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, while lactase non-persistence or “adult-type hypolactasia” is more frequent in most other populations. 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 phenotype and it is this change that is proposed as a DNA test for both Europeans and Africans. We agree that presence of a T 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. 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 European groups.

I particularly draw readers’ attention to our recent study. 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 ±25%. We found the T allele in Cameroonian 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.

D M Swallow

Correspondence to: Professor D M Swallow, The Galton Laboratory, Biology Department, University College London, Woburn House, 4 Stephenson Way, London NW1 2HE, UK, dmswallow@hgp.mrc.ac.uk

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 us and others 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. 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. 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 76% to 94%. In children in particular, the lactose tolerance test has not been ideal due to the high rate (up to 30%) of false positive results. The specificity of the breath hydrogen test varies between 89% and 100% and sensitivity from 69% to 100%. There is evidence that the breath hydrogen test may be an indicator of bacterial overgrowth rather than lactose malabsorption.

(2) Definition of ethnic origin was based on self definition and spoken language. As the groups 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, 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.

When conducting phenotype-genotype correlation studies in lactase persistence/non-persistence, detailed clinical studies are essential. The studies are difficult as it is difficult 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.

K-L Kolho

Hospital for Children and Adolescents, University of Helsinki, Helsinki, Finland

I Järvellä

Department of Medical Genetics, University of Helsinki and Laboratory of Molecular Genetics, Helsinki University Central Hospital, Helsinki, Finland

Correspondence to: Dr K-L Kolho, Hospital for Children and Adolescents, Box 281, FIN-00029, Finland; kaapo.leena.kolho@helsinki.fi

Conflict of interest: None declared.

References


Author’s reply
I write as the statistician on the paper by Mulcare and colleagues, which was criticised by Kolho and Jarvella above. I wish to correct two assertions made by Kolho and Jarvela. The first is their claim that with our statistical procedure “there is a risk of wrong conclusions being drawn” because “adult-type hypocalcitasia is difficult to assess due to inaccurate diagnostic tests.” This would indeed be true had we applied a “naïve” test (for example, a \( \chi^2 \) test) in which we had assumed the diagnoses of hypocalcitasia to be without error. In fact, not only did we assume that diagnoses occurred with error, but we did not even presume to know exactly what that level of error was. Instead, our uncertainty about the true level of error was modelled in a Bayesian framework and the uncertainty about the true level of error was indeed be true had we applied a “naïve” test (for example, a \( \chi^2 \) test) in which we had assumed the diagnoses of hypocalcitasia to be without error. In fact, not only did we assume that diagnoses occurred with error, but we did not even presume to know exactly what that level of error was. Instead, our uncertainty about the true level of error was modelled in a Bayesian framework and the uncertainty about the true level of error was

abdominal symptoms leading to dehydration, in combination with repetitive bathing behaviour (Gut 2004;53:1566–70). They have concluded that these symptoms are due to cannabis use.

Cannabis has been consumed for many centuries and is currently used by millions of people in many countries. It is hard to believe that a distinctive syndrome caused by cannabi

ny has never been reported before by users or clinicians.

The authors assert that cannabis laws are particularly liberal in South Australia. Four Australian jurisdictions now have a cannabis expiation notice system which South Australia first introduced in 1986. The other four Australian jurisdictions have variations on a bond system. Several European countries have far more lenient legislative arrangements. After over a generation of liberalisation of cannabis laws in many countries around the world, there is little evidence of a subsequent increase in cannabis use.

In a comparative study using the same methodology, the prevalence of cannabis use in more “liberal” Amsterdam was lower than in the more “punitive” San Francisco.

The title of the paper, “Cannabinoid hyperemesis” is unduly presumptive. Some of these cases appeared to improve with abstinence and then relapsed when patients were “rechallenged” with cannabis, but neither the patients nor the authors appear to have been blinded in the rechallenge. The proposed biological explanation is weak.

We suggest that alternative explanations need to be sought for these cases. This syndrome should not be accepted as being caused by cannabis without additional reports and other evidence.

A Byrne, R Hallinan
Byrne Surgery, Sydney, Australia

A Wojdak
St Vincent’s Hospital, Darlinghurst, New South Wales, Australia

Correspondence to: Dr A Byrne, Byrne Surgery, 75 Redfern St, Redfern, 2016, Sydney, Australia; ajbymere@ozemail.com.au

Dr Wojdak is President of the Australian Drug Law Reform Foundation which supports the taxation and regulation of cannabis.

Reference

Authors’ reply
We would like to thank Byrne et al for their interest in our paper (Gut 2004;53:1566–70). They should be noted that we undertook an observational study by necessity. Cannabis is an illegal drug and double blind control trials with illicit substances are prohibited and unethical. The assertion that cannabis has been “consumed for many centuries” needs to be tempered with the fact that cannabis has been grossly under-researched clinically and, as we have shown with this syndrome, nowhere near fully understood in its neuropharmacology or paradoxical actions. Since the publication of our article, other authors have published similar findings to ours and drawn the same conclusions.1

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 \( \alpha \) (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 \(^{13}C\)-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—307C polymorphisms were in almost complete linkage disequilibrium in all three groups (p<0.05). We thus restricted further analyses to IL-1B—31. No polymorphism remained associated with non-complicated DU, but the IL-1RN2 allele was associated with risk of perforated DU. Among interleukin 1 (IL-1) cluster and tumour necrosis factor \( \alpha \) (TNFA)—307 polymorphisms were genotyped by PCR, PCR/restriction fragment length polymorphism (RFLP), or PCR/ confronting two pair primers. 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—307C polymorphisms were in almost complete linkage disequilibrium in all three groups (p<0.05). We thus restricted further analyses to IL-1B—31. No polymorphism remained associated with non-complicated DU, but the IL-1RN2 allele was associated with risk of perforated DU. Among intron 2, carriage of the IL-1RN2 allele was associated with increased risk of perforated DU (OR 2.13; 95% CI 1.04–4.37; p=0.04).

Conflict of interest: None declared.

References

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Conflict of interest: None declared.

References
Table 1 Univariate and multivariate analysis of the cytokine loci between patients with perforated duodenal ulcer (n = 29) and all blood donors (n = 539), and between patients with perforated duodenal ulcer (n = 29) and Helicobacter pylori positive blood donors (n = 369)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>IL-1B</th>
<th>IL-1RN</th>
<th>TNFA</th>
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<tr>
<td></td>
<td>Allelic</td>
<td>Allelic</td>
<td>Allelic</td>
</tr>
<tr>
<td></td>
<td>Status</td>
<td>Status</td>
<td>Status</td>
</tr>
<tr>
<td></td>
<td>p Value</td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>casA/g positive status</td>
<td>1/1</td>
<td>ref</td>
<td></td>
</tr>
<tr>
<td>IL-1B 31</td>
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<td>1.00</td>
<td></td>
</tr>
<tr>
<td>IL-1RN 31C</td>
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<td>0.02-0.90</td>
<td></td>
</tr>
<tr>
<td>TNFA 307</td>
<td>2/2</td>
<td>ref</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.00</td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>

Uni, univariate; OR, odds ratio; 95% CI, 95% Cornfield confidence intervals; Ref, reference (wild) genotype; IL, interleukin; TNF, tumour necrosis factor.

Conflicts of interest: None declared.

References


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

Long term exposure to gluten in coeliacs, and coeliac disease (CD) for those under 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 auto-antibodies. Similarly, in the absence of overt clinical symptoms of T1D, some coeliac children produce diabetes auto-antibodies in a gluten dependent manner with a delayed intestinal challenge with gluten produces mucosal recruitment of lymphocytes, similar to that in CD patients. In diabetics, 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 8.1–10.2%)). Following the patient developed treated diabetic retinopathy and microalbuminuria, with an average albumin excretion rate (AER) of 230 μg/min, despite treatment with angiotensin converting enzyme inhibitors. In 2001, ‘burning’ epigastric pain appeared with abdominal distension. Duodenal biopsy and number of intraepithelial lymphocytes were normal.

Patient No 2 was a two year old boy at risk of CD and T1D (diabetic father and coeliac brother) who tested positive for two out of six HLA T1DM specific genotypes (DR1*0301, DQ6*0201 and DRB1*0401, DQ6*0101, DQ6*0102 and DQ6*0103). Tests for anti-tTG serum antibodies were negative while anti-islet cell antibodies (ICA) became positive at 20 months. Informed of the potential risks, the child’s parents consented to intestinal biopsy to detect possible silent CD. Duodenal biopsy and number of intraepithelial lymphocytes were normal.

In both subjects, positive tTG antibody clones (table 1) were isolated only from the intestinal lymphocyte libraries. Two control subjects aged 10 and 45 years, suffering from Helicobacter pylori gastritis and with no family history of CD or T1D, tested negative for intestinal anti-tTG clones. The diabetic adult and the child’s parents agreed to a GFD for 12 months, after which laboratory tests and biopsy were repeated. In the adult, control of diabetes was unchanged but AER was markedly improved (20 μg/min). The boy tested negative to ICA. In both subjects, biopsies were normal, and analysis of new phage antibody libraries showed complete elimination of anti-tTG clones in the adult and 90% reduction in both positivity and diversity in the child (table 1). Both patients remain on a...
GFD; AER is still normal in the diabetic adult and the child is still negative for ICA.

In the context of genetic predisposition to gluten intolerance, in line with Maki’s data on the gradual development of CD in diabetics, we found a gluten dependent immunological response, apparently only in the intestine. In the boy, reduced response to tTG and elimination of ICA after GFD may have been due to very early intervention, indicating temporary protection from the disease. In the diabetic adult, reduction of anti-tTG, in line with Maki’s data and the child is still negative for ICA.

GFD; AER is still normal in the diabetic adult and confirm the benefits of a GFD.

Similar larger scale studies are needed to prove that gluten is harmful in these subjects. DQ2/8 may develop intestinal anti-tTG antibodies in these subjects, and confirm the benefits of a GFD.

Subject Age (y) HLA ICA % of tTG positive clones Different antibodies

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (y)</th>
<th>HLA 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>
</tr>
<tr>
<td>Adult during GFD</td>
<td>2</td>
<td>DQ2/8</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td>Child</td>
<td>2</td>
<td>DQ2/8</td>
<td>+</td>
<td>50%</td>
</tr>
<tr>
<td>Child During GFD</td>
<td>10</td>
<td>DQ2/8</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td>Control subject</td>
<td>45</td>
<td>DQ2/8</td>
<td>–</td>
<td>0</td>
</tr>
</tbody>
</table>

GFD, gluten free diet; ICA, anti-tTG 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 Ness found 126 published cases of factor V inhibitor. The inhibitor emerged after major surgery, haemostatic 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. 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 walsalvea and anticoagulant therapy only with meshing followed by local cauterisation with silver nitrate. Laboratory tests did not show seen clotting factor consumption (platelet count: 68 x 10^9/l, D-dimers <500 ng/ml, fibrinogen 3 g/l). Valproic acid was replaced by phenobarbital while propranolol was maintained.

Three months after prothrombin level was 33%, factor V 14%, factor II 72%, factor VII 70%, factor IX 92%, and factor X 65% of control. Factor VIII, XI, and XII levels were normal, as was thrombin time. He was screened for factor V inhibitor by measuring residual factor V after one hour of incubation at 37°C of equal parts of pooled normal plasma and patient plasma. Antibody titre was determined with the Bethesda method used to titre factor VIII inhibitor. Factor V inhibitor titre was 1.0 Bethesda units. Protein immunoelectrophoresis was normal, and tests for antinuclear, antinative DNA, anti-beta2-glycoprotein 1, anticardiolipin, antithyoperoxidase, antithrombocodrin, and antismooth muscle antibodies were negative. No positive aminotransferases were normal. Hypercoagulability 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 Ness’ occurred during the last decade, and two thirds of cases followed bovine thrombin exposure. Antibovine 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 streptomycin, major surgery (usually in patients having received transfusions or betalactam agents), and autoimmune disorders (coeliac disease, bullous pemphigoid, Sjogren’s syndrome, 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 antibodies can appear at all ages but most are detected in cases occurring 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 hepatic failure. Rare cases of cutaneous or lupus-like syndrome have been linked to valproic acid or its produg valpromide. Factor V inhibitors have occasionally been detected in patients with such syndromes, but our patients 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 hypocoagulability 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 cytology, the presence an acquired factor V inhibitor and a possible drug related cause should be sought.

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, 6, and 24 hours post injection (fig 1). In the fasting experiment, we also injected 0.03 nmol/kg 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 found in previous studies of alcoholic hepatitis have required liver biopsy evidence of alcoholic hepatitis as part of the case definition. Some of the previous studies of alcoholic hepatitis have required liver biopsy evidence of alcoholic hepatitis that range between 35% and 50%.

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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M N Neary, M R Druise, J C Small, S R Bloom
Department of Metabolic Medicine, Hammersmith Hospital, Imperial College London, London, UK

Conflict of interest: None declared.

References


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. The 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 corollary 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.

I N Guha, W M Rosenberg
University of Southampton, Liver Unit, Southampton, UK
Correspondence to: Dr I N Guha, Mail point 811, Level D, Southampton General Hospital, Trenoma Rd, Southampton S016 6YD, UK; guhaneil@hotmail.com

IN Guha received grant support from Pfizer.
W M Rosenberg is a consultant for Schering-Plough, Roche, Gildead, 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, Trichurus 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 but mostly inactive disease and three RDs each received a carefully measured inoculum (table 1). Subsequently, four additional CD subjects with chronic and mostly active disease were inoculated with L3i cultured from faeces provided by an RD, and the original CD cohort were reinoculated from week 27 to week 30. Ethics approval was granted by the Townsville Health Service District Institutional Ethics Committee. Haematological and clinical measurements are expressed as mean (95% confidence interval).

The inoculation caused a mild itch within five minutes that dissipated after a few days in eight CD subjects and a pruritic rash that lasted two weeks in the RDs, who also developed a painful transient enteropathy. Neither respiratory symptoms nor detectable aberrant migration occurred. In the CD cohort, blood eosinophilia developed from week 5 (mean 2.60 × 10^9/l (1.89) v week 1 0.18 × 10^9/l (0.10) v week 20 0.39 (0.20)). Patent infection had established by week 20 in all cases. CD activity index (CDAI) remained unchanged until week 17, possibly in part due to a hookworm related enteropathy recognisable because of blood eosinophilia and faceal Charcot-Leydon crystals. By week 20, the CDAI questionnaire was improved (mean 151 (14) v 179 (20)) and the four week cumulated CDAI scores was decreased (mean 141 (31) v 87 (15)). Haemoglobin fell marginally (week 1 mean 135.6 (7.8) g/l v week 20 129.3 (4.1) g/l). Reinoculation of the five CD subjects first exposed caused no apparent adverse effect. Disease reactivation, as defined by a CDAI
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 IE or above do not respond to H pylori eradication.4 However, the prognostic value of staging in stage IE cases is very limited, although tumours that involve the muscularis propria or serosa (stage IIE) showed a higher failure rate than those restricted to the mucosa and submucosa (stage IE1).3 Paradoxically, the majority of gastric MALT lymphomas at diagnosis are at stage IE 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, including 26/43 (60%) at stage IE.9 In contrast, translocation was detected in only 2/40 responsive cases and the two translocation positive cases showed a temporary response to H pylori eradication.9 Based on the same series of cases, we examined the value of t(1;14)(p22;q32)/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).9 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 IIE 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.

### References


### Table 1

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</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.

### Strong BCL10 nuclear expression identifies gastric MALT lymphomas that do not respond to H pylori eradication

Approximately 75% of gastric mucosa associated lymphoid tissue (MALT) lymphomas

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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**

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

<table>
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<tr>
<th>Case No</th>
<th>Age</th>
<th>Sex</th>
<th>Primary site</th>
<th>Genetic investigations</th>
<th>BCL10 nuclear staining</th>
<th>IHC</th>
<th>Dissemination</th>
<th>Clinical follow up</th>
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<td>71</td>
<td>M</td>
<td>Stomach</td>
<td>Karotyping, interphase FISH, molecular cloning</td>
<td>(1;14)(p22;q32)</td>
<td>Strong nuclear staining</td>
<td>IV E</td>
<td>Perigastric lymph nodes, omentum, spleen, pleural effusion, blood and bone marrow involvement</td>
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<td>2</td>
<td>48</td>
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<td>Karotyping, interphase FISH</td>
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<td>n/a</td>
<td>IVE</td>
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* 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
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6 Ye H, Gong L, Liu H, et al. MALT lymphoma with t(1;14)(q32;q31)/IGH-MALT1 is characterized by strong cytoplasmic MALT1 and BCL10 expression. J Pathol 2005;205:293–301.

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 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 in 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). 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 in no patients in the interferon-β group. Leucocyte, neutrophil, and platelet counts and haemoglobin concentrations were similar in two groups. Therapy was discontinued because of serious adverse events (including depression) in three patients in the interferon-α group. All 12 patients in the interferon-β group completed 24 weeks of treatment. The dose of ribavirin was reduced because of anaemia in eight patients in the interferon-α group and in four in the interferon-β group. 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

| Table 1 Proportions of patients without detectable hepatitis C virus RNA in serum |
|---------------------------------|-------------------|-------------------|-------------------|
|                                | Interferon-α group | Interferon-β group |
|                                | (n = 15)           | (n = 12)          | p Value (2 test)  |
| Week 4                         | 4 (27%)           | 3 (25%)          | 0.92 |
| Week 12                        | 7 (47%)           | 10 (83%)         | 0.049 |
| Week 24 (end of therapy)       | 10 (67%)          | 9 (75%)          | 0.64 |
| Week 48                        | 0 (0%)            | 3 (25%)          | 0.040 |

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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,3 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.4 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 response5 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.

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M Enomoto, A Tamori, N Kawada, H Jomura
Department of Hepatology, Osaka City University Medical School, Osaka, Japan

S Nishiguchi
Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya, Japan

T Saibara, S Onishi
Department of Gastroenterology and Hepatology, Kochi Medical School, Kochi, Japan

S Mochida, K Fujimura
Division of Gastroenterology and Hepatology, Internal Medicine, Saitama Medical School, Saitama, Japan

Correspondence to: Dr S Nishiguchi, Division of Hepatobiliary and Pancreatic Diseases, Department of Internal Medicine, Hyogo College of Medicine, 1-1 Mukogawa, Nishinomiya, Hyogo 663-8501, Japan; nishiguc@hyo-med.ac.jp
doi: 10.1136/gut.2005.081935
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Cryptic gluten intolerance in type 1 diabetes: identifying suitable candidates for a gluten free diet

D Sblattero, A Ventura, A Tommasini, L Cattin, S Martelossi, F Florian, R Marzari, A Bradbury and T Not

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