controls that had not come into contact with an SST tube, control limits were not exceeded for the overwhelming majority of results (70 of 71 results, with 1 missing value).

Other factors, such as the volume of the control material, length of incubation (~2 h) in the tube, and contact with the stopper and its lubricant, may influence the magnitude of tube effects. We filled tubes with only 1 mL of control material so as to produce high concentrations of additive and thus maximize any possible effects of the additives on the assays investigated in this study.

Our results emphasize the benefit of strict adherence to one of the basic tenets of QC, namely, that control specimens should be treated identically to specimens from patients. This strategy has revealed the interference attributable to the additives in the variant lot of SST tubes and, had it been in place, would have alerted the laboratory to the interference. We recommend that laboratories consider including this strategy in their QC plan. Periodic or routine processing of controls in blood collection tubes should provide a timely warning of possible interferences by additives in blood collection tubes.

References

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Tissue Differences in the Expression of Mutations and Polymorphisms in the GRHPR Gene and Implications for Diagnosis of Primary Hyperoxaluria Type 2

To the Editor:
Primary hyperoxaluria type 2 (PH2; OMIM 260000) is an inherited disease of endogenous oxalate overproduction arising from mutations in the GRHPR gene encoding glyoxylate reductase. The disease typically presents with urolithiasis or recurrent urinary tract infections and increased urinary oxalate (1). The diagnosis may be supported by t-glyceraciduria, although this does not occur in all cases (2). Definitive diagnosis is currently based on demonstration of diminished glyoxylate reductase activity in a liver biopsy (3), although DNA analysis offers a noninvasive method.

The GRHPR gene maps to the centromeric region of chromosome 9 (4) and, from Northern blot analysis (5), is ubiquitously expressed, although the bulk of enzyme activity is found in the liver (3, 5). Among the described mutations and polymorphisms (5, 6), c.103delG accounts for 37% of mutant alleles, allowing diagnosis of PH2 to be made by genetic testing (5). One of the polymorphisms, c.579G>A, occurs in exon 6, and the G allele has been shown to have a frequency of 0.68 in genomic DNA from PH2 patients (5).

While evaluating leukocyte cDNA for identifying mutations and demonstrating potential splice defects in this gene, we found a lack of expression of mutations in leukocyte cDNA, in contrast to liver cDNA and genomic DNA from the same individual.

We studied liver and blood samples from a patient who presented clinically with features of PH and who subsequently was found, on liver enzyme analysis, to have PH2 (2). Genomic DNA was prepared from EDTA-whole blood with the QiAamp DNA Blood Mini Kit (Qiagen). RNA was isolated from liver by homogenization in RNA isolator (Sigma Genosys) and from blood with the QiAamp Blood Mini Kit (Qiagen), according to the manufacturer’s instructions. Reverse transcriptase-PCR was carried out using a Sensiscript Reverse Transcriptase Kit (Qiagen). Genomic DNA was amplified with intronic primers flanking exons 6 and 7, and cDNA was amplified with primers designed to amplify across intron–exon boundaries (5). Sequencing of PCR products was performed on an ABI 3100 (Applied Biosystems) sequencer either directly after purification using the QIAquick PCR Purification Kit (Qiagen) or after cloning into TA-vector (Invitrogen).

Analysis of GRHPR genomic DNA showed a homozygous 2-bp deletion (c.608-609delCT) mutation and a G allele at position c.579 of cDNA (Fig. 1A). The same genotype was observed in liver cDNA (Fig. 1B). Leukocyte cDNA, however, showed a heterozygous pattern for the mutation as well as the c.579G>A polymorphism. After cloning, 2 transcripts were identified. One of these had the CT deletion mutation and c.579G (Fig. 1C); the other transcript had no mutation and contained the A allele at c.579 (Fig. 1D). The discrepancy was verified by repeat analysis and by analysis of other family members. All controls, including reverse transcriptase-negative control and DNA-free PCR reactions, were negative.

The most likely explanation for these findings, which affect expression of mutations and a polymorphic site, is the presence of 2 highly homologous genes with different expression profiles. A BLAST search (http://
We have now extended our studies to an additional 6 patients with PH2, with a variety of genotypes, and we have found the same mismatch between leukocyte cDNA and genomic DNA. Further work, including sequence analysis of the 5'- and 3'-untranslated regions of liver and leukocyte GRHPR cDNA and of cosmids containing GRHPR genomic DNA, is in progress. However, until a satisfactory explanation is found, it would be ill advised to use GRHPR enzyme or cDNA derived from tissues other than liver for the diagnosis of PH2.

References

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Increased Lipid Concentration Is Associated with Increased Hemolysis

To the Editor:

The relationship between serum lipids and erythrocyte membrane fragility is complex (1). As seen in routine laboratory practice, samples with hypertriglyceridemia or mixed hypercholesterolemia and hypertriglyceridemia can present as visibly turbid (“milkshake” appearance), but more often they present as “strawberry milkshake” because of increased hemolysis. The amount of hemolysis appears to increase as the lipid concentration increases, although the extent of this effect is not well characterized.

We investigated whether a relationship exists between increasing total lipid concentration (cholesterol plus triglycerides) and hemolysis in actual patient samples. To determine this relationship, we assessed all samples collected for lipid analysis during 2003 and 2004. Samples from the hospital emergency department were excluded because such samples have a high incidence of being grossly hemolyzed (2).

As a routine procedure, hyperlipidemic samples with a milkshake appearance and optical turbidity (i.e., it was not possible to see through the sample) were analyzed for cholesterol, triglycerides, and amount of hemolysis [hemolytic index (HI)] on the Hitachi Modular System with Roche reagents (Roche Diagnostics). The HI was determined by measuring the difference between the absorbance of the sample diluted in isotonic saline at 570 and 600 nm. The hemolysis absorbance value was converted to mg/dL by a multiplication factor. All optically turbid samples were ultracentrifuged on an Airfuge (Beckman Airfuge, Beckman Coulter Diagnostics) for 15 min at 20 psi air pressure and 107 000 g (90 000 rpm). The samples were divided into 6 groups based on increasing lipid concentrations (triglycerides and cholesterol; Table 1). The mean HI for each group was then determined. For lipemic samples containing triglyceride concentrations >15 mmol/L, HI was evaluated after ultracentrifugation. Lipemia did not affect the HI when the triglyceride concentration was <15 mmol/L, but when the triglyceride concentration was >15 mmol/L, we observed an increase in spectrophotometric artifacts (data not shown).

We used the association between hyperlipidemia and hemolysis [ln(HI)] to normalize results for all samples. Multiple linear regression analysis of ln(HI) values indicated that both cholesterol (P < 0.001) and triglycerides (P < 0.001) were linearly related to the HI. The data at higher lipid concentrations (Table 1) suggest that the triglyceride concentration has a greater influence on the HI than the cholesterol concentration. Interestingly, for paired groups with similar triglyceride concentrations, those with a higher cholesterol concentration had a slightly lower HI. A detailed discussion as to possible mechanisms for this observation is beyond the scope of this letter.

These results demonstrate that increasing hyperlipidemia (particularly hypertriglyceridemia) is associated with increased hemolysis. Whether this association is causal is debatable. It is possible that increased lipid concentrations alter the lipid composition of the erythrocyte membrane, leading to increased erythrocyte fragility, with subsequent leakage of cellular content such as hemoglobin. This erythrocyte fragility may be exacerbated as blood moves through collection needles and gel pores, increasing the ease with which these cells can be lysed, with a resulting increase in hemolysis. Such an association has been noted in patients with inherited metabolic diseases in which hyperlipidemia is related to primary lipoprotein lipase deficiency (3), Tangier disease (4), and lecithin:cholesterol acyltransferase deficiency (5). In experimental systems, erythrocyte membrane fluidity has decreased as cholesterol increases (1, 6, 7).

In summary, hyperlipidemic samples are associated with increased hemolysis, possibly the result of increased erythrocyte membrane fragility induced by alterations in membrane lipid content.

Table 1. Range of HI observed with increasing total lipid (triglycerides plus cholesterol) concentrations.

<table>
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<tr>
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<tr>
<td>&gt;12</td>
<td>&gt;7</td>
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References


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