LETTERS

Genetic association between EPHX1 and Crohn's disease: population stratification, genotyping error, or random chance?

We read with interest the article by de Jong and colleagues (Gut 2003;52:547–51) reporting studies of genetic associations between DNA polymorphisms in xenobiotic metabolising genes and Crohn's disease (CD). The authors employed a case control study design to test seven polymorphisms in five candidate genes to identify potential mutation status to identify potential genetic associations between and Crohn's disease. The authors noted that 12% of SNPs tested found that one of the most commonly cited explanations for non-replication of genetic associations is stratification, through population admixture, and variability in disease 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 ile462val (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.

Table 1 Allele and genotype frequencies between cases and controls

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>n</th>
<th>T/T</th>
<th>C/T</th>
<th>T/C</th>
<th>H/H</th>
<th>C/C</th>
<th>Tyr allele frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>344</td>
<td>167</td>
<td>146</td>
<td>31</td>
<td>31</td>
<td>31</td>
<td>69.8%</td>
</tr>
<tr>
<td>CD ALL</td>
<td>307</td>
<td>155</td>
<td>152</td>
<td>25</td>
<td>33</td>
<td>33</td>
<td>71.7%</td>
</tr>
<tr>
<td>CD 0 CARD15 DSA</td>
<td>202</td>
<td>99</td>
<td>83</td>
<td>20</td>
<td>9</td>
<td>9</td>
<td>59.6%</td>
</tr>
<tr>
<td>CD 1 CARD15 DSA</td>
<td>69</td>
<td>33</td>
<td>33</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>71.7%</td>
</tr>
<tr>
<td>CD 2 CARD15 DSA</td>
<td>20</td>
<td>12</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>55.0%</td>
</tr>
</tbody>
</table>

DSAs, disease susceptibility alleles; CD, Crohn's disease.
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. Upper gastrointestinal endoscopy was performed and confirmed Barrett’s oesophagus (defined as a length >3 cm and presence of specialised intestinal epithelium containing alcian blue staining goblet cells) was present in the distal oesophagus. 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 alcian 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. 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 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 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 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. Following successful antireflux surgery, proliferative indices in surface epithelial cells and crypts of Barrett’s oesophagus are significantly lower compared with a failed procedure. 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

### Reference


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. 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,"1 in patients with Barrett’s oesophagus on PPI therapy.

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×).

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www.gutjnl.com
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 a hepatitis C awareness week. The event was attended by 1500 attendees and media attendance was estimated to be 15 million. A random survey of 100 attendees found that 30% were previously unaware of HCV and 64% wished to learn more about it. A project in Southwark aimed to identify new cases and assist in their treatment. The project was roll out nationally and is expected to identify new cases and provide patient care.

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References

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

Intestinal microbiota development begins immediately following birth.1 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 presence of facultative anaerobes causing diarrhoea and food poisoning.2 Bifidobacteria are considered useful for health promotion. Reported effects are related to the individual “balance” of the gut microbiota and prevention of aberrations within the gastrointestinal tract. Clostridia are generally considered harmful toxin producing species causing diarrhoea and food poisoning.3

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.4 Such differences may be related to external environmental factors.

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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Normally delivered</th>
<th>Caesarean born</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Closstridia</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>Lactobacteris/enterococci</td>
<td>11.56 (11.46–11.7)</td>
<td>11.59 (11.5–11.68)</td>
<td>0.61</td>
</tr>
<tr>
<td>Lactobacteris/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>Bifidobacteria/enterococci</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 (16–253)</td>
<td>65 (25–160)</td>
<td>0.85</td>
</tr>
</tbody>
</table>

Values are median (interquartile range).
factors (for example, mode of delivery and early feeding practices). The results of this study, showing that clostridial numbers in normally born children seven years after delivery are significantly higher than in caesarean born children, demonstrate that abnormal development of the intestinal microbiota reported following caesarean section delivery may continue even beyond infancy. These findings call for further assessment of microbiota composition throughout childhood when dietary interventions may still offer a rational means of health improvement. It is of importance to characterise the optimal clostridial numbers and species composition at different ages following normal and caesarean delivery.

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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 in a patient who developed fulminant Crohn’s colitis after allogeneic stem cell transplantation (ASCT) (Gut 2003;52:1518–21). Although the donor had no history of Crohn’s disease (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 for primary sclerosing cholangitis. She had a family history of a maternal uncle and grandfather with colon cancer. Following liver transplantation, the patient was diagnosed with 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 colonoscopy 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 gastric 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).

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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 in the submucosal plexus. In our hands, 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 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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In fig 1 of the paper of von Boyen et al, the nature of the GFAP immunoreactivity is not fibrinous, but granular, while the predominant labelling of S-100 is nuclear. In our hands 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.

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References

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