Gene-gene interactions. Allele and genotype frequencies were compared between cases and controls using a \( \chi^2 \) test for difference in proportions. Likewise, a \( \chi^2 \) test was used to assess Hardy-Weinberg equilibrium (HWE) across genotypes.

We found no significant differences in allele or genotype frequencies between cases and controls (table 1). Stratification of the data by CARD15 mutation status showed no significant differences in Tyr113His allele frequencies in CD cases with none, one, or two CARD15 mutations. Genotypes in our cases and controls were in HWE (\( \chi^2 > 0.5 \)).

Case control based studies of genetic association assume that differences in allele frequencies relate directly to the phenotype under investigation, and that no unobserved confounding factors exist which may be attributable to the associated allele. While having greater power than family based studies to detect associations through linkage disequilibrium mapping, case control analysis is susceptible to type I errors (false positives). One of the most commonly cited explanations for non-replication of genetic associations is stratification, through population admixture, and variability in disease frequencies between and within component subpopulations. However, relatively few instances of this have been clearly established. Stratification may be identified and potentially controlled for by incorporating anonymous genetic markers into the study design. However, the efficacy of this approach depends on the level of stratification present, and the difference in SNP frequency and disease prevalence in the normal and affected populations. We noted that in de Jong et al the distribution of genotypes in controls for SNP Tyr113His was not in HWE (\( \chi^2 = 5.67, \ p = 0.017 \)). It is possible that this may have generated a type I error in their analysis. A degree of population admixture in their control cohort could account for the deviation from HWE and give rise to the observed association between the normally common T allele (as we observed) and Crohn’s disease. Alternative explanations are genotyping error and random chance. We examined the genotype distribution for the seven SNPs tested by de Jong et al and found that in addition to Tyr113His, the Ille62Val (1506A>G) SNP in CYP1A1 was not in HWE (\( \chi^2 = 7.87, \ p = 0.005 \)). A recent review of published association studies by Xu and colleagues found that 12% of SNPs tested were inconsistent with HWE in control subjects.

Our findings highlight the value of testing genetic association data for normal genotype distribution, and for rigorous replication of genetic associations with adequate statistical power.

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References


Use of cyclosporin in pregnancy

Cyclosporin has been established in the management of steroid resistant severe ulcerative colitis. We read the letter by Dor and Blanshard (Gut 2003;52:1070) regarding the severe side effects of cyclosporin used in a patient with steroid resistant severe ulcerative colitis after undergoing emergency Caesarean section. We would like to report our experience of a pregnant patient with steroid resistant severe distal ulcerative colitis in whom remission was induced with cyclosporin. She delivered a healthy baby at 34 weeks.
A 36 year old woman presented for the first time with a five week history of bloody diarrhoea and mucus discharge in the 12th week of her first pregnancy. Ulcerative colitis was confirmed on flexible sigmoidoscopy and histology. She was started on mesalazine (Pentasa) 1 g twice daily orally and Pentasa enema was added subsequently. She failed to respond well to oral prednisolone (40–60 mg daily) for five weeks or to subsequent intravenous prednisolone given for a further two and a half weeks. Azathioprine (oral 150 mg daily) was also added. Repeat sigmoidoscopy confirmed severe distal colitis with ulceration. At the 23rd week of pregnancy, she was started on intravenous cyclosporin (2 mg/kg) with careful monitoring of serum levels. Significant improvement was noted in two weeks, after which cyclosporin was changed to the oral route. Steroids were gradually tapered to 2.5 mg daily. At 34 weeks she underwent an emergency Caesarean section because of antepartum haemorrhage and a healthy baby girl (birth weight 2.07 kg) was delivered. Two weeks later, cyclosporin was weaned off after minimal rise of serum creatinine that coincided with high serum cyclosporin levels. Her serum creatinine normalised four weeks later. She and baby remained well on azathioprine and mesalazine 14 weeks after delivery.

Intravenous cyclosporin induced remission in our pregnant patient who had failed to respond to high dose oral and intravenous prednisolone. Colectomy and the associated potential complications in pregnancy were avoided. There is only one other case report in the literature where cyclosporin was used in similar circumstances. While we would agree that cyclosporin should be used cautiously in pregnancy, our positive experience, and that of others, supports the view of our colleagues, that cyclosporin may induce remission and avoid colectomy during pregnancy.

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Is symptom control the correct end point for proton pump inhibitor treatment in Barrett’s oesophagus?

We have recently reported that abnormal acid reflux persists in up to 50% of patients with long segment Barrett’s oesophagus, despite good control of symptoms of gastro-oesophageal reflux disease (GORD) with proton pump inhibitor (PPI) therapy.1 The critical question is whether such persistence of abnormal acid reflux alters the risk of progression to adenocarcinoma. We investigated this issue by studying cellular proliferation and expression of cyclin D1, which is an important marker of neoplastic progression,” in patients with Barrett’s oesophagus on PPI therapy.

A prospective cross-sectional survey of 20 patients with long segment Barrett’s oesophagus (defined as a length ≥3 cm and presence of specialised intestinal epithelium containing alician blue staining goblet cells) was conducted. In all cases, GORD symptoms had been well controlled with PPI therapy (omeprazole n = 13 patients, median dose 20 mg (range 10–40); lansoprazole n = 5, 30 mg; or rabeprazole n = 2, 20 mg). Patients had received PPI therapy for a median duration of 30 months (12–66). Oesophageal manometry, 24 hour ambulatory pHmetry, and Bilitec 2000 monitoring were conducted on all patients, without interruption of their usual PPI therapy. Representative endoscopic biopsy specimens of Barrett’s oesophagus from each patient were studied for expression of cyclin D1 protein (primary antibody 1:50 dilution; Novocastra Lab) and Ki-67 protein (primary antibody 1:75; Dako Lab), by standard immunohistochemistry. The histopathologist was blinded to clinical information. A proliferative index was computed for each patient by scoring the percentage of Ki-67 labelled specialised columnar epithelial cells, as previously described.1 Cyclin D1 expression was semi-quantitatively assessed. The mean percentage of positive cells in areas of intestinal-type specialised columnar epithelium was assigned to one of three categories: 0, <5%; 1, 5–50%; or 2, >50%. The intensity of cyclin D1 immunostaining was scored as: week = 1, moderate = 2, or intense = 3. The percentage category of positive cells and staining intensity were multiplied to produce a weighted score for each patient. All cases with weighted scores ≥1 were designated positive.

Despite PPI therapy and absence of GORD symptoms, pHmetry detected abnormal acid reflux in nine (45%) patients (pH <4 for median (range) 19.2% (range 4.6–32.1) of 24 hours; DeMeester score 49.5 (20.2–109.8)). The remaining 11 patients had acid reflux within the normal range (pH <4 for ≤4.5% of 24 hours). Proliferative indices (mean (SD)) for patients with abnormal acid reflux and those with normal acid reflux were similar (56.5 (8.7) v 37.4 (5.5), respectively; p = 0.3). Cyclin D1 expression was positive in seven (78%) patients with abnormal acid reflux and in seven (64%) patients with normal acid reflux (p = 0.4) (fig 1). The weighted score of cyclin D1 expression was identical (median 2 (range 2–6)) for patients with abnormal acid reflux and those with normal acid reflux.

These data imply that the risk of neoplastic progression was independent of the status of control of acid reflux by PPI therapy. We also examined the association between acid reflux and bile reflux. Absorbance ≥0.14 for ≤1.8% of the 24 hour monitoring period was considered the normal range for bile reflux in this study. Despite PPI therapy, abnormal bile reflux was detected in 12 (60%) patients, including six (55%) with normal acid reflux (absorbance ≥0.14 for 13.0% (2.5–46.5)) and six (66%) with normal acid reflux (absorbance ≥0.14 for 17.4% (3.5–63.7)). Such persistent bile reflux may explain the similarity in expression of Ki-67 or cyclin D1 in the two groups with different control of acid reflux.

In contrast with PPI therapy, antireflux surgery that is successful in controlling acid reflux also controls bile reflux.3 Following successful antireflux surgery, proliferative indices in surface epithelial cells and crypts of Barrett’s oesophagus are significantly lower compared with a failed procedure.3 In the light of the present data, we propose the need for a novel clinical trial of PPI therapy versus antireflux surgery. Patients who are randomised to PPI therapy should undergo

Figure 1 [A] Ki-67 immunoreactive cells are localised at the base of the crypts and glandular zones in specialised intestinal-type columnar epithelium. Arrowheads indicate some goblet cells (original magnification 200×). [B] Specialised intestinal-type columnar epithelium exhibits moderate nuclear cyclin D1 staining in the majority of epithelial cells lining the crypts (weighted score 4). Arrowheads indicate some goblet cells (original magnification 200×).
serial pH and Bilitec monitoring, with appropriate therapeutic modification to achieve predetermined end points. Patients who are randomized to laparoscopic fundoplication should have additional PPI therapy, as required, to achieve the same end points. Outcomes should be measured by standard endoscopic assessment and also by examination of a panel of molecular and cellular markers that is important in the pathogenesis of Barrett’s adenocarcinoma.

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Improving hepatitis C services across the UK: response to a walk-in HCV testing service

The Department of Health (DH) estimates that approximately 0.4% of the UK population are chronically infected with hepatitis C virus (HCV) (that is, 200 000 people). As few as 10% of these individuals, who are at risk of end stage liver disease, are thought to be aware of their infection. Clearly action is required to identify and treat these patients with current drugs (pegylated interferons and ribavirin) that can cure over 50% of infected patients.

The UK voluntary sector have responded to the government identified need for more public information about HCV by organising public information about HCV by organising open access confidential hepatitis C testing and may facilitate public education about this important treatable infection. However, these clinics are labour intensive and, in our experience, unlikely to provide a cost effective solution to the identification of people with treatable, sometimes fatal, infection.

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Conflict of interest: Dr Foster acts as a consultant to companies who sell drugs for the treatment of viral hepatitis and has received research funding from such companies. He has received fees from companies who market antiviral therapeutics.

Influence of mode of delivery on gut microbiota composition in seven year old children

Intestinal microbiota development begins immediately following birth. The composition of the infant’s evolving microbiota is initially defined by the mother, the source of the newborn’s first microbial inoculum. Colonising bacteria rapidly adapt to breast milk and epithelial mucins as sources of nutrients. The prevalence of caesarean section delivery in Western countries is increasing. Caesarean born babies are deprived of contact with the maternal/vaginal microbiota and the first exposure is characterised by a lack of facultative anaerobes such as Clostridium species. Caesarean born infants have a more slowly diversifying microbiota, with differences reported from normally born infants, even after six months of age. Abaracnians in early microbiota acquisition can affect immunophysiological development with a heightened disease risk. This study assessed microbiota composition in seven year old children and compared the respective effects of normal delivery and caesarean section.

In all, 60 seven year old children were randomly selected from Southwestern Finland, representing caesarean and vaginal deliveries. The children were invited to attend a clinical examination, including skin prick testing and determination of serum total and antigen specific IgE antibodies. Perinatal data were derived from hospital medical records. Questionnaires were completed by the parents to verify a history of allergic symptoms.

Fecal samples were produced at clinical examination and frozen at –70°C for microbiota assessment. Fecal microbiota profiles were determined using the culture independent fluorescent in situ hybridisation method. Probes specific for bifidobacteria, lactobacilli/enterococci, bacteroides, clostridia, and total bacterial numbers were applied. Written informed consent was obtained from parents and the study was approved by the ethics committee of the university.

Of the study population, 31 children had been delivered by caesarean section and 29 by vaginal delivery. At seven years of age, significantly higher numbers of clostridia were found in children delivered vaginally compared with caesarean born children (p = 0.0055) (table 1). No differences were observed in other faecal bacteria or total numbers of bacteria (table 1).

Children with asthma diagnosed by a physician (n = 6) had lower numbers of clostridia in their faecal specimens while healthy children (n = 54) had higher clostridial numbers.

Early colonisation guides subsequent microbiota development which may later impact on health, to the extent of predisposing some infants towards specific diseases. Bifidobacteria are considered useful for health promotion. Reported effects are related to the individual “balance” of the gut microbiota and prevention of abberancies within the gastrointestinal tract. Clostridia are generally considered harmful toxin producing species causing diarrhoea and food poisoning.

Our results show that bifidobacterial levels in the faeces of cohort children were comparable at seven years of age, independent of the mode of delivery at birth, while numbers of clostridia were significantly higher in normally born children seven years after birth. Differences in neonatal gut microbiota in particular the balance between Bifidobacterium species and Clostridium species, have been reported to precede heightened production of antigen specific IgE antibodies, a hallmark of the atopic responder type. Such differences may be related to external environmental factors.

<table>
<thead>
<tr>
<th>Parameter (concn of specific microbe or total IgE)</th>
<th>Normally delivered</th>
<th>Caesarean born</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clostridia</td>
<td>9.29 (9.06–9.51)</td>
<td>8.83 (8.6–9.06)</td>
<td>0.0055</td>
</tr>
<tr>
<td>Bifidobacteria</td>
<td>10.32 (10.13–10.5)</td>
<td>10.29 (9.99–10.59)</td>
<td>0.87</td>
</tr>
<tr>
<td>Total bacteria</td>
<td>11.56 (11.46–11.7)</td>
<td>11.59 (11.5–11.68)</td>
<td>0.61</td>
</tr>
<tr>
<td>Lactobacilli/enterococci</td>
<td>9.07 (8.85–9.3)</td>
<td>9.05 (8.86–9.2)</td>
<td>0.85</td>
</tr>
<tr>
<td>Bacteroides</td>
<td>9.95 (9.67–10.24)</td>
<td>9.84 (9.52–10.17)</td>
<td>0.63</td>
</tr>
<tr>
<td>Total IgE</td>
<td>79 (76–255)</td>
<td>65 (25–160)</td>
<td>0.85</td>
</tr>
</tbody>
</table>

Values are median (interquartile range).

Table 1 Numbers of faecal bacteria (log 10 number of bacteria/g faeces) and total serum IgE concentration, and number of children with asthma or atopic dermatitis among seven year old children with a history of normal birth or caesarean section.
We read with interest the case described by a patient who developed fulminant Crohn’s colitis after allogeneic transplantation from a living related donor for liver transplant from a donor with known history of CD. The recipient tested negative for any of the three common CD associated NOD2/CARD15 variants (R702W, G908R, 1007fsinsC) but unfortunately we were unable to screen the liver donor for these polymorphisms. Our case, similar to that described by Sonwalkar et al, raises the intriguing possibility that CD susceptibility may have been transferred to the recipient with liver transplantation as well. Collins et al have reported complete and stable replacement of recipient haematopoiesis and B lymphopoiesis with donor derived cells approximately six weeks following orthotopic liver transplantation for haemochromatosis. They have also shown that CD susceptibility may be present in the recipient. Whether liver donor selection should exclude those with a known diagnosis of CD is unclear and is still premature to answer.

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References

Crohn’s ileitis after liver transplantation from a living related donor with Crohn’s disease

We read with interest the case described by Sonwalkar et al of a patient who developed fulminant Crohn’s colitis after allogeneic stem cell transplantation (ASCT) (Gut 2003;52:1518–21). Although the donor had no known history of CD (CD) and did not carry the IBD3 or IBD5 haplotypes associated with CD, HLA class III mismatches at IBD3 and a CD associated polymorphism of the 5′UTR of NOD2/CARD15 were present in the donor and in the reconstituted immune cell population of the recipient post ASCT. The authors hypothesised that adoptive transfer of CD susceptibility may have occurred between ACST donor and recipient.

Herein, we report a case of a patient who developed CD after receiving a living related liver transplant from a donor with known CD. A 24 year old female received a liver transplantation with a living related donor for decompensated cirrhosis secondary to vertically transmitted chronic hepatitis C infection. The family history was significant for a maternal aunt diagnosed with CD, who served as the liver donor, and a maternal uncle and grandfather with colon cancer. Following liver transplantation, the patient was maintained on an immunosuppressive regimen consisting of tacrolimus 3 mg twice daily, sirolimus 5 mg daily, as well as TMP-SMZ prophylaxis. Her initial post-transplant course was uneventful but she later developed recurrent hepatitis C infection, treated with pegylated interferon and ribavirin. She presented with symptoms consistent with intermittent small bowel obstruction 11 months post-transplant. She was also receiving prednisone 15 mg daily at that time. A computed tomography scan of the abdomen and pelvis (see fig 1A on the Gut website: www.gutjnl.com) and an upper gastrointestinal with small bowel follow through study (see fig 1B on the Gut website: www.gutjnl.com) demonstrated marked fold thickening of the distal ileum. An enteroscopy demonstrated patchy ulcerations in the jejunum and Roux-en-Y limb of the small bowel. Biopsies showed focal ulceration and mild active inflammation without evidence of granuloma or viral inclusions. Wireless capsule endoscopy demonstrated multiple erosive and ulcerative changes in the distal small intestine (see fig 1C, 1D on the Gut website: www.gutjnl.com).

Because of persistent symptoms and concern for possible lymphoproliferative disorder, the patient underwent an open laparoscopy which revealed nodularity of the terminal ileum. Intraoperative colono-scopy demonstrated nodularity and three ulcers in the distal ileum. Histopathological examination of the resected ileal specimen demonstrated focal villous blunting, expansion of the lamina propria with acute and chronic inflammatory cells, reactive crypt changes, and occasional crypt abscesses and focal gastritic metaplasia (arrow and insert). SM, submucosa.

Figure 1: Histopathological examination of a resected ileal specimen demonstrated focal villous blunting, expansion of the lamina propria with acute and chronic inflammatory cells, reactive crypt changes, and occasional crypt abscesses and focal gastric metaplasia (arrow and insert). SM, submucosa.
Enteric glia

von Boyen et al recently reported a study of glial fibrillary acidic protein (GFAP) expression in enteric glia (Gut 2004;53:222–8). Their new data are very interesting and add to our understanding of the possible role of enteric glia in gastrointestinal pathophysiology. However, we must take issue with some of the data presented that show extensive nuclear labelling with S-100 and with the submucosal plexus. This is not the case (see our fig 1) and so we feel this calls into question whether the extensive nuclear labelling observed in both fig 1 and fig 2 is really reflective of the distribution of S-100. Moreover, in the paper cited by the authors in support of nuclear localisation, Ferri et al state that “only cytoplasmic localisation (of S-100) was consistently demonstrated in enteric glia”, contrary to von Boyen et al’s assertion that S-100 labelling is largely nuclear.

Finally, it should also be noted that GFAP expression in culture may reflect an altered state of differentiation as an adaptation to in vitro conditions. Hence some of the observed changes in GFAP expression may be explained by processes reflecting changes in the culture conditions rather than a pathophysiological response to cytokines.

The issues of glial heterogeneity and the role of enteric glia in inflammation raised in the paper are very interesting, and of considerable importance in understanding the physiology and pathophysiology of the gastrointestinal tract. By analogy with the brain, it is likely that enteric glia play an important role in the function of the gut. However, we feel that the extensive glial heterogeneity suggested in the paper by von Boyen et al may be overestimated and we urge caution in extrapolation of these data based on the immunohistochemistry presented in this manuscript.

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References


www.gutjnl.com

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In the paper by Wang et al (Gut 2004;53:1096–1101), the acknowledgement and correct email address were not presented. The acknowledgement should have read as follows: "The authors thank senior technician Shu-Hao Wen for her assistance in processing the tissue slides, and Drs Jian-Ming Qian, Gang Sun, and Xiao-Hong Liu for their help in collecting the biopsy samples for the study project. In addition, the correct email address for Professor G-Z Pan is: pgz@public3.bta.net.cn."

An author was omitted from the paper by Francés et al (Gut 2004;53:860–4), entitled "Bacterial DNA activates cell mediated immune response and nitric oxide overproduction in peritoneal macrophages from patients with cirrhosis and ascites." This paper was published in the June issue and the missing author is E Rodriguez, Immunology Department, Hospital General Universitario, Alicante, Spain.

doi: 10.1136/gut.2003.027425corr1
Genetic association between EPHX1 and Crohn's disease: population stratification, genotyping error, or random chance?

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