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Genetic variation in *PNPLA3* confers susceptibility to nonalcoholic fatty liver disease

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Nonalcoholic fatty liver disease (NAFLD) is a burgeoning health problem of unknown etiology that varies in prevalence among ancestry groups. To identify genetic variants contributing to differences in hepatic fat content, we carried out a genome-wide association scan of nonsynonymous sequence variations (n = 9,229) in a population comprising Hispanic, African American and European American individuals. An allele in *PNPLA3* (rs738409[G], encoding I148M) was strongly associated with increased hepatic fat levels ($P = 5.9 \times 10^{-10}$) and with hepatic inflammation ($P = 3.7 \times 10^{-4}$). The allele was most common in Hispanics, the group most susceptible to NAFLD; hepatic fat content was more than twofold higher in *PNPLA3* rs738409[G] homozygotes than in noncarriers. Resequencing revealed another allele of *PNPLA3* (rs6006460[T], encoding S453I) that was associated with lower hepatic fat content in African Americans, the group at lowest risk of NAFLD. Thus, variation in *PNPLA3* contributes to ancestry-related and inter-individual differences in hepatic fat content and susceptibility to NAFLD.

In humans, adipose tissue serves as a reservoir to limit the deposition of triglyceride in the liver and other metabolically active tissues[1]. The effectiveness of this buffer in protecting against the accumulation of fat in the liver varies widely among individuals: hepatic fat content ranges from less than 1% to more than 50% of liver weight in the general population[2]. The accumulation of excess triglyceride in the liver, a condition known as hepatic steatosis (or fatty liver), is associated with adverse metabolic consequences, including insulin resistance and dyslipidemia[3,4]. In a subset of individuals, hepatic steatosis promotes an inflammatory response in the liver, referred to as steatohepatitis, which can progress to cirrhosis and liver cancer[3,5]. Nonalcoholic fatty liver disease (NAFLD) is the most common form of liver disease in Western countries[6]. Approximately 10% of liver transplants done in the United States are for cirrhosis related to NAFLD[4].

Factors promoting deposition of fat in the liver include obesity, diabetes, insulin resistance and alcohol ingestion[3,6]. The propensity to develop hepatic steatosis differs among ancestry groups, with African Americans having a lower (24%) and Hispanics a higher (45%) frequency of the disorder than European Americans (33%) in a large US urban population[2]. Hispanics also have a higher prevalence of steatohepatitis and cirrhosis, whereas African Americans are less prone to develop liver failure[2,7–9]. The factors responsible for these ancestry-related differences in prevalence of hepatic steatosis and liver injury are not known.

To identify DNA sequence variations that contribute to inter-individual differences in NAFLD, we carried out a genome-wide survey of nonsynonymous sequence variations in a population-based study comprising individuals of various ancestries, the Dallas Heart Study[10]. We limited our analysis to nonsynonymous sequence variations to focus on variants with a higher likelihood of affecting gene function. Hepatic fat content was measured in the Dallas Heart Study using proton magnetic resonance spectroscopy (^{1}H-MRS), the most accurate, quantitative, noninvasive method available[2,11,12]. Of the 12,138 nonsynonymous variants assayed using chip-based oligonucleotide hybridization[13], 9,229 exceeded the quality control threshold for the study (see Methods) and were included in the analysis.

We tested each variant for association with hepatic fat content in 1,032 African American, 696 European American and 383 Hispanic study participants in the Dallas Heart Study who obtained ^{1}H-MRS of the liver[2]. To maximize statistical power, we pooled the three ancestry groups, and included a global ancestry score (calculated using a panel of 2,270 ancestry-informative SNPs) in the model to control for population stratification (see Methods). The quantile-quantile plot of P values showed no systematic deviation from the null distribution (**Fig. 1a**).

A single variant in *PNPLA3* (rs738409) was strongly associated with hepatic fat content ($P = 5.9 \times 10^{-10}$; **Fig. 1b**). The variant is a cytosine to guanine substitution that changes codon 148 from isoleucine to methionine; this residue is highly conserved in vertebrates (**Fig. 2a**).

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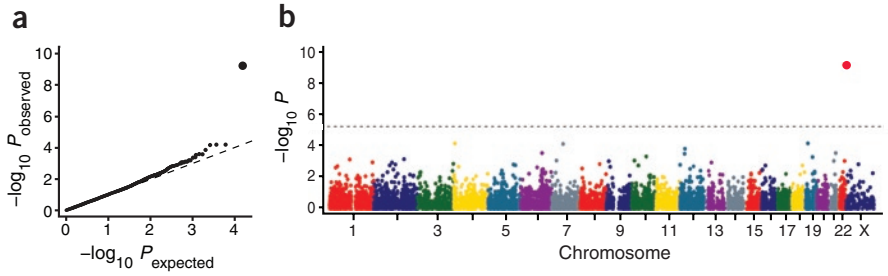


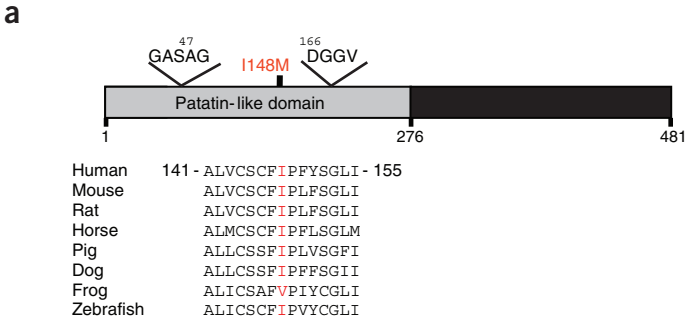
Figure 1 Genome-wide scan of liver triglyceride content measured by proton magnetic resonance imaging in the Dallas Heart Study ($n = 2,111$). (**a**) Quantile-quantile plot of P values. (**b**) Scatter plot of P values. The dashed line denotes the Bonferroni-corrected significance threshold ($P = 5.4 \times 10^{-6}$).

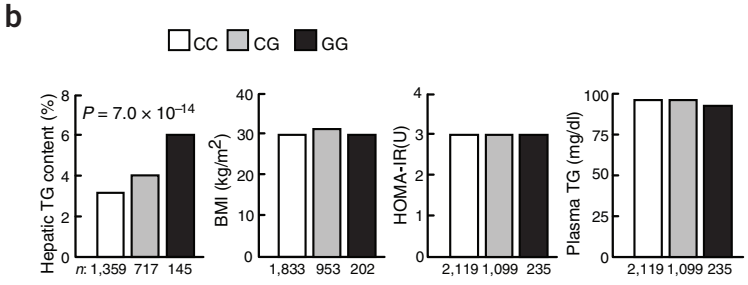
PNPLA3 encodes a 481 amino acid protein of unknown function that belongs to the patatin-like phospholipase family[14]. The progenitor of this family, patatin, is a major protein of potato tubers and has non-specific lipid acyl hydrolase activity[15,16]. None of the other nonsynonymous sequence variants tested in the genome-wide scan exceeded the Bonferroni-corrected threshold for significance ($P = 5.4 \times 10^{-6}$; **Fig. 1b**).

The association between *PNPLA3* rs738409 and hepatic fat content remained highly significant ($P = 7.0 \times 10^{-14}$) after adjustment for body mass index (BMI), diabetes status, ethanol use, as well as global and local ancestry (**Fig. 2b**), and was apparent in all three ancestry groups (**Fig. 2c** and **Supplementary Table 1** online). Thus, the association between rs738409 and hepatic fat content was not attributable either to the effect of known risk factors for liver fat accumulation or to population stratification.

The frequencies of the *PNPLA3* rs738409[G] allele were concordant with the relative prevalence of NAFLD in the three ancestry groups[2]; the highest frequency of the allele was in Hispanics (0.49), with lower frequencies observed in European Americans (0.23) and African Americans (0.17). Accordingly, we examined the relationship between *PNPLA3* rs738409 and evidence of hepatic inflammation, as indicated by release of liver enzymes into the circulation, and found a significant elevation in serum concentrations of alanine aminotransferase (ALT) in association with the *PNPLA3* rs738409[G] allele ($P = 3.7 \times 10^{-4}$). Analysis of the three ancestry groups revealed that the association with ALT was limited to Hispanics ($P = 1.3 \times 10^{-5}$), the group with the greatest prevalence of hepatic steatosis and susceptibility to cirrhosis (**Supplementary Table 1**)[2,7]. The *PNPLA3* rs738409[G] allele was also associated with serum aspartate aminotransferase concentrations in Hispanics ($P = 0.002$). These findings are consistent with our prior observation that a higher proportion of Hispanics with hepatic steatosis have evidence of hepatic inflammation[7], and suggests that the *PNPLA3* rs738409[G] allele adversely affects liver function.

Increased hepatic fat content is associated with insulin resistance and dyslipidemia (increased plasma levels of triglyceride and lower levels of high-density-lipoprotein cholesterol), but the causal nature of these relationships remains poorly defined[3]. We did not find any association between the *PNPLA3* rs738409[G] allele and BMI or indices of insulin sensitivity, including fasting glucose and insulin concentrations or homeostatic model assessment of insulin resistance (HOMA-IR), in the entire Dallas Heart Study (**Fig. 2b** and **Supplementary Table 1**). No associations were observed between *PNPLA3* genotype and plasma concentrations of triglyceride (**Fig. 2b**), total cholesterol, high-density-lipoprotein cholesterol or low-density-lipoprotein cholesterol (**Supplementary Table 1**). A corresponding analysis in a larger sample ($n = 14,821$), the Atherosclerosis Risk in Communities Study (ARIC)[17], also showed no association of *PNPLA3* rs738409 with BMI, indices of insulin sensitivity, or plasma concentrations of triglyceride or high-





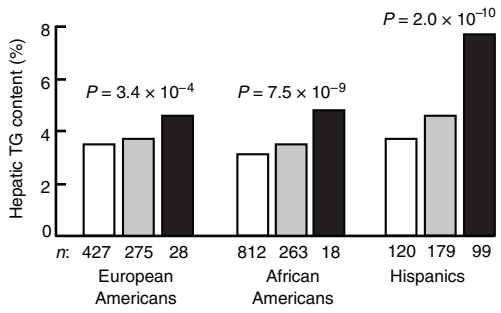


Figure 2 Association between a sequence variant in *PNPLA3* (rs738409) and hepatic triglyceride content. (**a**) PNPLA3 is a 481-residue protein that contains a patatin-like domain at the N terminus with consensus sequences for a Ser-Asp catalytic dyad (Gly-X-Ser-X-Gly and Asp-X-Gly/Ala)[14]. The I148M substitution (rs738409) is located between the consensus sequences for the catalytic dyad and is highly conserved. (**b**) Median hepatic triglyceride (TG) content, body mass index (BMI), homeostatic model assessment of insulin resistance (HOMA-IR) and plasma TG levels for individuals by *PNPLA3* genotype in the Dallas Heart Study. Values for hepatic fat content were compared using ANOVA. Age, sex, BMI, diabetes status, ethanol use and global ancestry were included as covariates in the model. (**c**) Median hepatic fat contents and *PNPLA3* rs738409 genotypes in European Americans, African Americans and Hispanics in the Dallas Heart Study. Associations between hepatic fat content and *PNPLA3* rs738409 genotypes were tested using ANOVA with age, local ancestry, sex, diabetes status, ethanol intake and BMI as covariates.

density-lipoprotein cholesterol (**Supplementary Table 2** online). On the basis of the observed associations between the SNP and hepatic fat and between hepatic fat and HOMA-IR in the Dallas Heart Study, we calculated the power to detect an association with HOMA-IR to be >96% in the African Americans and 91% in the European Americans in ARIC.

The data from these studies indicate that the *PNPLA3* rs738409[G] allele is associated with a systematic increase in hepatic fat content but not with major alterations in glucose homeostasis or lipoprotein metabolism. Thus, increased hepatic fat content does not inevitably lead to insulin resistance, which is consistent with recent observations in some animal models[18,19].

To determine whether other sequence variations in *PNPLA3* contribute to differences in hepatic fat content, we resequenced the coding region of *PNPLA3* in the 80 men (32 African Americans, 32 European Americans and 16 Hispanics) and 80 women who had the highest levels of hepatic fat in the Dallas Heart Study ('high group'), and in a sex- and ancestry-matched group with the lowest levels of hepatic fat ('low group')[2]. The number of individuals with nonsynonymous variants found only in the high group ($n = 8$) was similar to the number of individuals with nonsynonymous variants specific to the low group ($n = 9$), but the three individuals with likely null mutations (FS-Y21 (p.Tyr21fs, 64delC) and IVS7+1 (c.1112+1G>T)) were all in the high group (**Fig. 3a**), which is consistent with the hypothesis that loss of function of PNPLA3 causes an increase in hepatic triglyceride content.

Eight variants were present in both the low and the high hepatic fat groups (**Fig. 3a** and **Supplementary Table 3** online), and the six most common of these sequence variations were genotyped in the entire sample. One variant, *PNPLA3* rs6006460[T], which substitutes an isoleucine for serine in codon 453, was common in African Americans (MAF = 0.104) but rare in European Americans (0.003) and Hispanics (0.008) (**Supplementary Table 3**) and was associated with a significantly lower liver fat content. Median hepatic triglyceride content was 18% lower in African Americans with the *PNPLA3* rs6006460[T] when compared to African Americans homozygous for the wild-type allele (3.3% versus 2.7%, $P = 6.0 \times 10^{-4}$; **Fig. 3b**). Further evidence that the variant was associated with lower hepatic fat content was provided by the finding that, of individuals with the *PNPLA3* rs6006460[T] allele, a significantly greater number had a hepatic fat content in the lowest decile of the population than in the highest decile (**Fig. 3b**). We did not find any significant difference in the number of individuals identified in the extremes for any of the other SNPs (data not shown).

The effect of *PNPLA3* rs6006460[T] on hepatic fat content was independent of the *PNPLA3* rs738409 polymorphism. Both variants were statistically significant when included in a multiple regression model, and the *PNPLA3* rs6006460[T] allele was significantly associated with hepatic fat in African Americans homozygous for the *PNPLA3* rs738409[C] allele (data not shown). The identification of a second allele of *PNPLA3* that was independently associated with hepatic fat content further supports a role for PNPLA3 in determining hepatic triglyceride levels, and indicates the presence of both loss-of-function and gain-of-function alleles at this locus. The mechanisms by which these alleles affect hepatic fat content are not known.

The frequencies of both *PNPLA3* rs738409[G] and rs6006460[T] in the three ancestry groups represented in the Dallas Heart Study are concordant with ancestry-related differences in hepatic fat content[2]. Exclusion of the individuals carrying either of these two alleles substantially attenuated the differences in hepatic fat content between the ancestry groups; regression analysis indicated that these two sequence variations accounted for 72% of the observed ancestry-related differences in hepatic fat content in the Dallas Heart Study. Thus, genetic variation in *PNPLA3* accounts for a large fraction of the ancestry-related differences in the propensity to accumulate excess fat in the liver in this study population.

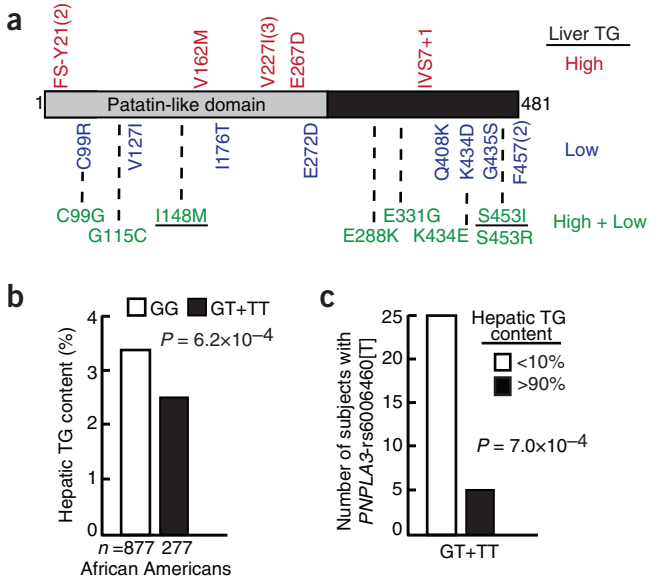


Figure 3 Identification of a *PNPLA3* allele (rs6006460, encoding S453I) associated with lower hepatic fat content in African Americans in the Dallas Heart Study. (**a**) Exons and flanking introns of *PNPLA3* were sequenced in the 32 European American and 32 African American men and women and in the 16 Hispanic men and women with the lowest and highest hepatic triglyceride (TG) content determined using proton magnetic resonance imaging[2]. The substitutions encoded by the nonsynonymous variations identified in individuals in only the high, only the low and in both the high and low groups are shown. All the variants not found in both groups were present in only a single subject unless otherwise indicated by a number in parentheses.The rs numbers for polymorphisms and the oligonucleotides used for PCR-sequencing of the coding regions are provided in **Supplementary Tables 3** and **4**. (**b**) Median hepatic triglyceride content in African Americans homozygous for the wild-type *PNPLA3* allele (rs6006460[G], GG) or carrying the rs6006460[T] allele (GT + TT). (**c**) Number of individuals with *PNPLA3* rs6006460[T] in the upper and lower deciles of hepatic fat content.

The physiological substrate(s) of PNPLA3 has not been defined. Expression of *PNPLA3* is under metabolic control in adipose tissue and liver; mRNA levels are low in the fasted state and increase markedly with carbohydrate feeding[20,21]. PNPLA3 structurally resembles calcium-independent phospholipase A_2, but the recombinant protein has low phospholipase activity when expressed in insect (Sf9) cells[22]. PNPLA3 has more robust activity against triglyceride *in vitro* and can also transfer fatty acids to and from mono- and diacylglycerol[22]. It is not known whether the primary effect of PNPLA3 in the liver is to hydrolyze triglyceride or to transfer fatty acids between lipids (transacetylation). Studies are in progress to determine the specific effects on lipid metabolism of the nonsynonymous variants in *PNPLA3* identified in this study.

Currently, we cannot accurately predict which individuals with fatty liver will develop steatohepatitis and progress to cirrhosis and liver failure. The finding that markers of liver inflammation (serum levels of liver-derived enzymes) were elevated in *PNPLA3* rs738409[G] carriers, which was also observed in an independent genome-wide association study[23], suggests that this genetic variant may confer increased susceptibility to hepatic injury. Patatin-like phospholipase family mem-

bers in other organisms are upregulated in response to environmental insults[24]. The sequence variations in *PNPLA3* reported here may provide predictive information regarding the risk of developing hepatic steatosis and liver injury in response to environmental stresses such as caloric excess, infections or drugs.

METHODS

Study populations. The Dallas Heart Study is a population-based probability sample of Dallas County. The sampling frame and the study design have been described in detail[10]. African Americans were oversampled (52% African American, self-identified as 'black'; 29% European American, self-identified as 'white'; 17% Hispanic self-identified as 'hispanic'; and 2% other ancestries). The institutional review board of University of Texas Southwestern Medical Center approved the study, and all study subjects provided written informed consent. We determined alcohol consumption according to answers to previously validated questions[2]. Blood pressure, height, weight and BMI and calculated variables were measured as described[10]. We obtained fasting blood samples from 3,551 subjects (ages 30–65), and 2,971 of these individuals completed a clinic visit; we measured hepatic triglyceride content using ^{1}H-MRS in 2,240 African Americans, European Americans and Hispanics[7,12].

The association between *PNPLA3* rs738409 and metabolic phenotypes was also examined in the Atherosclerosis Risk in Communities Study (ARIC), a large prospective study that focuses on cardiovascular disease in European Americans and African Americans. Details of the ARIC study design and the methods used to measure plasma lipid levels have been published previously[17,25]. The data used in this analysis were collected from the baseline examination.

Genome-wide association and other statistical methods. Genome-wide association analysis was done using 12,138 nonsynonymous sequence variations from dbSNP and the Perlegen SNP database (available on request). We assayed SNPs in 3,383 Africans American, European American and Hispanic participants of the Dallas Heart Study using high-density oligonucleotide arrays (Perlegen Sciences). SNPs that met any of the following criteria were excluded (n = 2,623): error probability >20%, genotype call rate <0%, or a significant deviation from Hardy-Weinberg equilibrium ($P < 0.0001$). Of the 9,515 SNPs that were successfully assayed, 286 were monomorphic in the Dallas Heart Study sample. We tested the remaining 9,229 variants for association with hepatic fat content in the 2,111 African American, European American and Hispanic subjects in the Dallas Heart Study who underwent ^{1}H-MRS of the liver[2] and in whom ancestry-informative SNPs had been assayed previously; global and local ancestries were inferred for each individual using STRUCTURE[26] under a linkage model with 2,270 ancestry-informative SNPs[27]. We pooled all participants together and inferred global ancestry (the probability of an individual belonging to a given cluster), setting the number of clusters, K, equal to 3. Although the ancestry-informative SNP panel was primarily designed for African Americans (mean multipoint information content[28] ($\overline{IC}$) = 0.82), it was adequately informative in European Americans ($\overline{IC}$ = 0.63) and Hispanics ($\overline{IC}$ = 0.66). We also inferred local ancestry as the probability that a particular genomic region belonged to a given cluster. The results were almost identical when ancestry adjustment was done with the same SNPs using principal-component analysis (data not shown).

The statistical significance of 9,229 SNPs in the genome-wide association study was assessed using analysis of variance (ANOVA). To accommodate confounding factors, we included age, sex and global ancestry as covariates in the model. The additive effect of each variant was tested by encoding the genotype variable as 0, 1 or 2. Because the distribution of hepatic fat levels is highly skewed, we applied a power transformation ($\lambda = 1/4$) to the trait before the analysis. To account for multiple testing, we adjusted the significance threshold for the number of tests done using the Bonferroni method. SNPs with a nominal $P < 5.4 \times 10^{-6}$ were considered significant on a genome-wide scale.

We tested the association between *PNPLA3* variants and hepatic fat content within each ancestry group using ANOVA, including age, sex, BMI, diabetes status, ethanol use and local ancestry as covariates. Individuals whose genetic ancestry was not consistent with their self-reported ancestry (n = 11, 5 and 16 for African Americans, European Americans and Hispanics, respectively) and who had a fractional ancestry that was more than three times the interquartile range below the 25th percentile for their reference group were excluded from the analysis. Because the distribution of hepatic triglyceride content is skewed, we reported medians and interquartile ranges.

We analyzed the association of *PNPLA3* rs738409 with BMI, HOMA-IR and plasma triglyceride levels in the African Americans, Europeans Americans and Hispanics together using ANOVA including age, sex and local ancestry as covariates. HOMA-IR was adjusted for BMI, and plasma triglyceride levels were adjusted for BMI and diabetes.

To determine the contribution of the sequence variations we identified in *PNPLA3* to the ancestry-related differences in hepatic fat content, we examined the proportion of variance explained by ancestry ($R1$) using a linear model. We then determined the proportion of variance explained by ancestry after adjustment for the *PNPLA3* alleles (rs738409[G] and rs6006460[T]) ($R2$). The proportion of variance due to ancestry and explained by these two alleles was determined from $(R1 - R2)/R1$.

Resequencing *PNPLA3*. The exons and flanking introns of *PNPLA3* were sequenced as described previously[29] in the African American, European American and Hispanic men and women in the Dallas Heart Study with the highest and lowest hepatic triglyceride content. Oligonucleotide primers used for sequencing are shown in **Supplementary Table 4** online. All sequence variants identified were verified by manual inspection of the chromatograms, and missense changes were confirmed by an independent resequencing reaction.

Genotyping assays. We developed fluorogenic 5′-nucleotidase assays for *PNPLA3* rs738409 and for the sequence variants identified in both the high and low hepatic triglyceride groups in the resequencing experiments. Sequence variations in *PNPLA3* were assayed using the TaqMan assay system (Applied Biosystems) on a 7900HT Fast Real-Time PCR instrument. Probes and reagents were purchased from Applied Biosystems.

Accession codes. GenBank: *PNPLA3*, NM_025225; PNPLA3, NP_079501.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

H.H.H., J.C.C., E.B., L.A.P. and D.C. conceived, designed and directed the study; J.K., S.R., C.X. and A.P. performed and interpreted the genetic analysis. All the authors approved the final manuscript and contributed critical revisions to its intellectual content.

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1. Wang, M.Y. *et al.* Adipogenic capacity and the susceptibility to type 2 diabetes and metabolic syndrome. *Proc. Natl. Acad. Sci. USA* **105**, 6139–6144 (2008).
2. Browning, J.D. *et al.* Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* **40**, 1387–1395 (2004).
3. Browning, J.D. & Horton, J.D. Molecular mediators of hepatic steatosis and liver injury. *J. Clin. Invest.* **114**, 147–152 (2004).
4. McCullough, A.J. The clinical features, diagnosis and natural history of nonalcoholic fatty liver disease. *Clin. Liver Dis.* **8**, 521–533 (2004).
5. Adams, L.A. *et al.* The natural history of nonalcoholic fatty liver disease: a population-based cohort study. *Gastroenterology* **129**, 113–121 (2005).
6. de Alwis, N.M. & Day, C.P. Non-alcoholic fatty liver disease: the mist gradually clears. *J. Hepatol.* **48** (Suppl. 1), S104–S112 (2008).
7. Browning, J.D., Kumar, K.S., Saboorian, M.H. & Thiele, D.L. Ethnic differences in the prevalence of cryptogenic cirrhosis. *Am. J. Gastroenterol.* **99**, 292–298 (2004).
8. Clark, J.M., Brancati, F.L. & Diehl, A.M. The prevalence and etiology of elevated aminotransferase levels in the United States. *Am. J. Gastroenterol.* **98**, 960–967 (2003).

9. Caldwell, S.H., Harris, D.M., Patrie, J.T. & Hespenheide, E.E. Is NASH underdiagnosed among African Americans? *Am. J. Gastroenterol.* **97**, 1496–1500 (2002).
10. Victor, R.G. *et al.* The Dallas Heart Study: a population-based probability sample for the multidisciplinary study of ethnic differences in cardiovascular health. *Am. J. Cardiol.* **93**, 1473–1480 (2004).
11. Longo, R. *et al.* Proton MR spectroscopy in quantitative *in vivo* determination of fat content in human liver steatosis. *J. Magn. Reson. Imaging* **5**, 281–285 (1995).
12. Szczepaniak, L.S. *et al.* Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. *Am. J. Physiol. Endocrinol. Metab.* **288**, E462–E468 (2005).
13. Hinds, D.A. *et al.* Whole-genome patterns of common DNA variation in three human populations. *Science* **307**, 1072–1079 (2005).
14. Wilson, P.A., Gardner, S.D., Lambie, N.M., Commans, S.A. & Crowther, D.J. Characterization of the human patatin-like phospholipase family. *J. Lipid Res.* **47**, 1940–1948 (2006).
15. Rydel, T.J. *et al.* The crystal structure, mutagenesis, and activity studies reveal that patatin is a lipid acyl hydrolase with a ser-asp catalytic dyad. *Biochemistry* **42**, 6696–6708 (2003).
16. Strickland, J.A., Orr, G.L. & Walsh, T.A. Inhibition of *Diabrotica* larval growth by patatin, the lipid acyl hydrolase from potato tubers. *Plant Physiol.* **109**, 667–674 (1995).
17. National Heart, Lung, and Blood Institute. *Atherosclerosis Risk in Communities (ARIC) Study. Operations Manual No 2: Cohort Component Procedures.* (ARIC Coordinating Center, School of Public Health, University of North Carolina, Chapel Hill, North Carolina, 1987).
18. Minehira, K. *et al.* Blocking VLDL secretion causes hepatic steatosis but does not affect peripheral lipid stores or insulin sensitivity in mice. *J. Lipid Res.* **49**, 2038–2044 (2008).
19. Monetti, M. *et al.* Dissociation of hepatic steatosis and insulin resistance in mice overexpressing DGAT in the liver. *Cell Metab.* **6**, 69–78 (2007).
20. Baulande, S., Lassnier, F., Lucas, M. & Pairault, J. Adiponutrin, a transmembrane protein corresponding to a novel dietary- and obesity-linked mRNA specifically expressed in the adipose lineage. *J. Biol. Chem.* **276**, 33336–33344 (2001).
21. Lake, A.C. *et al.* Expression, regulation, and triglyceride hydrolase activity of adiponutrin family members. *J. Lipid Res.* **46**, 2477–2487 (2005).
22. Jenkins, C.M. *et al.* Identification, cloning, expression, and purification of three novel human calcium-independent phospholipase A_2 family members possessing triacylglycerol lipase and acylglycerol transacylase activities. *J. Biol. Chem.* **279**, 48968–48975 (2004).
23. Yuan, X. *et al.* Population-based genome-wide association studies reveal six loci influencing plasma levels of liver enzymes. *Am. J. Hum. Genet.* (in the press).
24. Rietz, S., Holk, A. & Scherer, G.F. Expression of the patatin-related phospholipase A gene AtPLA IIA in *Arabidopsis thaliana* is up-regulated by salicylic acid, wounding, ethylene, and iron and phosphate deficiency. *Planta* **219**, 743–753 (2004).
25. Brown, S.A. *et al.* Plasma lipid, lipoprotein cholesterol, and apoprotein distributions in selected U.S. communities. *Arterioscler. Thromb.* **13**, 1139–1158 (1993).
26. Falush, D., Stephens, M. & Pritchard, J.K. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* **164**, 1567–1587 (2003).
27. Smith, M.W. *et al.* A high-density admixture map for disease gene discovery in African Americans. *Am. J. Hum. Genet.* **74**, 1001–1013 (2004).
28. Zhu, X. *et al.* Admixture mapping for hypertension loci with genome-scan markers. *Nat. Genet.* **37**, 177–181 (2005).
29. Romeo, S. *et al.* Population-based resequencing of *ANGPTL4* uncovers variations that reduce triglycerides and increase HDL. *Nat. Genet.* **39**, 513–516 (2007).