LEADING ARTICLE

# TPMT in the treatment of Crohn's disease with azathioprine

**L Lennard**

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Azathioprine induced profound myelosuppression linked to TPMT deficiency has now been documented in many patient groups, including those with Crohn's disease. At the start of azathioprine or mercaptopurine therapy, measurement of TPMT activity has a role in identifying the 1 in 300 patients who are at risk of severe myelosuppression when treated with standard thiopurine dosages. During the initial months of azathioprine therapy a knowledge of TPMT status warns of early bone marrow toxicity. In patients established on azathioprine these is no clear evidence to suggest that TPMT is predictive of clinical response or drug toxicity, indicating a role for TPMT in the prediction of early events rather than long term control. In patients with Crohn's disease on long term azathioprine therapy, it is clear that myelosuppression, particularly leucopenia, is caused by other factors in addition to variable TPMT activity and therefore monitoring of blood cell counts throughout treatment is essential.

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## THE IMPORTANCE OF TPMT

The first indication that thiopurine methyltransferase (TPMT) deficiency was associated with profound myelosuppression came from observations in adults taking azathioprine as an immunosuppressive agent. Accumulation of grossly elevated concentrations of mercaptopurine derived thioguanine nucleotide (TGN) cytotoxic metabolites[1 2] was linked to a lack of red blood cell TPMT activity.[3] These observations of azathioprine induced profound myelosuppression linked to TPMT deficiency have now been documented in many patient groups and confirmed by numerous reports.[4–7]

## HOW AZATHIOPRINE WORKS

Azathioprine has no indigenous immunosuppressive activity; it is a prodrug. The first step in biotransformation is non-enzymic cleavage to form mercaptopurine which in turn undergoes extensive metabolism.[8] Mercaptopurine can be oxidised, methylated, or formed into a variety of active thionucleotide metabolites. It is drug derived thioguanine nucleotide which is eventually incorporated into DNA as a false base.[9]

The thionucleotide metabolites of mercaptopurine compete with their endogenous counterparts in many biochemical pathways. Nucleotides play a variety of important roles in all cells: they are precursors of DNA and RNA, they are essential carriers of energy (for example, ATP and GTP), and they also function as cellular second messengers.[10] It is the importance of these nucleotide dependent processes to functioning and dividing cells that has endowed the thiopurine antimetabolite azathioprine with both immunosuppressive and cytotoxic properties.

## THE ROLE OF TPMT IN THE CLINICAL PHARMACOLOGY OF THIOPURINE DRUGS

TPMT methylates azathioprine metabolites at the expense of TGN formation. Both mercaptopurine and mercaptopurine nucleotide (thioinosine monophosphate) are good substrates for TPMT, but TGNs are poor substrates.[11] In the mercaptopurine treatment of childhood leukaemia, TPMT activities show a significant negative correlation to red blood cell TGN concentrations[12] and TPMT deficiency is associated with grossly elevated TGN concentrations and profound myelosuppression.[13] Multivariate analysis has confirmed that higher TGN concentrations[3] and lower TPMT activity[14] tend to be associated with better outcomes. Therefore, in children with lymphoblastic leukaemia, TPMT activity has been shown to reflect mercaptopurine efficacy and toxicity.[12–15]

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During chemotherapy, one reason for mercaptopurine "resistance" is very high TPMT activities; extensive methylation results in suboptimal cytotoxic TGN formation.[12] A second cause of low TGN concentrations is simply not taking the tablets. The two cases of low TGNs can be distinguished by measurement of methylmercaptopurine nucleotide metabolites (products of the TPMT reaction) alongside TGNs.[16] Reports based on therapeutic drug monitoring suggest that 10% of patients fail to take their mercaptopurine or azathioprine tablets.[16 17]

Thiopurine drugs are potential inducers of liver damage and this is reflected in the abnormal liver function tests which are frequently reported in

**Abbreviations:** TPMT, thiopurine methyltransferase; TGN, thioguanine nucleotide; IBD, inflammatory bowel disease; MeMPs, methylmercaptopurine nucleotide metabolites; HBI, Harvey Bradshaw index.

children undergoing mercaptopurine or thioguanine long term maintenance chemotherapy for lymphoblastic leukaemia.[18] The exact mechanisms of induced hepatotoxicity are unknown but TPMT produced methylated mercaptopurine metabolites have been implicated in the resultant liver damage[19]; children with higher TPMT activities develop more hepatotoxicity.[15]

## TPMT: THE BASICS

TPMT activity in the red blood cell and other human tissues is under the control of a common genetic polymorphism.[20] The frequency distribution of TPMT activity in Caucasian populations is trimodal: approximately 89% of the population have high enzyme activity and are homozygous for the wild-type allele (*TPMT*$^{H}$), 11% inherit intermediate levels of enzyme activity with one wild-type and one variant allele (heterozygous *TPMT*$^{H}$/*TPMT*$^{L}$), while 1 in 300 subjects have no functional activity (two variant alleles, homozygous *TPMT*$^{L}$).

A number of variant TPMT alleles have been described,[21] and ethnic differences in the incidence of these variant alleles may be important in the clinical use of thiopurines. *TPMT*3A*, a double mutant, is the most frequently occurring variant allele (*TPMT*$^{L}$) in white Caucasians but each mutation can occur independently (*TPMT*3B* and *TPMT*3C*). In African-Americans the mutant allele frequency was the same as recorded in Caucasians, but *TPMT*3C* was the most prevalent mutant allele.[22] In a Korean population *TPMT*3C* was the most frequent variant allele, and the *TPMT*3A* allele was absent.[21]

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Variant alleles were only detected in 2.0% of South West Asians (*TPMT*3C*) and 4.7% of Chinese (*TPMT*3C*) compared with 10% of Caucasians (*TPMT*2*, **3A,* and **3C*), indicating that *TPMT*3C* is the oldest mutation and *TPMT*2* the most recent, while variant alleles *TPMT*4* to *TPMT8* are thought to be isolated mutations.[23]

## TPMT AND CROHN'S DISEASE

The thiopurine drugs azathioprine and mercaptopurine are well established in the treatment of Crohn's disease.[24] Non-allergic clinical toxicities appear to be dose dependent and may correlate with aspects of azathioprine metabolism.[24] Leucopenia is a frequently reported side effect in patients with inflammatory bowel disease (IBD), and the observations made with respect to the TPMT genetic polymorphism and bone marrow toxicity in childhood leukaemia were initially translated to the treatment of Crohn's disease in 1996 by Cuffari and colleagues.[25] This study investigated mercaptopurine efficacy and toxicity in 25 adolescent patients with Crohn's disease. TPMT activity was investigated indirectly by measurement of the products of the TPMT reaction the methylmercaptopurine nucleotide metabolites (MeMPs). Remission (assessed by a modified Harvey Bradshaw index (HBI)) correlated with TGN concentrations ($p<0.5$) but not MeMPs. Mercaptopurine complications however were "generally associated" with increased MeMPs. However, Cuffari observed that although a lack of clinical response (high HBI) was associated with low TGNs, a satisfactory clinical response (low HBI) was associated with a wide range of red blood cell TGNs. In a larger patient group (n=82), disease remission was shown to correlate with TGNs over 250 pmol/$8\times10^{8}$ red blood cells.[26 27] However, MeMPs, potentially an indirect measure of TPMT activity, did not correlate with TGN concentrations.[27] The question now was, can direct measurement of TPMT phenotypic activity or genotype assist clinicians in optimising the therapeutic response to mercaptopurine?

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A dramatic amplification of interest in the TPMT genetic polymorphism arose with the commercial availability of the thiopurine enzyme and metabolite assays.[28 29] This, coupled with a number of institutions capable of "in house" pharmacogenetic analysis, has produced a plethora of abstracts in recent years, but many of these have yet to be translated into peer reviewed articles. Dubinsky and colleagues[28] in a study of 92 paediatric IBD patients, 79 of whom had Crohn's disease, reported that patients heterozygous for TPMT (8/92) had higher TGN concentrations ($p<0.001$) and all responded to therapy. This study confirmed an association between high red blood cell TGNs and clinical response to mercaptopurine. The best probability of patient response was not significantly increased until red blood cell TGN concentrations were >235 pmol. The odds ratio of a therapeutic response for red blood cell TGNs greater than 235 pmol was 5.0 (95% confidence interval 2.6–9.7; $p<001$). The products of the TPMT reaction (the MeMPs) did not correlate with disease activity but patients generating high MeMP concentrations did however experience more hepatotoxicity. Although hepatotoxicity was experienced at low MeMP concentrations, the risk increased threefold at MeMPs above the third quartile (>5700 pmol).

In this study,[28] analysis of TPMT genotypes showed that only one of 13 patients who experienced leucopenia was heterozygous for TPMT. The vast majority (97%) of patients with drug related toxicity had a wild-type TPMT genotype. In addition, the range of mercaptopurine dosages were similar in the heterozygotes and wild-type TPMT genotypes.[28] Subsequently, this group analysed TPMT activities in 51 IBD patients, of whom 35 had Crohn's disease.[29] Again, there was no significant relationship between TPMT activity and thiopurine dosage. In a subgroup of 42 patients, seven had intermediate TPMT activity (that is, presumed heterozygotes) and all were non-responders. In the group as a whole, red blood cell TGNs were positively correlated with therapeutic efficacy and MeMPs with drug related toxicity which, in the majority of cases, was hepatotoxicity.

Colombel and colleagues[30] reported TPMT genotype analysis in 41 Crohn's disease patients. All of this patient group had experienced leucopenia (white cell count $<3.0\times10^{9}$/l) or thrombocytopenia (platelets $<100\times10^{9}$/l) leading to a reduction in thiopurine dose (17% of patients) or withdrawal (83% of patients). Four patients (10%) were TPMT deficient (homozygous variant allele), seven (17%) were heterozygotes, and the reminder had wild-type activity. From the start of thiopurine therapy, the time to bone marrow toxicity was less than 1.5 months in the four TPMT deficient patients, ranged from 1 to 18 months in the heterozygotes, and from 0.5 to 87 months in patients with wild-type TPMT. Variant alleles associated with lack of or lower TPMT activity are over represented in this small study group compared with the general population but it was clear that myelosuppression was caused by other factors in these patients with Crohn's disease.[30]

By far the largest and perhaps the most extensive study on TPMT activities in IBD patients was reported by Lowry and colleagues.[31] Of 170 patients studied, 130 had Crohn's disease and the duration of constant dose azathioprine (n=115) or mercaptopurine (n=55) therapy ranged from 3.5 to 102 months. All patients had responded to, and were tolerant of, thiopurines. Patients with intermediate TPMT activity (n=23, 13.5%) had significantly higher red blood cell TGN concentrations, but for the group as a whole there was no difference in red blood cell TGNs between those in clinical remission

(n=114, 67%) and those with active disease. In Lowry's report,[31] active disease was recorded in 33% of those with wild-type homozygous "normal" TPMT activity and 33% of patients with a heterozygous intermediate TPMT activity. Neither pretreatment or on-therapy TPMT activities were associated with leucopenia but the self selected nature of this cohort would have excluded those individuals with early leucopenia.

A recent report by Campbell and colleagues[32] of TPMT activities in 113 IBD patients (61 with Crohn's disease) investigated those who were currently taking azathioprine (n=63), those who had discontinued azathioprine (n=24), and those who had never taken azathioprine (n=26). TPMT activities were similar in all three groups (range 9–41 units; median 25). TPMT activity correlated ($r$=0.41, $p<0.001$) with the lowest neutrophil count in the first four months of azathioprine therapy. In the intolerant group, those experiencing neutropenia had significantly lower TPMT activities than those experiencing other toxicities (for example, pancreatitis, hepatitis, dermatological problems). Survival analysis, based on time to first relapse, was performed on a subgroup of patients taking low dose (<2.0 mg/kg) azathioprine. Those individuals with lower TPMT activities (<20 units) had a statistically significant relapse free advantage (log rank $\chi^2$=4.0; $p<0.05$).[32] In this low dose azathioprine cohort, mean TPMT activities in patients with stable disease (n=20) were significantly lower (19.8 *v* 27.6 units) than those measured in patients who had experienced active disease (n=14).

Similar apparently contradictory results have emerged when assessing the clinical value of TPMT in the context of azathioprine immunosuppression for other patient groups. In the long term treatment of systemic lupus erythematosus with azathioprine, TPMT genotyping failed to predict the majority of thiopurine induced neutropenias.[33] In contrast, in rheumatic disease, TPMT genotype predicted therapy limiting toxicity induced by azathioprine, five of the six patients with variant alleles experienced leucopenia within one month of starting thiopurines. Within two months of starting azathioprine, seven patients had abnormal liver function tests. Six of the seven had wild-type TPMT alleles.[34] Taken together, the results of all of the studies detailed above indicate a potential role for TPMT in the prediction of early events rather than long term control.

## DRUG-DRUG INTERACTIONS

The clinician should be aware that a number of compounds, which could be coadministered with azathioprine or mercaptopurine, may potentially influence TPMT activity. After a therapeutic dose of aspirin, plasma concentrations of salicylic acid are within the range for TPMT inhibition[35] and the loop diuretic frusemide inhibits TPMT at concentrations within the therapeutic range for frusemide.[36]

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Balsalazide, olsalazine, sulphasalazine, and 5-aminosalicylic acid are potent inhibitors of TPMT in vitro.[37 38] In a large long term clinical study of IBD, mercaptopurine was withdrawn in 10% of patients because of the occurrence of adverse reactions. Over half of the patients in that study were treated simultaneously with mercaptopurine and sulphasalazine.[39] A possible drug-drug interaction, linked to TPMT inhibition, was reported in a patient with refractory Crohn's disease who developed bone marrow suppression while receiving daily mercaptopurine and olsalazine.[40]

In the report of Dubinsky and colleagues,[28] 48 patients (52%) received concomitant mesalamine therapy but coadministration of mesalamine was reported not to influence TGN or MeMP concentrations. This was later confirmed in a subsequent study reporting TPMT activities in 51 IBD patients (35 with Crohn's disease).[29] Concurrent mesalamine did not influence TPMT activity or mercaptopurine derived metabolite concentrations. This report is in contrast with the findings of Lowry and colleagues[41] who compared red blood cell TGNs in the same patient prior to and during coadministration of 5′ aminosalicylic acids. Mesalamine, balsalazide, and sulphasalazine all produced increased red blood cell TGNs in the 29 Crohn's disease patients studied. This was taken to indicate the possibility of in vivo TPMT inhibition.[41] Inhibition of an enzyme in vivo will not necessarily be reflected by lower TPMT activities because TPMT is measured in vitro in the absence of the inhibitor.

Patients receiving concurrent mesalamine, sulphasalazine, or olsalazine had significantly lower median white cell counts compared with patients not taking aminosalicylates.[31] In addition to the possible myelosuppressive influence of increased TGN concentrations, there are other factors that can influence leucocyte count in these patients. An additional variable in the occurrence of leucopenia is the ability of the individual to metabolise aminosalicylates by acetylation. Variations in the ability to form the major metabolite N-acetyl 5′aminosalicycylic acid (that is, "slow acetylator" status) have been linked to leucopenia. This may also contribute to the myelosuppression observed with sulphasalazine.[41 42]

## CONCLUSIONS

When reflecting on TPMT activity in the treatment of Crohn's disease with azathioprine, it is clear that one is dealing with a mixed cohort of patients receiving a wide variety of thiopurine dosages supplemented to various degrees by other immunosuppressive agents.[24] At the start of azathioprine or mercaptopurine therapy, measurement of TPMT activity has a role in identifying the 1 in 300 patients who are at risk of severe myelosuppression when treated with standard thiopurine dosages. In addition, identification of the heterozygote intermediate TPMT individual identifies those prone to early leucopenic episodes. During the initial four months of thiopurine therapy, lower TPMT activities correlate with low neutrophil counts.[32] Thus a knowledge of TPMT status warns of early bone marrow toxicity. Indications are that identification of the heterozygote would indicate those patients who could be safely managed on lower (<2.0 mg/kg) azathioprine dosages.[32] We await a formal analysis of TPMT activities with respect to the frequency of active disease recurrence in those patients on long term thiopurine immunosuppression.

> "A knowledge of TPMT status warns of early bone marrow toxicity"

In patients established on azathioprine, TPMT was not predictive of clinical response or drug toxicity.[31] These observations are perhaps due in part to the self selected nature of this group of patients in whom early events had already occurred. Thus during long term thiopurine therapy, it appears that by careful titration of dosages to clinical response by frequent monitoring of blood cell counts and liver function tests the TPMT genetic polymorphism could have been circumvented. As yet, there is no evidence to suggest a specific role for TPMT in the management of patients already established on azathioprine/mercaptopurine immunosuppression. However, taken together the results of the studies detailed in this article indicate a role for TPMT in the prediction of early events rather than long term control.

In the patient with Crohn's disease on long term azathioprine therapy it is clear that myelosuppression, particularly leucopenia, is caused by other factors in addition to variable TPMT activity and therefore monitoring of blood cell counts throughout treatment remains essential.[15]

## ACKNOWLEDGEMENT

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# PostScript

## LETTERS

### *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 (*Gut* 2002;**51**:143–6), it is clear that myelosuppression may be caused by other factors in addition to variable TPMT.

Since the identification of the molecular basis of inosine triphosphate pyrophosphatase (ITPAse) deficiency,[1] a clinically benign condition characterised by abnormal accumulation of inosine triphosphate in erythrocytes, the possibility of a correlation between thiopurine toxicity and ITPAse deficiency has been raised. Complete ITPase deficiency was found to be associated with a homozygous missense 94C>A mutation that encodes a $Pro^{32}Thr$ exchange, whereas an intronic IVS2+21A>C polymorphism was shown to have a less severe effect, homozygotes retaining 60% ITPAse activity. It was then postulated that in ITPAse deficient patients treated with thiopurine drugs, a 6-thio-ITP metabolite could accumulate resulting in toxicity.[1] A recent study in 62 patients with inflammatory bowel disease reported a significant association between the *ITPA* 94C>A polymorphism and AZA related adverse effects, specifically flu-like symptoms, rash, and pancreatitis.[2] 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 leucopenia during AZA/6-MP therapy.[3] 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 leucopenia (white blood cell count $<3000/mm^3$; n = 24) or thrombocytopenia (platelets $<100\ 000/mm^3$; 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.[1] 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.[1] 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.

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

| *ITPA* genotype | CD patients (n = 41) | Control population† (n = 100) |
|---|---|---|
| Wt/Wt | 26 (0.63)* | 64 (0.64) |
| Wt/94C>A | 6 (0.15) | 10 (0.10) |
| Wt/IVS2+21A>C | 7 (0.17) | 24 (0.24) |
| 94C>A/94C>A | 0 (0.00) | 0 (0.00) |
| IVS2+21A>C/IVS2+21A>C | 1 (0.02) | 0 (0.00) |
| 94C>A/IVS2+21A>C | 1 (0.02) | 2 (0.02) |

*Values in parentheses represent genotype frequencies.
†The control population comprised 100 healthy Caucasians who were genotyped in a previous study.[1]

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### 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 3:1), 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.[1] 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 by 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 than that of patients overall, emphasising that the risk of a neoplasm increases with longstanding coeliac disease.

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

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## 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 acidity[1] as well as *Helicobacter pylori* infection,[2] and that patients with *H pylori* infection treated with PPI have additive hypergastrinaemia.[3] Hypergastrinaemia predisposes to gastric carcinoids in animals[4 5] and humans[6 7] as well as to malignant ECL cell derived tumours (gastric carcinomas) in animals[8] and humans.[9 10]

Interestingly, the carcinogenic effect of *H pylori* infection may be completely explained by its hypergastrinaemic effect,[11] 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* infection[12] may also be caused by increased hypergastrinaemia in infected mice.[13]

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

We commend the British Society of Gastroenterology and the authors for the excellent publication of guidelines for the management of inflammatory bowel disease in adults (*Gut* 2004;**53**(suppl V):vi–16). However, we feel that their recommendation for routine terminal ileal biopsying is inappropriate. Although it is important 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 Creutzfeld-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 ileocaecal valve or ileal mucosa is preferable.

It is worth emphasising that prion protein may be present in any part of the gastrointestinal tract[2] and random biopsy of gastrointestinal mucosa for reasons other than confirming an endoscopic abnormality or excluding microscopic colitis is not acceptable. 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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## IgG food antibodies should be studied in similarly treated groups

The recent paper by Atkinson and colleagues (*Gut* 2004;**53**:1459–1464) 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 produce, fungi, meat products (hamburgers, sausages, and cooked meats made with bread or breadcrumbs), yeast extracts (Bisto, Marmite, Oxo, Bovril, Vegemite, gravy browning, and all similar extracts), all B vitamin preparations, and sometimes, most worryingly, ''sugar foods'' (sugar, sucrose, fructose, maltose, lactose, glycogen, glucose milk, sweets, chocolate, sweet biscuits, cakes, candies, cookies, puddings, desserts, canned food, packaged food, hamburgers, honey, mannitol, sorbitol, galactose, monosaccharides, polysaccharides, date sugar, turbinado sugar, molasses, maple syrup, most bottled juices, all soft drinks, tonic water, milkshakes, raisins, 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 IgE antibodies to the same antigen.

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Competing Interests: None declared.

## 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 for yeast, milk, whole egg, and wheat were very low (0%, 1.3%, 26.7%, 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,[1] 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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## 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 stricturing Crohn's disease in an adult female patient with high stoma output.[1] She was treated solely 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 $\alpha 2$. The mechanism for inducing remission in our patient was possibly inhibition of expression of MHC class II protein in downregulating the inflammatory response.[2]

Previous studies have shown that there is a decrease in plasma antioxidant defences in all types of inflammatory bowel disease.[3] 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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## 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,[3] is 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 relatively 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.[4 5] 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 3145 µg/l. Her serum iron (17.1 µmol/l) and transferrin saturation (29%) were normal. Liver functions 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 within 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.

Venesection 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.[6] 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.[6–9]

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 unclear.

In conclusion, we have identified the 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 the study of iron overload disorders in this region and may be the basis of hitherto unexplained cases of iron overload.

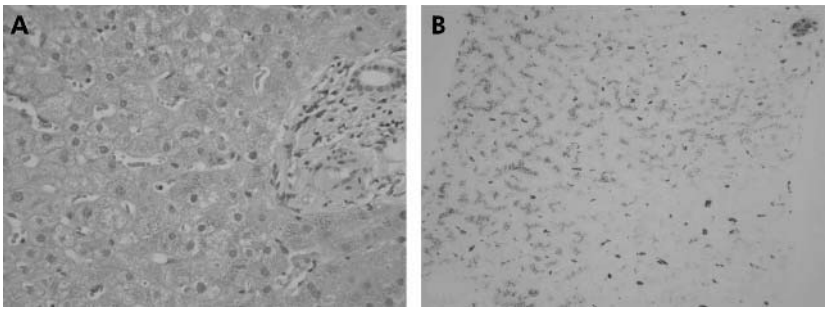


**Figure 1** Liver biopsy sections from our patient stained with (A) haematoxylin and eosin and (B) Perls' Prussian blue (magnification 100×). Grade 3–4 iron is prominent in hepatocytes and Kupffer cells.

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# BOOK REVIEW

## Morson and Dawson's Gastrointestinal Pathology, 4th edn

Edited by D W Day, J R Jass, A B Price, *et al*. Massachusetts: Blackwell Publishing, 2003, £175.00, pp 692. ISBN 0-632-04204-4

Why do people buy s 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

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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.

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In the December issue of *Gut* fig 1 in the paper by AJG Bell *et al* (Human lymphocyte stimulation with pouchitis 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.