PostScript

LETTERS

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.[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 phenotype[2] 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,[3] and there is evidence that this nucleotide resides in a functional element.[4 5] 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.[6 7]

I particularly draw readers' attention to our recent study.[6] 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 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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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[1–3] and others[4 5] 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.[6] Their doubt is based on several assumptions that make it difficult to evaluate the significance of the findings. These assumptions can be listed as following:

(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.[7–10] 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.[11] The specificity of the breath hydrogen test varies between 89% and 100% and sensitivity from 69% to 100%.[12] There is evidence that the breath hydrogen test may be an indicator of bacterial overgrowth rather than lactose malabsorption.[13]

(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,[6] 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.[6]

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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Author's reply

I write as the statistician on the paper by Mulcare and colleagues,[1] which was criticised by Kolho and Järvelä above. I wish to correct two assertions made by Kolho and Järvelä. The first is their claim that with our statistical procedure "there is a risk of wrong conclusions being drawn" because "adult-type hypolactasia is difficult to assess due to inaccurate diagnostic tests". This would indeed be true had we applied a "naïve" test (for example, a χ^2 test) in which we had assumed the diagnoses of hypolactasia 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 trained using available published data on comparisons of "true" diagnoses (for example, based on biopsy results) and "indirect" diagnoses (for example, based on breath hydrogen). To accomplish this, a novel statistical method was developed, which was described by Mulcare and colleagues.[1] The fact that we incorporated these additional sources of error into our method means that the p values we obtained were not as low as they would have been had we applied a naïve test such as a χ^2 test. Our remarkable finding was that, despite this, we still found significant departures in multiple sub-Saharan African populations. This led us to reject the null hypothesis that the presence of the C/T−13910 variant alone, even with diagnostic error, could explain the published data on lactase persistence in Africa. Other reasons must be sought to explain our results, one of which is the possibility that additional genetic variants influence lactase persistence.

The second assertion by Kolho and Järvelä was that there were "no statistics shown against the C/T−13910 variant, only speculation presented". The meaning is unclear here. Certainly, statistics both in the sense of "data" and in the sense of "inference" were presented in our paper (see above). Our conclusions regarding the C/T−13910 variant were derived from carefully constructed statistical inference, and not mere "speculation".

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"Cannabis hyperemesis" causation questioned

The authors describe a number of cases of a bizarre syndrome of severe vomiting, 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 cannabis has never been noted 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.[1]

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.

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Authors' reply

We would like to thank Byrne *et al* for their interest in our paper (*Gut* 2004;**53**:1566–70). It 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 publication of our article, other authors have published similar findings to ours and drawn the same conclusions.[1]

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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 ^{13}C-urea breath test. *cag*A status was assessed by PCR. In the blood donors, *H pylori* status and *cag*A status were determined by serology. *IL-1B*−511/−31, *IL-1RN*, and *TNFA*−307 polymorphisms were genotyped by PCR, PCR/restriction fragment length polymorphism, or PCR/confronting 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*−31C polymorphic alleles 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 after correcting for age and sex, but the *IL-1RN*2 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 analysis, *IL-1B*−31C and *TNFA*−307A alleles remained inversely associated with the disease, even after inclusion of confounding factors (table 1). *cag*A status remained 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

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)

		All subjects			*H pylori* positive subjects		
		Uni	Multivariate		Uni	Multivariate	
Genotype		p Value	OR	95% CI	p Value	OR	95% CI
*cag*A positive status	1/1				0.00	24.72	3.29–185.66
IL-1B−31	T/T	Ref			Ref.		
	C carrier	0.04	0.42	0.20–0.90	0.05	0.33	0.15–0.73
IL-1RN	1/1	Ref			Ref.		
	2 carrier	0.60			0.70		
TNFA−307	G/G	Ref			Ref.		
	A carrier	0.03	0.21	0.05–0.88	0.03	0.21	0.05–0.96

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

regarding perforated DU, as measured by surgical interventions in emergency services.[3] Knowing who, among all *H pylori* infected subjects, will develop a perforated DU is therefore an important issue in treatment.

Garcia-Gonzales and colleagues[4] and Zambon and colleagues,[5] evaluating Spanish and Italian populations, respectively, did not find associations between single *IL-1* polymorphisms and DU. Conversely, Furuta and colleagues[6] found that *IL-1RN* allele 2 and *IL-1B*−511T/T were protective factors for DU in a Japanese population.

In this investigation, in accordance with previous studies,[4 5] no role could be established for *IL-1B*−511T or *IL-1B*−31C alleles in non-complicated DU. However, *IL-1B*−31C and *TNFA*−307A carriage was negatively associated with perforated DU. Thus the same *IL-1B* and *TNFA* polymorphisms which were associated with atrophy and increased gastric carcinoma risk in Caucasian populations[7–9] were found to be inversely associated with perforated DU.

The mechanism by which overproduction of IL-1β and TNF-α due to *IL-1B*−31 and *TNFA*−307 polymorphisms protects from DU perforation may not differ from that associated with gastric carcinoma. The prevailing mechanism is probably inhibition of gastric acid production. Consequently, bacteria spread to the corpus where they accentuate the inflammation, lowing acid production, with the net effect of diminishing the risk of DU perforation.

Even though our results are biologically plausible, several factors may contribute to geographical specificities, as already seen in studies on other gastrointestinal diseases.[7–10] Also, we have previously demonstrated[1] in our population that the distribution of the inflammatory alleles at *IL-1* loci is intermediate between Asians and Caucasians.[1]

In conclusion, one of the questions that motivated the studies associating host cytokine polymorphisms with *H pylori* associated diseases was the possibility of explaining why some infected individuals develop gastric carcinoma, others peptic ulcer, and the majority remain otherwise without complications. These polymorphisms may play a role in the genesis of *H pylori* associated diseases but are probably insufficient to completely answer this question. Our study demonstrated independent inverse associations between *IL-1B*−31C and *TNFA*−307A polymorphic alleles and perforated DU, but no association with non-complicated DU.

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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 coeliacs,[1] and coeliac disease (CD) diagnosis after 16 years of age[2] may induce type 1 diabetes (T1D) and other autoimmune disorders. Increased prevalence of CD among diabetics and their relatives is well documented.[3] Early introduction of gluten to children at high risk for T1D produces T1D associated islet autoantibodies.[4] Similarly, in the absence of overt clinical symptoms of T1D, some coeliac children produce diabetes autoantibodies in a gluten dependent manner.[5] In diabetics, intestinal challenge with gluten produces mucosal recruitment of lymphocytes,[6] similar to that in CD patients.[7] In diabetics, however, there is no production of CD related anti-tissue transglutaminase antibodies (anti-tTG).[6]

We have used a phage display assay[8] 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[8] 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 8.1–6.1) but the patient developed surgically 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 of six HLA T1DM specific genotypes (DR1 *0301, DQA1*0501, DQB1*0201 and DRB1*0401, DQA1*0301, DQB1*0302). 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

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

Subject	Age (y)	HLA	ICA	% of tTG positive clones	Different antibodies
Adult	35	DQ2/8	−	50	3
Adult during GFD			−	0	0
Child	2	DQ2/8	+	50.3	10
Child During GFD			−	5.4	5
Control subject	10	DQ2/8	−	0	0
Control subject	45	DQ2	−	0	0

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

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,[9] 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 microalbuminuria may have indicated that while a GFD is of little benefit to the pancreas, improvements can still be obtained in other organs.

In conclusion, at risk subjects with HLA DQ2/8 may develop intestinal anti-tTG antibodies on extended exposure to gluten. Similar larger scale studies are needed to prove that gluten is harmful in these subjects and confirm the benefits of a GFD.

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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 recent review of the literature, Streiff and Ness[1] 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 telangiectasia and was controlled by meshing followed by local cauterisation with silver nitrate. Laboratory tests showed no signs of clotting factor consumption (platelet count 68×10^9/l, D-dimers <500 ng/ml, fibrinogen 3 g/l). Valproic acid was replaced by phenobarbital while propranolol was maintained.

Three months later 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℃ 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, anti-thyroperoxidase, antimitochondria, and anti-smooth muscle antibodies were negative. Aspartate aminotransferase was 73 IU/l (normal <40 IU/l), albuminaemia 35.2 g/l, and α fetoprotein 9.6 ng/ml. Abdominal sonography and colonoscopy were normal. Gastroduodenal endoscopy showed grade 1 oesophageal varices. Eighteen months after admission, 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 Ness[1] 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, Sjögren's syndrome, Hashimoto thyroiditis) associated with congenital factor V deficiency. No cause was found in nearly 20% of cases.[2] 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 reported cases occurred after age 65 years.[2] 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%.[2]

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.[3] 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,[4] 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.[2] In patients with no identified cause, the inhibitor only disappeared in 62% of cases after a mean of 23 weeks,[2] 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.[2] 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 series 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 cytolysis, the presence an acquired factor V inhibitor and a possible drug related cause should be sought.

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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)[1] 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.[1] As the authors suggest, their results might indicate the presence of an alternative receptor through which desacylated ghrelin acts.

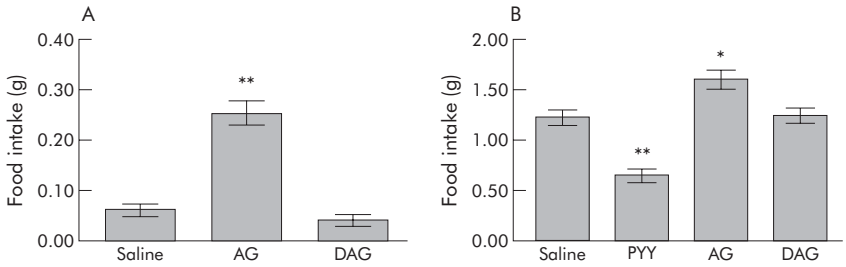

Figure 1 Cumulative two hour food intake under (A) fed and (B) fasting states following intraperitoneal saline, 0.3 nmol/g acylated ghrelin (AG), 0.3 nmol/g desacylated ghrelin (DAG), and 0.03 nmol/g $PYY_{3–36}$ (PYY). *$p<0.05$ versus saline and DAG; **$p<0.005$ versus saline.

We were interested in investigating whether DAG would modulate feeding. We injected saline, 0.3 nmol/g AG, and 0.3 nmol/g 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/g $PYY_{3–36}$ 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,[2] AG stimulated feeding in the fed state. However, DAG had no significant effect on food intake in the fed state. In the fasting study, $PYY_{3–36}$ 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 320%, 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 7.5 nmol *v* 3 nmol per 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.

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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 that 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%.[1–3] 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.[1 4] 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.[5]

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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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.[1 2] A gradual decline in endoparasites is but one argument that might explain this phenomenon.[3] Weinstock and colleagues have successfully tested the pig whipworm, *Trichuris suis*, in patients with inflammatory bowel disease (IBD).[4 5] However, repeated inoculation was required and concern has been raised that aberrant migration could occur.[6] 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.[7] Auto-reinfection, 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 anthelminthic. 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 disappeared 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 0.59 (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 faecal Charcot-Leydon crystals.[8] After 20 weeks, the IBD questionnaire was improved (mean 151 (14) *v* 179 (20)) and the four week cumulated CDAI scores was decreased (mean 141 (31) *v* 87 (15)).[9] 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 >150, occurred in two (CD4, CD5; table 1) after the doses of long term immune suppressive drugs had been reduced. The subject (CD3–7) driven trend was to reduce immune suppression as health improved, a strategy often associated with worsening of symptoms. The five CD subjects first inoculated were in remission at week 45 (fig 1).

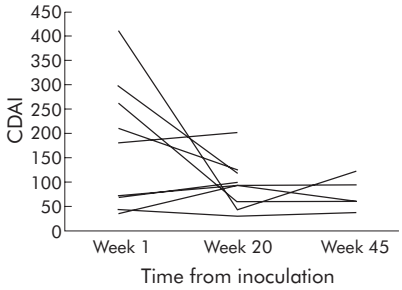

Figure 1 Initial Crohn's disease activity index (CDAI) score for each CD patient versus score at week 20 and at week 45 for the first five inoculated cases (mean 165 (95% confidence interval 145) *v* 64 (25), p = 0.132; mean 165 *v* 75 (29), p = 0.246).

Our pilot study has established a potential for NA, already a fact of life for many millions, as a candidate parasite to inoculate those with autoimmune disease. The natural advantages are lifecycle and migration predictability, ability to control the size of and eliminate a colony, and the parasite's longevity. Inoculation proved safe, even in immune suppressed patients. Our hope that NA would suppress autoreactivity sufficiently to allow immune suppressive therapy to be stopped was unrealistic. Recent and compelling evidence has shown that IBD is self sustaining.[10] It may be that after remission is achieved, endoparasites will offer an alternative or adjunct to immune suppressive therapy, a priority for some people with CD.

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Table 1 Crohn's disease activity index (CDAI) in CD subjects inoculated with infective larvae (L3i). Subsequently, the five CD subjects first inoculated were reinoculated from week 27 to week 30 and four CD subjects with chronic and mostly active disease were inoculated with larvae sourced from one of the authors

	Initial inoculation trial					Reinoculation trial				
	Time (weeks)					Time (weeks)				
ID, age (y), sex	0–4	5–8	9–12	13–16	17–20	27	30	35	39–41	45
CD1 55 M										
Inoculum therapy	25 L3i					25 L3i				
CDAI	79	60	89	77	68		96		93	62
CD2 46 M										
Inoculum therapy	25 L3i					25 L3i				
CDAI	38	114	20	68	48		30			36
CD3 41 F										
Inoculum therapy	25 L3i P5 M15	P5 M15	P5 M7.5			25 L3i P5 M10	P5 M10	P5 M10	M10	M10
CDAI	46	71	85	83	90		36	30	92	95
CD4 34 M										
Inoculum therapy	50 L3i P38 M30	P38 M30	P50 M30	P25 M30	P5 M30	50 L3i P5 M20	P2.5 M20	M20	P25 M30	P20 M30
CDAI	260	230	232	264	118		60	442	60	122
CD5 21 F										
Inoculum therapy	50 L3i P10 M20	P10 M20	P13 M20	P10	P7.5	P5	25 L3i P5	P25 M20	P15 M20	P10 M20
CDAI	144	151	103	79	73		410	180	44	61
CD6 33 F										
Inoculum therapy	50 L3i P15 M20	P10 M20	P5 M20	P5 M20	P7.5 M20					
CDAI	49	32	6	29	100					
CD7 33 M										
Inoculum therapy	50 L3i A150	P25 A150	P5 A150	P5 A150	P8 A150					
CDAI	260	114	96	125	118					
CD8 46 M										
Inoculum therapy	50 L3i M20	M20	M20	M20	M20					
CDAI	145	159	171	152	186					
CD9 44 F										
Inoculum therapy	100 L3i P5 M20	P10 M20	P5 M20	P5 M20	P10 M20					
CDAI	173	127	106	76	125					

L3i, n 3rd stage *N americanus* larvae inoculated percutaneously; P, prednisone n mg/day; A, azathioprine n mg/day; M, methotrexate n mg/week.

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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 can be cured by *Helicobacter pylori* eradication.[1] 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 II_E or above do not respond to *H pylori* eradication.[2–4] However, the prognostic value of staging in stage I_E cases is very limited, although tumours that involve the muscularis propria or serosa (stage I_{E2}) show a higher failure rate than those restricted to the mucosa and submucosa (stage I_{E1}).[2–4] 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, including 26/43 (60%) at stage I_E.[5] In contrast, translocation was detected in only 2/48 responsive cases and the two translocation positive cases showed a temporary response to *H pylori* eradication.[5] Based on the same series of cases, we examined the value of t(1;14)(p22;q32)/*IGH-BCL10* in prediction of the response of gastric MALT lymphomas 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 cells,[6] 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.[5]

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, *IGκ* break-apart probes, and *BCL10/IGH* dual colour dual fusion translocation probes were performed.[6 7] 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 ($\Delta Ct = 3.4$) 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)).[6]

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_E 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,

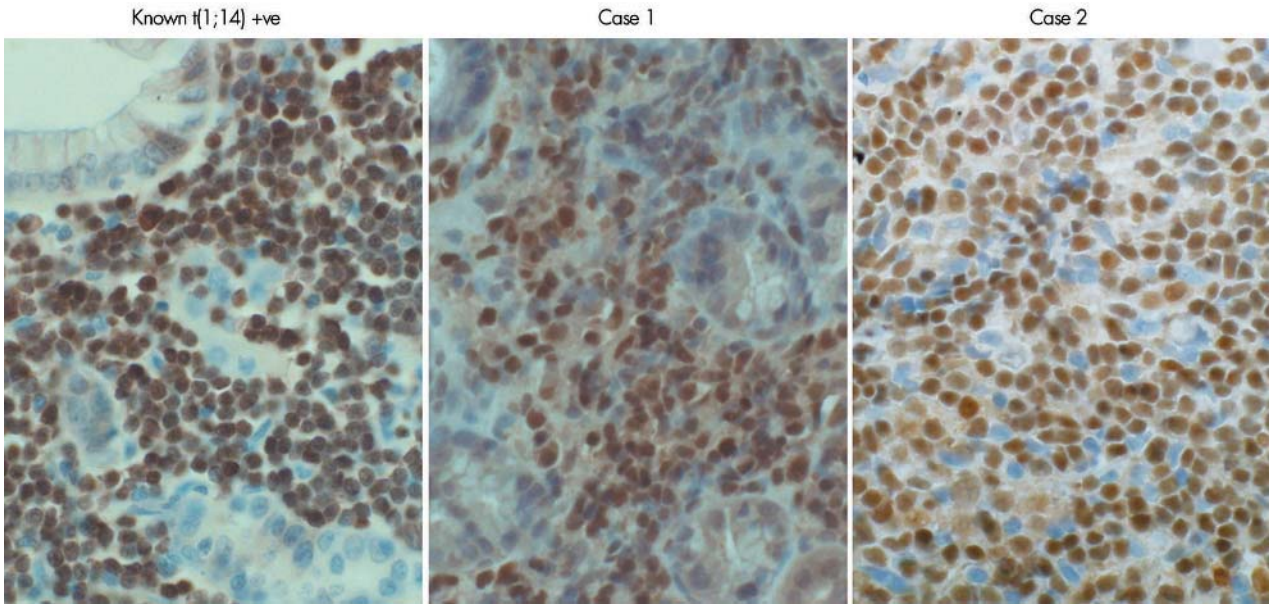

Figure 1 BCL10 immunohistochemistry. Both cases 1 and 2 show strong BCL10 nuclear staining in virtually all tumour cells, similar to that seen in tumour cells with t(1;14)(p22;q32).

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.

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

Case No	Age	Sex	Primary site	Genetic investigations	BCL10 involved chromosomal translocation	BCL10 IHC	Staging*	Dissemination	Clinical follow up
1[8]	71	M	Stomach	Karotyping, interphase FISH, molecular cloning	t(1;14)(p22;q32)	Strong nuclear staining	IV_E	Perigastric lymph nodes, omentum, spleen, pleural effusion, blood	n/a
2	48	F	Stomach	Karotyping, interphase FISH	t(1;14)(p22;q32)	n/a	IV_E	Perigastric and splenic hilus lymph nodes, pleural involvement, bone marrow involvement?	4 cycles of MCP chemotherapy, partial remission, alive at two year follow up
3	67	M	Stomach	Karotyping, interphase FISH	t(1;2)(p22;q12)	Strong nuclear staining	III_E	Perigastric lymph nodes and spleen	Chemotherapy with chlorambucil, complete remission in three year follow up
4	73	F	Stomach	Interphase FISH	Yes	Strong nuclear staining	III_E	Perigastric and mesenteric lymph nodes,	Low dose chlorambucil chemotherapy, complete remission at 1.5 year follow up
5	49	M	Stomach	Interphase FISH	Yes	Strong nuclear staining	IV_E	Lung	n/a
6	n/a	n/a	Stomach	Karotyping, interphase FISH	t(1;14)(p22;q32)	Strong nuclear staining	II_E	n/a	n/a
7[9]	63	F	Lung	Karotyping, interphase FISH	t(1;14)(p22;q32)	n/a	IV_E	Blood, bilateral pulmonary involvement, pleural and ascitic effusions, retroperitoneal lymph node	8 year low grade B cell lymphoma, then presented an aggressive clinical course presenting with lymphocytosis, pleural and ascitic effusions, partially response to chemotherapy, died of disease
8[9]	49	F	Lung	Karotyping, interphase FISH	t(1;14)(p22;q32)	n/a	II_E	n/a	n/a
9	57	F	Lung	Interphase FISH	IGH-BCL10 fusion	Strong BCL10 nuclear staining	I_E	No clinical evidence	Data on treatment not available, but patients alive without evidence of disease for 16 years
10	32	F	Breast	Karotyping, interphase FISH	t(1;14)(p22;q32)	n/a	II_E	Axillary lymph nodes	6 cycles of CHOP therapy followed by surgery, complete remission in two year follow up
11	75	F	Breast	Interphase FISH	IGH-BCL10 fusion	Strong BCL10 nuclear staining	I_E	No clinical evidence	n/a

*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.

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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 I 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-α2b (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-α2b 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 groups (table 1). The proportion of patients without 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 (n = 15)	Interferon-β group (n = 12)	p Value (χ^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

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 response[5] 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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EDITOR'S QUIZ: GI SNAPSHOT

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Answer

From question on page 122

At explorative laparotomy, the pancreatic tumour involving the head and proximal body of the pancreas was judged to be resectable. Pylorus preserving proximal pancreaticoduodenectomy 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.[1] 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.[2] 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.[3]

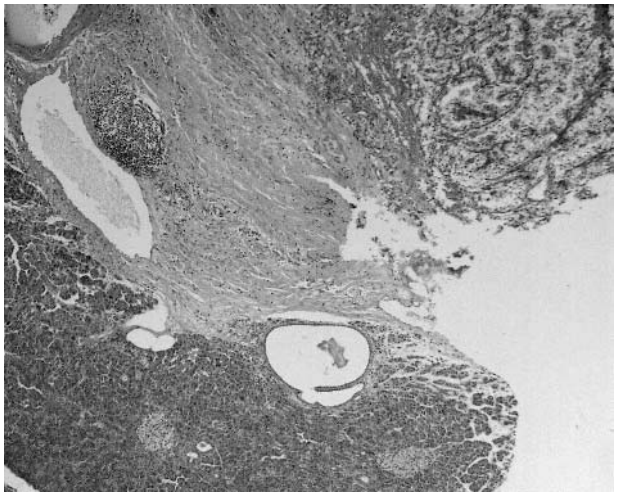

Figure 2 Histomorphological appearance of the pancreatic tumour (haematoxylin-eosin, ×40). From the lower left to the upper right corner, normal pancreatic glandular tissue, desmoplastic capsule, and clear cell carcinoma are visible.

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