

patients with genotype 3. In another retrospective analysis of 174 patients with chronic hepatitis C, obesity (and not steatosis) was a negative predictor of SVR.<sup>9</sup> It could be that obesity (with increased plasma free fatty acid) causes steatosis, and then each independently diminishes the response to treatment. Obesity decreases interferon bioavailability and impairs the immune response to HCV. The opposite was found in a study involving non-diabetic European patients with HCV genotype 1 at low risk for the metabolic syndrome where the prevalence of steatosis was nearly 60%.<sup>10</sup> Insulin resistant was a risk factor for moderate/severe steatosis, especially in men. Moderate/severe steatosis was found to be associated with hyporesponsiveness to treatment.

We obviously agree on the need for larger studies which could explain the mechanisms that promote the occurrence of steatosis in chronic hepatitis C, and the relationship with response to treatment.

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## Do ITPA and TPMT genotypes predict the development of side effects to AZA?

In a retrospective study of patients with inflammatory bowel disease, van Dieren *et al* recently reported the absence of a correlation between genotypes for both inosine triphosphate pyrophosphatase (ITPA) and thiopurine methyltransferase (TPMT), with any side effects to azathioprine (AZA) (*Gut* 2005;**54**:1664). This contrasts with two other studies. A rigorous prospective study, published recently, has demonstrated a significant association between ITPA genotype and early dropout from AZA therapy.<sup>1</sup> Our original publication implicated ITPA in a number of adverse effects, which were independent of myelosuppression.<sup>2</sup> Another letter has reported non-association of ITPA with myelosuppression<sup>3</sup> but thiopurine induced myelosuppression has been well documented over the past 25 years as associated with TPMT, not ITPA, status.<sup>4</sup>

However, we draw attention to a peculiar feature of the TPMT results of van Dieren *et al* that one patient—who suffered severe myelosuppression—was reported as TPMT\*3B/\*3B genotype. We previously published a meta-analysis of the incidence of the TPMT\*3B (G460A) mutation,<sup>5</sup> discovering that it is rare, and this has been confirmed by a recent large study, making the chance of homozygosity negligible.<sup>6</sup> Indeed, our evidence suggested that even the few cases of TPMT\*3B may be overreported as a result of a technical problem in TPMT genotyping by polymerase chain reaction-restriction fragment length polymorphism. Table 1 shows the frequency of TPMT mutant alleles, including TPMT\*3B, showing the low frequency of the TPMT\*3B allele and apparent cases of overreporting. The TPMT\*3A allele, the most common mutant polymorphism among Caucasians, is a double mutant combining an A719G mutation and G460A mutation. The A719G mutation is usually typed by restriction endonuclease digestion that relies on creation of an AccI recognition site, and we have shown that this enzyme is prone to failure.<sup>5</sup> Failure of A719G recognition will thus result in misreading the TPMT\*3A allele as G460A only—that is, as the TPMT\*3B allele (and the TPMT\*3C allele will be misread as wild-type TPMT\*1).

Curiously, van Dieren *et al* state that pretherapy TPMT genotyping is “of limited clinical value” but their results do not support this statement, as two cases of severe and potentially life threatening myelosuppression in their patient group were predicted by TPMT genotyping. In the absence of sequencing evidence, some doubt remains over the other TPMT genotype results presented. TPMT requesting has become routine in some large scale clinical practices, particularly in combination with thioguanine nucleotide monitoring.<sup>6–8</sup> But until extensive and preferably prospective studies are accumulated in the literature it seems too early to dismiss the full pharmacogenetic value of ITPA and TPMT. We keenly await further publications elucidating genetic regulation of thiopurine metabolism as a valuable pharmacogenetic model.

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## Authors' reply

In response to the comments of Duley *et al* to our letter (*Gut* 2005;**54**:1664), we would like to make some additional remarks.

Firstly, Duley *et al* concluded that our study had a retrospective character but this is not correct. Ours was a longitudinal cohort study in which all patients with inflammatory bowel disease visiting our outpatient department were included. These patients were followed prospectively for the development of azathioprine (AZA) associated side effects. Thiopurine methyltransferase (TPMT) and inosine triphosphate pyrophosphatase (ITPA) genotypes were determined during the follow up period.

Next, Duley *et al* questioned the quality of our polymerase chain reaction (PCR) sensitivity for the detection of the A719G TPMT mutation. Indeed, we found an undigested band in this PCR which involved restriction endonuclease digestion that relies on creation of an AccI recognition site with an adequate positive (digested) control. Moreover, DNA sequencing by an independent laboratory also showed the wild-type genotype in the 719 site. However, following the criticism by Duley *et al*, we repeated the PCR with another Taq polymerase and we have now observed a