Allelic variation in *Helicobacter pylori*: progress but no panacea

*Helicobacter pylori* colonisation in the stomach is associated with increased risk for the development of peptic ulcer disease and non-cardia gastric adenocarcinoma. However, the incidences of these diseases vary in different parts of the world, and these rates have been changing over the past century. It is now clear that the mere presence of *H pylori* is insufficient to account for this variation. Alternative hypotheses to explain differing outcomes include variation in bacterial strains, in host related factors, or in the particular interactions governing the long term equilibrium between *H pylori* strain populations and the colonised host. In this issue, Kidd et al (see page 499) explore whether *H pylori* strain differences are related to illness occurrence in South African patients undergoing endoscopy. Why was such a study undertaken?

Despite overall conservation of most genes, *H pylori* are a highly diverse bacterium species. Their population structure indicates that they are freely recombining, which tends to eliminate clonal or allelic variation. Yet, in accordance with previous work, Kidd et al found that important clonal differences exist, even within the single geographical locale studied. Three important allelic differences are the presence or absence of the cag island, the m1 or m2 alleles of *vacA*, and the independent s1 and s2 alleles of *vacA*; s1 can now be divided into s1a, s1b, and s1c. Although the boundaries of these alleles are not fully defined and probably shift, their very existence, against the strong pressure of the recombinational process, indicates their important biological roles for *H pylori* populations.

It is unlikely that the differential ability to cause disease in humans is responsible for this bacterial variation, as ulcer disease and gastric cancer chiefly occur late in life. Thus, these diseases per se are unlikely to have an important role in disease and gastric cancer chiefly occur late in life. Thus, humans is responsible for this bacterial variation, as ulcer cancer risk. Alternative explanations for variation include whether *H pylori* presence or absence of the cag island, the m1 or m2 alleles of *vacA*, and the independent s1 and s2 alleles of *vacA*; s1 can now be divided into s1a, s1b, and s1c. Although the boundaries of these alleles are not fully defined and probably shift, their very existence, against the strong pressure of the recombinational process, indicates their important biological roles for *H pylori* populations.

It is unlikely that the differential ability to cause disease in humans is responsible for this bacterial variation, as ulcer disease and gastric cancer chiefly occur late in life. Thus, these diseases per se are unlikely to have an important role in aiding the transmission of *H pylori* to new hosts that is in any way analogous to the ability of *Mycobacterium tuberculosis* to facilitate its own transmission by causing cavitary lung disease. More likely, differential disease risk associated with particular *H pylori* alleles is a consequence of particular bacterial adaptations that facilitate colonisation. For example, adaptation (lifestyle) differences that exist between cag+ and cag− strains now have been defined experimentally. It may be that the lifestyle of the colonising bacterial population reflects the summation of all the particular allelic variations. Van Doorn and colleagues have used the combination of cag, *vacA*, and *iceA* allelic differences to provide a very rough approximation of disease risk.

Kidd and colleagues have added to our understanding of *H pylori* strain variation and disease in several ways. Firstly, they present evidence indicating that at least 12 (35%) of 34 patients studied were colonised by more than a single *H pylori* strain, which extends our understanding of the reservoir for recombination under contemporary circumstances, and further indicates that study of only a single isolate from a patient is suboptimal. Secondly, they confirm that *H pylori* genotype distribution has geographic clustering, with a very high proportion of South African isolates being cag+ and *vacA* s1b. Thirdly, they show that particular genotypes are associated with particular diseases. Their results parallel those from the United States and Europe, but the association of s1b/m1 strains with gastric cancer is new. In particular, this finding emphasises that the particular associations found to date (and those not found), are human population specific. In coming years, investigators will need to categorise such associations in each major population group. For example, although the presence of cag+ *H pylori* strains is associated with both increased ulcer and gastric cancer risk in the USA, Europe, and Latin America, their role in Asia is much more obscure, and their ubiquity in this African population indicates their insufficiency to account for disease occurrence differences. Nevertheless, compared with the absence of *H pylori*, colonisation by s2 strains seems to have little impact on disease occurrence in this South African population, and in other groups studied.

Fourthly, Kidd and colleagues studied size heterogeneity of the cagA 3′ region, a new genotype in relation to disease occurrence. Their work, confirming observations in German and Japanese studies, suggests that certain cagA 3′ variants may be markers of particular diseases.

However, although helpful for stratifying risk, just as *H pylori* presence is helpful, the presently known *H pylori* allelic differences are insufficient to explain fully variation in disease occurrence, and do not sufficiently account for geographical variation or temporal changes in disease rates. Rather, the key to understanding *H pylori* related diseases is likely to be the interaction term between host genotype, host environment, and gastric microbial populations. I have hypothesised that the age at which *H pylori* is acquired, and the multiplicity of different organisms colonising the stomach have important bearing on gastric microecology, and thus, ultimately, on disease risk. Nevertheless, those looking for simple answers about the relations of *H pylori* and disease undoubtedly will be disappointed; the complexity likely is older than the human race. However, the challenge is great, and the clinic is our laboratory. Clinical researchers, microbiologists, experimental pathologists, and mathematicians each can contribute to solving the puzzle.

Conflict of interest: M J Blaser holds patents relating to *H pylori* genotypes, serology, and vaccines.

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Short bowel, short answer?

The paper by Jeppsen et al (see page 559) shows that glucagon-like peptide-2 (GLP-2) concentrations are low in patients lacking an ileum and colon. This is not an unexpected finding as the L cells that produce GLP-2 are situated in the ileum and colon. GLP-2 is an enterocyte specific growth hormone that in mice causes small and large bowel villus/crypt growth and increases small and large bowel length and weight. In mice it also reduces body weight and restores mucosal integrity after dextran induced colitis. In pigs it reduces gastric antral motility. The deficiency of GLP-2 in patients with a jejunostomy may explain why these patients show no evidence of structural or functional intestinal adaptation over time. As it stimulates mucosal growth and slows gastric emptying. Thus GLP-2 serum values could become a useful measure of the amount of intestinal adaptation. If GLP-2 values are low, then it will be important to study the therapeutic effects of administering Peptide YY, which like GLP-2 is produced by the L cells of the ileum and colon, slows gastric emptying and small bowel transit and may be responsible for the “ileal” and “colonic” brakes. Peptide YY serum values are high in patients with a retained colon and low in patients with a jejunostomy. Thus peptide YY may be responsible for part of the functional adaptation that occurs in patients with a retained colon; however, it is unlikely to induce structural changes.

Data are not yet available about GLP-2 values in patients with a jeuno-colic anastomosis. If high, this may represent an adaptive mechanism by which GLP-2 increases intestinal growth and slows gastric emptying. Thus GLP-2 serum values could become a useful measure of the amount of intestinal adaptation. If GLP-2 values are low, then it will be important to study the therapeutic effects of administering it. There may even be a congenital defect in absorption related to a deficiency of GLP-2.

The importance of GLP-2 is just starting to be recognised. Its role in promoting intestinal growth makes it potentially useful treatment for patients with a short or damaged bowel. The role of GLP-2 (and peptide YY) in inducing jejunal structural and functional adaptation will only become clearer after they (or analogues) have been infused/injected into patients with a short bowel, and the short and long term effects documented.

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Pseudo-obstruction in children: transplant or wait?

With advances in immunosuppression intestinal transplantation has emerged as a viable option for patients with chronic intestinal failure and life threatening complications. With a one year graft survival rate of around 60% over the past few years, the current situation compares favourably with the pre-1990 results when 30% survival rates were achieved (data from the Intestinal Transplant Registry, www.intestinaltransplant.net). At the 7th Meeting of the European Intestinal Transplantation Study Group (Brussels, 1998), one year survival rates up to 80–90% were reported by some very experienced centres.

In children, chronic intestinal pseudo-obstruction is often a primary disorder, either congenital or acquired. Secondary pseudo-obstruction is related to various systemic diseases and is more common in older patients. According to the pathological findings, the primary disease is classified as neuropathic, myopathic, or idiopathic if no specific pattern is recognised. The disease always involves the small bowel and may occur in any other (or all) regions of the digestive tract. Signs and symptoms of obstruction, but without anatomical obstruction being evident on conventional imaging, dominate the clinical picture. The first symptoms may appear as early as the neonatal period or later in life and range from occlusion and complete intolerance of the digestive tract. Signs and symptoms of obstruction, but without anatomical obstruction being evident on conventional imaging, dominate the clinical picture. From infancy to late childhood, the presence of both signs and symptoms is frequently seen. The disease is uncommon and because it usually progresses slowly, the poor long term prognosis has probably been underestimated in the past.

Sigurdsson et al's experience with 27 young patients (1–19 years old) (see page 570) is of major importance for several reasons. As the patients were referred for transplant, this group is probably not representative of the general population but confirms, however, that severe complications can occur during childhood. Multidisciplinary, perioperative management is mandatory and surgery must be tailored to each individual because the disease can affect other portions of the digestive system and other organ systems. With expert care, good results can be achieved in this difficult group of patients, as illustrated in this series.

Of more concern is that of the 22 patients registered for transplantation, eight were transplanted, eight died before a graft became available and six were still awaiting transplant. This is of course a result of the current shortage of donors but also reflects the poor clinical condition of the patients at the time of registration. Poor clinical condition and pre-existing complications are clearly related to an increased risk of death. Moreover, worsening of the clinical condition while on the waiting list leads to increased post-transplant morbidity and a higher death rate. Taking into account that the long term results of transplants are still not as good as those for long term parenteral nutrition, the timing of referral and the criteria for small bowel transplantation continue to be debated. The current general indication for bowel replacement is still the development of life threatening problems in the context of gut failure (mostly progressive liver disease and/or central venous access problems). Sigurdsson et al propose a reasonable approach—patients who do not present with cholestasis or hepatic dysfunction should be managed conservatively. These patients should benefit from the best appropriate care, minimising the risk of progressive liver disease and preventing line infections. This strategy adheres to the current consensus guidelines: transplantation should be avoided by adequate medical management but patients who develop complications (cholestasis, portal hypertension, vascular access problems) should be referred for transplantation.

Earlier referral and thus registration of patients while still in reasonable health may help to decrease both pre- and post-transplant risks. In a recent study, early referral for liver transplantation led to better results which in turn had a positive effect on patient selection. Bueno et al reported that a bilirubin concentration above 3 mg/l, a prothrombin time of more than 15 seconds or bridging fibrosis at liver biopsy were significantly related to poor outcome. Also, 80% of the children under 1 year of age when referred for transplantation died within two years. Thus, patients should be referred to transplant centres as early as possible before liver dysfunction or portal hypertension has developed, or at least before complications occur. Then, after a full evaluation of the patient’s condition, the appropriate management strategy should be discussed with the referring team, including the option for possible future transplantation and its ideal timing.

Early referral and early interactive management between local and transplant centres is essential if the general outcome of patients in potential need of bowel replacement is to be improved.

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Screening for colorectal cancer: the heart of the matter

All screening interventions have the potential to do harm and screening by faecal occult blood testing (FOBT) is no exception. The harm arises not from the test itself, although the psychological impact is not negligible, but mainly from the investigation and treatment of test positives. The benefits of FOBT have been studied extensively and it seems that among those who comply with biennial testing, colorectal cancer mortality is significantly reduced and that the reduction is greater when the test is used annually. But there is a price to pay. Firstly, there is the anxiety engendered by performing the test itself and the greater anxiety induced in those who are found to be positive requiring further investigation. Although this has been shown to be short-lived, lasting mainly until the completion of diagnostic investigations, cumulatively it might be expected to affect around 10% of those complying with the test biennially over 20 years.

Secondly, there are the well documented complications associated with colonoscopy, which is considered the gold standard for the diagnostic workup of test positives. Overall mortality rates are reported to average around 0.02%, varying between 0 and 0.06%. Perforation can be expected in 0.3% and haemorrhage in around 1% of people undergoing polypectomy. A variety of other complications have been reported, the most serious of which are cardiovascular. Finally, there is the significant morbidity associated with the surgical treatment of colorectal cancer and large adenomas, with published 30-day mortality rates varying between 1 and 8%.

Based on the promising findings of the randomised trials, pilot studies of FOBT screening are about to start in two sites in the UK in preparation for possible implementation in a national screening programme. These studies aim to examine feasibility and acceptability rather than efficacy and adverse effects. Therefore the paper in this issue reporting on the adverse effects of screening in the Nottingham study is timely. Robinson et al (see page 588) specifically tackle three issues raised by Ahlquist in a paper questioning the wisdom of implementing screening by FOBT. Ahlquist argues: (1) that false negative results falsely reassure patients, leading to delayed diagnosis and poorer outcome; (2) that colonoscopic mortality compounded over several years could counter any gains in reducing deaths from colorectal cancer; (3) that true positive FOBT results may lead to overdiagnosis and treatment of asymptomatic colorectal cancers which might have an innocuous natural history, perhaps leading to a shortened lifespan. To counter the first point, Robinson et al show that there is no difference in the stage at diagnosis in interval and control cancers, suggesting that false negative FOBTs probably do not delay diagnosis. In response to the second point, they show that there were no colonoscopy deaths although their perforation rate (5 of 1474, 0.3%) was exactly as that reported by Ahlquist. Moreover, they restrict their analysis only to baseline colonoscopies even though those who were found to have adenomas were entered into a colonoscopic surveillance programme. If we assume that the 40% of patients undergoing colonoscopy who were found to have adenomas underwent an average of two more colonoscopies, this approximately doubles the exposure of the cohort to colonoscopy.

One point not tackled by Robinson et al was the observation by Ahlquist that, in the Minnesota study, the reduction in colorectal mortality was precisely offset by an increase in mortality from cardiac ischaemia. An increase in cardiovascular deaths, of similar magnitude to the reduction in colorectal cancer mortality, was also observed in the Danish study. The non-colorectal cancer mortality rate was also increased in Nottingham, more than offsetting any reduction in deaths from colorectal cancer, therefore it would have been of interest to have examined deaths from ischaemic heart disease in Nottingham (table 1).

### Table 1 Proportion of deaths due to various causes by screening arm in randomised trials of faecal occult blood testing

<table>
<thead>
<tr>
<th></th>
<th>Screened</th>
<th></th>
<th>Control</th>
<th></th>
<th>Screened/Control</th>
<th>Difference (n)</th>
<th>Difference (%)</th>
</tr>
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<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
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<tr>
<td>Harzesteal and colleagues</td>
<td>75253</td>
<td>16.78</td>
<td>74998</td>
<td>16.69</td>
<td>+109</td>
<td>+0.09</td>
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<tr>
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<td>12624</td>
<td>0.48</td>
<td>12515</td>
<td>0.56</td>
<td>−60</td>
<td>−0.08</td>
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<tr>
<td>CRC</td>
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<td>16.30</td>
<td>12095</td>
<td>16.13</td>
<td>+169</td>
<td>+0.17</td>
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<tr>
<td>Non-CRC</td>
<td>7069</td>
<td>0.53</td>
<td>6972</td>
<td>0.53</td>
<td>−97</td>
<td>−0.24</td>
<td></td>
</tr>
<tr>
<td>IHD</td>
<td>6228</td>
<td>20.11</td>
<td>6303</td>
<td>20.35</td>
<td>−75</td>
<td>−0.24</td>
<td></td>
</tr>
<tr>
<td>Kronborg and colleagues</td>
<td>30967</td>
<td>19.52</td>
<td>30966</td>
<td>19.61</td>
<td>−27</td>
<td>−0.09</td>
<td></td>
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<tr>
<td>All causes</td>
<td>182</td>
<td>0.59</td>
<td>230</td>
<td>0.74</td>
<td>−48</td>
<td>−0.16</td>
<td></td>
</tr>
<tr>
<td>CRC</td>
<td>6046</td>
<td>19.52</td>
<td>6073</td>
<td>19.61</td>
<td>+54</td>
<td>+0.17</td>
<td></td>
</tr>
<tr>
<td>Non-CRC</td>
<td>2497</td>
<td>8.06</td>
<td>2443</td>
<td>7.89</td>
<td>+54</td>
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<tr>
<td>Cardiovascular</td>
<td>15570</td>
<td>21.59</td>
<td>15394</td>
<td>21.70</td>
<td>+21</td>
<td>+0.11</td>
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<tr>
<td>Mandel and colleagues</td>
<td>3361</td>
<td>0.53</td>
<td>121</td>
<td>0.79</td>
<td>−39</td>
<td>−0.26</td>
<td></td>
</tr>
<tr>
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<td>20.91</td>
<td>+60</td>
<td>+0.15</td>
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<tr>
<td>CRC</td>
<td>1079</td>
<td>6.93</td>
<td>1025</td>
<td>6.66</td>
<td>+54</td>
<td>+0.27</td>
<td></td>
</tr>
<tr>
<td>IHD</td>
<td>82</td>
<td>0.53</td>
<td>121</td>
<td>0.79</td>
<td>−39</td>
<td>−0.26</td>
<td></td>
</tr>
</tbody>
</table>

CRC, colorectal cancer; IHD, ischaemic heart disease.
So what should we make of this? If there is an increase in deaths from coronaries, when do they occur and what is causing them? Robinson et al state that none of 1778 patients who were not being treated for detected colorectal neoplasia, died within 30 days of colonic investigation. It is not clear how many suffered a cardiac event within that time which might have led to death at a later time. Myocardial infarction during colonoscopy seems to be rare with a large US survey reporting three (0.012%) events in 25 298 colonoscopies, but the incidence of infarction within a few hours or days of colonoscopy is unknown.

The possibility of an increase in coronary mortality observed in the published randomised trials of colorectal screening suggests that lower gastrointestinal endoscopy and surgery should be undertaken judiciously, balancing in each patient the risks and benefits. Haemoccult, the FOBT used in the European randomised trials, when used in the unrehydrated form is a specific test with a predictive value for cancer of a positive test of around 10%. There is no other indication for colonoscopy in which the yield of cancers is so high. However around 40% of those undergoing colonoscopy as a result of a positive FOBT are found to have adenomas and it is customary to enter them into an endoscopic surveillance programme where the benefits are less clearly defined and, perhaps more importantly, the harm has yet to be quantified.

W S ATKIN

**HCV in hepatic failure: West and East do not meet**

Despite the identification of hepatitis C virus (HCV) as an aetiological agent of blood borne non-A, non-B (NANB) hepatitis a decade ago, its role in fulminant NANB hepatitis is still uncertain. In the West, HCV is only infrequently associated with NANB fulminant hepatitis. Thus, in studies from the USA, positive serum HCV RNA was reported in none of 15 cases by Wright and colleagues and in only two (12%) of 17 cases by Liang et al. Similarly, none of the 23 and 30 NANB cases in the French and English series, respectively, was positive for HCV infection. In contrast, the prevalence of serological markers of HCV infection in NANB fulminant hepatitis is much higher in the Oriental countries. In an early study from Japan, Muto et al found that seven (58%) of 12 patients with NANB fulminant hepatitis were anti-HCV (anti-C100–3) positive; findings which were later confirmed by Yangi et al who reported detectable serum HCV RNA in 43% (3/7) of cases of NANB fulminant hepatitis. In Taiwan—geographically close to Japan—HCV RNA was detected in 45–50% of patients with NANB fulminant hepatitis and in the Republic of China, more than 50% of patients with various types of fulminant viral hepatitis had anti-HCV.

The cause of this discrepancy between findings in the West and East is uncertain. Views of the pathogenesis of fulminant viral hepatitis suggest that an aggressive early host immune response to the virus is the cause of massive hepatocellular necrosis. Levels of the causative virus in the serum and liver may be high before the "peak" of liver damage and due to immune clearance, the viral load could be below the detection limit of currently available assays when the patients are first seen at the time of severe liver damage. Hence, in the absence of serial serum samples, in particular those preceding the patient’s admission to hospital, early HCV viraemia may have been missed. In many series the diagnosis has been based on the presence of anti-HCV rather than HCV RNA in the serum and unlike HCV RNA, anti-HCV is usually not detectable for two to three months after the initial exposure and infection. With the short time course of illness, patients with fulminant hepatic failure may not survive long enough to develop detectable anti-HCV in the serum. In most of the studies tackling the issue of HCV aetiology in NANB fulminant hepatitis, serial serum samples during and after the illness are lacking. However, in a recent study by an NIH group, the level of HCV viraemia, as measured by branched-chain DNA assay, increased in parallel with serum alanine aminotransferase concentrations and the degree of hepatocellular necrosis, indicative perhaps of a direct cytopathic effect of the virus. Accordingly, a single negative test for HCV RNA, by PCR, in a patient with fulminant hepatitis would rule out HCV as the cause. This finding certainly requires further validation.

As the various HCV genotypes have geographical preferences: 1b in Oriental countries and 1a in the USA, another possibility would be that the differences between East and West in fulminant NANB hepatitis are related to distinct HCV genotypes. This possibility was explored in the prospective study reported in this issue by Chu et al (see page 613). One hundred and nine consecutive HCV RNA positive patients with acute hepatitis admitted to a Taiwan hospital were investigated. Eleven patients (10.1%) developed fulminant hepatic failure but no correlation could be detected between the occurrence of fulminant hepatitis and HCV genotype. Other viral factors such as HCV viral load and coinfection with hepatitis G virus (HGV) were also investigated and were not found to be related to the occurrence of a fulminant course. One shortcoming was the lack of cases with genotype 1a in the current series which could have weakened the significance of the findings. In a recent human-to-chimpanzee transmission experiment, the same HCV strain, genotype
HBV infection and even displace HBsAg from serum.14 15 In fulminant hepatic failure in the East, it is clear that in the West there is less frequently be the cause of fulminant hepatic failure. Whatever the inter-relation between HCV and HBV is likely in areas of high prevalence as in the East—HCV will without the co-factor of HBV infection—much more may be attributed to HCV alone. It is also possible that markers may not be detectable and the severe liver damage be directly related to the occurrence of severe liver damage. 15

One important finding in the study by Chu et al was the presence of concomitant chronic hepatitis B virus (HBV) infection, as defined by positive HBV surface (HBsAg) and negative IgM anti-HBV core (HBc) antigens in nine (82%) of 11 subjects who developed fulminant hepatic failure compared with only 30/98 (31%) of those with non-fulminant acute HCV hepatitis (p<0.05). This is in keeping with a study from France which showed that eight (47%) of 17 HBsAg positive patients with fulminant hepatic failure were also HCV RNA positive, although on no occasion in that series was HCV infection alone demonstrable. Furthermore, Wright et al detected HBV DNA by PCR in the liver specimen from six of 12 patients with “NANB” fulminant hepatic failure treated by transplantation who had negative HBV serological markers and undetectable serum HBV DNA in the serum by PCR.2 It has also been shown that acute hepatitis C infection in patients with chronic HBV can suppress markers of HBV infection and even displace HBsAg from serum.14 15 Hence, in some instances of HCV superinfection of HBV carriers or concomitant dual infection, HBV serological markers may not be detectable and the severe liver damage may be attributed to HCV alone. It is also possible that without the co-factor of HBV infection—much more likely in areas of high prevalence as in the East—HCV will less frequently be the cause of fulminant hepatic failure. Whatever the inter-relation between HCV and HBV infection in the East, it is clear that in the West there is still a significant percentage of cases of fulminant hepatic failure from presumed viral hepatitis where no markers of hepatitis viral infection can currently be identified.

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Allelic variation in *Helicobacter pylori*: progress but no panacea

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*Gut* 1999 45: 477-478  
doi: 10.1136/gut.45.4.477

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