take place on 7–10 April 2005 in Milwaukee, Wisconsin, USA, at The Pfister Hotel, 424 E. Wisconsin Avenue, Milwaukee, Wisconsin 53202 (tel: +1 414 273 8222; toll free tel: +1 800 558 8222; fax: +1 414 273 5025; email: info@thepfisterhotel.com; web: http://www.iffgd.org/symposium2005.html).

CORRECTIONS

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In reference 38 of the paper by C Gasche and P Grundtner, published in the January issue (Genotypes and phenotypes in Crohn's disease: do they help in clinical management? *Gut* 2005;**54**:162–7), the page span is incorrect, it should read 1658–64.

In the paper by Sheu *et al* in the July 2003 issue of *Gut* (B-S Sheu, S-M Sheu, H-B Yang, A-H Huang, and J-J Wu. Host gastric Lewis expression determines the bacterial density of *Helicobacter pylori* in *babA2* genopositive infection. *Gut* 2003;**52**:927–932), the B and C slides of figure 1 have been transposed and the arrow on D should be labelled Le^x not Le^b.