IRRITABLE BOWEL SYNDROME

Bacillary dysentery as a causative factor of irritable bowel syndrome and its pathogenesis

L-H Wang, X-C Fang, G-Z Pan

Background and aims: The incidence of irritable bowel syndrome (IBS) or functional bowel disorders (FBD) after bacillary dysentery (BD) has not been extensively evaluated, and little is known of the pathogenesis of post-infective (PI) IBS. Therefore, we investigated the incidence of IBS and FBD in a Chinese patient population who had recovered from BD. To further elucidate its pathogenesis, neuroimmunological changes, including interleukins (IL), mast cells, neuropeptides, and the relationship between mast cells and intestinal nerves, were investigated.

Methods: A cohort study of 295 patients who had recovered from BD (shigella identified from stool in 71.4%) and 243 control subjects consisting of patient siblings or spouses who had not been infected with BD were included in the study. All subjects were followed up using questionnaires for 1–2 years to explore the incidence of FBD and IBS, as defined by the Rome II criteria. In 56 cases of IBS (PI and non-PI) from another source, the number of mast cells in biopsy specimens from the intestinal mucosa were stained with antihistaope antibody and counted under light microscopy. Also, the relationship of mast cells to neurone specific enolase (NSE), substance P (SP), 5-hydroxytryptamine (5-HT), or calcitonin gene related peptide positive nerve fibres was observed using double staining with alcin blue and neuropeptide antibodies. In 30 cases of IBS (PI-IBS, n = 15) taken at random from the 56 cases, expression of interleukin (IL)-1α, IL-1β, and IL-1 receptor antagonist (IL-1ra) mRNAs in intestinal mucosa were identified using reverse transcription-polymerase chain reaction. The above results were compared with 12 non-IBS controls.

Results: In the BD infected cohort, the incidences of FBD and IBS were 22.4% and 8.1% (in total)-10.2% (among those in who shigella were identified) respectively, which were significantly higher (p<0.01) than the incidences of BD (7.4%) and IBS (0.8%) in the control cohort. A longer duration of diarrhoea (>7 days) was associated with a higher risk of developing FBD (odds ratio 3.49 (95% confidence interval 1.7–7.13)). Expression of IL-1β mRNA in terminal ileum and rectosigmoid mucosa was significantly higher in PI-IBS patients (p<0.01). The number of mast cells in the terminal ileum mucosa in PI-IBS (11.19 (2.83)) and non-PI-IBS patients (10.78 (1.23)) was significantly increased compared with that (6.05 (0.51)) in control subjects (p<0.01). Also, in the terminal ileum and rectosigmoid mucosa of IBS patients, the density of NSE, SP, and 5-HT positively stained nerve fibres increased (p<0.05) and appeared in clusters, surrounding an increased number of mast cells (p<0.01 compared with controls).

Conclusions: BD is a causative factor in PI-IBS. The immune and nervous system may both play important roles in the pathogenesis of PI-IBS.

PATIENTS AND METHODS

Study design/patients

Source I: follow up study

This was a cohort study comprising patients who had suffered from BD and who attended the Dysentery Clinic of the Peking Union Medical College Hospital (PUMCH), from April to October 1998, and their family members who had not been infected with BD. All subjects were adult Chinese, followed up for a period of 1–2 years, interviewed mainly by

Abbreviations: BD, bacillary dysentery; FBD, functional bowel disorder; IBS, irritable bowel syndrome; IL, interleukin; IL-1ra, interleukin 1 receptor antagonist; PI, post-infective; RT-PCR, reverse transcription-polymerase chain reaction; NSE, neurone specific enolase; SP, substance P; CCRP, calcitonin gene related peptide; 5-HT, 5-hydroxytryptamine; SA-HRP, streptavidin-horseradish peroxidase; PBS, phosphate buffered saline

www.gutjnl.com
Bacillary dysentery and IBS

Patients and sources are shown in fig 1. During the same period were enrolled as controls. Numbers of patients and sources are shown in fig 1.

In the cohort study, patients’ siblings or spouses who did not have episodes of dysentery or acute infectious diarrhoea during the same period were enrolled as controls. Numbers of patients and sources are shown in fig 1.

Source II: subjects involved in mast cell/nerve fibre, and interleukin studies

Patients were from the Gastroenterology Clinic, PUMCH (fig 1). Fifty six cases of diarrhoea-type IBS who fulfilled the Rome II criteria.8 FBD included other functional bowel disorders as well as IBS (see footnote to table 1).

Questionnaire

Patients and control subjects in the follow up study answered the same questionnaire, as reported by Neal and colleagues 2 with minor modifications. Questions concerned the presence of symptoms after the episode of dysentery, such as: abdominal discomfort or pain, form of stool, change in bowel habit, sense of urgency, straining or incomplete evacuation at the passage of stool, passage of slime or mucus, bloating, or feeling of abdominal distension. Question also concerned the patient’s general health, previous medical history, and bowel habits a year before dysentery. All questions were followed by “if yes, from what time, on how many days a week, and for how many months a year”?

The majority (90%) of patients and their family members were interviewed by telephone, and the remainder by mail. Diagnosis of FBD or IBS was made according to the Rome II criteria. FBD included other functional bowel disorders as well as IBS (see footnote to table 1).
Identification of expression of IL mRNAs

IL-1α, IL-1β, and IL-1 receptor antagonist (IL-1ra) mRNAs were identified as follows. Extraction of RNA from mucosal biopsies by Trizol (Gibco) reagent was based on the procedures provided by the company, and reverse-transcription polymerase chain reaction (RT-PCR) was according to the procedure of Jobin and Gauthier with minor modifications. The RT-PCR kit was from Boda Company (Beijing, China).

The RT reaction was as the follows: 1 μl dNTP, 1 μl M-MuLV, 1 μl 5×M-MuLV buffer, 1 μl random primer, 0.3 μl RNase inhibitor, and 4 μl RNA sample, and then ddH₂O was added to make a final volume of 20 μl. The reaction was set at 75°C for two hours and then at 95°C for five minutes.

For PCR, 4 μl of RT product were incubated with 1 μl dNTP, 0.5 μl Taq polymerase, 5 μl 10× PCR reaction buffer, 5 μl 15 mM MgCl₂, and one of the following primers: 4 μl IL-1α (P1: 5’-GTC TCT GAA TCA GAA ACT TCA-3’; P2: 5’-ATT GCC GCT GAA AGG AGA GA-3’, 436 bp product); 4 μl IL-1β (P1: 5’-ATA AGC CAC TCT ACA GCT-3’; P2: 5’-ATG GGC CCT GAA AGG AGA GA-3’, 420 bp product); 4 μl IL-1ra (P1: 5’-GGC TTC CGT GCC AGT CAC ATA ACT ATC-3’; P2: 5’TAC TAC TCG TCC TCC TGG-AAG TAG AA-3’, 521 bp product); or 1 μl β-actin (P1: 5’GAA TTC ATG TTG GAC ACC TTC A3’; P2: 5’CCG GAT CCA TTC GCT GCT CGA AGT CCA-3’, 318 bp product). dd H₂O was added to make a final volume of 50 μl. The conditions for the PCR reactions for IL-1α, IL-1β, and β-actin were: 94°C for five minutes, then 35 cycles at 94°C for 50 seconds, 55°C for 50 seconds, and 72°C for one minute; re-extension was carried out at 72°C for four minutes. The conditions for the PCR reaction for IL-1ra were: 94°C for five minutes, 35 cycles at 94°C for one minute, 55°C for one minute, and 72°C for three minutes; re-extension was carried out at 72°C for eight minutes.

After agarose gel electrophoresis of the PCR products, photos of the mRNAs of IL-1α, IL-1β, IL-1ra, and β-actin bands were expressed as their complementary cDNAs and were measured by Phoretix ID Advanced software. The intensity of the cDNA for IL-1α, IL-1β, IL-1ra, and β-actin of each sample was normalised by the intensity of β-actin (relative IL-1 intensity = IL-1 intensity/β-actin intensity).

Immunohistochemical study of mast cells and nerve fibres

Rabbit antimast cell tryptase (Maixin Co., Fuzhou, China) was employed for the staining of mast cells in the mucosa. Tissue sections (4 μm) were incubated with rabbit antimast cell tryptase overnight at 4°C. Then, goat antirabbit biotinylated IgG added for 10–30 minutes at 37°C. Slides were treated with SA-HRP. Staining was developed by 3-amino-9-ethylcarbazole. All slides were rinsed with PBS between each step. PBS was used instead of the primary antibody in the processing of control slides.

Mast cell counting and scoring of neuropeptide staining were performed single blind (that is, the examiner read the slides without knowing the patient’s status or diagnosis). Every patient’s sample was encoded with a number assigned at random; the code was broken after cell counting. The counting and scoring systems were as follows:

Biostatistical analysis

The frequencies of all items analysed were compared between the study group and the control group using the χ² test. Risk factors were explored using regression analysis and analysis of variates and then at 95°C for five minutes.

Cell counts were expressed as the number of cells per field under high power microscopy (mean (SD)). Positive nerve fibre staining was expressed as the numeric score assessed per field under high power microscopy (mean (SD)). One way ANOVA was used in the comparison. Significant difference was taken as p<0.05; p<0.01 indicated very significant difference.

RESULTS

The follow up study

A total of 329 patients participated in the study (response rate 73.1%). Thirty four of the responders with a history of FBD were excluded. The remaining 295 cases without a history of FBD were enrolled in the study. Shigella were identified from stool culture in 71.4% of cases. As the incidence of IBS or FBD in the final analysis was not significantly different between the bacteria identifiable and non-identifiable groups (p>0.05), data were pooled in the analysis.

In the 295 cases of infectious diarrhoea, the male to female ratio was 1:1.34. Although the male to female ratio in the 243 controls was higher (1:5:3:1), there was no significant difference in the incidence of FBD or IBS between the sexes (p>0.05). Mean age of the infectious diarrhoea group was 40.74 (SD 14.94) years compared with 41.26 (14.76) years in the control group. Both groups were comparable in terms of age and economic status.

Incidence of FBD and IBS in infectious diarrhoea and control groups

In the BD group, after a follow up period of 1–2 years, 66 cases (22.4%) were found to have FBD and 24 cases (8.1%) in total developed IBS. The incidence of IBS was 10.2% (24/235) in patients with shigella infection. In the control group, the incidence of FBD was 7.4% and of IBS 0.8%, which was significantly lower (p<0.01) than that in the BD group.

Risk factors for FBD during infectious diarrhoea

Regression analysis of variants indicated that prolongation of the duration of infectious diarrhoea resulted in an increase in

<table>
<thead>
<tr>
<th>Duration (days)</th>
<th>n</th>
<th>FBD cases (%)</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–7</td>
<td>125</td>
<td>13 (10.40)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>8–14</td>
<td>104</td>
<td>30 (31.18)†</td>
<td>3.49</td>
<td>1.71–7.13</td>
</tr>
<tr>
<td>&gt;15</td>
<td>66</td>
<td>23 (34.85)†</td>
<td>4.61</td>
<td>2.14–9.91</td>
</tr>
</tbody>
</table>

Prolongation of the duration of BD over seven days significantly increased the incidence of FBD (**p<0.01). FBD includes: IBS, n = 24; functional diarrhoea, n = 18; functional abdominal bloating, n = 17; functional constipation, n = 4; and unspecified functional bowel disorder, n = 3.

OR, odds ratio; 95% CI, 95% confidence interval.
the incidence of FBD (table 1). If the duration of infection exceeded seven days, the patient had a 2.5-fold increased risk of future development of FBD compared with those whose duration was less than a week. Therefore, we conclude that duration of infectious diarrhoea is an important risk factor in FBD.

Sex or age had no significant effect on the incidence of FBD or IBS in our study (both p>0.05).

Expression of IL-mRNAs in intestinal mucosa

In PI-IBS patients, expression of IL-1β mRNA (expressed as cDNA identified at 436 bp by RT-PCR) in the terminal ileum mucosa or in the rectosigmoid mucosa was significantly higher (p<0.01) than in non-PI-IBS patients or in control subjects (table 2, fig 2).

There were no differences in expression of IL-1α or IL-1αa mRNAs between the IBS groups (p>0.05), or between the IBS and control group (p>0.05).

Number of mast cells and the relationship between mast cells and nerve fibres in the intestinal mucosa of IBS patients

Number of mast cells in the intestinal mucosa of IBS patients and controls

The number of mast cells in the mucosa of the terminal ileum in PI-IBS (11.98 (2.83)) and non-PI-IBS (10.78 (1.23)) patients was significantly higher than that of 6.05 (0.51) in controls (p<0.01) (table 3). There was no significant difference in the number of mast cells in the rectosigmoid mucosa when either IBS group was compared with controls (p>0.05). In either location, there was no significant difference in the number of mast cells in PI-IBS and non-PI-IBS patients (p>0.05).

Scores for expression of NSE, SP, 5-HT, and CGRP positive fibres in intestinal mucosa and the relationship between nerve fibres and mast cells in IBS patients

As indicated in table 3, significantly higher scores for expression of NSE, SP, and 5-HT positive fibres in the mucosa of the terminal ileum or at the rectosigmoid junction were found in PI-IBS and non-PI-IBS patients compared with controls (p<0.05). But expression of CGRP positive fibres in the mucosa at either location was not significantly different between either IBS group and the control group (p>0.05). There was no difference in mucosal expression of NSE, SP, 5-HT, or CGRP positive fibres between PI-IBS and non-PI-IBS patients at either location (p>0.05).

Using the technique of double staining, it was possible to identify the relationship between mast cells and antibody stained nerve fibres. Nerve fibres stained bright red and appeared in clusters closely surrounding mast cells (fig 3). In IBS patients, positively stained nerve fibres around mast cells were significantly increased in density and also mast cells surrounded by those fibres were increased in number in the terminal ileum mucosa compared with controls (6.73 (1.02) v 4.25 (0.50); 6.84 (0.85) v 4.28 (0.40); 6.72 (0.81) v 4.00 (0.63); and 6.73 (0.82) v 4.33 (0.54), respectively, for NSE, SP, 5-HT, and CGRP stained fibres; all p<0.01). However, no difference was observed between the PI-IBS and non-PI-IBS groups (p>0.05).

DISCUSSION

Our study in 295 patients recovering from BD with no previous history of FBD indicated that after a follow up period of 1–2 years, 22.4% of patients developed symptoms of FBD, and 8.1% (in total)–10.2% (among whom shigella were identified) of cases developed IBS-like symptoms, as defined by the Rome II criteria. In the control cohort, consisting of 243 subjects with no episode of BD, and comparable in age, and economic and social status with the study cohort, the development of symptoms of FBD and IBS occurred in only 7.4% and 0.8% of cases, respectively, which was significantly lower than that of the study group (p<0.01). The results of our study are similar to those reported by Neal et al in the UK (FBD 25%, IBS 7%),5 despite the fact that their patients had food poisoning, chiefly resulting from campylobacter or salmonella infection, and our patients had suffered from BD, the majority of whom were infected with shigella. We also noticed that a higher incidence of FBD tended to occur in patients who had a longer duration of BD. This may be explained by delayed or inappropriate initial treatment, or to a more severe inflammation which resulted in deeper impairment of the underlining nerve fibres.10–11 It is suggested from the present study that treatment for acute enteric infection should be given initially and efficiently to prevent susceptible cases from subsequent development of FBD or IBS.

Table 2 Interleukin 1β mRNA expression in the intestinal mucosa of post-infective irritable bowel syndrome (PI-IBS) and non-PI-IBS patients and controls

<table>
<thead>
<tr>
<th>Location</th>
<th>PI-IBS (n = 15)</th>
<th>Non-PI-IBS (n = 15)</th>
<th>Control (n = 12)</th>
<th>F</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ileum</td>
<td>2.73 (0.64)</td>
<td>1.38 (0.26)</td>
<td>1.50 (0.32)</td>
<td>27.06</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Rectosigmoid</td>
<td>2.32 (0.42)</td>
<td>1.24 (0.28)</td>
<td>1.27 (0.27)</td>
<td>26.69</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values shown in the table are relative intensities of expression of each sample calculated over the intensity of β-actin expression. Expression of interleukin 1β mRNA in PI-IBS patients was significantly higher than that in non-PI-IBS patients or in controls (p<0.01). Expression was not significantly different (p>0.05) between non-PI-IBS patients and controls.

Figure 2 Interleukin 1β (IL-1β) mRNA expression in the intestinal mucosa of irritable bowel syndrome (IBS) patients and controls.

Electrophoresis of reverse transcription-polymerase chain reaction products; IL-1β mRNAs are expressed as their complementary cDNAs identified at 436 bp. Mucosal specimens were from: lane 1, rectosigmoid junction of a post-infective (PI)-IBS patient; lane 2, terminal ileum of a PI-IBS patient; lane 3, rectosigmoid junction of a non-PI-IBS patient; lane 4, terminal ileum of a non-PI-IBS patient; lane 5, rectosigmoid junction of a control subject; and lane 6, terminal ileum of a control subject. Expression of IL-1β mRNAs in PI-IBS patients (as represented in lanes 1 and 2) was significantly higher that that in non-PI-IBS patients and controls.
The notion that PI-IBS results from an enhanced inflammatory response is further supported by our observation that expression of IL-1β mRNA in the intestinal mucosa was significantly increased in PI-IBS patients. Our observation is similar to that reported by Gwee and colleagues. They found increased expression of IL-1β mRNA in the rectal mucosa of their patients who developed IBS symptoms at three months after acute gastroenteritis but our study provided evidence for a longer duration of inflammatory response after infection. We also detected an increase in the number of mast cells within the lamina propria in the terminal ileum of our IBS patients. This is consistent with the study of Sullivan and colleagues. They found increased expression of IL-1β mRNA in the rectal mucosa of their patients who developed IBS symptoms at three months after acute gastroenteritis but our study provided evidence for a longer duration of inflammatory response after infection.

Also, we detected an increase in the number of mast cells within the lamina propria in the terminal ileum of our IBS patients. This is consistent with the study of Sullivan and colleagues. In another study, we found that activation of the mucosal immune system as an inflammatory response may play an important role in the pathogenesis of PI-IBS. Our study provides further evidence that a close attachment exists between mast cells and enteric nerves in the intestinal mucosa of IBS patients. Stead and colleagues indicated over a decade ago that there was a close relationship between mast cells and nerves in human mucosa from the ileum to the sigmoid colon in a number of colonic structural diseases. In the present study, we are able to further demonstrate in IBS patients that a higher density of positively stained nerve fibres (such as NSE, SP, and 5-HT fibres) were closely attached to mast cells in the intestinal mucosa. Also, mast cells surrounded by nerve fibres were significantly increased in number in IBS patients (in both PI-IBS and non-PI-IBS) compared with normal controls (p < 0.01). Spiller and colleagues also identified increased numbers of enteroendocrine cells (including 5-HT containing cell) and T lymphocytes in the rectal mucosa of their PI-IBS patients following campylobacter enteritis. This linkage between immune cells/mast cells and enteric nerves is important as it provides the structural requirement for a dynamic interaction between immune cells and nerves to form integrated neural-immune regulation on gut function in IBS. This was speculated by other investigators recently. The hypothesis is further supported by identification and excitation of 5-HT3 receptors on sensory neurones in the rat colon. Neuropeptides, such as SP and CGRP, could modulate the activity of mast cells in the opposite direction. Therefore, factors influencing the nervous system may also participate in the pathogenesis of IBS through this pathway.

In conclusion, our study provides new evidence in support of BD as a causative factor of PI-IBS. Our observation that an enhanced inflammatory response occurred in PI-IBS, and the appearance of clustering of nerves and mast cells in the intestinal mucosa of IBS patients, indicates that the immune system and the nervous system both play important roles in the pathogenesis of PI-IBS. However, we cannot explain the fact that there was also an increased number of mast cells and the same increased expression in positively stained nerve fibres in non-PI-IBS and PI-IBS patients. We do not know

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>PI-IBS patients (n = 27)</th>
<th>Non-PI-IBS (n = 29)</th>
<th>Controls (n = 12)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terminal ileum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mast cell</td>
<td>11.19 (2.63)*</td>
<td>10.78 (1.23)*</td>
<td>6.05 (0.51)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>NSE</td>
<td>3.59 (0.50)*</td>
<td>3.34 (0.61)</td>
<td>2.73 (0.90)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>SP</td>
<td>2.78 (0.89)</td>
<td>2.69 (0.85)</td>
<td>1.91 (0.70)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>5-HT</td>
<td>2.85 (0.82)*</td>
<td>2.76 (0.91)</td>
<td>2.09 (0.83)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>CGRP</td>
<td>2.26 (1.20)</td>
<td>2.14 (0.69)</td>
<td>2.09 (1.04)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Rectosigmoid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mast cell</td>
<td>6.14 (0.43)</td>
<td>5.96 (0.43)</td>
<td>5.79 (0.48)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>NSE</td>
<td>3.37 (0.56)</td>
<td>3.28 (0.80)</td>
<td>2.55 (1.21)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>SP</td>
<td>2.89 (0.80)*</td>
<td>2.62 (1.73)</td>
<td>2.00 (0.77)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>5-HT</td>
<td>2.74 (0.71)*</td>
<td>2.72 (0.96)</td>
<td>2.61 (1.03)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>CGRP</td>
<td>2.44 (1.05)</td>
<td>2.38 (1.01)</td>
<td>2.27 (1.35)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*p < 0.05 compared with control subjects.

NSE, neurone specific enolase; SP, substance P; CGRP, calcitonin gene related peptide; 5-HT, 5-hydroxytryptamine.

**Figure 3** Expression of neurone specific enolase (NSE) stained nerve fibres around mast cells in the mucosa (×1000) of the terminal ileum in an irritable bowel syndrome (IBS) patient (A) and a control subject (B). Mast cells stained blue and NSE containing fibres stained red. There is a high density of dark red NSE fibres appearing in clusters around multiple mast cells in the mucosa of the IBS patient compared with very limited staining fibres around the few mast cells in the control subject.
whether PI-IBS represents a special form of IBS or if the development of all forms of IBS has to undergo the same pathogenetic mechanism. Further studies are needed to elucidate this question.

**Authors’ affiliations**
L-H Wang, X-C Fang, G-Z Pan, Department of Gastroenterology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing 100730, China

**REFERENCES**


Bacillary dysentery as a causative factor of irritable bowel syndrome and its pathogenesis

L-H Wang, X-C Fang and G-Z Pan

Gut 2004 53: 1096-1101
doi: 10.1136/gut.2003.021154

Updated information and services can be found at:
http://gut.bmj.com/content/53/8/1096

References
This article cites 21 articles, 6 of which you can access for free at:
http://gut.bmj.com/content/53/8/1096#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Errata
An erratum has been published regarding this article. Please see next page or:
/content/53/9/1390.2.full.pdf

Topic Collections
Articles on similar topics can be found in the following collections
Irritable bowel syndrome (327)
Campylobacter, Salmonella, Shigella, Escherichia coli (242)
Gastrointestinal hormones (848)
Diarrhoea (663)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/
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 for disease association. Evidence was found for a significant association of a single nucleotide polymorphism (SNP), Tyr113His (348T>C), in the microsomal epoxide hydrolase 1 gene (EPHX1), with CD. Homozygosity for the T (Tyr 113) allele was significantly higher in cases than in healthy controls ($\chi^2 = 23.7$, $p<0.0001$, odds ratio 2.9). The observed frequency of the T allele in controls was 41%, which is outside the range of frequencies (58–94%) reported in other control populations (reviewed in de Jong et al.).

Its frequency in CD cases was 67%. In view of the strength of reported association, we wondered whether we might observe a frequency of the T allele in controls comparable with the frequency in cases. Such a frequency might be explained by chance. Alternatively, it is possible that an unobserved confounding factor exists 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 ile162Val (1506A>G) SNP in CIPT1 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.

A P Cuthbert, S A Fisher, C M Lewis, C G Mathew
Division of Genetics and Development, Guy’s, King’s, and St Thomas’ School of Medicine, King’s College London, Guy’s Hospital, London SE1 9RT, UK

J Sanderson
Department of Gastroenterology, St Thomas’ Hospital, London SE1 7EH, UK

A Forbes
St Mark’s Hospital, Northwick Park, Watford Rd, Harrow, Middlesex HA1 3UJ, UK

Correspondence to: Professor C G Mathew, Department of Medical and Molecular Genetics, GKT School of Medicine, 8th floor Guy’s Tower, London SE1 9RT, UK, christopher.mathew@kcl.ac.uk

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 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 mesalazine (40–60 mg daily) for five weeks or to subsequent intravenous mesalazine 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 mesalazine. Colectomy was strongly considered for this patient. Intraoperative biopsy specimens were not available. Improvement of her distal colitis was noted within two weeks of cyclosporin treatment. At 36 weeks of pregnancy, she was started on 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 mesalazine. Colectomy was strongly considered for this patient. Intraoperative biopsy specimens were not available. Improvement of her distal colitis was noted within two weeks of cyclosporin treatment. At 36 weeks of pregnancy, she was started on 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.

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 alcin blue staining goblet cell) 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; Novocarsa 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 intensity of cyclin D1 immunostaining was scored as: weak = 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) 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) vs 37.4 (5.3), 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 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 abnormal 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. 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...
serial pH and Bili-tec monitoring, with appropriate therapeutic modification to achieve predetermined end points. Patients who were randomly assigned to laparoscopic fundoplication should have additional PPI therapy, as required, to achieve the same end points. Outcomes should be measured by standard serial endoscopic assessment and also by examination of a panel of molecular and cellular markers that is important in the pathogenesis of Barrett's adenocarcinoma.

A I Sarela, C S Verbeke, C Pring, P J Guilow
University of Leeds School of Medicine, Leeds, UK

Correspondence to: Mr A Sarela, B 37, Clarendon Wing, Department of Surgery, The General Infirmary at Leeds, Leeds LS1 3EX, UK; a.sarela@leeds.ac.uk

References


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 day. We took advantage of the publicity around hepatitis C awareness day to assess the value of a walk-in HCV testing clinic.

Our clinic was held over four days (4–7 July 2003) and was widely publicised in the local press and television. Patients who wished to be tested were invited to attend a clinic in the Minor Injuries Unit at St Bartholomew’s Hospital in the City of London. The clinic was manned between 8am until 11am for counselling and informed testing (hepatitis C antibody test and over function tests). Results were available the next day and patients were informed in person 24 hours later.

Nineteen people attended and two were infected. One of these patients had been lost to follow up due to non-attendance at a local liver clinic 12 years ago.

Open access confidential hepatitis C testing clinics may play an important role in encouraging people to come forward for HCV 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 this treatable, sometimes fatal, infection.

R F C’Souza, M J Glynn, E Alstead, G R Foster
Hepatology Group, Barts and The London, Queen Mary’s School of Medicine and Dentistry, London, UK

I Ushiro-Lumb
Department of Virology, Barts and The London, Queen Mary’s School of Medicine and Dentistry, London, UK

Correspondence to: Professor G R Foster, Hepatology Group, Department of Gastroenterology, DDIC, Turner St, London E1 2AD, UK; g.r.foster@qmul.ac.uk

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 strict anaerobes and the presence 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. Aberrancies 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, lactobacillli/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 guidance subserves 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 aberrancies 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.

In our cohort the 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 conditions.

<p>| 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 |</p>
<table>
<thead>
<tr>
<th>Parameter (concent 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 (11.46–11.68)</td>
<td>11.59 (11.5–11.68)</td>
<td>0.61</td>
</tr>
<tr>
<td>Lactobacillus/enterococci</td>
<td>9.07 (8.85–9.31)</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 (71–255)</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.

S Salminen
Department of Biochemistry, University of Turku, 20014 Turku, Finland

G R Gibson, A L McCartney
Food Microbial Sciences Unit, School of Food Biosciences, University of Reading, Reading, UK

E Isolauri
Department of Paediatrics, University of Turku, 20014 Turku, Finland

Correspondence to: Professor S Salminen, Functional Foods Forum, University of Turku, 20014 Turku, Finland; seppo.salminen@utu.fi

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, 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 replace- ment of recipient haematopoiesis and B lymphopoiesis with donor derived cells approximately six weeks following orthotopic liver transplantation for haemochromatosis. T lineage reconstitution also occurred and derived almost exclusively from expansion of mature memory/effector T cells from the transplanted liver. One possibility is that the expanded immune cells have become tolerant to the graft but not to the intestinal luminal antigens leading to the development of CD. Whether liver donor selection should exclude those with a known diagnosis of CD is unclear and is still premature to answer.

K A Papadakis, R Matsuk, M T Abreu, E A Vasilieoukas, P R Flesner, J Latchege, T Tran, F F Poordad, P Martin, J Vierling, S R Targan
Cedars-Sinai Medical Center, UCLA School of Medicine, Los Angeles, California, USA

www.gutjnl.com
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 data presented that show extensive nuclear labelling with 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 culturing. 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.

K A Sharkey, Y Nassar
Gastrointestinal Research Group, Department of Physiology and Biophysics, University of Calgary, Calgary, Alberta, Canada

A Ruhl
Department of Human Biology, Technical University Munich, Freising-Weihenstephan, Germany

Correspondence to: Professor K A Sharkey, Gastrointestinal Research Group, Department of Physiology and Biophysics, University of Calgary, 3330 Hospital Drive, NW Calgary, Alberta, Canada T2N 4N1; ksharkey@ucalgary.ca

References


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.

CORRECTIONS
doi: 10.1136/gut.2003.021154corr1

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.

doi: 10.1136/gut.2003.027425corr1

An author was omitted from the paper by Francès 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.