ITPA genotyping test does not improve detection of Crohn’s disease patients at risk of azathioprine/6-mercaptopurine induced myelosuppression

The thiopurine drugs azathioprine (AZA) and 6-mercaptopurine (6-MP) are effective for the treatment of inflammatory bowel disease (IBD) and their prescription is increasing. Haematotoxicity, which can lead to potentially life threatening bone marrow suppression, represents the most serious side effect of thiopurine therapy. It has been attributed to the accumulation of active cytotoxic metabolites of AZA/6-MP, collectively called 6-thioguanine nucleotides, resulting from a deficiency in thiopurine catabolism specifically catalysed by the thiopurine S-methyltransferase (TPMT) enzyme. Genotyping tests are now available to identify deficient and intermediate methylators who are, respectively, homozygous and heterozygous for non-functional alleles of the TPMT gene. As pointed out by Lennard in the leading article, homozygous and heterozygous for intermediate methylators who are, respectively, homozygous and heterozygous for non-functional alleles of the TPMT gene, represents the most serious side effect of thiopurine therapy. Due to the retrospective nature of the study, no correlation was observed with occurrence of neutropenia but only 11 patients were studied. We previously reported TPMT genotype analysis in 41 Crohn’s disease (CD) patients who had experienced leukopenia during AZA/6-MP therapy.² Even though this study confirmed the efficiency of TPMT genotyping in identifying patients at risk of developing myelosuppression, it also highlighted its limitations, as only 27% of patients carried mutant alleles of the TPMT gene that were associated with enzyme deficiency. This prompted us to investigate the occurrence of ITPA mutations in this series of patients in order to evaluate whether genotyping of the ITPase gene could improve the detection rate of patients at risk of thiopurine myelotoxicity.

Our population comprising 41 patients with CD has been described in detail previously.³ Briefly, all patients had either leukopenia (white blood cell count <3000/ mm³; n = 24) or thrombocytopenia (platelets <100 000/mm³; n = 30), or both (n = 14), leading either to discontinuation of treatment or reduction of dose by 50% or more during AZA (n = 33) or 6-MP (n = 8) treatment. Patients were genotyped for the ITPA 94C>A and IVS2+21A>C mutations according to a previously described procedure based on endonuclease digestion of polymerase chain reaction products. Distribution of the 41 patients according to their ITPA genotype is presented in table 1 and compared with that of a previously published control population of 100 healthy Caucasians.⁴ Allele frequencies in the CD population were 0.085 for the 94C>A mutation and 0.12 for the IVS2+21A>C mutation, similar to frequencies observed in the control population (0.06 and 0.13, respectively). There was no significant difference in the genotypes distribution between the two populations, which confirmed the lack of association between ITPase deficiency and myelosuppression during thiopurine therapy. Due to the retrospective nature of the study, no correlation with other side effects could be investigated. In conclusion, application of ITPA genotyping tests does not seem to improve the identification of patients at risk of myelosuppression with AZA/6-MP therapy. Although we believe that conventional TPMT genotyping tests should still be applied before the initiation of thiopurine treatment, further work is needed on the role of other candidate genes that may be involved in thiopurine haematotoxicity.

Acknowledgements

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Table 1 Distribution of ITPA genotypes in 41 Crohn’s disease (CD) patients and 100 healthy Caucasians

<table>
<thead>
<tr>
<th>ITPA genotype</th>
<th>CD patients (n = 41)</th>
<th>Control population (n = 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt/Wt</td>
<td>26 (0.63)</td>
<td>64 (0.64)</td>
</tr>
<tr>
<td>Wt/94C&gt;A</td>
<td>6 (0.15)</td>
<td>10 (0.10)</td>
</tr>
<tr>
<td>Wt/IVS2+21A&gt;C</td>
<td>7 (0.17)</td>
<td>24 (0.24)</td>
</tr>
<tr>
<td>94C&gt;A/94C&gt;A</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>IVS2+21A&gt;C/IVS2+21A&gt;C</td>
<td>1 (0.02)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>94C&gt;A/IVS2+21A&gt;C</td>
<td>1 (0.02)</td>
<td>2 (0.02)</td>
</tr>
</tbody>
</table>

*Values in parentheses represent genotype frequencies.
†The control population comprised 100 healthy Caucasians who were genotyped in a previous study.

Small bowel malignancy at diagnosis of coeliac disease

We were very interested in the paper by Rampertab et al (Gut 2003;52:121–14) and the correspondence by Hawdle et al (Gut 2004;53:470). Their data are quite similar to ours, from the Italian Registry of Complications of Coeliac Disease. We collected information on 1968 patients over 18 years of age (mean age at diagnosis: 36.7 years; female/male ratio 5:1), diagnosed with coeliac diseases between January 1982 and December 2002 at 20 Italian clinical centres specialised in gastrointestinal disease. The diagnosis was made according to revised ESPGHAN criteria.⁵ We found five (0.25%) patients with a small bowel malignancy at the time of diagnosis of coeliac disease. Age range was 49–69 years (mean 59 years) with a predominance of females (4:1). Survival rate was very poor as three patients died within 36 months of diagnosis.

These results indicate that there is an increased risk of developing small bowel malignancy in patients with coeliac disease. This correlation was confirmed in the female/male ratio. In fact, while small bowel neoplasms are usually more frequent in males, in our population four of five cases were female. Moreover, mean age at diagnosis of these cases was higher that than of patients overall, emphasising that the risk of a neoplasm increases with longstanding coeliac disease.

References


In conclusion, early diagnosis of coeliac disease should be made to prevent small bowel neoplasms from developing, and so this cancer should be carried out at diagnosis of coeliac disease, especially in patients diagnosed during adulthood.

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Reference

Hypergastrinaemia in patients infected with Helicobacter pylori treated with proton pump inhibitors

We read with interest the commentary by McColl on Helicobacter pylori infection and long term proton pump inhibitor (PPI) therapy (Gut 2004;53:5–7). It is remarkable that he did not mention gastrin although hypergastrinaemia is a result of reduced gastric acid1 as well as Helicobacter pylori infection,2 and that patients with H pylori infection treated with PPI have additive hypergastrinaemia.3 Hypergastrinemia predisposes to gastric carcinoids in animals4 and humans,5,6 as well as to malignant ECL cell derived tumours (gastrointestinal carcinomas) in animals7 and humans.8

Interestingly, the carcinogenic effect of H pylori infection may be completely explained by its hypergastrinaemic effect, a work where McColl was one of the authors. Furthermore, the increased gastric cancer frequency in moderate hypergastrinaemic INS-GAS mice concomitantly infected by H pylori infection9 may also be caused by increased hypergastrinaemia in infected mice.10

To conclude, it is odd that gastrin was not taken into consideration when discussing the risk of gastric cancer following treatment with PPI in patients infected with H pylori. Animal as well as human studies linking gastrin to gastric cancer give support for a strategy where H pylori is eradicated in patients on long term PPI treatment.

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References

Terminal ileal biopsies should not be used to document extent of colonicoscopic examination

We commend the British Society of Gastroenterology and the authors for the excellent publication of guidelines for the management of inflammatory bowel disease (Gut 2004;53(suppl V):vi–16). However, we feel that their recommendation for routine terminal ileal biopsy is inappropriate. Although it is convenient to biopsy the terminal ileum if there is macroscopic evidence of an abnormality, their statement that ‘‘a terminal ileal biopsy performed at colonoscopy documents the extent of examination’’ is not recommended practice, due to the potential risk of variant Creutzfeldt-Jacob disease transmission from prion proteins which are prevalent in the lymphoid tissue of Peyer’s patches in the ileum. Although the use of disposable forceps may reduce the risk of transmission, there could still be contamination of the intubation channel of the colonoscope and prion protein is resistant to the standard endoscopic cleaning process.1 If the extent of examination needs to be documented, then a photograph of the ileocecal valve or ileal mucosa is preferable.

It is worth emphasising that prion protein may be present in any part of the gastrointestinal tract and random biopsy of gastrointestinal mucosa for reasons other than confirming an endoscopic abnormality or excluding microscopic colitis is not accepta-

ble. Similarly, for surveillance colonoscopy where multiple biopsy is recommended, the risk benefit ratio of this policy must be supported by the clinical indications.

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References

IgG food antibodies should be studied in similarly treated groups

The recent paper by Atkinson and colleagues (Gut 2004;53:1459–464) regarding IgG food antibodies and irritable bowel syndrome (IBS) fails to compare like with like. Regardless of the IgG results, the treatment group excluded significantly different foods to the control group, particularly those foods which appear to exacerbate symptoms of IBS. Of particular concern is the ‘‘yeast exclusion’’ diet. A low yeast diet is not a recognised diet in standard textbooks of dietetics and nutrition. However, alternative practitioners offering such a ‘‘yeast exclusion diet’’ sometimes recommend exclusion of a wide range of foods, such as: bakery products, alcoholic beverages, many other beverages including commercial fruit juices, cereals, condiments, dairy products, fungi, meats, nuts, and fruits (bananas, prunes, dates, dried apricots, dates, prunes, dried figs, and other dried fruit).

Therefore, regardless of IgG antibody status, the dietary restrictions in one group are not controlled for by the other group, and hence the conclusion may not be valid.

It would also be helpful to know if any of the patients with IgG antibodies to a particular antigen also had IgG antibodies to the same antigen.

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IgG antibodies to foods in IBS

We read with interest the article by Atkinson et al (Gut 2004;53:1459–64). The authors describe an important advance in our understanding of the putative role of inflammation in irritable bowel syndrome (IBS). However, we wonder whether their conclusion that assay of IgG antibodies may have a role in identifying candidate foods for elimination to treat patients with IBS may be a step too far.

The four foods to which the patients most commonly formed antibodies and hence the four foods most commonly eliminated from the “true diet” were yeast (86.7%), milk (84.3%), whole egg (58.3%), and wheat (49.3%). The “sham diet” involved eliminating foods to which the patients had not formed antibodies and, therefore, in the sham group the exclusion rates were for yeast, milk, whole egg, and wheat were very low (0%, 1%, 36.7%, and 8% respectively). It is therefore difficult to assess whether a diet excluding these foods would have led to symptomatic improvement in all patients, regardless of their antibody status.

Furthermore, the foods to which the study group commonly formed antibodies were similar to those already identified as leading to symptomatic benefit in patients with IBS when excluded from their diet. In a review cited by Atkinson and colleagues, it was noted that in eight trials of exclusion diets in IBS, seven identified dairy products and five identified wheat as worsening symptoms. It is not clear whether the difference in improvement in symptoms seen in the current study between true and sham groups can be explained simply by the omission of these foods. This could in practice eliminate the need for antibody testing.

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Reference


Influence of dietary factors on the clinical course of inflammatory bowel disease

Jowett et al reported in their elegant study on the role of diet in maintaining remission in patients with ulcerative colitis (Gut 2004;53:1479–84). Surely the effect of diet has an essential, but often forgotten, role in altering the course of disease in all types of inflammatory bowel diseases. This role does not necessarily act by maintaining patients in remission clinically, but perhaps more importantly by minimising the activities of the disease and rendering it quiescent.

We have recently reported a case of active strictureting Crohn’s disease in an adult female patient with high stoma output. She was treated initially with casein base formula (Modulen IBD-Nestle, Vevey, Switzerland) for three weeks. Her stoma output was reduced from 2800 ml to 400 ml per day by day 10. Serum albumin and serum protein significantly increased also. She subjectively felt better and pain free and stopped her opiate and non-opiate formula. The casein based formula is a nutritionally complete formulation containing a natural anti-inflammatory growth factor, transforming growth factor β2. The mechanism for inducing remission was possibly inhibition of expression of MHC class II protein in downregulating the inflammatory response.

Previous studies have shown that there is a decrease in plasma antioxidant defences in all types of inflammatory bowel disease. This is mirrored by an increase in free radical peripheral leucocyte DNA damage. It is therefore possible that the casein based formula acts as an antioxidant to minimise the oxidative stress that occurs in patients with active Crohn’s disease. Another possible mechanism is that this formula may have a role as a prebiotic by stimulating the activity of bacteria which are already present in the gut.

Remission induced in our case study highlights the part played by a casein based formula in the management of adult Crohn’s disease. The encouraging result demonstrates the need to treat similar cases with dietary measures first. This opportunity should not be missed as it may well obviate the need for surgical intervention or administration of potent pharmacotherapeutic agents which carries the risk of several comorbidities.

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References


Identification of ferroportin disease in the Indian subcontinent

Haemochromatosis is a common inherited disorder of iron metabolism, characterised by excessive iron absorption and deposition in tissues. The majority of cases are associated with mutations in the HFE gene and inherited in an autosomal recessive manner.1 Autosomal dominant forms of haemochromatosis have been reported, mainly associated with mutations in the ferroportin 1 gene.2 This syndrome, termed type 4 haemochromatosis or more recently ferroportin disease,1 usually characterised by an early increase in serum ferritin with normal transferrin saturation. Iron accumulation is most prominent in Kupffer cells and other macrophages, in addition to hepatocytes. Some patients do not tolerate venesection therapy well and can develop anaemia. Hereditary iron overload disorders appear to be uncommon in Asia. Secondary iron overload due to beta thalassaemia is common in the Indian subcontinent. However, primary iron overload disorders and HFE mutations appear to be rare and cases have not been well characterised in this region.3 We identified a patient from the Indian subcontinent with features typical of ferroportin disease.

A 36 year old female of Sri Lankan origin presented for a routine medical examination in December 2003. She was found to have an elevated serum ferritin of 4344 μg/l and transferrin saturation (29%) were normal. Liver function tests, blood glucose, and thyroid studies were all normal. Physical examination was normal and she had no significant past medical history or risk factors for iron overload.

C282Y, H63D, and S65C HFE gene mutations were all negative and she had no family history of iron overload. Her mother and three siblings all had normal serum ferritin levels. Her father died of ischaemic heart disease aged 48 years.

A magnetic resonance imaging scan showed hepatic iron overload. Liver biopsy showed grade 3–4 iron deposition with periportal iron hepatocytes and Kupffer cells; no fibrosis or cirrhosis was evident (fig 1). The hepatic iron concentration was 17 700 μg/g dry weight and hepatic iron index was 9.1.

Venecesion therapy was initially poorly tolerated with the development of anaemia following the first two 500 ml venesections. Her haemoglobin is now stable on a programme of 300–500 ml venesections every three weeks.

The features of ferroportin disease in this patient led us to sequence the ferroportin 1 gene, as previously described.4 Analysis of the DNA sequence revealed a heterozygous three base pair deletion (TTG) in exon 5. This is the same deletion, V162del, described by us and others in haemochromatosis patients from Australia, the UK, Italy, and Greece.5

This is the first report to identify V162del or indeed any ferroportin 1 mutation in an individual from the Indian subcontinent. Identification of V162del in an Asian patient confirms that this mutation is likely to be the most common mutation of ferroportin 1 and the most common cause of non-HFE associated haemochromatosis. The wide geographical distribution of this mutation suggests that it is a recurrent mutation that has repeatedly arisen in distinct populations, probably by slippage mispairing.

Iron overload in this patient was typical of ferroportin disease. At the time of diagnosis she was asymptomatic and had no fibrosis on liver biopsy. Whether fibrosis or clinical complications will develop with age if iron stores are not depleted is unknown.

In conclusion, we have identified V162del mutation of ferroportin 1 in a fifth geographical location, emphasising that this mutation is the most common and widely distributed mutation which causes non-HFE haemochromatosis. We have identified V162del in a region where iron overload disorders have not been well characterised. Analysis of this and other ferroportin 1 mutations may be useful in identifying iron overload disorders in this region and may be the basis of hitherto unexplained cases of iron overload.

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References

BOOK REVIEW

Morson and Dawson’s Gastrointestinal Pathology, 4th edn

Why do people buy a book such as this, which involves a not inconsiderable financial outlay (even if you box clever and make it tax deductible)? I think for two main reasons—firstly, for use as a bench book, and secondly, for information on the pathological basis of gastrointestinal disease for interest, teaching, or indeed research purposes.

On the first criterion, this book succeeds, usually quite brilliantly. As a vade mecum on gastrointestinal pathology it should be on the shelf of every pathologist who engages in the reporting of such material. In my view, the book is more user friendly than the competition—Fenoglio-Preiser and Goldman to name but two—and is certainly more readable. I would therefore extol its virtues unreservedly in this respect.

On the second criterion, as a source book, I suppose the correct word is patchy. Some sections, for example that on colorectal tumours, is admirable in this respect, whereas other sections are more limited in scope and even cursory in their treatment of the pathobiology. There is also the problem of the unavoidable intrinsic delay in producing such a book, resulting in reference lists which are some years away from the publication date. I am aware however that my personal outlook is not that of most individuals who will purchase this volume so I am probably being over critical. It is, after all, quintessentially a bench book, and excellent at that.

However, I do have one real beef. In any multiauthor work there is bound to be variation, but here we are not told which one of the stellar caste were responsible for which section or chapter. Of course we can make informed guesses about the Barrett’s or colorectal carcinoma sections, but who did the GIST bit? Because of some (minor) errors in the criteria for the diagnosis of malignancy, I have tried to berate a number of authors who have all denied responsibility, and blamed someone else—usually the author(s) absent at the time. Not good enough.

I have to concede however that the authors have succeeded in producing perhaps the text in gastrointestinal pathology, which is a credit to both themselves and the discipline in the UK. I congratulate them.

N A Wright

CORRECTIONS

do: 10.1136/gut.2003.025494corr1

do: 10.1136/gut.2003.025494corr1

do: 10.1136/gut.2003.026807corr1

do: 10.1136/gut.2003.026807corr1

In the January 2005 issue of Gut, one of the author’s names of the paper entitled Human peripheral and gastric lymphocyte responses to Helicobacter pylori NapA and APhC differ in infected and uninfected individuals (H J Windle, Y S Ang, V A Morales, R McManus, and D Kelleher. Gut 2005;54:25–32) was cited incorrectly. V A Morales should read V Athie-Morales. The journal apologises for this mistake.

In the December issue of Gut fig 1 in the paper by AJG Bell et al (Human lymphocyte stimulation with pohcitis flora is greater than with flora from a healthy pouch but is suppressed by metronidazole. Gut 2004;53: 1801–1805) is incorrect. The labels for fig 1C are inverted; the squares should have been labelled HetNon and the triangles HetPM. The legend is also incorrect because the label for flora grown on agar without metronidazole is HetNon, not HetP as stated.