DNA test for hypolactasia premature

I write in response to the article by Rasinperä and colleagues (Gut 2004;53:1571–6) in which a DNA test was proposed for “adult-type hypolactasia.”

The ability to digest the milk sugar lactose as an adult (lactase persistence) is a variable genetic trait in human populations, lactase persistence being the most frequent phenotype in Northern Europe, whereas lactase non-persistence or “adult-type hypolactasia” is more frequent in most other populations.1 In Sub-Saharan Africa, for example, lactase persistence is found only at low frequency in the majority of populations that have been tested, but in some populations, particularly pastoralist groups, it is significantly more frequent.

A CT polymorphism located 13.9 kb upstream of exon 1 of the lactase gene (LCT) was previously shown in a Finnish population to be tightly associated with the lactase persistence phenotype2 and it is this change that is proposed as a DNA test for both Europeans and Africans. We agree that presence of a CT at this polymorphic site is indeed a fairly good predictor of lactase persistence in Northern Europeans, and there is evidence that this nucleotide resides in a functional element.3 However, the presence of the alternative allele C at this site is not a good predictor of lactase non-persistence or “adult hypolactasia” in many non-Northern Europeans.4 Particularly many draws readers’ attention to our recent study.5 We typed this polymorphism in 1671 individuals from seven African countries, which included 20 distinct cultural groups. In seven cases it was possible to match the groups tested with groups from the literature for whom phenotypic information was available. In five of these groups the published frequencies of lactase persistence were >85%. We found the T allele in Cameroon but it was so rare elsewhere that it cannot explain the frequency of the lactase persistence phenotype throughout Africa and we devised a statistical test to show that these results were unlikely to have been obtained by chance.

Our ongoing results support this published information and we urge the community to refrain from using DNA tests on Africans and probably other non-Northern Europeans until an appropriate DNA change has been identified.

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Conflict of interest: None declared.

References


Authors’ reply

Dr Swallow raises a question about another DNA variant underlying adult-type hypolactasia in sub-Saharan populations and does not recommend analysis of the C/T –13910 variant as a genetic test in African and non-Northern European populations. Although the studies performed by us4,5 and others6 do not support the existence of another variant, we agree with Dr Swallow that well conducted studies are needed to confirm this.

The significance of the C/T –13910 variant underlying adult-type hypolactasia was questioned in the article by Mulcare.7 Their doubt is based on several assumptions that make it difficult to evaluate the significance of the findings. These assumptions can be listed as follows:

1. It is not known whether or not the study subjects presented with adult-type hypolactasia. Thus, there is a risk of wrong conclusions being drawn. It is well documented that the clinical diagnosis of adult-type hypolactasia is difficult to assess due to inaccurate diagnostic tests and variable, usually mild, symptoms.8,9 The diagnosis is usually based on indirect tests (lactose tolerance test or breath hydrogen test) whose specificity has been reported to range from 77% to 96% and sensitivity from 69% to 100%.10 There is evidence that the breath hydrogen test may be an indicator of bacterial overgrowth rather than lactose malabsorption.11

2. Definition of ethnic origin was based on self definition and spoken language. As the authors themselves clarified, African populations have complex demographic histories. Many of the analysed groups were very small, and hence chance may have played a role. In contrast with the findings of Mulcare,4 our genotyping data in nomadic pastoralists, Fulani-Sudanese were in agreement with the previously published figures of lactase persistence in this population.

3. There was no statistics shown against the C/T –13910 variant, only speculation presented in Mulcare’s paper.12

When conducting phenotype-genotype correlation studies in lactase persistence/non-persistence, detailed clinical studies are essential. The studies are difficult as it is unethical to take an intestinal biopsy from a healthy subject that would give the most reliable diagnosis. Measurement of lactase activity from hospitalised patients with a clinical indication for intestinal biopsy may reflect a disease in the gut and the result obtained may not correlate with the genotype. These uncertainties should be taken into account when interpreting the genotyping results in adult-type hypolactasia.

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Conflict of interest: None declared.

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Conflict of interest: None declared.

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IL-1 gene cluster and TNFA−307 polymorphisms in the risk of perforated duodenal ulcer
Helicobacter pylori virulence markers have been associated with duodenal ulcer (DU) but there are few studies evaluating host factors such as cytokine polymorphisms and, to the best of our knowledge, no study has evaluated these polymorphisms as risk factors for perforated DU. We investigated associations among interleukin 1 (IL-1) cluster and tumour necrosis factor α (TNFA)−307 polymorphisms, and DU and perforated DU in a non-Caucasian population. We included 223 patients with DU, 29 patients with perforated DU, and 541 blood donors. H pylori status was investigated by culture, preformed urease test, stained smear, polymerase chain reaction (PCR), and the 13C-urea breath test. cagA status was assessed by PCR. In the blood donors, H pylori status and cagA status were determined by serology, IL-1B−511T and IL-1B−512C polymorphisms were genotyped by PCR, PCR/restriction fragment length polymorphism (RFLP) typing two pair primers.1 Data were analysed in logistic models. The loci did not deviate significantly from the expected Hardy-Weinberg distribution in the control group. IL-1B−511T and IL-1B−512C polymorphisms were in almost complete linkage disequilibrium in all three groups (p<10−6). We thus restricted further analyses to IL-1B−31. No polymorphism remained associated with non-complicated DU predicting for age and sex, but the IL-1RN2 carrier showed a trend towards increasing DU risk (p=0.06; odds ratio (OR) 1.43 (95% confidence interval (CI) 0.99−2.05)). Regarding perforated DU, in the multivariate analyses, IL-1B−31C and TNFA−307A alleles remained inversely associated with the disease, even after inclusion of confounding factors (table 1). cagA status was the strongest factor associated with either uncomplicated (p=0.00; OR 4.29 (95% CI 2.63−6.98)) or perforated DU. The other polymorphisms were not associated with perforated DU (table 1).

Although morbidity from peptic DU has greatly decreased since early studies on H pylori infection,2 little change was observed

Reference

H pylori infection,2 little change was observed
regarding perforated DU, as measured by surgical interventions in emergency services. Knowing who, among all *H pylori* infected subjects, will develop a perforated DU is therefore an important issue in treatment.

Garcia-Gonzales and colleagues and Zambon and colleagues, evaluating Spanish and Italian populations, respectively, did not find associations between single polymorphisms and DU. Conversely, Furuta and colleagues found that *IL-1RN* allele 2 and *IL-1B* 511T or *IL-1B* 307A polymorphisms were associated with atrophy and increased gastric carcinoma risk in Caucasian populations were found to be inversely associated with perforated DU. The mechanism by which overproduction of *TNFα* in non-complicated DU. However, *IL-1B* 511T was protective factors for DU in a Japanese population. In this investigation, in accordance with previous studies, no role could be established for *IL-1B* or *TNFα* in non-complicated DU. However, *IL-1B* 31C and *TNFα*—307A carriage was negatively associated with perforated DU. Thus the same *IL-1B* and *TNFα* polymorphisms which were associated with atrophy and increased gastric carcinoma risk in Caucasian populations were found to be inversely associated with perforated DU.

Table 1

<table>
<thead>
<tr>
<th>Genotype</th>
<th>IL-1B polymorphism (alleles)</th>
<th>IL-1RN polymorphism (alleles)</th>
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<tr>
<td>1/1</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>1/2</td>
<td>C carrier 0.04</td>
<td>A carrier 0.03</td>
</tr>
<tr>
<td>2/2</td>
<td>C carrier 0.60</td>
<td>A carrier 0.03</td>
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</table>

<table>
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<tr>
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<th>H pylori positive subjects</th>
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<tbody>
<tr>
<td>Genotype</td>
<td>Uni</td>
</tr>
<tr>
<td>cagA positive status</td>
<td></td>
</tr>
<tr>
<td>1/1</td>
<td>0.00</td>
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<tr>
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<td>Ref</td>
</tr>
<tr>
<td>2/2</td>
<td>0.05</td>
</tr>
</tbody>
</table>

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doi: 10.1136/gut.2005.077362

Conflict of interest: None declared.

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Cryptic gluten intolerance in type 1 diabetes: identifying suitable candidates for a gluten free diet

Long term exposure to gluten in coeliaics, and coeliac disease – which after 16 years of age may induce type 1 diabetes (T1D) and other autoimmune disorders. Increased prevalence of CD among diabetes and their relatives is well documented. Early introduction of gluten to children at high risk for T1D produces T1D associated islet autoantibodies. Similarly, in the absence of overt clinical symptoms of T1D, some coeliac children produce diabetes autoantibodies in a gluten dependent manner. Non-classic intestinal challenge with gluten induces mucosal recruitment of lymphocytes, similar to that in CD patients. In diabetes, however, there is no production of CD related anti-tissue transglutaminase antibodies (anti-tTG).

We have used a phage display assay to show that in CD patients, production of anti-tTG is limited to the intestine. Here, we monitored the effects of a gluten free diet (GFD) on anti-tTG antibody synthesis in the intestinal mucosa of a diabetic adult and a boy at high risk of diabetes, both carrying HLA DQ2/DQ8, but lacking serum anti-tTG. Intestinal specimens from both subjects and samples of peripheral blood lymphocytes were used to make phage-antibody libraries to look for lymphocytes synthesising anti-tTG antibodies.

Patient No 1 was a 35 year old man who had T1D for 20 years. During 1998–2001, serum anti-tTG responses were negative, and clinical control of T1D was good (mean glycosylated haemoglobin 6.8% (range 8.1–7.3%). The patient developed a perforated peptic ulcer and underwent surgical interventions in emergency services.

The boy at high risk of diabetes, both carrying HLA DQ2/DQ8, but lacking serum anti-tTG. Intestinal specimens from both subjects and samples of peripheral blood lymphocytes were used to make phage-antibody libraries to look for lymphocytes synthesising anti-tTG antibodies.
Table 1  Clones isolated from intestinal biopsies and number of anti-
transglutaminase positive clones before and after 12 months of a gluten free diet
in the adult diabetic, in the at risk child, and in the controls

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (y)</th>
<th>HLA</th>
<th>ICA</th>
<th>% of tTG positive clones</th>
<th>Different antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>35</td>
<td>DQ2/8</td>
<td>–</td>
<td>50</td>
<td>3</td>
</tr>
<tr>
<td>Adult during GFD</td>
<td>2</td>
<td>DQ2/8</td>
<td>+</td>
<td>50.3</td>
<td>5</td>
</tr>
<tr>
<td>Child</td>
<td>10</td>
<td>DQ2/8</td>
<td>–</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Child During GFD</td>
<td>45</td>
<td>DQ2</td>
<td>–</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

GFD; gluten free diet; ICA, islet-cell antibodies; anti-tTG, anti-transglutaminase antibodies; Different, number of different clones determined by sequencing.

References

Acquired factor V inhibitor associated with valproic acid use in a cirrhotic patient

Acquisition of factor V inhibitor is a rare event. The inhibitor most frequently encountered in clinical practice is directed against factor VIII. In a review of the literature, Streiff and Nesser found 126 published cases of factor V inhibitor. The inhibitor emerged after major surgery, hae-
motasic therapy with bovine thrombin, malignancies, autoimmune disorders, blood transfusion, antibiotic therapy, or for unknown reasons. We report the emergence of factor V inhibitor in a cirrhotic patient receiving valproic acid for seizure control. At a 50 year old man treated for alcoholic cirrhosis was admitted for epistaxis. He had no history of autoimmune disorders or blood transfusion. For three years he had been taking valproic acid 1 g/day orally for seizures, and propranolol 60 mg/day. On admission, prothrombin level was 5% of control, factor V was 1%, and factor II was 49%. Two months previously prothrombin level had been 83% of control on two occasions one month apart. Physical examination showed compensated cirrhosis. Epistaxis was linked to valproic acid and warfarin was stopped. Anti-factor V antibody was measured by a functional assay using factor V-depleted plasma and patient plasma. Antibody titre was determined with the Bethesda method using human leukocyte factor V as antigen. Factor V inhibitor titre was 1.0 Bethesda units. Protein immunoelectrophoresis was normal, and tests for antinuclear, anti-DNA, anti-2-glycerophosphate 1, anticardiolipin, anti-
thromboplastin, antithromboplastin, and antithromboplastin antibodies were negative. Aspartate aminotransferase was normal, albuminaemia 35.2 g/l, and 8-fetoprotein 9.6 g/ml. Abdominal sonography and colonoscopy were normal. Gastrointestinal endoscopy showed grade 1 oesophageal varices. Eighty minutes monitored, the patient was asymptomatic and epistaxis had not recurred. Factor V was 41% and weak factor V inhibitor activity persisted (0.6 Bethesda units). Hypocoagulability due to factor V inhibitor is rare and can be difficult to diagnose in a patient with cirrhosis. Eighty seven of the 126 cases described by Streiff and Nesser occurred during the last decade, and two thirds of cases followed bovine thrombin exposure. Anti-bovine factor V and antihuman factor V antibodies can interact, potentially inactivating human factor V in vivo. Other noteworthy causes are blood transfusion, cancer, treatment with betalactam antibiotics or streptomy
cin, major surgery (usually in patients having received transfusions or betalactam agents), and autoimmune disorders (coeliac disease, bullous pemphigoid, Sjögren’s syn-
drome, Hashimoto thyroiditis) associated with congenital factor V deficiency. No cause was found in nearly 20% of cases. To date, no cases have been linked to dental extraction or other minor surgeries. Anti-factor V antibo-
dies can appear at all ages. Most reported cases occurred after age 65 years. The inhibitor was discovered fortuitously in nearly 40% of cases following an isolated increase in prothrombin time. Bleeding was the main presenting sign in 60% of cases, and was life threatening in 22%.

To our knowledge, this is the first reported case of factor V inhibitor associated with valproic acid therapy. It is noteworthy that valproic acid inhibits fatty acid beta oxidation, potentially leading to life threatening microvesicular steatosis. However, our patient had no clinical or biological signs of hepatitis. Rare cases of cutaneous vasculitis or lupus-like syndrome have been linked to valproic acid or its prodrug valpromide. Factor V inhibitors have occasionally been detected in patients with such syndromes, but our patient had no clinical or biological signs of an autoimmune process. Factor V inhibitor appeared after three years of treatment with valproic acid, and prothrombin...
level improved partially after drug withdrawal. In previously reported cases, the inhibitor disappeared in 88% of patients overall, after a mean of 10 weeks. In patients with no identified cause, the inhibitor only disappeared in 62% of cases after a mean of 23 weeks, although this did not affect outcome. Bleeding is difficult to treat in patients with factor V inhibitor. Various approaches have been tried, such as infusion of fresh frozen plasma or, better, platelet concentrates. Plasmapheresis has been used to lower antibody titre and high dose immunoglobulin to neutralise the antibodies. Steroids and immunosuppressants (azathioprine, cyclophosphamide), alone or in combination, have been used for long term inhibition of factor V inhibitor synthesis. However, the results are difficult to interpret as the studies were small and included patients with heterogeneous manifestations. There is no consensus treatment.

In conclusion, the onset of hypoacu- gability linked to a decline in factor V level in a cirrhotic patient should not be systematically attributed to hepatocellular insufficiency; in the absence of marked cytolysis, the presence of an acquired factor V inhibitor and a possible drug related cause should be sought.

References

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References


Acylated ghrelin stimulates food intake in the fed and fasted states but desacylated ghrelin has no effect

We were interested to read the article of Asakawa et al (Gut 2005;54:18–24) which reported that intracerebroventricular and peripheral administration of desacylated ghrelin inhibited food intake in mice in the fasted state. Acylated ghrelin (AG) has a unique biological structure with an acyl side chain on the third amino acid residue. AG is an endogenous ligand for the growth hormone secretagogue receptor (GHS-R1a) and stimulates feeding and growth hormone release. In contrast, desacylated ghrelin (DAG), which does not have the acyl side chain, has no affinity for the GHS-R1a. As the authors suggest, their results might indicate the presence of an alternative receptor through which desacylated ghrelin acts.

We were interested in investigating whether DAG would modulate feeding. We injected saline, 0.3 nmol/kg AG, and 0.3 nmol/kg DAG into C57Bl6 male mice intraperitoneally on two occasions, firstly in the fed state and secondly following a 20 hour fast, and measured food intake at 1, 2, 4, and 24 hours post injection (fig 1). In the fasting experiment, we also injected 0.03 nmol/g PYY3–36 as a positive control. All animal procedures were approved by the British Home Office Animals (Scientific Procedures) Act 1986 (project license No 70/5281). Results were analysed using a one way repeated measures ANOVA. As previously reported, AG stimulated feeding in the fed state. However, DAG had no significant effect on food intake in the fed state. In the fasting study, PYY3–36 significantly inhibited feeding. AG stimulated cumulative food intake in fasted mice for up to six hours post injection although the percentage increase compared with saline was less than in the fed state (per cent increase two hours following ghrelin injection: fed state 32%, fasted state 30%). In contrast with the findings of Asakawa et al, DAG had no effect on food intake at any time point examined. We used a higher dose of DAG than that administered by Asakawa et al (approximately 2.5 nmol s−1 g−1 mouse) and therefore the absence of a feeding effect associated with DAG is unlikely to be explained by differences in dosing.

In conclusion, we have observed that acylated ghrelin stimulated food intake in the fasting as well as in the fed state. In contrast with the findings of Asakawa et al, there was no alteration in feeding in either the fed or fasting state following desacylated ghrelin. Our results suggest that circulating acylated ghrelin stimulates feeding independently of desacylated ghrelin.

Acknowledgements

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Figure 1 Cumulative two hour food intake under (A) fed and (B) fasting states following intraperitoneal saline, 0.3 nmol/g acylated ghrelin (AG), 0.3 nmol/g desacylated ghrelin (DAG), and 0.3 nmol/g PYY3–36 (PYY). **p<0.05 versus saline and DAG; *p<0.005 versus saline.

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Conflict of interest: None declared.

Future use of the Glasgow alcoholic hepatitis score

We read with interest the findings of Forrest and colleagues (Gut 2005;54:1174–9) regarding their prognostic algorithm for alcoholic hepatitis, the Glasgow alcoholic hepatitis score (GAHS). The study uses robust clinical end points to develop an algorithm which has diagnostic advantages over the modified discriminant function score (DFS). We would like to discuss some of the future implications of this important study.

The overall death rate in the study was 23% at 28 days and the death rate of patients with a DFS >32 was 29% at 28 days in the derivation population. The latter figure is lower than the placebo arms of many of the randomised controlled trials of alcoholic hepatitis that range between 35% and 50%. This difference compared with the published literature may be attributable to case definition. It is possible that there were a fewer number of patients in the derivation cohort for GAHS with true alcoholic hepatitis. Some of the previous studies of alcoholic hepatitis have required liver biopsy evidence of alcoholic hepatitis as part of the case definition. This was not the case for entry into the derivation cohort for the GAHS study and the case definition was based solely on clinical and biochemical evidence of liver dysfunction in patients with heavy alcohol consumption. In the validation population there was biopsy evidence of alcoholic hepatitis in only 33%.

While this may invalidate the GAHS as a means of identifying cases of alcoholic hepatitis, it does not invalidate its use in identifying patients at risk of death when admitted to hospital with liver dysfunction on a background of heavy alcohol use. This makes it far more pragmatic than tests based on biopsies as many hospitals do not have access to specialised services to perform transjugular liver biopsies in the acute setting. Furthermore, there are published
randomised controlled trials which have not required histological evidence of alcoholic hepatitis before allocating treatment. The controversy to this is that although alcoholic hepatitis often presents with clinical features of fever, leucocytosis, and hyperbilirubinaemia, there remains a differential diagnosis which may require a biopsy to resolve.

It is important to differentiate between true alcoholic hepatitis and severe liver dysfunction in patients with heavy alcohol consumption because it will influence the choice of intervention. Randomised controlled trials that use GAHS to identify patients with alcoholic hepatitis might be greatly underpowered if the therapy (for example, steroids) is effective in alcoholic hepatitis but ineffective or harmful in other clinical conditions where abnormal clinical parameters might be associated with heavy alcohol consumption. Selection of risk stratification models should be determined by the severity of the adverse effects of the therapy under trial. Those with more severe adverse effects will warrant models with high specificity whereas drugs with minimal side effects will benefit from a model with a high sensitivity. Compared with the DFS, the GAHS has an increased specificity, decreased sensitivity, and improved accuracy, making it suited to the selection of subjects in studies using more toxic therapies.

The utility of the GAHS will depend on the effect of its use in the care of patients. We suggest that the next step in the evaluation of GAHS should be a clinical trial to see if patients randomised to risk stratification with GAHS followed by appropriate interventions have a better outcome than those managed conventionally.

We believe this is an excellent study using robust clinical end points. It is a practical model which can be used easily at the bedside to give valuable prognostic information. Success of future therapeutic trials in alcoholic hepatitis will not only depend on the efficacy of the drug but also the appropriate selection of patients by models and their respective cut off points.

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IN Guha received grant support from Pfizer.
W M Rosenberg is a consultant for Schering-Plough, Roche, Gilead, Bayer, and Pfizer. He is the Chief Scientific Officer for HepCGen.

Conflict of interest: None declared.

References

A proof of concept study establishing Necator americanus in Crohn’s patients and reservoir donors
The emergence of autoimmunity, including Crohn’s disease (CD) where the immune relationship with commensal bacteria is corrupted, has been linked to hygiene. A gradual decline in endoparasites is but one argument that might explain this phenomenon. Weinstock and colleagues have successfully tested the pig whipworm, Trichuris suis, in patients with inflammatory bowel disease (IBD). However, repeated inoculation was required and concern has been raised that aberrant migration could occur. The haematophagous hookworm, Necator americanus (NA), is proposed as an alternative. We have tested if CD patients tolerate hookworm infection, and the practical issues associated with establishing reservoir donors (RDs).

Over 700 million people remain infected with hookworms. Infective larvae (L3i) are acquired through skin contact with contaminated soil. Auto-infection, direct person to person infection, aberrant migration, and hypobiosis do not occur. Adult worms live in the host small intestine for an average of five years. Infection can be easily terminated with an anthelmintic. Anaemia is the only disease of consequence but is an unusual outcome in properly nourished individuals. Using L3i originally obtained from Madang, Papua New Guinea, but maintained in a healthy researcher in the UK, five CD subjects with longstanding in Crohn’s disease activity index (CDAI) score for each CD patient versus score at week 20 and at week 45 for the first five inoculated cases (mean 165 (95% confidence interval 145) v 64 (25), p = 0.132; mean 165 v 75 (29), p = 0.246).
Approximately 75% of gastric mucosa associated lymphoid tissue (MALT) lymphomas can be cured by Helicobacter pylori eradication. It would be very useful to identify, at the time of diagnosis, the 25% of cases of gastric MALT lymphoma that will not respond to H pylori eradication. In general, lymphomas at stage IIE or above do not respond to H pylori eradication. However, the prognostic value of staging in stage I E cases is very limited, although tumours that involve the muscularis propria or serosa (stage IIE) show a higher failure rate than those restricted to the mucosa and submucosa (stage I E).^4^ Paradoxically, the majority of gastric MALT lymphomas at diagnosis are at stage I E but 20% of these cases will not respond to H pylori eradication.

In a previous study, we have examined the value of t(11;18)(q21;q21) in prediction of the response of gastric MALT lymphoma to H pylori eradication. Among the 111 cases of gastric MALT lymphoma studied, t(11;18)(q21;q21) was present in 42/63 (67%) non-responsive cases and the two translocation (6.94 (1.72)), well above that in those without eradication (2.37 (1.72)). The level of IGH-BCL10 mRNA. Based on the same series of cases, we examined the value of t(11;18)(p22;q21) in prediction of the response of gastric MALT lymphoma to H pylori eradication. Of the 111 cases examined, 75 including 35 from the complete regression group and 40 from the non-responsive group, had adequate tissue specimens for evaluation of BCL10 staining. Two cases showed strong BCL10 nuclear staining in virtually all tumour cells (fig 1), similar to that seen in t(1;14)(p22;q32) positive cases,^2^ while the remaining cases displayed either weak cytoplasmic or weak nuclear staining. Both cases with strong BCL10 nuclear staining were from the H pylori eradication non-responsive group; one case (case No 1) had stage II E disease and showed no response 12 months after H pylori eradication while the other (case No 2) had stage I E disease and showed no response eight months after H pylori eradication. As shown in our previous study, both cases were t(11;18)(q21;q21) negative. To ascertain whether the two cases that showed strong BCL10 nuclear staining were positive for t(1;14)(p22;q32) or variant, interphase fluorescence in situ hybridisation (FISH) with BCL10 break-apart dual colour probes, IGH break-apart probes, IxG break-apart probes, and BCL10/IGH dual colour dual fusion translocation probes were performed. Both cases failed to show evidence of BCL10 gene break or amplification. Case No 2 showed an IGH break, but FISH with BCL10/IGH dual colour dual fusion translocation probes failed to show evidence of BCL10/IGH translocation. To further investigate these cases, we performed real time quantitative reverse transcription-polymerase chain reaction of BCL10 mRNA. Unfortunately, adequate tissue materials were available only in case No 2. The level (ACI = 3.4) of BCL10 mRNA expression in this case was compatible with that in MALT lymphoma with t(1;14)(p22;q32) (mean 1.60 (SD 2.37)), well above that in those without the translocation (6.94 (1.72)).

To further assess the impact of t(1;14)(p22;q32) on the clinical behaviour of MALT lymphoma, we retrospectively reviewed the clinical presentation of 11 cases, including six from the stomach with known BCL10 involved translocation (table 1). Of these cases, nine including all those from the stomach, were at stage II or above. Although clinical presentation and follow up data were not available in each case, three cases (Nos 1, 2 and 7) presented unusual wide dissemination.

### Table 1

<table>
<thead>
<tr>
<th>ID, age (y), sex</th>
<th>Initial inoculation trial</th>
<th>Re inoculation trial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time (weeks)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0–4</td>
<td>5–8</td>
</tr>
<tr>
<td>CD 1 55 M</td>
<td>Inoculum therapy</td>
<td>CDAI 95</td>
</tr>
<tr>
<td></td>
<td>CDAI</td>
<td></td>
</tr>
<tr>
<td>CD 2 46 M</td>
<td>Inoculum therapy</td>
<td>CDAI 96</td>
</tr>
<tr>
<td></td>
<td>CDAI</td>
<td></td>
</tr>
<tr>
<td>CD 3 41 F</td>
<td>Inoculum therapy</td>
<td>CDAI 93</td>
</tr>
<tr>
<td></td>
<td>CDAI</td>
<td></td>
</tr>
<tr>
<td>CD 4 54 M</td>
<td>Inoculum therapy</td>
<td>CDAI 62</td>
</tr>
<tr>
<td></td>
<td>CDAI</td>
<td></td>
</tr>
<tr>
<td>CD 5 21 F</td>
<td>Inoculum therapy</td>
<td>CDAI 62</td>
</tr>
<tr>
<td></td>
<td>CDAI</td>
<td></td>
</tr>
<tr>
<td>CD 6 33 F</td>
<td>Inoculum therapy</td>
<td>CDAI 62</td>
</tr>
<tr>
<td></td>
<td>CDAI</td>
<td></td>
</tr>
<tr>
<td>CD 7 33 M</td>
<td>Inoculum therapy</td>
<td>CDAI 62</td>
</tr>
<tr>
<td></td>
<td>CDAI</td>
<td></td>
</tr>
<tr>
<td>CD 8 46 M</td>
<td>Inoculum therapy</td>
<td>CDAI 62</td>
</tr>
<tr>
<td></td>
<td>CDAI</td>
<td></td>
</tr>
<tr>
<td>CD 9 44 F</td>
<td>Inoculum therapy</td>
<td>CDAI 62</td>
</tr>
</tbody>
</table>

L3i, n 3rd stage N americanus larvae inoculated percutaneously; P, prednisone n mg/day; A, azathioprine n mg/day; M, methotrexate n mg/week.
including pleural effusion, and blood and bone marrow involvement (Table 1).

Taken together, our results suggest that gastric MALT lymphomas with strong BCL10 nuclear expression or t(1;14)(p22;q32) are mostly likely resistant to *H pylori* eradication.

**Acknowledgements**

This study was supported by research grants from Leukaemia Research Fund, UK and Deutsche Krebshilfe. The authors thank J Audouin, L Bedenne, O Bouche, Marie-Christine Copin, Y Bouhnik, J Fournet, A De Mascarel, Ph Moreau, J Lafon, A Parientie, and F Piard of the Groupe d’Etude des Lymphomes Digestifs (GELD), France; T. Thomas and P. Zinzani of Università degli Studi di Bologna, Italy; and M Stolte of Institut fur Pathologie, Klinikum Bayreuth, Germany, for contribution of part of the specimens used for this study.

**Figure 1** BCL10 immunohistochemistry. Both cases 1 and 2 show strong BCL10 nuclear staining in virtually all tumour cells, similar to that seen in tumour cells with t(1;14)(p22;q32).

**Table 1** Clinical feature of mucosa associated lymphoid tissue (MALT) lymphoma with t(1;14)(p22;q32) or variants

<table>
<thead>
<tr>
<th>Case No</th>
<th>Age</th>
<th>Sex</th>
<th>Primary site</th>
<th>Genetic investigations</th>
<th>BCL10 involved chromosome translocation</th>
<th>BCL10 IHC</th>
<th>Staging*</th>
<th>Dissemination</th>
<th>Clinical follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>81</td>
<td>M</td>
<td>Stomach</td>
<td>Karotyping, interphase FISH</td>
<td>t(1;14)(p22;q32)</td>
<td>Strong nuclear staining</td>
<td>IV</td>
<td>Perigastric lymph nodes, omentum, spleen, pleural effusion, blood</td>
<td>n/a</td>
</tr>
<tr>
<td>2</td>
<td>48</td>
<td>F</td>
<td>Stomach</td>
<td>Karotyping, interphase FISH</td>
<td>t(1;14)(p22;q32)</td>
<td>n/a</td>
<td>IV</td>
<td>Perigastric and splanchnic lymph nodes, pleural involvement, bone marrow involvement</td>
<td>n/a</td>
</tr>
<tr>
<td>3</td>
<td>67</td>
<td>M</td>
<td>Stomach</td>
<td>Karotyping, interphase FISH</td>
<td>t(1;22)(p11;q11)</td>
<td>Strong nuclear staining</td>
<td>IIIE</td>
<td>Perigastric lymph nodes and spleen</td>
<td>n/a</td>
</tr>
<tr>
<td>4</td>
<td>73</td>
<td>F</td>
<td>Stomach</td>
<td>Interphase FISH</td>
<td>Yes</td>
<td>Strong nuclear staining</td>
<td>IIIE</td>
<td>Perigastric and mesenteric lymph nodes</td>
<td>n/a</td>
</tr>
<tr>
<td>5</td>
<td>67</td>
<td>M</td>
<td>Stomach</td>
<td>Interphase FISH</td>
<td>Yes</td>
<td>Strong nuclear staining</td>
<td>IV</td>
<td>Lung</td>
<td>n/a</td>
</tr>
<tr>
<td>6</td>
<td>n/a</td>
<td>n/a</td>
<td>Stomach</td>
<td>Karotyping, interphase FISH</td>
<td>t(1;14)(p22;q12)</td>
<td>Strong nuclear staining</td>
<td>IIIE</td>
<td>Chemotherapy with chlorambucil, complete remission in three year follow up</td>
<td>n/a</td>
</tr>
<tr>
<td>7</td>
<td>63</td>
<td>F</td>
<td>Stomach</td>
<td>Karotyping, interphase FISH</td>
<td>t(1;14)(p22;q32)</td>
<td>Strong nuclear staining</td>
<td>IIIE</td>
<td>Low dose chlorambucil chemotherapy, complete remission at 1.5 year follow up</td>
<td>n/a</td>
</tr>
<tr>
<td>8</td>
<td>83</td>
<td>F</td>
<td>Lung</td>
<td>Karotyping, interphase FISH</td>
<td>t(1;14)(p22;q32)</td>
<td>Strong nuclear staining</td>
<td>IIE</td>
<td>Blood, bilateral pulmonary involvement, pleural and ascitic effusions, retroperitoneal lymph node</td>
<td>n/a</td>
</tr>
<tr>
<td>9</td>
<td>57</td>
<td>F</td>
<td>Lung</td>
<td>Interphase FISH</td>
<td>No evidence</td>
<td>Strong BCL10 nuclear staining</td>
<td>IIE</td>
<td>No clinical evidence</td>
<td>Data on treatment not available, but patients alive without evidence of disease for 16 years</td>
</tr>
<tr>
<td>10</td>
<td>32</td>
<td>F</td>
<td>Breast</td>
<td>Karotyping, interphase FISH</td>
<td>t(1;14)(p22;q12)</td>
<td>n/a</td>
<td>IIE</td>
<td>Anillary lymph nodes</td>
<td>6 cycles of CHOP chemotherapy followed by surgery</td>
</tr>
<tr>
<td>11</td>
<td>75</td>
<td>F</td>
<td>Breast</td>
<td>Interphase FISH</td>
<td>IGH-BCL10 fusion</td>
<td>Strong BCL10 nuclear staining</td>
<td>IIE</td>
<td>No clinical evidence</td>
<td>Complete remission in two year follow up</td>
</tr>
</tbody>
</table>

*Ann Arbor-Musshoff staging system for extranodal lymphoma; the clinical stage was likely to have been underestimated as appropriate staging was unlikely to be carried out in each of these archival cases.*

IHC, immunohistochemistry; FISH, fluorescence in situ hybridisation; n/a, not available.
Conflict of interest: None declared.

References

Interferon-β plus ribavirin for patients with hepatitis C virus genotype 1: a randomised pilot trial

The rate of sustained eradication of hepatitis C virus (HCV) in response to a combination of interferon-α and ribavirin remains unsatisfactory in patients with genotype 1 infection.1 No effective alternative treatment is currently available for non-responders. Interferon-β is also a type 1 interferon commonly used to treat chronic HCV infection in Japan. A previous study showed that a 24 week course of therapy with interferon-β plus ribavirin resulted in sustained loss of HCV in three of nine patients with chronic hepatitis C.2 However, the efficacy and safety of interferon-β combined with ribavirin has yet to be fully evaluated.

We report the results of a randomised pilot trial comparing interferon-β plus ribavirin with interferon-α plus ribavirin in patients with HCV genotype 1 who poorly responded to interferon-α plus ribavirin. A total of 28 patients with HCV genotype 1 were given 6 MU of recombinant interferon-2b (Schering-Plough, Kenilworth, New Jersey, USA) by intramuscular injection daily for four weeks. Twenty seven patients (16 men and 11 women; mean age 47 ± 8 years) in whom HCV RNA was detected in serum on polymerase chain reaction at week 2 were included in this study and randomly assigned to receive one of two regimens from week 5. Fifteen patients continued to receive 6 MU interferon-2b intramuscularly, given daily from week 5 to week 8, and three times weekly from week 9 to week 24 (interferon-α group). The other 12 patients were assigned to 6 MU natural interferon-β (Toray Industries Inc., Tokyo, Japan), given by intravenous injection daily from week 5 to week 8, and three times weekly from week 9 to week 24 (interferon-β group). Ribavirin (Schering-Plough) was concurrently administered at a daily dose of 600 to 900 mg to patients who weighed 60 kg or less and 800 mg to those who weighed more than 60 kg. At the time of this study, a 24 week course of interferon-α plus ribavirin was commonly used in Japan. The data were analysed according to intention to treat.

Baseline characteristics of the patients in the treatment groups were similar. At week 4 of therapy, when treatment was randomly assigned, the proportion of patients without detectable HCV RNA in serum did not differ between the interferon-α group and interferon-β group (table 1). The proportion of patients with HCV RNA in serum was higher in the interferon-β group than in the interferon-α group at week 12, but did not differ between the groups at the end of treatment (week 24). However, 24 weeks later (week 48), the proportion of patients with a sustained virological response was significantly higher in the interferon-β group than in the interferon-α group. During treatment, neutralising antibodies to interferon were detected in two patients in the interferon-α group and no patients in the interferon-β group (table 1). The proportion of patients with HCV RNA in serum was higher in the interferon-β group than in the interferon-α group at week 12, but did not differ between the groups at the end of treatment (week 24).

Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Interferon-α group (n = 12)</th>
<th>Interferon-β group (n = 15)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 4</td>
<td>4 (27%)</td>
<td>3 (25%)</td>
<td>0.92</td>
</tr>
<tr>
<td>Week 12</td>
<td>7 (47%)</td>
<td>10 (83%)</td>
<td>0.049</td>
</tr>
<tr>
<td>Week 24 (end of therapy)</td>
<td>10 (67%)</td>
<td>9 (75%)</td>
<td>0.64</td>
</tr>
<tr>
<td>Week 48</td>
<td>0 (0%)</td>
<td>3 (25%)</td>
<td>0.040</td>
</tr>
</tbody>
</table>

The data were analysed according to intention to treat.

We enrolled patients who did not have a favourable early response to treatment with interferon-α and ribavirin. Antibodies to interferon, which sometimes develop in

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patients given recombinant interferon-α, can cause resistance to therapy. Both interferon-α and -β bind to a common type I interferon receptor but utilise different regions of the receptor subunits for specific signalling pathways, potentially leading to distinct biological responses. An oligonucleotide array study has shown that some interferon stimulated genes are preferentially induced by interferon-β, but not by interferon-α. We thus believe that interferon-β might be beneficial for some patients who are resistant to interferon-α. A large randomised trial of peginterferon-α plus ribavirin versus interferon-β plus ribavirin for 48 weeks is being conducted in patients with HCV genotype 1 who do not have a virological response to 12 weeks of treatment with peginterferon-α and ribavirin.

In summary, a combination of interferon-β and ribavirin produced a significantly better sustained virological response than a combination of interferon-α and ribavirin in patients with HCV genotype 1 who were resistant to interferon-α plus ribavirin. Although the overall safety profiles of the two regimens were similar, the rates of treatment discontinuation and of reduction in the dose of ribavirin were lower in patients receiving interferon-β and ribavirin than in those receiving interferon-α and ribavirin.

Acknowledgement
Grant support was received from Ministry of Health, Labour, and Welfare, Japan.

References

Editor’s Quiz: GI Snapshot

Answer
From explorative laparotomy, the pancreatic tumour involving the head and proximal body of the pancreas was judged to be resectable. Pylorus preserving proximal pancreateoduodenectomy was performed. Histology of the tumour was consistent with a diagnosis of renal cell cancer (RCC) metastasis to the pancreas (fig 2). Metastases were not detected in peripancreatic lymph nodes. The patient did not receive any further adjuvant therapy and was discharged from hospital without any serious perioperative morbidity.

The vast majority of pancreatic carcinomas are primary, and among these, more than 90% are of ductal origin. Solitary pancreatic masses can be classified as secondary tumours to the pancreas in only 2% of all cases. In the latter group, RCC seems to be the most common cancer. Within the last three years, 43 new cases of RCC metastases to the pancreas have been reported (Medline review). Median interval from nephrectomy to diagnosis of pancreatic metastases is 83 months, but time intervals as long as 10–20 years were also reported. Complete resection of pancreatic metastases from RCC are associated with long term survival, particularly in cases of single tumours and/or a long disease free interval.

References

Figure 2. Histomorphological appearance of the pancreatic tumour (haematoxylin-eosin, ×40). From the lower left to the upper right corner, normal pancreatic glandular tissue, desmoplastic capsule, and clear cell carcinoma are visible.

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