Genome-Wide Association Study of Liver Fat: The Multiethnic Cohort Adiposity Phenotype Study

S. Lani Park,1 Yuqing Li,2 Xin Sheng,1 Victor Hom,1 Lucy Xia,1 Loreall Pooler,1 V. Wendy Setiawan,1 Unhee Lim,1 Kristine R. Monroe,1 Lynne R. Wilkens,3 Bruce S. Kristal,4,5 Johanna W. Lampe,6 Meredith Hullar,6 John Shepherd,3 Lenora L. M. Loo,3 Thomas Ernst,7 Adrian A. Franke,3 Maarit Tiirikainen,3 Christopher A. Haiman,1 Daniel O. Stram,1 Loïc Le Marchand,3 and Iona Cheng2

The global rise in fatty liver is a major public health problem. Thus, it is critical to identify both global and population-specific genetic variants associated with liver fat. We conducted a genome-wide association study (GWAS) of percent liver fat and nonalcoholic fatty liver disease (NAFLD) assessed by magnetic resonance imaging in 1,709 participants from the population-based Multiethnic Cohort Adiposity Phenotype Study. Our participants comprised older adults of five U.S. racial/ethnic groups: African Americans (n = 277), Japanese Americans (n = 424), Latinos (n = 348), Native Hawaiians (n = 274), and European Americans (n = 386). The established missense risk variant rs738409 located in patatin-like phospholipase domain containing 3 (PNPLA3) at 22q13 was confirmed to be associated with percent liver fat ($P = 3.52 \times 10^{-15}$) but more strongly in women than men ($P_{heterogeneity} = 0.002$). Its frequency correlated with the prevalence of NAFLD across the five ethnic/racial groups. Rs738409 was also associated with homeostasis model assessment of insulin resistance (HOMA-IR) ($\beta = 0.028$; $P = 0.009$) and circulating levels of insulin ($\beta = 0.022$; $P = 0.020$) and alanine aminotransferase ($\beta = 0.016$; $P = 0.030$). A novel association of percent liver fat with rs77249491 (located at 6q13 between limb region 1 domain containing 1 [LMBRD1] and collagen type XIX alpha 1 chain [COL19A1] ($P = 1.42 \times 10^{-8}$) was also observed. Rs7724941 was associated with HOMA-IR ($\beta = 0.12$; $P = 0.005$), insulin ($\beta = 0.11$; $P = 0.003$), triglycerides ($\beta = 0.059$; $P = 0.01$), high-density lipoprotein ($\beta = −0.046$; $P = 0.04$), and sex hormone binding globulin ($\beta = −0.084$; $P = 0.0012$). This variant was present in Japanese Americans (minor allele frequency [MAF], 8%) and Native Hawaiians (MAF, 2%). Conclusion: We replicated the PNPLA3 rs738409 association in a multiethnic population and identified a novel liver fat risk variant in Japanese Americans and Native Hawaiians. GWASes of percent liver fat in East Asian and Oceanic populations are needed to replicate the rs77249491 association. (Hepatology Communications 2020;4:1112-1123).

Fatty liver is a major public health problem. High levels of liver fat are associated with dysmetabolism, metabolic syndrome, and insulin resistance that can progress to adverse clinical conditions, such as chronic liver disease, cirrhosis, and hepatocellular carcinoma. (1,2) Nonalcoholic fatty liver disease (NAFLD), defined as the accumulation of liver fat not caused by heavy alcohol use, is projected to exceed viral hepatitis as the primary cause of liver disease. (1) The primary risk factors for high levels of

Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; COL19A1, collagen type XIX alpha 1 chain; CRP, C-reactive protein; DXA, dual energy X-ray absorptiometry; GCKR, glucokinase regulator; GWAS, genome-wide association study; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; LD, linkage disequilibrium; LDL, low-density lipoprotein; LMBRD1, limb region 1 domain containing 1; MAF, minor allele frequency; MEC-APS, Multiethnic Cohort Adiposity Phenotype Study; MRI, magnetic resonance imaging; NAFLD, nonalcoholic fatty liver disease; OR, odds ratio; PNPLA3, patatin-like phospholipase domain containing 3; QC, quality control; ROI, region of interest; SHBG, sex hormone binding globulin; SNP, single-nucleotide polymorphism; TM6SF2, transmembrane 6 superfamily member 2.

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liver fat include obesity, sedentary lifestyle, diet, and genetics. Body weight reduction, diet modifications, and increased physical activity are the only means to reduce liver fat and limit progression to adverse liver conditions. Thus, identifying populations at high risk for liver fat that may benefit from early detection and targeted lifestyle interventions would aid in the prevention of fatty liver conditions.

Fatty liver is a heritable trait with reported heritability estimates ranging from 22% to 38%. Prior genome-wide association studies (GWASes) of liver fat have mostly included patients diagnosed with NAFLD based on elevated liver enzymes, computed tomography (CT), magnetic resonance imaging (MRI), magnetic resonance spectroscopy imaging of hepatic steatosis, or liver biopsy and have treated liver fat as a binary trait as opposed to a continuous measure from CT or MRI. While approximately 14 variants have been associated with nonalcoholic fatty liver,(5-7) only three independent risk loci (P < 5 × 10⁻⁸) have been consistently replicated to date.(5) The patatin-like phospholipase domain containing 3 (PNPLA3) missense variant rs738409 (I148M) is the best established NAFLD risk variant.(6,8,9) PNPLA3 encodes for triacylglycerol lipase that is expressed in adipocytes and hepatocytes, and the variant G allele in rs738409 (I148M) reduces PNPLA3 enzymatic lipase activity. The transmembrane 6 superfamily member 2 (TM6SF2) missense variant rs58542926 (E167K) was first identified in an exome-wide association study of patients with NAFLD.(8) TM6SF2 is a regulator of liver fat metabolism, and lower activity of this enzyme has been associated with reduced secretion of triglyceride-rich lipoproteins.(10) The T allele of rs58542926, which results in a change in amino acid from glutamic acid to lysine, has been associated with higher liver triglyceride content and lower circulating triglyceride levels.(8) The glucokinase regulator (GCKR) gene variant rs780094 has been associated with NAFLD and metabolic traits.(7,11) GCKR encodes for a regulatory protein that inhibits glucokinase in both the liver and pancreatic islet cells.

The majority of prior GWASes of liver fat have focused on patients with NAFLD based on single ancestral populations of Europeans or East Asians.(7,12-14) The most racially/ethnically diverse study was conducted in the Dallas Heart Study and included 1,324 African Americans, 882 European Americans, 467 Latinos, and 63 others.(6,8) A GWAS of liver fat among a multiethnic sample of the general population with wide ranges in liver fat and genetic variation is a powerful and efficient approach to discover novel risk variants.(15) In a report from the Multiethnic Cohort Adiposity Phenotype Study (MEC-APS), we observed striking differences in the distribution of percent liver fat across five racial/
ethnic groups ($P$ heterogeneity $< 1.6 \times 10^{-12}$).\cite{16} For instance, we found that the total fat-adjusted mean percent liver fat for Japanese American men and women (5.8% and 7.1%, respectively) was 2-fold greater than for African American men and women (3.6% and 3.2%, respectively), the group with the lowest amount of liver fat.\cite{16} After correction for sampling fractions, Latinos had the highest prevalence of NAFLD (52.0%) and African Americans had the lowest prevalence (20.2%).\cite{16}

In this report, we describe a GWAS of liver fat and NAFLD in the MEC-APS, a comprehensive population-based study of adiposity phenotypes and obesity-associated biomarkers (measured in serum or plasma) conducted among African Americans, Japanese Americans, Latinos, Native Hawaiians, and European Americans. Liver fat and NAFLD (liver fat $>5.5\%$) were quantified by abdominal MRI, a noninvasive reference assessment method.\cite{17} With a focus on investigating the genetic determinants of liver fat as a continuous trait, we improved our ability (study power) to detect modest effects and to quantify the effects by allelic dose across and within specific populations. Furthermore, with our comprehensive measures of body size, such as clinically measured height and dual energy X-ray absorptiometry (DXA)-measured total fat mass, we were able to account for confounding and/or effect modification due to variation in adiposity across populations. Lastly, for the most significant variants, we examined their relationships with circulating concentrations of obesity-related clinical biomarkers to evaluate their possible biological effects.

Participants and Methods

STUDY POPULATION

The MEC study was established in 1993-1996 to examine diet, lifestyle factors, and genetic susceptibility in relation to cancer.\cite{18} It is an ongoing prospective study of more than 215,000 adult men and women primarily of African American, Japanese American, Latino, Native Hawaiian, and European American ancestry. In 2013-2016, the MEC-APS was conducted to identify predictors of body fat distribution and to examine their associations with obesity-related cancers. Study recruitment and methods have been described in detail.\cite{16} In brief, 1,861 men and postmenopausal women who were healthy, not currently smoking, no history of chronic hepatitis, aged 60-77 years, and a body mass index (BMI) between 17.1-46.2 kg/m² were enrolled in the study with stratified sampling by sex, race/ethnicity, and six BMI categories. All MEC-APS participants provided written informed consent that was approved by the respective institutional review boards (IRBs) at the University of Hawaii (UH) or University of Southern California (USC). The IRBs at UH (CHS-#17200), USC (#HS-12-00623), and University of California, San Francisco (#17-23399) approved the study protocol, which conforms to the 1975 Helsinki Declaration. Study participants underwent an abdominal MRI and body composition assessment by whole-body DXA and completed an in-person interview, blood collection, and self-administered questionnaire. Ninety-one participants were excluded after quality control (QC) filtering of the genotype data (see below), and 61 were excluded for invalid MRI scans (due to motion artifacts or presence of intravisceral masses). Thus, the final study population for analysis was comprised of 1,709 participants (Supporting Fig. S1).\cite{16}

PERCENTAGE LIVER FAT, NAFLD, TOTAL FAT MASS

The MRI scanners Siemens TIM Trio at UH and General Electric HDx at USC, both with a 3-tesla magnetic field strength, were used to quantify liver fat. The MRI protocol assessed four abdominal intervertebral segments of the intra-abdominal cavity (L1-L2, L2-L3, L3-L4, L4-L5) and did not include the intrathoracic or intrapelvic cavity. Percent liver fat was estimated from a series of axial triple gradient-echo Dixon-type scans (10-mm slices, no gap; echo time, 2.4, 3.7, and 5.0 milliseconds; repetition time, 160 milliseconds; 25-degree flip angle) by analyzing in-phase, out-of-phase, and in-phase signals in a circular region of interest (ROI; 20 cm²) in the lateral right lobe of the liver that was manually selected to avoid intrahepatic vessels and bile ducts.\cite{19} Additional details regarding the protocol can be found in Lim et al.\cite{16} We selected two ROIs for each participant and repeat scanned each ROI, the average of which was used for the estimation of liver fat. NAFLD was defined as percent liver fat of 5.5% or greater excluding subjects with excessive alcohol consumption defined as $>30$ g/day of alcohol in men and $>20$ g/day
of alcohol in women in the past year. Body fat distribution was determined by a whole-body DXA scan (Hologic Discovery A densitometer; Bedford, MA). Total fat mass (kg) was estimated for the whole body, as described.

**OBESITY-RELATED, HORMONAL, AND LIVER ENZYME BIOMARKERS**

Fasting blood samples were collected at the time of body composition measurement, processed into components, and stored at −80°C. A Cobas MiraPlus chemistry autoanalyzer (Roche, Indianapolis, IN) was used to measure concentrations of serum total and high-density lipoprotein (HDL) cholesterol (kits H7510 and H7545; Pointe Scientific), glucose (kit GL1611; Random Laboratories), high-sensitivity C-reactive protein (CRP; kit C7568; Core Lab Supplies), triglycerides (kit T7532, Pointe Scientific), and the liver enzyme alanine aminotransferase (ALT; kit AL1205; Randox Laboratories). Enzyme-linked immunosorbent assays were used to measure insulin (kit EZH1-14K; EMD Millipore) and sex hormone binding globulin (SHBG; kit GWB-D85DE; GenWay). Homeostasis model assessment of insulin resistance (HOMA-IR) and HOMA-beta were derived from glucose and insulin data, low-density lipoprotein (LDL) cholesterol was derived from the Friedewald equation using triglyceride, and total and HDL cholesterol concentrations were derived for a valid range of triglyceride concentrations.

**GENOTYPING**

DNA extraction from blood samples for the MEC-APS participants was performed using the Qiagen QIAamp DNA kit (Qiagen Inc., Valencia, CA). DNA samples were genotyped by the Illumina MEGAEx array. Single-nucleotide polymorphisms (SNPs) with a call rate <0.95, a replicate concordance <1.00% based on 39 QC replicate samples, and those with poor clustering after visual inspection were removed. Problematic samples with a call rate <0.95 or gender/sex mismatches were removed. From an initial 2,046,060 genotyped SNPs, there were 1,847,764 genotyped SNPs available.

**IMPUTATION**

Additional QC steps were implemented before SNP imputation. These included removing all monomorphic SNPs in the overall data set, SNPs with call rate <0.98, SNPs with estimated minor allele frequencies (MAF) that deviated by ±20% in comparison to the corresponding ancestral group in the 1000 Genomes Project (phase 3), and insertion/deletion polymorphisms not identified within the Haplotype Reference Consortium (HRC). These additional QC steps resulted in 1,417,570 SNPs with typed genotypes that were available for imputation. Imputation (including both haplotype phasing and SNP imputation) was performed using the HRC imputation server (version r1.1 2016) with a cosmopolitan reference panel of all 1000 Genome Project samples in addition to the panel described by McCarthy et al. Postimputation imputed SNPs with MAF <1% or an imputation quality score <0.4 were filtered out for a total of 9,789,995 SNPs available for analysis.

**STATISTICAL ANALYSIS**

QQ plots and lambdaGC were used to assess genomic inflation, comparing the empirically observed distribution of the test statistic to the expected median for percent liver fat and NAFLD with lambdas of 0.939 and 1.007, respectively. For percent liver fat, SNP associations (additive genetic model) were estimated using a linear regression of log-transformed percent liver fat, adjusting for age, sex, and eight genetic ancestry proportions (African, Southern and Northern European, Amerindian, Japanese, Okinawan, Native Hawaiian, and Native Hawaiian/Chinese/Filipino) to account for population stratification. Ancestry proportion estimates were computed using Admixture software based on multilocus genotype data of a random sample of 20,000 SNPs and a cross-validation parameter (K) of 8 based on a cross-validation procedure. For NAFLD, SNP associations (additive genetic model) were estimated using a logistic regression model, adjusting for the same above-mentioned variables. A P-value threshold of $5 \times 10^{-8}$ was used to determine genome-wide statistical significance. For top associations, statistical models were also adjusted for total fat mass. To evaluate independent SNP effects, conditional analysis was conducted that included less correlated lead SNPs ($\rho^2 < 0.9$; $P < 5 \times 10^{-8}$). Stratified analyses for top SNP associations were conducted by self-reported race/ethnicity, sex, and total fat mass. Heterogeneity in effects by self-reported race/ethnicity, sex, and total fat mass were examined by including a cross-product term in our statistical models of the top
SNPs with race/ethnicity, sex, or total fat mass, respectively. Sensitivity analyses were conducted for the top SNPs with additional adjustment for visceral fat.

We examined the association of the top SNPs with 11 obesity-related, hormonal, and liver enzyme biomarkers (HDL, LDL, total cholesterol, glucose, insulin, HOMA-beta, HOMA-IR, CRP, SHBG, triglycerides, and ALT). Each log-transformed biomarker was regressed on the top SNPs adjusted for age, sex, genetic ancestry proportions, and total fat mass.

Results

Study characteristics of the 1,709 MEC-APS participants (n = 277 African Americans, 424 Japanese Americans, 348 Latinos, 274 Native Hawaiians, and 386 European Americans) are presented in Table 1. The mean overall age at clinic visit was 69.2 years. Without adjusting for the sampling fractions, Japanese Americans (mean percent liver fat, 6.8 ± 5.3%; prevalence of NAFLD, 44.5%) and Latinos (mean, 6.4 ± 4.3%; NAFLD prevalence, 43.5%) had the highest level of percent liver fat and prevalence of NAFLD. This was followed by Native Hawaiians (mean, 5.8 ± 4.7%; NAFLD prevalence, 35.0%) and European Americans (mean, 4.9 ± 4.6%; NAFLD prevalence, 21.5%). African Americans had the lowest percent liver fat and NAFLD prevalence (mean, 4.0 ± 2.7%; NAFLD prevalence, 14.5%). Mean percent liver fat and NAFLD prevalence did not differ by sex; however, mean total fat mass was greater in women than men (27.6 vs. 22.7 kg) (Supporting Table S1). The participants’ genetic ancestry estimates were consistent with self-reported race/ethnicity (Supporting Table S2).

In the association analysis with percent liver fat, we identified 10 SNPs that reached a genome-wide significance threshold of \( P < 5 \times 10^{-8} \), with seven SNPs located in chromosome 22q13 and three SNPs located in chromosome 6q13 (Fig. 1; Table 2). All variants associated with percent liver fat with \( P < 10^{-6} \) without adjustment for total fat mass are presented in Supporting Table S3. None of the variants deviated from Hardy-Weinberg equilibrium (threshold for deviation \( P < 1.0 \times 10^{-6} \)).

For 22q13, the seven SNPs associated with percentage liver fat are located in the known PNPLA3 risk locus (Table 2). Five of the seven variants were originally genotyped and two were imputed. Imputed genotypes were used for all analyses; here, all seven had an imputation score of >0.97 (Table 2). Six of the 22q13 SNPs were highly correlated \((r^2 > 0.90)\), and rs16991236 was

### TABLE 1. MEC-APS STUDY CHARACTERISTICS* BY RACE/ETHNICITY (N = 1,709)

|                      | African Americans (n = 277) | Japanese Americans (n = 424) | Latinos (n = 348) | Native Hawaiians (n = 274) | European Americans (n = 386) |
|----------------------|-----------------------------|------------------------------|-------------------|---------------------------|-------------------------------|
| Age, years; mean (SD)| 69.9 (2.7)                  | 68.9 (2.5)                   | 69.7 (2.7)        | 68.6 (3.4)                 | 69.0 (2.3)                    |
| Education, n (%)     |                             |                              |                   |                           |                               |
| High school          | 38 (13.8)                   | 35 (8.4)                     | 147 (42.6)        | 58 (21.2)                  | 28 (7.3)                      |
| Some college         | 130 (47.1)                  | 116 (27.7)                   | 120 (34.8)        | 102 (37.4)                 | 75 (19.5)                     |
| College              | 61 (22.1)                   | 161 (38.4)                   | 42 (12.2)         | 70 (25.6)                  | 107 (27.8)                    |
| Graduate and professional school | 47 (17.0) | 107 (25.5) | 36 (10.43) | 43 (15.8) | 175 (45.5) |
| Smoking status, n (%)|                             |                              |                   |                           |                               |
| Never                | 169 (61.0)                  | 249 (58.7)                   | 235 (67.53)       | 167 (60.9)                 | 242 (62.7)                    |
| Former               | 108 (39.0)                  | 175 (41.3)                   | 113 (32.47)       | 107 (39.1)                 | 144 (37.3)                    |
| Alcohol, g/day; mean (SD) | 6.2 (14.5) | 4.7 (9.8) | 7.3 (13.0) | 7.0 (14.2) | 10.9 (17.5) |
| BMI, kg/m²; mean (SD) | 29.2 (4.9)                  | 26.2 (4.03)                  | 29.01 (4.79)      | 28.6 (4.8)                 | 26.9 (4.6)                    |
| % liver fat, mean (SD) | 4.0 (2.7) | 6.8 (5.3) | 6.4 (4.3) | 5.8 (4.7) | 4.9 (4.6) |
| NAFLD, yes; n (%)†   | 37 (14.5)                   | 177 (44.5)                   | 140 (43.5)        | 85 (35.0)                  | 67 (21.5)                     |
| Total fat mass, kg; mean (SD)‡ | 31.4 (9.3) | 24.5 (7.6) | 20.4 (5.9) | 28.9 (8.0) | 23.3 (7.7) |

*All characteristics were assessed at time of clinic visit except education information, which was obtained from the MEC study baseline questionnaire (1993-1996).
†NAFLD was defined as liver fat >5.5%, and 180 participants were excluded due to excessive alcohol consumption (<30 g/day in men or < 20 g/day in women). Total NAFLD sample size was n = 1529.
‡Total sample size of MEC-APS Study subjects with GWAS, % liver fat and total fat mass information was n = 1690.
modestly correlated with these SNPs ($r^2 \sim 0.6$). The most significant association ($P = 3.52 \times 10^{-15}$) was observed with the established NAFLD missense variant rs738409 ($I148M$) and was the primary signal in a conditional analysis that included the only modestly correlated SNP (rs16991236). The G allele of rs738409 was associated with a 1.20-fold increase (beta = 0.08) in (geometric) mean percent liver fat with evidence of heterogeneity in effects by sex ($P_{\text{heterogeneity}} = 0.043$). Interestingly, the effect estimate for percent liver fat was greater and more significant in women than in men ($P_{\text{heterogeneity}} = 0.002$). The G allele was associated with approximately a 1.23-fold increase (beta = 0.09) in (geometric) mean percent liver fat in women ($P = 3.35 \times 10^{-10}$) and a 1.15-fold increase (beta = 0.06) in men ($P = 1.16 \times 10^{-6}$) (Table 2). As women on average have a higher total fat mass than men (Supporting Table S1), we tested the interaction of rs738409 with total fat mass category (< and $\geq$ median of 24.45 kg) on percent liver fat in each sex and found the suggestion of an interaction between the SNP and total fat mass in women ($P_{\text{interaction}} = 0.04$ and 0.25 for women and men, respectively, Supporting Table S4). With additional adjustment for visceral fat, the association between rs738409 and percent liver fat was highly significant ($P = 8.88 \times 10^{-16}$; data not shown).

The MAF for the rs738409 PNPLA3 variant differed across racial/ethnic groups; it was most frequent in Latinos (46%) and Japanese Americans (43%) then followed by Native Hawaiians (26%), European Americans (24%), and African Americans (14%). In race/ethnicity-specific analyses, the largest effect estimate and most significant association for rs738409 and percent liver fat was in European Americans (beta = 0.10; $P = 1.40 \times 10^{-7}$) followed by Japanese Americans (beta = 0.09; $P = 7.30 \times 10^{-7}$) (Table 3; Supporting Fig. S2A,B). Modest associations were found in African Americans (beta = 0.07; $P = 0.017$), Latinos (beta = 0.06; $P = 0.002$), and Native Hawaiians (beta = 0.05; $P = 0.101$) (Table 3).
| SNP          | Chrom Position | Imputed Information score | Ref. Allele | Minor Allele | MAF | Beta† SE | PValue | Beta† SE | PValue | Beta† SE | PValue |
|--------------|----------------|---------------------------|-------------|--------------|-----|---------|--------|---------|--------|---------|--------|
| rs738409    | 22q13 44324727 | 0.98                      | C           | G            | 0.32 | 0.08    | 0.010 | 3.52 × 10^{-15} | 0.06  | 0.013 | 1.16 × 10^{-6} | 0.09  | 0.014 | 3.35 × 10^{-10} |
| rs738408    | 22q13 44324730 | 0.98                      | C           | T            | 0.32 | 0.08    | 0.010 | 3.66 × 10^{-15} | 0.06  | 0.013 | 1.20 × 10^{-6} | 0.09  | 0.014 | 3.35 × 10^{-10} |
| rs3747207   | 22q13 44324855 | 0.97                      | A           | G            | 0.32 | 0.08    | 0.010 | 7.65 × 10^{-15} | 0.06  | 0.013 | 2.24 × 10^{-6} | 0.09  | 0.014 | 4.00 × 10^{-10} |
| rs2294915   | 22q13 44340904 | >0.99                     | C           | T            | 0.33 | 0.07    | 0.009 | 6.06 × 10^{-15} | 0.07  | 0.013 | 1.32 × 10^{-7} | 0.08  | 0.014 | 9.44 × 10^{-9}  |
| rs16691236  | 22q13 44358997 | >0.99                     | A           | G            | 0.19 | 0.08    | 0.012 | 1.30 × 10^{-11} | 0.08  | 0.016 | 1.21 × 10^{-6} | 0.08  | 0.018 | 3.50 × 10^{-6}  |
| rs1833349   | 22q13 44331943 | 0.99                      | G            | A            | 0.29 | 0.07    | 0.010 | 6.88 × 10^{-11} | 0.05  | 0.013 | 5.81 × 10^{-5} | 0.07  | 0.015 | 3.32 × 10^{-7}  |
| rs1977080   | 22q13 44330031 | 0.99                      | C            | T            | 0.29 | 0.07    | 0.010 | 4.63 × 10^{-11} | 0.06  | 0.013 | 3.70 × 10^{-5} | 0.08  | 0.015 | 3.12 × 10^{-7}  |
| rs77249491  | 6q13 70539253  | 0.98                      | G            | A            | 0.02 | 0.18    | 0.031 | 1.42 × 10^{-8}  | 0.18  | 0.043 | 1.45 × 10^{-4} | 0.16  | 0.044 | 2.02 × 10^{-4}  |
| rs146418612 | 6q13 70542348  | 0.98                      | C            | T            | 0.02 | 0.17    | 0.031 | 2.14 × 10^{-6}  | 0.18  | 0.043 | 2.50 × 10^{-5} | 0.16  | 0.044 | 2.02 × 10^{-4}  |

*Additive genetic model, adjusting for age, sex, and genetic ancestry proportions and total fat mass were used to estimate the associations between genetic variants and percent liver fat.
†Log based 10 unit change per allele increase.
‡rs738409 P heterogeneity by sex = 0.043; rs77249491 P heterogeneity by sex = 0.470.

Abbreviations: chrom, chromosome; ref., reference.

| SNP       | Chr | MAF  | Beta† | SE  | PValue | MAF  | Beta† | SE  | PValue | MAF  | Beta† | SE  | PValue |
|-----------|-----|------|-------|-----|--------|------|-------|-----|--------|------|-------|-----|--------|
| rs738409  | 22q13 | 0.14 | 0.07  | 0.03 | 0.017 | 0.43 | 0.09  | 0.02 | 7.30 × 10^{-7} | 0.46 | 0.06  | 0.02 | 0.002 |
| rs738408  | 22q13 | 0.14 | 0.07  | 0.03 | 0.017 | 0.43 | 0.09  | 0.02 | 7.94 × 10^{-7} | 0.46 | 0.06  | 0.02 | 0.002 |
| rs3747207 | 22q13 | 0.14 | 0.08  | 0.03 | 0.007 | 0.43 | 0.09  | 0.02 | 8.58 × 10^{-7} | 0.46 | 0.05  | 0.02 | 0.004 |
| rs2294915 | 22q13 | 0.20 | 0.07  | 0.02 | 0.005 | 0.42 | 0.09  | 0.02 | 8.41 × 10^{-7} | 0.46 | 0.06  | 0.02 | 0.001 |
| rs16691236 | 22q13 | 0.03 | 0.13  | 0.05 | 0.024 | 0.35 | 0.09  | 0.02 | 2.33 × 10^{-5} | 0.20 | 0.06  | 0.02 | 0.009 |
| rs1833349 | 22q13 | 0.15 | 0.08  | 0.03 | 0.004 | 0.42 | 0.09  | 0.02 | 1.48 × 10^{-6} | 0.41 | 0.04  | 0.02 | 0.026 |
| rs1977080 | 22q13 | 0.15 | 0.08  | 0.03 | 0.003 | 0.42 | 0.09  | 0.02 | 1.02 × 10^{-6} | 0.41 | 0.05  | 0.02 | 0.016 |
| rs77249491 | 6q13  | –    | –     | –    | –     | 0.08 | 0.16  | 0.04 | 5.21 × 10^{-6} | –    | –     | –    | –     |
| rs146418612 | 6q13  | –    | –     | –    | –     | 0.08 | 0.16  | 0.04 | 7.16 × 10^{-6} | –    | –     | –    | –     |
| rs78276535 | 6q13  | –    | –     | –    | –     | 0.08 | 0.16  | 0.04 | 4.89 × 10^{-6} | –    | –     | –    | –     |

*Additive genetic model, adjusting for age, sex, and genetic ancestry proportions and total fat mass were used to estimate the associations between genetic variants and percent liver fat.
†Log based 10 unit change per allele increase.
‡rs738409 P heterogeneity by race/ethnicity = 0.46; rs77249491 P heterogeneity by race/ethnicity = 0.95.
Abbreviation: Chr, chromosome.
Heterogeneity by race/ethnicity was not detected ($P$ heterogeneity = 0.46; Table 3; Supporting Table S5).

For 6q13, three genome-wide associations were observed for rs77249491, rs146418612, and rs78276535 with percent liver fat (Fig. 1; Supporting Table S3) that remained statistically significant with adjustment for total fat mass (Table 2). These three variants were imputed with an imputation score of >0.98 (Table 2) and are in high linkage disequilibrium (LD) ($r^2 > 0.95$). Thus, we could not distinguish the primary signal and only considered the strongest association (rs77249491; $P = 1.42 \times 10^{-8}$) in further analyses. The A allele of rs77249491 was associated with a 1.51-fold increase (beta = 0.18) in (geometric) mean percent liver fat (Table 2). This association was primarily driven by Japanese Americans (beta = 0.16; $P = 0.03$ and $P = 0.05$), and was in high LD with in Geico (beta = 0.028; $P = 0.030$). For rs77249491 in 6q13, the A allele was positively associated with HOMA-IR (beta = 0.034; $P = 0.05$), and no association was seen with NAFLD (Supporting Tables S8 and S9).

Lastly, we evaluated the associations of rs738409 and rs77249491 with obesity-related circulating biomarkers (Table 4). For rs738409 in PNPLA3, the variant allele was positively associated with HOMA-IR (beta = 0.028; $P = 0.009$), insulin (beta = 0.022; $P = 0.020$), and ALT (beta = 0.016; $P = 0.030$). For rs77249491 in 6q13, the A allele was positively associated with HOMA-beta (beta = 0.089; $P = 0.023$), HOMA-IR (beta = 0.12; $P = 0.0005$), insulin (beta = 0.11; $P = 0.0003$), and triglyceride (beta = 0.059; $P = 0.013$) levels and inversely associated with HDL (beta = -0.046; $P = 0.039$) and SHBG (beta = -0.084; $P = 0.0012$). When restricting to only Japanese Americans and Native Hawaiians combined, the association for rs77249491 remained statistically significant for the same biomarkers except for HDL ($P = 0.078$) (Supporting Table S10). These associations were no longer statistically significant after adjustment for percent liver fat, suggesting that liver fat is the pathway by which these variants influence these metabolic phenotypes.

**Discussion**

In this GWAS conducted in a multiethnic population-based cohort, we found two risk loci associated with MRI-assessed percent liver fat that were independent of total adiposity. The strongest association was with rs738409 in PNPLA3, a known missense NAFLD risk variant,\(^6\,^8\,^9\); this variant demonstrated consistent effects in the five racial/ethnic groups studied. In addition, the rs738409 and percent liver fat association was found to be significantly stronger in women than men. The second risk variant was a novel locus, rs77249491 in 6q13. This association was driven primarily by Japanese Americans as the minor risk allele is common in East Asians yet rare or absent in the other four racial/ethnic groups. Our data are also consistent with reported associations for two other NAFLD risk variants in...
**TABLE 4. ASSOCIATION BETWEEN rs738409 AND rs77249491 WITH OBESITY-RELATED, HORMONAL, AND LIVER ENZYME BIOMARKERS IN MEC-APS**

| Variant  | n   | Beta | PValue† |
|----------|-----|-----|---------|
| rs738409 |     |     |         |
| HDL (mg/dL) | 1,741 | -0.003 | 0.68 |
| LDL (mg/dL)  | 1,736 | -0.009 | 0.16 |
| Total cholesterol (mg/dL) | 1,742 | -0.007 | 0.10 |
| Glucose (mg/dL) | 1,740 | 0.004 | 0.24 |
| HOMA-beta (%) | 1,718 | 0.016 | 0.19 |
| HOMA-IR | 1,727 | 0.028 | 0.009 |
| CRP (mg/L) | 1,518 | 0.004 | 0.82 |
| Insulin (μU/mL) | 1,729 | 0.022 | 0.020 |
| SHBG (nmol/L) | 1,735 | 0.010 | 0.20 |
| Triglycerides (mg/dL) | 1,742 | -0.009 | 0.22 |
| ALT (U/L) | 1,738 | 0.016 | 0.030 |
| rs77249491 |     |     |         |
| HDL (mg/dL) | 1,741 | -0.046 | 0.039 |
| LDL (mg/dL)  | 1,736 | -0.032 | 0.12 |
| Total cholesterol (mg/dL) | 1,742 | -0.017 | 0.23 |
| Glucose (mg/dL) | 1,740 | 0.0098 | 0.41 |
| HOMA-beta (%) | 1,718 | 0.089 | 0.023 |
| HOMA-IR | 1,727 | 0.12 | 0.0005 |
| CRP (mg/L) | 1,518 | 0.082 | 0.16 |
| Insulin (μU/mL) | 1,729 | 0.11 | 0.0003 |
| SHBG (nmol/L) | 1,735 | -0.084 | 0.0012 |
| Triglycerides (mg/dL) | 1,742 | 0.059 | 0.0133 |
| ALT (U/L) | 1,738 | 0.032 | 0.18 |

*Additive genetic model, adjusting for age, sex, and genetic ancestry proportions and total fat mass were used to estimate the associations between genetic variants and obesity-related, hormonal, and liver enzyme biomarkers.

†P < 0.05 is considered significant.

GCKR and TM6SF2; however, these associations did not reach statistical significance. In addition, rs738409 and rs77249491 were associated with several circulating obesity-related biomarkers, supporting the biological relevance of these variants.

Our study observed consistent patterns of associations for rs738409 in PNPLA3 with percent liver fat and NAFLD across race/ethnicity and found that the population-specific frequency of the minor allele correlated with the population-specific prevalence of NAFLD in the MEC. PNPLA3 encodes for triacylglycerol lipase that is expressed in adipocytes and hepatocytes. The G allele (global frequency, 32%) in rs738409 leads to a change in the amino acid isoleucine to methionine, which results in a reduction of liver lipase activity. In addition, rs738409 and NAFLD found that studies with a greater proportion of men were correlated with a lower mean difference across rs738409 genotypes. Our study observed consistent patterns of associations for rs738409 in PNPLA3 with percent liver fat and NAFLD across race/ethnicity and found that the population-specific frequency of the minor allele correlated with the population-specific prevalence of NAFLD in the MEC. PNPLA3 encodes for triacylglycerol lipase that is expressed in adipocytes and hepatocytes. The G allele (global frequency, 32%) in rs738409 leads to a change in the amino acid isoleucine to methionine, which results in a reduction of liver lipase activity. Lower liver lipase activity may result in reduced breakdown of liver fatty acids and the accumulation of fat in the liver. In addition, the PNPLA3 s738409 variant has been found in a mouse model to result in the accumulation of triglycerides from hepatic lipid droplets. The potential functional relevance of rs738409 was reported to be deleterious using wANNOVAR (http://wannovar.wglab.org). Additionally, this study is the first to suggest that PNPLA3 rs738409 may have a greater effect in women than men. In support of our finding, a meta-analysis study of rs738409 and NAFLD found that studies with a greater proportion of men were correlated with a lower mean difference across rs738409 genotypes.

To our knowledge, there have been no other GWASes of percent liver fat among East Asians. Thus, our findings remain to be replicated. Three prior GWASes of NAFLD in East Asian populations did not report associations with variants in 6q13, despite this variant being present in those of East Asian ancestry (MAF, 7% in East Asians; 1000 Genomes Project). This may be due to the use of NAFLD as a binary trait, which is less powerful for detecting associations than a continuous measure of percent liver fat. Additionally, two of these three studies focused on patients with NAFLD, that may miss asymptomatic subjects with NAFLD who may be present in the general population. In our study, the association of rs77249491 with percent liver fat as a continuous measure reached genome-wide significance; yet a more modest association was observed for NAFLD as a binary trait with our relatively limited sample size.

The rs77249491 variant is located ~32 kb upstream and ~36 kb downstream from two genes, limb region 1 domain containing 1 (LMBRD1) and collagen type XIX alpha 1 chain (COL19A1), respectively. The other two variants in LD (rs146418612 and rs78276535) are <3 kb from the top hit (rs77249491) and also located between these two genes. LMBRD1 encodes for the protein LMBD1, a membrane protein involved in intracellular lysosomal-mediated transport and metabolism of cobalamin (vitamin B12). Mouse knockdown experiments show that heterozygous loss of LMBRD1 leads to an up-regulation of the insulin receptor pathway activation, which can in turn activate lipogenesis in the liver. In addition, LMBD1 localizes to the plasma membrane functioning to regulate the levels of membrane-bound insulin receptor. Mouse knockdown experiments show that heterozygous loss of LMBRD1 leads to an up-regulation of the insulin receptor pathway activation, which can in turn activate lipogenesis in the liver. Variants in LMBRD1 have been associated with triglyceride levels and BMI. COL19A1 encodes for the alpha chain of type XIX collagen, which is a member of the fibril-
Variants of PNPLA3 have been associated with immunoglobulin G (IgG) glycosylation and interleukin-18 (IL-18) levels. N-glycosylation of IgG may serve as a biomarker for nonalcoholic steatohepatitis, and IL-18 in mice is involved in hepatic homeostasis. No potential functional activity for rs77249491 was reported using ANNOVAR. Also, we queried an East Asian eQTL data set that included blood samples from 298 Japanese subjects and found no significant cis-eQTLs for rs77249491 with its neighboring genes LMBRD1 or COL19A (P > 0.01). However, two potential trans-eQTLs (P < 1 × 10⁻⁵) were identified, the G protein-coupled receptor 148 (GPR148) on chromosome 2 and glucosamine-6-phosphate deaminase 1 (GNPDA1) on chromosome 5. GPR148 belongs to the super family of GPCRs, which are primarily involved in cyclic adenosine monophosphate (cAMP)-dependent signaling pathways. GNPDA1 is involved in pathways related to amino sugar and nucleotide sugar metabolism, specifically where it has been found to catalyze the reversible conversion of D-glucoasamine-6-phosphate into D-fructose 6 phosphate and ammonium.

NAFLD often presents in individuals who are overweight or obese and have elevated fasting blood glucose, triglycerides, and liver enzymes and increased insulin resistance as measured by HOMA-beta, HOMA-IR, insulin, and triglycerides and inversely associated with HDL and SHBG. Our study found suggestive associations for the previously identified liver fat risk loci rs780094 in GCKR and rs58542926 in TM6SF2 in directions that are consistent with prior studies. For rs780094, associations have been reported among European ancestry populations (MAF, 41%), with only modest or weak associations among Asian populations (MAF, 48%). Rs58542926 in TM6SF2 was previously identified in an exome-wide study of NAFLD in African Americans (MAF, 3%), Hispanics (MAF, 6%), and European Americans (MAF, 7%). This variant has been replicated in two Chinese populations (MAF, 9%) but not in two other studies with populations from China and Latin America. The reasons for these discrepancies are unclear and may be due to differences in the methods used to identify NAFLD. For rs72613567, the insertion of an A allele in hydroxysteroid 17-beta dehydrogenase 13 (HSD17B13) had an inverse relationship with both NAFLD risk and ALT. This inserted allele was found to alter messenger RNA splicing so that the truncated protein reduced enzymatic activity. In our study, rs72613567 was not available in our data set for replication; however, rs6834314, which is in high LD with rs72613567 (r² = 0.94), was not associated with NAFLD (OR, 1.02; P = 0.58) or ALT (P = 0.79). Our null findings may be a result of our inclusion of participants with mostly asymptomatic NAFLD as NAFLD was detected from MRI in healthy individuals. This aligns with findings that rs6834314 is associated with increased liver steatosis but decreased liver inflammation and enzymes. This study has several strengths. We used the Illumina MEGA genotyping array, which to date provides the largest coverage of SNPs across the genome for diverse ancestral populations. In addition, our population-based design of five major U.S. racial/ethnic groups with substantial variation in the distribution of percent liver fat and genetic variants provides broad representation. This allows for an effective approach in detecting liver fat risk variants and assessing heterogeneity of effects across populations. Notably, this is the first study to assess genetic determinants of liver fat among Native Hawaiians, an understudied population that experiences a disproportionately high burden of obesity and metabolic diseases. We used
highly sensitive methods to assess percent liver fat (MRI) and total fat mass (DXA). These two measures allowed for testing whether associations with liver fat were independent of total fat mass.

There were a few study limitations worth considering. While the total study population with MRI-assessed percent liver fat was large (n = 1,709), the sample size was modest for each of the five racial/ethnic groups (ranging from 269 to 424) and our analysis for NAFLD (n = 1,529) had limited statistical power. In addition, we did not have information on NAFLD-related conditions and disease severity, such as liver fibrosis.

In summary, rs738409 in PNPLA3 and rs77249491 at 6q13 were identified as statistically significant risk loci for percent liver fat and were associated with circulating metabolic biomarkers in this multiethnic study. The novel association with rs77249491 in Japanese Americans and Native Hawaiians warrants further replication and may represent a population-specific variant that contributes to the higher risk of NAFLD in East Asians. Additional efforts are needed to translate knowledge on genetic susceptibility into behavioral and therapeutic interventions that would reduce the metabolic consequences and significant morbidities associated with liver fat.

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Author names in bold designate shared co-first authorship.

Supporting Information

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