People with fatty liver are more likely to have the *PEMT* rs7946 SNP, yet populations with the mutant allele do not have fatty liver

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We reported that individuals with nonalcoholic fatty liver were significantly more likely to have a single nucleotide polymorphism (SNP) in the gene encoding for phosphatidylethanolamine *N*-methyltransferase (*PEMT*). This SNP (rs7946, +5465 G → A) leads to a V175M substitution and is a loss of function mutation (1). Drs. Romeo, Cohen, and Hobbs conducted an interesting study further investigating our observation. They asked whether individuals with the mutant allele (A) were more likely to have fatty liver, and concluded that this is not the case.

At first glance, the two reports appear to be arriving at opposite conclusions. Populations with fatty liver are more likely to have the mutant allele (Song *et al.*), yet populations with the mutant allele do not, on average, have fatty liver (Romeo *et al.*). However, there is an important difference in the questions being asked. When the underlying mechanisms are considered, it is apparent that both observations can be true.

PEMT catalyzes *de novo* synthesis of phosphatidylcholine in liver (2, 3). Loss of function of this enzyme can result in fatty liver because phosphatidylcholine is required for hepatic secretion of triacylglycerol in very low density lipoproteins (VLDL) (4–6). Thus, the rs7946 SNP in *PEMT* slows the rate of removal of fat from the liver. If the rate of production of triacylglycerol exceeds the rate of removal, fatty liver occurs. It is possible that under normal circumstances the rate of triacylglycerol synthesis is slow enough that even the mutant *PEMT* activity is sufficient to sustain adequate export of this fat. Only under conditions of rapid triacylglycerol production (*i.e.*, excess calorie intake) would the defect in *PEMT* become rate-limiting and cause fatty liver. Thus, the rs7946 SNP in *PEMT* could be necessary but not sufficient to cause fatty liver. If this were the case, then individuals with fatty liver would be more likely to have the *PEMT* SNP, but not every individual with the *PEMT* SNP would have fatty liver.

Romeo *et al.* report data from 2349 subjects. We studied only 87 subjects. Romeo included many more African American and Hispanic subjects, and in these two populations a third are wild type, a third heterozygous for the rs7946 SNP, and a third homozy-

gous for the rs7946 SNP. This is very different from the distribution in Caucasians. Our study included mainly Caucasian subjects, so it is most appropriate to compare our results with those observed in Romeo's Caucasian subjects.

These results (Table 1) are remarkably similar. To ascertain whether the A allele is over-represented in subjects with fatty liver, we can use the Fisher's Exact Test (7) (Table 2).

We conclude that the study of Romeo *et al.*, in a very large population, supports our finding that the rs7946 SNP in *PEMT* is more common in Caucasians with fatty liver. The Romeo *et al.* study provides important additional information: 1) Caucasians have a different distribution of this SNP than do Hispanics and African Americans; 2) having this SNP is not sufficient to cause fatty liver, as many individuals with the SNP have normal liver fat. It is likely that this SNP slows export of fat from liver, and results in fatty liver only when triacylglycerol synthesis is accelerated.

TABLE 1. Comparison of genotypes in the Song and Romeo studies^a

	GG	GA	AA
Fatty liver	2 (7.1%) 3 (2.5%)	7 (25%) 45 (36.9%)	19 (67.9%) 74 (60.7%)
Control	11 (18.6%) 36 (5.9%)	24 (40.7%) 457 (42.4%)	24 (40.7%) 313 (51.7%)

^a Numbers of subjects in the Song *et al.* study are indicated in plain typeface; numbers of Caucasian subjects in the Romeo study are indicated in boldface. The percentage of the groups represented by the genotype is shown in parentheses.

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TABLE 2. Probability that the A allele is over-represented in subjects with fatty liver^a

	Song <i>et al.</i>		Romeo <i>et al.</i>	
	G alleles	A alleles	G alleles	A alleles
Fatty liver	11	45	51	193
Control	46	72	329	883
Probability		0.015		0.05

^a Number of alleles present in each group. Probability that A allele is over-represented in the population with fatty liver was calculated using the Fisher's Exact Test (7).

References

1. Song, J., da Costa, K. A., Fischer, L. M., Kohlmeier, M., Kwock, L., Wang, S., and Zeisel, S. H. (2005) Polymorphism of the PEMT gene

and susceptibility to nonalcoholic fatty liver disease (NAFLD). *FASEB J.* **19**, 1266–1271

2. Bremer, J., and Greenberg, D. (1961) Methyl transferring enzyme system of microsomes in the biosynthesis of lecithin (phosphatidylcholine). *Biochim. Biophys. Acta* **46**, 205–216

3. Vance, D. E., Walkey, C. J., and Cui, Z. (1997) Phosphatidylethanolamine *N*-methyltransferase from liver. *Biochim. Biophys. Acta* **1348**, 142–150

4. Yao, Z. M., and Vance, D. E. (1988) The active synthesis of phosphatidylcholine is required for very low density lipoprotein secretion from rat hepatocytes. *J. Biol. Chem.* **263**, 2998–3004

5. Yao, Z. M., and Vance, D. E. (1989) Head group specificity in the requirement of phosphatidylcholine biosynthesis for very low density lipoprotein secretion from cultured hepatocytes. *J. Biol. Chem.* **264**, 11373–11380

6. Yao, Z. M., and Vance, D. E. (1990) Reduction in VLDL, but not HDL, in plasma of rats deficient in choline. *Biochem. Cell. Biol.* **68**, 552–558

7. (2004) Analyze-It for Microsoft Excel. Analyze-It for Microsoft Excel, Ltd., Leeds, UK