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Association between *PNPLA3* (rs738409), *LYPLAL1* (rs12137855), *PPP1R3B* (rs4240624), *GCKR* (rs780094), and elevated transaminase levels in overweight/obese Mexican adults

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Abstract

Purpose—There is scarce information about the link between specific single-nucleotide polymorphisms (SNPs) and risk of liver disease among Latinos, despite the disproportionate burden of disease among this population. Our aim was to investigate nine SNPs in or near the following genes: *PNPLA3, LYPLAL1, PPP1R3B, GCKR, NCAN, IRS1, PPARG,* and *ADIPOR2* and examine their association with persistently elevated alanine aminotransferase (ALT) or aspartate aminotransferase (AST) levels in Mexican adults.

Materials and Methods—Data and samples were collected from 741 participants in the Mexican Health Worker Cohort Study, in Cuernavaca, Mexico. We identified 207 cases who had

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persistently elevated levels of ALT or AST (≥40 U/L) and 534 controls with at least two consecutive normal ALT or AST results in a six month period, during 2006–2010 and 2011–2013. TaqMan assays were used to genotype the SNPs.

Results and Discussion—The risk allele of *PNPLA3* rs738409 was found to be associated with persistently elevated levels of ALT or AST, adjusting for age, sex, BMI, type 2 diabetes, and ancestry: (OR = 2.28, 95% CI= 1.13, 4.58). A significant association was found between the *LYPLAL1, PPP1R3B,* and *GCKR* risk alleles and elevated ALT or AST levels among overweight/obese adults.

Conclusion—These results suggest that among Mexicans, the *PNPLA3* (rs738409), *LYPLAL1* (rs12137855), *PPP1R3B* (rs4240624), and *GCKR* (rs780094) polymorphisms may be associated with a greater risk of chronic liver disease among overweight adults. This study is the first to examine these nine SNPs in a sample of Mexican adults.

Keywords

Alanine aminotransferase (ALT); aspartate aminotransferase (AST); candidate gene study; Latinos; Mexican adults; Nonalcoholic fatty liver disease

INTRODUCTION

The term non-alcoholic fatty liver disease (NAFLD) refers to the extra fat that accumulates in liver cells, but is not due to excessive alcohol consumption. If a liver's weight is greater than 5–10% due to fat, it is diagnosed as fatty liver or steatosis. Worldwide, the prevalence of NAFLD is approximately 9–46%, and an estimated 30% in the general U.S. population [1]. NAFLD is a spectrum of progressive liver disease that ranges from simple hepatic steatosis to non-alcoholic steatohepatitis (NASH), which is the more severe form of fatty liver disease. NASH can progress to cirrhosis and is associated with hepatic failure and hepatocellular carcinoma (HCC) [1]. By 2020, NASH is predicted to be the leading cause of liver transplantation in the U.S. [2]. NAFLD can also progress to HCC without a prior diagnosis of cirrhosis [3].

In the U.S., the prevalence of NAFLD and NASH is highest among Latinos, followed by whites and African Americans [4–8]. Known risk factors for NAFLD and NASH include obesity, metabolic syndrome, diabetes mellitus, and insulin resistance [1, 9–11]. NAFLD is found in up to 80–90% of obese adults, in 30–50% of diabetics, and in up to 90% of patients with hyperlipidemia [12]. A study of liver disease trends in Mexico indicates that by 2050, 90% of chronic liver disease cases will be caused by alcohol and/or obesity, with fewer than 10% of cases due to hepatitis B (HBV) or hepatitis C (HCV) infection [13].

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are considered reliable tests of liver damage, although NAFLD can also be found in persons with normal liver blood tests [4]. As measures of liver cell injury, ALT and AST levels can help identify asymptomatic liver diseases [14]. Elevated ALT or AST levels may be caused by alcohol, hepatitis B (HBV), hepatitis C (HCV), NAFLD or NASH [15]. Having elevated ALT or AST levels does not necessarily mean that a person has or will develop liver disease, but cirrhosis and other forms of advanced liver disease can be identified from these tests [5, 16, 17].

Studies have also found that Latinos are more likely to have elevated aminotransferase levels [5, 18, 19].

There is evidence to support the notion that NAFLD may be a heritable disease, in which the interaction between genetic variants and environmental factors determine the progress and severity of disease [2]. A genome-wide association study (GWAS) of 9,229 nonsynonymous single-nucleotide polymorphism (SNPs), which was performed as part of the Dallas Heart Study, found that the rs738409 (I148M) variant in *PNPLA3* was strongly associated with liver fat [20]. The prevalence of this SNP was 49% in Latinos, 23% in whites, and 17% in African Americans. The association between the *PNPLA3* variant, rs738409 (I148M), and hepatic fat has been validated [21, 22] and to date it is the most robust and consistent association between hepatic steatosis and a single SNP [23–25]. The Genetics of Obesity-related Liver Disease (GOLD) Consortium also found variants associated with hepatic steatosis and histologic NAFLD in or near genes *NCAN* (neurocan), *GCKR* (glucokinase regulatory protein), *LYPLAL1* (lysophospholipase like 1) and *PPP1R3B* (protein phosphatase 1, regulatory subunit 3b) [21].

Our primary aim was to perform a candidate gene case-control association study to examine nine SNPs located in or near the following genes: *PNPLA3, LYPLAL1, PPP1R3B, GCKR, NCAN, IRS1, PPARG,* and *ADIPOR2* and their association with persistently elevated ALT or AST levels, in 741 adults from Mexico. We chose these specific SNPs based on previous research that has identified variants in or near these genes as potential genetic modifiers that are associated with NAFLD and NASH [21–26]. *PNPLA3, PPP1R3B,* and *PPARG* are believed to affect lipid metabolism [23, 24, 27], *LYPLAL1, GCKR, NCAN,* and *ADIPOR2* are related to the role of inflammation and development of NAFLD and NASH [23, 24], while *GCKR, IRS1,* and *PPARG* have been linked to insulin resistance [23, 24]. Additionally, SNPs in or near *PPP1R3B, GCKR,* and *NCAN* have been linked to abnormal serum lipid levels, and variants near *PPP1R3B* and *GCKR* have been shown to affect glycemic traits [23, 24]. These pathways have been found to play an important role in the progression of liver damage among patients with NAFLD and NASH [23, 24]. Only rs738409 (I148M) in *PNPLA3* has been previously investigated in a sample of Mexican adults.

SUBJECTS AND METODS

The *Mexican Health Worker Cohort Study* (MHWCS) is a long-term study of workers from two organizations located in Cuernavaca, Mexico, the Mexican Institute of Social Security (IMSS) and the National Institute of Public Health (INSP), and their immediate family members. From 2004–2006 (Wave 1), approximately 4,000 health workers between 20 to 85 years of age were enrolled in the MHWCS, and 1,026 of these participants were followed-up during 2011 to 2013 (Wave 2). Study participants completed several self-reported questionnaires that collected information about demographics, overall health status, and behavioral factors (e.g. diet, physical activity, alcohol consumption), at each follow-up period. They also underwent a complete physical examination and blood tests following an overnight fast, including transaminase (ALT and AST), cholesterol (total, HDL and LDL), triglycerides, glucose, body fat proportion (DEXA), etc. at every follow-up phase. During

Wave 2, the participants also provided a blood sample for genetic testing, after an overnight fast. Study activities, such as clinical procedures, data coding and entry, and participant follow-up practices, have been standardized and validated [28, 29].

A nested case-control study was conducted using a sample of 207 cases of NAFLD and 534 healthy controls from the MHWCS. Controls were selected from participants in the MHWCS who had a minimum of two consecutive normal alanine aminotranserase levels (ALT ≤ 40 UI/L) results in both Wave 1 (2004–2006) and Wave 2 (2011–2013). The cases of NAFLD were confirmed by ultrasound to identify the accumulation of fat in the liver. Participants who self-reported as heavy or binge drinkers, were infected with HBV or HCV, or had a prior liver disease diagnosis were excluded from this study.

Genotyping of SNPs in Candidate Genes

A commercial isolation kit (QIAGEN systems Inc., Valencia, CA) was used to extract the genomic DNA from the peripheral blood of the study participants. Commercial predesigned TaqMan Probes in a StepOne Plus RT PCR system (Applied Biosystems, Foster City, CA, USA) were used to genotype the following nine SNPs: rs738409 (*PNPLA3*), rs12137855 (*LYPLAL1*), rs4240624 (*PPP1R3B*), rs780094 (*GCKR*), rs2228603 (*NCAN*), rs2943634 and rs2972146 (*IRS1*), rs1801282 (*PPARG*), and rs767870 (*ADIPOR2*), which have been examined in various studies [21–26], and associated in Hispanic and/or Mexican American populations. The call rate was greater than 97% for the SNPs that were tested and we did not observe any discordant genotypes in 20% of duplicate samples. Since the Mexican-Mestizo population is admixed, we used ancestry informative markers (AIMs) to rule out false associations due to population stratification. The GoldenGate BeadArray (Illumina) was used to genotype a set of 96 AIMs distributed across the genome. These AIMs have been validated in other studies of the Mexican population to primarily distinguish between the American, European and African populations [30, 31].

Clinical and Anthropometric Measurements

Body mass index (BMI) and the following clinical measures: cholesterol (total, HDL and LDL), and triglycerides, were examined as part of this study. Subjects were classified based on BMI according to the guidelines established by the National Heart, Lung and Blood Institute: normal weight (18.5–24.9 kg/m2), overweight (25.0–29.9 kg/m2), and obesity (≥30.0 kg/m2) [32].

Statistical Analyses

Student's *t*-tests and Pearson chi-square tests were used to evaluate the socio-demographic and clinical differences between the cases and controls. For each SNP, maximum likelihood estimates of allele frequencies were tested for departures from Hardy-Weinberg Proportions using the chi-square goodness of fit tests among the 534 study controls. In a case-control study design, controls are meant to be representative of the general population, under a rare disease assumption. If genotypes are associated with different disease risks, then the genotypes of cases may not be in HWE.

Our primary analysis was to determine the individual association between each SNP and persistent elevated aminotransferase levels, using an additive model. Unconditional univariate and multivariate logistic regression analyses were used to calculate the crude and adjusted odds ratios (ORs) for risk of persistently elevated aminotransferase levels, and their 95% confidence intervals (CIs). Age, sex, BMI, and ancestry were included as potential confounder variables in the multivariate logistic regression models that examined the association between case-control status and genotype. Logistic regression has considerable flexibility in hypothesis testing and allows for the adjustment of covariates or computation of interactions between genotypes and other environmental exposures. To correct for multiple testing and address the problem of Familywise Type I Error (FWER), we applied the Bonferroni correction method [33] by dividing α by the number of genes/SNPs we examined (0.05/8), which resulted in a significance threshold of $P = 0.006$. Multiple linear regression models were used to explore the interaction between BMI and selected SNPs on aminotransferase levels, adjusting for the aforementioned potential confounders.

Quanto1.1 software was used to calculate statistical power for a significance level of 0.006 and MAF of 4.5 to 65% in 207 cases and 508 controls, considering a minimum power of 80% to detect differences in ALT levels, under an additive model. Stata 11 was used for the statistical analyses and a two-sided p-value of <0.05 was considered to be statistically significant.

The study protocol and informed consent forms were approved by the ethics committees of all participating institutions, and signed informed consent was provided by all participants. The research activities were conducted in accordance with the principles outlined in the Declaration of Helsinki. Additionally, we followed the Strengthening the Reporting of Genetic Association Studies (STREGA) guidelines to describe the study group selection and genetic association analysis [34].

RESULTS

Study Sample Characteristics

The participants included 207 cases and 534 controls, of which 35.7% and 21.9% were males, respectively. The mean age of the study subjects was 47.7 ± 11.6 and 50.0 ± 13.5 years and BMI was 29.1 ± 4.6 and 26.2 ± 4.4 Kg/m2 in case and controls, respectively. The demographic and clinical data that were observed among the cases and controls at baseline are presented in Table 1. No statistically significant differences were found between cases and controls in terms of education, hypertension, total cholesterol and LDL cholesterol ($P > 0.05$). Cases were significantly more likely to be male, younger, have a greater BMI, type 2 diabetes, have lower HDL cholesterol, higher triglyceride, ALT and AST levels, and an ALT/ALT >1 and >2, as compared to the controls ($P < 0.05$).

Association between SNPs and Risk of Persistent Elevated Aminotransferase Levels

All minor allele frequencies (MAFs) that were observed in this study are comparable to those found in the MXL (Mexican Ancestry in Los Angeles, California) samples from the 1000 genomes project. One of the nine SNPs (rs4240624, *PPP1R3B* gene) had a

substantially lower MAF among the CEU samples (6 vs. 30, respectively), whereas the MAF for the rs2228603 SNP (*NCAN* gene) was lower in the 1000 genomes-MXL samples and in the controls than among CEU (2 vs. 9, respectively). The genotype and allele frequency distributions observed for all SNPs did not differ from Hardy-Weinberg equilibrium among the controls (Table 2).

Table 3 reports the association between nine SNPs and risk of persistently elevated ALT levels, along with their adjusted odd ratios (ORs). Our unadjusted results indicate that the rs738409 (*PNPLA3*), rs12137855 (*LYPLAL1)*, rs4240624 (*PPP1R3B)*, rs780094 (*GCKR*), and rs2228603 *(NCAN)* SNPs are associated with persistently elevated aminotransferase levels. After adjusting for age, sex, BMI, type 2 diabetes, and ancestry estimates, only *PNPLA3* (rs738409) was found to be associated with persistently elevated aminotransferase levels (allelic OR = 2.28, 95% CI= 1.13, 4.58). Specifically, compared with the CC homozygous allele, carriers of the homozygous GG genotype had an over two-fold risk of persistently elevated ALT levels (adjusted OR = 2.02, 95% CI= 1.28, 3.19). We also observed a trend to association between *LYPLAL1* rs12137855 and persistently elevated aminotransferase levels (*P*=0.022 adjusted for age, sex, type 2 diabetes, and BMI). After applying a Bonferroni correction using a *P* value threshold of 0.006, the only associations that remained significant were the *PNPLA3* rs738409 SNP recessive model (*P*= 0.003) and the *P* for trend (*P* = 0.0052). (Table 3).

Interaction between selected SNPs, BMI, and Risk of Persistent Elevated Aminotransferase Levels

The *PNPLA3* M148M genotype was significantly more common among the cases in the total sample (P= 0.005) and among overweight/obese participants (P= 0.008). After stratification by sex, the association between the M148M genotype and elevated ALT levels remained significant among females (P= 0.031). The presence of elevated ALT or AST levels was also greater among female M148M carriers (P= 0.004), although sex differences were only significant in the total sample and among overweight/obese females (Table 4).

The homozygous TT *LYPLAL1* rs12137855 genotype was more common among cases in the total sample and among the overweight/obese, than the homozygous CC genotype (P= 0.066 and 0.021, respectively). This genotype was associated with elevated ALT in the total sample (P= 0.046) and among overweight/obese participants (P= 0.033). Additionally, the *LYPLAL1* T risk allele was associated with elevated ALT or AST levels in the total sample and among the overweight/obese (P= 0.020 and 0.009, respectively). (Supplementary Table 1).

Participants with the *PPP1R3B* G allele also had higher mean ALT levels than those with the A allele (42.1 vs. 28.7, respectively, P= 0.056). The *PPP1R3B* risk allele was also associated with elevated ALT or AST levels in the overweight/obese group and among the overweight/obese females (P= 0.035 and 0.042, respectively). (Supplementary Table 2).

Among the total sample, we observed that participants with the *GCKR* heterozygous TC genotype were more likely to have an ALT or AST level ≥40 U/L (P= 0.052), and this was also the case among females (P= 0.022). A higher mean ALT level was observed among

normal weight males with the *GCKR* T risk allele than those with the C allele (28.3 vs. 19.9, respectively). We also observed differences in the proportion of participants with ALT or AST levels ≥40 U/L among overweight or obese females with the T risk allele and those with the C allele (P= 0.011), as well as among all overweight/obese participants (P= 0.034). (Supplementary Table 3).

DISCUSSION

Our findings indicate an association between the *PNPLA3* "G" allele of rs738409 and persistently elevated transaminase levels in a sample of Mexican adults from central Mexico. Population structure of this admixed sample has no obvious effects on this association. These results support other studies that also report a higher prevalence of the *PNPLA3* "G" allele among Mexican Americans, Mexican-Mestizo and Mexican indigenous populations [21, 25] and adds to the evidence of an important determinant of inter-individual and ethnicity-related variations in elevated ALT levels [35, 36]. We found that among overweight or obese women, the *PNPLA3* "G", *GCKR* "T", and *PPP1R3B* "G" alleles were significantly related to persistently elevated ALT or AST levels. Although the proportion of obese or overweight males with elevated ALT levels was greater among those with the M148M genotype, the association was only found to be significant among females. Additionally, the significant associations observed between elevated aminotransferase levels and the *PNPLA3*, *LYPLAL1, PPP1R3B*, and the *GCKR* risk alleles were greater in females, even though males were consistently found to have higher mean ALT levels. The lack of significance observed among males could be explained in part by the lower percentage of males in the study sample (36%), as compared to females (66%). We did not find an association between the *IRS1*, *PPARG*, and *ADIPOR2* SNPs, which were previously associated with NAFLD in European populations. Potential reasons for this lack of association are that phenotype was not included in this study, racial/ethnic differences, and insufficient sample size.

While the environmental risk factors for NAFLD and NASH are well known, less is understood about the genetic basis of hepatic steatosis. Even less is known about the genetic factors that contribute to liver disease susceptibility among Mexicans and other Latino populations, despite the disproportionate burden of liver cancer among this group [37–39]. To date, there is scarce information about the link between specific candidate genes and the development of NAFLD and NASH in Latino populations [25, 40–42]. A study by Hernaez et al. used data from the National Health and Nutrition Examination Survey (NHANES) III to investigate the association between *PNPLA3* (rs738409), *LYPLAL1* (rs12137855), *PPP1R3B* (rs4240624), *GCKR* (rs780094), and *NCAN* (rs2228603) with hepatic steatosis among whites, blacks and Mexican-Americans. They found that only the G allele rs73849 in PNPLA3 was significantly associated with hepatic steatosis and increased levels of ALT in Mexican-Americans [26]. Further studies with Latinos are needed to elucidate the role of specific SNPs in NAFLD and NASH susceptibility.

To the extent that elevated ALT or AST levels could be indicative of sub-clinical liver inflammation, our findings suggest that the presence of the *PNPLA3*, *LYPLAL1, PPP1R3B,* or *GCKR* risk alleles among Mexicans who are overweight or obese, could lead to a greater

risk of developing chronic liver disease. In 2013, cirrhosis and other forms of chronic liver disease were the fifth leading cause of general mortality in Mexico [43], and in 2008, chronic liver disease was the second cause of deaths in the 15 to 64 year age group [44]. The prevalence of steatohepatitis and cirrhosis in the U.S. is higher among Latinos (45%), than among whites 33%, or African Americans (24%) [4]. In 2013, chronic liver disease was the sixth leading cause of death for all U.S. Latinos, and the third leading cause of death for Latino males, ages 55–64 [45]. Although this research was conducted in Mexico, our results may have implications for Mexican-Americans in the U.S., since prior studies have reported that ALT levels are generally greater in Mexican-American adults than among other races/ethnicities [5, 19, 46].

Our study has several limitations. First, the *Mexican Health Worker Cohort Study* (MHWCS) is not a population-based sample and the participants are predominantly female (75%). Subjects are mainly health workers, who are probably better educated and healthier than the general population, which could bias our results towards the null. The MHWCS participants are likely representative of employed, middle-class, urban adults from central Mexico, which corresponds to an estimated 34% of the population [47]. Second, the SNPs we examined were selected from previous reports based on populations that were predominantly European, and thus other SNPs in these genes could be associated with persistently elevated transaminase levels in our population. Third, our study was conducted with Mexicans, and does not include other Latino groups. Thus, our results might not be generalizable to other Latino groups due to the heterogeneity of health status among Latinos. Fourth, although histology is considered the “gold standard” to diagnose and stage NAFLD, we defined NAFLD cases as non- or moderate drinkers with least two elevated ALT or AST results and an ultrasound that identified liver fat. However, our results should be interpreted with caution since some studies have found that up to 75% of patients with hepatic steatosis can have normal ALT and AST levels [4], and there might also be misclassification bias if some participants reported that they were not drinkers when in fact they were. Additionally, ultrasonography is unable to detect liver fat below a threshold of 30% [1], and it cannot detect inflammation or fibrosis, which can indicate more advanced phases of NAFLD [48, 49]. Finally, the failure to observe an association between the *LYPLAL1*, *PPP1R3B*, *GCKR*, *NCAN*, *IRS1*, *PPARG* and *ADIPOR2* SNPs, and elevated ALT levels in our population could be attributed to a relatively small sample size, which resulted in limited statistical power. In order to replicate the previously observed association of these SNPs with ALT levels, considering a rank of MAF of 0.02 for the *NCAN* SNP to 0.32 for the *GCKR* SNP, a total of 1,769 cases and 4,422 controls would be needed to achieve at least 80% statistical power to detect a significant association. Taking into account the genotype frequencies we observed and our sample size, we had a statistical power of 80% (at $\alpha = 0.006$) to detect a genetic risk (odds ratio) of 1.6 for elevated ALT levels in additive mode of inheritance (Quanto software version 1.2.2) for rs738409 of the PNPLA3 gene. Despite these limitations, this study is the first to examine these nine SNPs in a sample of Mexican adults.

In conclusion, our results confirm that the risk allele of *PNPLA3* rs738409 is associated with persistently elevated ALT or AST levels, after adjusting for age, sex and BMI. We also found that the *LYPLAL1* (rs12137855) SNP is associated with elevated ALT levels among overweight/obese individuals, while the *PPP1R3B* (rs4240624) and *GCKR* (rs780094) SNPs

are associated with elevated ALT or AST levels among overweight/obese females. Our findings suggest that among Mexicans, the *PNPLA3*, *LYPLAL1*, *PPP1R3B*, and *GCKR* polymorphisms may be associated with a greater risk of developing chronic liver disease in overweight/obese adults. Recently, a genetic risk score constructed with the *PNPLA3*, *LYPLAL1*, *PPP1R3B*, and *GCKR* polymorphisms was associated with a higher hepatic triglyceride content and ALT levels in Mexican Mestizo subjects with severe obesity [50]. Based on previously reported studies, our replication results suggest that the *PNPLA3*, *LYPLAL1*, *PPP1R3B*, and *GCKR* SNPs may be useful genetic markers to help identify sub-clinical liver disease among Mexicans who are overweight/obese. This is particularly relevant because approximately 68% of males and 74% of females in Mexico are overweight or obese, and by 2050 these estimates are expected to increase to 88% and 91%, respectively [51]. Further fine mapping and GWAS confirmation studies are needed with a significantly larger sample of Mexicans to determine the main associated variants in this high-risk population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Socio-demographic and clinical characteristics of cases and controls at baseline. (n=741)

	Cases = 207		Controls = 534		
	%	Mean (SD)	%	Mean (SD)	P value[1]
Age (yrs)					
<40	25.12		23.97		0.001
40–49	26.57		24.72		
50–59	33.82		23.78		
60+	14.49		27.53		
Sex (Male)	35.75		21.91		0.000
Education					
≤ 6 years	15.92		21.52		0.066
≤ 12 years	46.27		37.52		
> 12 years	37.81		40.95		
BMI (kg/m^2)					
<25	12.64		87.36		< 0.001
≥25	36.25		63.75		
Hypertension					
No	69.08		72.85		0.307
Yes	30.92		27.15		
Diabetes					
No	81.64		89.70		0.003
Yes	18.36		10.30		
Weight (kg)		75.1 ± 15.5		65.3 ± 12.2	< 0.001
Height (cm)		160.2 ± 9.9		157.8 ± 8.2	< 0.001
Body Mass Index (BMI)		29.1 ± 4.6		26.2 ± 4.4	< 0.001
Total cholesterol (mg/dl)		199.3 ± 37.2		201.4 ± 41.4	0.524
Triglycerides (mg/dl)		194.9 ± 101.9		161.7 ± 126.5	< 0.001
HDL cholesterol (mg/dl)		36.3 ± 9.6		39.9 ± 12.7	< 0.001
LDL cholesterol (mg/dl)		124.3 ± 36.3		125.2 ± 37.9	0.757

	Cases = 207		Controls = 534		
	%	Mean (SD)	%	Mean (SD)	P value[1]
ALT (IU/L)		60.0 ± 44.1		19.4 ± 7.1	< 0.001
AST (IU/L)		42.8 ± 28.4		19.0 ± 5.2	< 0.001
ALT/AST ratio >1	85.02		45.13		0.000
ALT/AST ratio >2	7.73		0.94		0.000

[1] Differences between proportions were performed using chi-square tests of homogeneity; differences between means were performed using t-test.

Table 2

SNPs and minor allele frequencies observed in our study sample, as compared to those reported in other populations.

Gene	SNP	Minor Allele	Major Allele	Location	Minor Allele Frequency (%)[1]					
					CEU[2]	MEX[3]	CONTROLS n=534[4]	CASES n=207	P-value[5]	*P* for HWE[4]
PNPLA3	rs738409	G	C	22:43928847	22	55	54.97	65.22	**0.011**	0.991
LYPLAL1	rs12137855	T	C	1:219275036	21	14	10.06	15.37	**0.042**	0.855
PPP1R3B	rs4240624	G	A	8:9326721	6	30	26.15	32.67	0.076	0.601
GCKR	rs780094	T	C	2:27518370	41	34	32.35	35.54	0.408	0.152
NCAN	rs2228603	T	C	19:19219115	9	2	2.08	4.63	0.059	0.625
IRS1	rs2943634	A	C	2:226203364	32	17	15.75	15.22	0.858	0.197
IRS1	rs2972146	G	T	2:226235982	35	16	16.51	16.99	0.875	0.547
PPARG	rs1801282	G	C	3:12351626	10	13	13.66	13.90	0.932	0.057
ADIPOR2	rs767870	G	A	12:1780657	17	13	10.49	10.92	0.864	0.053

[1] Minor allele frequency is the frequency of the minor allele.

[2] Utah residents with Northern and Western European ancestry (CEU) obtained from 1000 Genomes.

[3] Mexican ancestry in Los Angeles, California (MEX) obtained from 1000 Genomes.

[4] Allele frequency and *P*-value for Hardy-Weinberg Equilibrium (HWE) were calculated among controls

[5] P-value for difference between cases and controls was calculated using tests of proportions

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Table 3

Association between SNPs and persistently elevated ALT levels. (n= 741)

Gene	SNP	Genotype	n		Crude Odds Ratio (95% CI)	*P*-value crude	Adjusted Odds Ratio[1] (95% CI)	*P*-value adjusted
			Cases	Controls				
PNPLA3	rs738409	CC	27	108	1.00 (Reference)		1.00 (Reference)	
		CG	90	264	1.36 (0.84–2.21)	0.210	1.17 (0.59–2.31)	0.654
		GG	90	161	**2.24 (1.36–3.67)**	**0.001**	**2.28 (1.13–4.58)**	**0.021**
P for trend[2]					**0.0004**		**0.0052** *	
Dominant model		CG + GG vs. CC	117	372	**1.69 (1.07, 2.67)**	**0.024**	1.56 (0.81,2.97)	0.181
Recessive model		GG vs. CC + CG	27	108	**1.78 (1.28, 2.48)**	**0.001**	**2.02 (1.28, 3.19)**	**0.003** *
LYPLAL1	rs12137855	CC	150	430	1.00 (Reference)		1.00 (Reference)	
		CT	47	97	1.39 (0.94, 2.06)	0.103	1.31 (0.76, 2.29)	0.334
		TT	8	5	**4.59(1.48, 14.24)**	**0.008**	4.90 (0.97, 24.83)	0.055
P for trend[2]					**0.0051**		**0.0228**	
Dominant model		CT + TT vs.CC	55	102	**1.55 (1.06, 2.25)**	**0.024**	1.47 (0.87, 2.50)	0.150
Recessive model		TT vs. CC + CT	8	5	**4.28 (1.38, 13.24)**	**0.012**	4.61 (0.91,23.23)	0.064
PPP1R3B	rs4240624	AA	94	287	1.00 (Reference)		1.00 (Reference)	
		AG	84	197	1.30 (0.92, 1.84)	0.135	1.16 (0.72, 1.87)	0.538
		GG	24	38	**1.93 (1.10, 3.38)**	**0.022**	1.77 (0.88, 3.57)	0.110
P for trend[2]					**0.0149**		0.1211	
Dominant model		AG + GG vs. AA	108	235	**1.40 (1.01, 1.94)**	**0.042**	1.28 (0.82, 2.00)	0.270
Recessive model		GG vs. AA + AG	24	38	**1.72 (1.00, 2.94)**	**0.049**	1.66 (0.85, 3.23)	0.138
GCKR	rs780094	CC	80	247	1.00 (Reference)		1.00 (Reference)	
		CT	103	215	**1.48 (1.05, 2.09)**	**0.026**	1.48 (0.92, 2.37)	0.105
		TT	21	62	1.05 (0.60, 1.82)	0.875	1.47 (0.73, 2.97)	0.277
P for trend[2]					0.2496		0.2832	
Dominant model		CT + TT vs.CC	124	277	1.38 (0.99, 1.92)	0.054	1.48 (0.94, 2.32)	0.090
Recessive model		TT vs. CC + CT	21	62	0.86 (0.51, 1.44)	0.558	1.19 (0.62, 2.26)	0.600

Gene	SNP	Genotype	n		Crude Odds Ratio (95% CI)	*P*-value crude	Adjusted Odds Ratio[1] (95% CI)	*P*-value adjusted
			Cases	Controls				
NCAN	rs2228603	CC	187	506	1.00 (Reference)		1.00 (Reference)	
		CT	17	22	**2.09 (1.09, 4.02)**	**0.027**	1.50 (0.58, 3.90)	0.405
		TT	1	0	--		--	
P for trend[2]					**0.0086**		0.0517	
Dominant model		CT + TT vs. CC	18	22	**2.21 (1.16, 4.22)**	**0.016**	1.62 (0.64, 4.10)	0.312
Recessive model		TT vs. CC + CT	1	0	--		--	
IRS1	rs2943634	AA	148	378	1.00 (Reference)		1.00 (Reference)	
		AC	55	132	1.06 (0.74, 1.54)	0.740	1.22 (0.75, 2.00)	0.418
		CC	4	17	0.60 (0.20, 1.82)	0.367	0.67(0.08, 5.71)	0.718
P for trend[2]					0.8036		0.6082	
Dominant model		AC + CC vs. AA	59	149	1.01 (0.71, 1.44)	0.951	1.19 (0.73, 1.93)	0.474
Recessive model		CC vs. AA + AC	4	17	0.59 (0.20, 1.78)	0.349	0.64 (0.08, 5.36)	0.678
IRS1	rs2972146	GG	145	363	1.00 (Reference)		1.00 (Reference)	
		GT	52	139	0.94 (0.65, 1.36)	0.730	1.05 (0.64, 1.73	0.843
		TT	9	16	1.41 (0.61, 3.26)	0.424	1.30 (0.36, 4.64)	0.688
P for trend[2]					0.8275		0.9351	
Dominant model		GT + TT vs. GG	61	155	0.99 (0.69, 1.40)	0.934	1.07 (0.66, 1.74)	0.772
Recessive model		TT vs. GG + GT	9	16	1.43 (0.62, 3.30)	0.397	1.28 (0.36, 4.54)	0.703
PPARG	rs1801282	GG	151	398	1.00 (Reference)		1.00 (Reference)	
		GC	51	114	1.18 (0.81, 1.72)	0.395	1.17 (0.69, 1.98)	0.555
		CC	3	15	0.53 (0.15, 1.85)	0.317	0.87 (0.20, 3.79)	0.854
P for trend[2]					0.9068		0.4430	
Dominant model		GC + CC vs. GG	54	129	1.10 (0.76, 1.60)	0.601	1.14 (0.69, 1.89)	0.615
Recessive model		CC vs. GG + GC	3	15	0.51 (0.15, 1.77)	0.287	0.84 (0.19, 3.63)	0.813
ADIPOR2	rs767870	GG	163	428	1.00 (Reference)		1.00 (Reference)	
		GA	41	91	1.18 (0.78, 1.78)	0.422	1.62 (0.91, 2.88)	0.104
		AA	2	10	0.53 (0.11, 2.42)	0.409	--	

Gene	SNP	Genotype	n		Crude Odds Ratio (95% CI)	*P*-value crude	Adjusted Odds Ratio[1] (95% CI)	*P*-value adjusted
			Cases	Controls				
P for trend[2]					0.8146		0.6943	
Dominant model		GA + AA vs. GG	43	101	1.12 (0.75, 1.67)	0.585	1.53 (0.86, 2.71)	0.147
Recessive model		AA vs. GG + GA	2	10	0.51 (0.11, 2.34)	0.386	--	

[1] Adjusted for age, sex, body mass index, diabetes, and ancestry.

[2] *P* for trend adjusted for age, sex, body mass index, and diabetes.

[*] Remain significant after a Bonferroni correction using a *P* value threshold of 0.006.

Table 4

Transaminase levels at baseline according to *PNPLA3* I148M genotypes, stratified by BMI.

All[2]	CC	CG	GG	
N=740	135 (18.2)	354 (47.8)	251 (33.9)	
Mean ALT (U/L)	27.5 (23.3–31.7)	29.8 (26.3–33.3)	33.8 (30.3–37.3)	0.251
Male	34.9 (26.1–43.6)	41.8 (30.3–53.2)	38.7 (31.7–45.8)	0.576
Female	25.7 (20.9–30.5)	25.7 (23.3–28.2)	31.827.8–35.8)	**0.031**
ALT or AST ≥ 40 U/L (%)	23 (13.1)	76 (43.4)	76 (43.4)	**0.014**
Male	10 (16.1)	27(43.6)	25 (40.3)	0.459
Female	13 (11.5)	49 (43.4)	51 (45.1)	**0.004** *
Cases (n=207)	27 (13.0)	90 (43.5)	90 (43.5)	**0.005**
Control (n=533)	108 (20.3)	264 (49.5)	161 (30.2)	
Normal weight				
N=261	44 (16.9)	127 (48.7)	90 (34.5)	
Mean ALT (U/L)	21.5 (18.6–24.5)	19.4 (17.4–21.5)	21.8 (19.4–24.2)	0.507
Male	29.5 (12.9–46.1)	22.5 (19.0–26.0)	22.4 (17.8–26.9)	0.452
Female	20.3 (17.7–22.9)	18.4 (15.9–20.9)	21.6 (18.7–24.5)	0.884
ALT or AST ≥ 40 U/L (%)	3 (13.0)	9 (39.1)	11 (47.8)	0.837
Male	2 (33.3)	2 (33.3)	2 (33.3)	0.314
Female	1 (5.9)	7 (41.2)	9 (52.9)	0.465
Cases (n=33)	4 (12.1)	13 (39.4)	16 (48.5)	0.680
Control (n=228)	40 (17.5)	114 (50.0)	74 (32.5)	
Overweight/Obesity				
N=479	91 (19.0)	227 (47.4)	161 (33.6)	
Mean ALT (U/L)	30.4 (24.4–36.4)	35.6 (30.5–40.8)	40.6 (35.5–45.6)	0.347
Male	36.4 (25.6–47.2)	52.4 (35.1–69.6)	45.7 (36.3–55.0)	0.784
Female	28.6 (21.4–35.8)	29.9 (26.5–33.2)	38.1 (32.1–44.1)	**0.018**
ALT or AST ≥ 40 U/L (%)	20 (13.2)	67 (44.1)	65 (42.8)	**0.022**
Male	8 (14.3)	25 (44.6)	23 (41.1)	0.874
Female	12 (12.5)	42 (43.8)	42 (43.8)	**0.004** *
Cases (n=174)	23 (13.2)	77 (44.3)	74 (42.5)	**0.008**
Control (n=305)	68 (22.3)	150 (49.2)	87 (28.5)	

Data are means (CI) or n (%)

[1] P-values were calculated by linear regression, adjusting for age, sex, diabetes, and ancestry in the continuous variables; the multiple logistic regression analyses adjusted for age, sex, diabetes, and ancestry in the categorical variables.

[2] The analyses with the total population were additionally adjusted for body mass index.

* Remain significant after a Bonferroni correction using a *P* value threshold of 0.006.