Genotyping of the UDP-Glucuronosyltransferase (UGT) 1A7 Gene Revisited

Dear Sir:

In 2001 and in 2003, we published 2 studies reporting the role of single nucleotide polymorphisms (SNPs) of the mutagen detoxifying UDP glucuronosyltransferase (UGT) 1A7 gene in hepatocellular carcinoma (HCC) and chronic pancreatitis and pancreatic cancer, concluding that a low catalytic activity variant of UGT1A7 (UGT1A7*3) represents a risk factor for these diseases.

In the first paper, 59 patients with HCC were compared with 70 controls regarding their UGT1A7 genotype (reported in Table 3 and Figure 3 of that paper). The genotyping data in Table 3 show a deviation from Hardy-Weinberg equilibrium (HWE). Although the observed frequency, for example, of UGT1A7*3/*3 was 10% in controls and 14% in HCC the expected frequency would have been 2.5% (4-fold lower) in controls and 19.4% (1.4-fold higher) in HCC, assuming HWE. Genotyping was conducted by using a single cDNA fragment derived from polymerase chain reaction (PCR) amplification of genomic patient DNA with a UGT1A7 first exon specific primer located from base pair −61 to −38 upstream of the ATG start codon (5’-ccgctgctagcgtacttattagagt-3’). The subsequent sequencing analysis and temperature gradient electrophoresis analysis were performed with this fragment.

In 2004, after publication of our study in 2001, we identified a separate SNP at position −57 (−57 $\triangleright$G) in the non-coding region of UGT1A7, which co-localizes with the above specified amplification primer. This coincidence, unknown at the time, is a possible explanation for a PCR amplification bias, and the observed deviation from HWE in our reported genotyping data, and has led us to change our genotyping methodology.

Although the data in Table 3 are reproducible with the same methodology, by using a different method (taqMan allelic discrimination analysis) that avoids an influence of −57G $\triangleright$C, different results were obtained. In an expanded collective of 125 HCC and 107 controls containing the originally analyzed cohort, allelic frequencies showed (control/HCC) UGT1A7*1: (36%/31%), UGT1A7*2: (26%/22%), UGT1A7*3: (38%/46%), UGT1A7*4: (1%/0%), which contrast the reported findings of UGT1A7*1: (56%/32%), UGT1A7*2: (24%/21%), UGT1A7*3: (16%/44%), UGT1A7*4: (4%/3%). This considerably drops the significance of an association of UGT1A7*3 with HCC from $P$ = .00038 to $P$ = .036 (Wilcoxon test).

Since publication of this study Tseng et al described UGT1A7 SNPs as a risk factor for HCC and an earlier onset of HCC in males, Stucker et al reported an association with a viral etiology of HCC but not in alcohol-related HCC, and Kong et al described an association with HCC risk in hepatitis B carriers. In all 3 of those studies using different cohorts from around the world, genotyping was performed with different methodologic approaches than that outlined above and used in our study 10 years ago. However, against the background of the technical issue of a SNP co-localizing with the employed amplification primer in our study, the data presented in Table 3 and Figure 4 of our paper and the conclusions drawn from them should be viewed with caution as outlined.

In the second paper published in 2003, the same genotyping methodology as described was also used in the absence of knowledge of the aforementioned non-coding variant at position −57. The data presented in Tables 2 and 3 of that paper are therefore affected similarly. Against this background, conclusions drawn from them should be viewed with caution. This does not affect the tissue distribution data presented in Figure 1. Since publication of this paper, 2 studies have failed to confirm an association of UGT1A7 genotype and pancreatic disease and it is, therefore, unlikely that UGT1A7 variants play a relevant role for pancreas diseases.

However, the risk associated with low activity variants of detoxifying enzymes continues to be an attractive hypothesis for tumor development as other studies have confirmed.

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Conflicts of interest

The authors disclose no conflicts.

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Ribavirin Concentration Is a More Important Predictor of Sustained Viral Response Than Anemia in Hepatitis C Patients

Dear Sir:

In a recent issue of GASTROENTEROLOGY, Sulkowski et al.1 retrospectively analyzed data from the IDEAL study and reported that anemia during pegylated interferon (PEG-IFN)/ribavirin (RBV) treatment in hepatitis C virus (HCV) genotype-1–infected patients was associated with higher rates of sustained viral response (SVR).2 Based on their results, the authors propose a “drug exposure hypothesis,” wherein anemia is a pharmacodynamic marker of RBV exposure, which correlates more closely with antiviral effect than the ingested RBV dose. However, the authors apparently do not consider the distinct possibility that serum RBV levels could predict SVR more accurately than ingested RBV dose or severity of anemia.

Several small studies have already examined the association between hemoglobin decline, plasma or serum RBV concentrations, and treatment response in HCV-monoinfected patients on (PEG)-IFN/RBV treatment. Unfortunately, these studies were either underpowered for multivariate analyses, or multivariate analyses were not performed at all.3–6 Therefore, it remained unclear until now whether both hemoglobin decline and RBV concentrations should be considered as separate entities independently predicting treatment response.

We have recently addressed this issue by performing additional analyses of data derived from the CIRA trial.7 In 242 treatment-naive HCV patients who were treated for ≥24 weeks with PEG-IFN/RBV in combination with amantadine or placebo, week 24 serum RBV concentrations were significantly higher in SVR patients compared with non-SVR patients (median 2.9 mg/L [range, 0.2–5.3] versus median, 2.4 mg/L [range, 0.2–4.0]; P < .001). In contrast, week 24 hemoglobin decline was not significantly different between the 2 groups (P = .22), although serum RBV concentrations significantly correlated with hemoglobin decline (r = 0.42; P < .001). On multivariate analysis, higher serum RBV concentrations were an independent predictor of SVR (adjusted odds ratio, 1.72 for each mg/L incline; 95% confidence interval, 1.13–2.63), whereas hemoglobin decline was not.

Based on these results, we conclude that higher serum RBV concentration rather than hemoglobin decline predicts treatment response. This is to some extent in line with the “drug exposure hypothesis” proposed by Sulkowski et al.,2 because serum RBV concentration is also a marker of RBV exposure. However, because differences in RBV pharmacokinetics and pharmacodynamics between individuals exist,8 anemia is not the ideal marker for RBV exposure. We speculate that differences in absorption and intracellular metabolism of RBV, possibly owing to genetic variation in RBV transporters, might explain why most patients with high RBV concentrations are anemic, whereas some others might not experience anemia at the same RBV concentrations. Future research on RBV concentrations should be aimed at identifying new correlates of higher concentrations and at interventions to increase RBV concentrations in HCV patients on antiviral therapy, such as individualized RBV dosing.

Before measurement of RBV concentration can be incorporated into daily clinical practice, prospective studies are needed to test interventions to increase RBV concentrations and thereby SVR rates of PEG-IFN/RBV therapy.

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