HHS Public Access

Author manuscript

Exp Mol Pathol. Author manuscript; available in PMC 2018 July 02.

Published in final edited form as:

Exp Mol Pathol. 2018 February ; 104(1): 50–58. doi:10.1016/j.yexmp.2018.01.001.

Genetic variants in *COL13A1, ADIPOQ* and *SAMM50,* in addition to the *PNPLA3* gene, confer susceptibility to elevated transaminase levels in an admixed Mexican population

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is the accumulation of extra fat in liver cells not caused by alcohol. Elevated transaminase levels are common indicators of liver disease, including NAFLD. Previously, we demonstrated that *PNPLA3* (rs738409), *LYPLAL1* (rs12137855),

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

PPP1R3B (rs4240624), and *GCKR* (rs780094) are associated with elevated transaminase levels in overweight/obese Mexican adults. We investigated the association between 288 SNPs identified in genome-wide association studies and risk of elevated transaminase levels in an admixed Mexican-Mestizo sample of 178 cases of NAFLD and 454 healthy controls. The rs2896019, rs12483959, and rs3810622 SNPs in *PNPLA3* and rs1227756 in *COL13A1* were associated with elevated alanine aminotransferase (ALT, ≥40 IU/L). A polygenic risk score (PRS) based on six SNPs in the *ADIPOQ*, *COL13A1*, *PNPLA3,* and *SAMM50* genes was also associated with elevated ALT. Individuals carrying 9–12 risk alleles had 65.8% and 48.5% higher ALT and aspartate aminotransferase (AST) levels, respectively, than those with 1–4 risk alleles. The PRS showed the greatest risk of elevated ALT levels, with a higher level of significance than the individual variants. Our findings suggest a significant association between variants in *COL13A1*, *ADIPOQ, SAMM50*, and *PNPLA3*, and risk of NAFLD/elevated transaminase levels in Mexican adults with an admixed ancestry. This is the first study to examine high-density single nucleotide screening for genetic variations in a Mexican-Mestizo population. The extent of the effect of these variations on the development and progression of NAFLD in Latino populations requires further analysis.

Keywords

Alanine aminotransferase (ALT); Aspartate aminotransferase (AST); Mexican adults; Polymorphisms; polygenic risk score

Introduction

Non-alcoholic fatty liver disease (NAFLD) is defined by the accumulation of fat in the liver (≥5% of fat in hepatocytes), which can develop into a more serious condition known as non-alcoholic steatohepatitis (NASH). Progression from NAFLD to NASH increases risk of cirrhosis, liver failure, and hepatocellular carcinoma (Wiegand et al., 2007). According to the World Gastroenterology Organization, NAFLD has been increasing during the past 20 years and is now one of the most common types of liver disease in Western countries (Wiegand et al., 2007). The gold standard for the diagnosis of NAFLD/NASH is the liver biopsy, but it is an invasive procedure that requires a highly experienced hepatopathologist. Since performing a liver biopsy can be impractical, unnecessary, and cost prohibitive, especially in underdeveloped countries, other clinical approaches including laboratory tests and imaging studies can be used to detect NAFLD (Chalasani et al., 2012; Nalbantoglu and Brunt, 2014). The most common tests to evaluate the degree of liver injury or liver disease are alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Kang, 2013; Thapa and Walia, 2007). Growing evidence suggests that ALT/AST levels can be considered biomarkers of liver metabolic function, which may be indicative of a normal response to high fat intake or liver damage, including NAFLD (Jadaho et al., 2004; Kechagias et al., 2008; Sookoian et al., 2016; Sookoian and Pirola, 2012). The AST/ALT ratio represents the time course and disease aggressiveness that can be predicted from the relatively short half-life of AST (18 h), as compared to ALT (36 h) (Botros and Sikaris, 2013).

As a lipid metabolism disorder, NAFLD has been associated with both strong environmental risk factors and a genetic component. The first genome-wide association study (GWAS) of

NAFLD identified the rs738409 G allele of the *patatin-like phospholipase domain-containing-3* (*PNPLA*3) gene as significantly associated with increased hepatic fat, inflammation, hepatic enzyme levels, and susceptibility to NAFLD (Romeo et al., 2008). These associations have been replicated in multiple populations (Chambers et al., 2011; Hotta et al., 2010; Kollerits et al., 2010; Kotronen et al., 2009; Zain et al., 2012). A subsequent study, which used data from the Genetics of Obesity-related Liver Disease (GOLD) Consortium, identified four additional NAFLD risk genetic variants located in or near the neurocan (*NCAN*), glucokinase regulatory protein (*GCKR*), lysophospholipase-like 1 (*LYPLAL1*), and protein phosphatase 1 regulatory subunit 3b (*PPP1R3B*) genes (Speliotes et al., 2011). However, most studies to date that report an association between specific genetic variants and increased risk of NAFLD or elevated transaminase levels have been performed in predominantly European and Asian populations, and it remains unclear if other loci contribute to the excess of NAFLD and elevated transaminase levels observed in the Mexican population. In a previous study, we demonstrated that *PNPLA3* (rs738409), *LYPLAL1* (rs12137855), *PPP1R3B* (rs4240624), *GCKR* (rs780094), are associated with elevated transaminase levels in overweight/obese Mexican adults (Flores et al., 2016).

Since Mexicans and other Latino populations have been underrepresented in GWAS, it is critical to investigate the genetic variants and genes that are shared in these diverse populations. Additionally, it is important to determine if the presence of these variants is correlated with readily detectable biomarkers, such as liver enzymes. Our aim was to extend the finding of our previous study and investigate the association between NAFLD and SNPs that were previously identified by GWAS in other populations, in an admixed sample of Mexican adults.

Materials and Methods

Human subjects and phenotype data

Subjects—The Mexican Health Worker Cohort Study (MHWCS) is a long-term study of workers from the Mexican Institute of Social Security (IMSS) and the National Institute of Public Health (INSP) in Cuernavaca, Morelos (located in central Mexico) that focuses on the association between certain lifestyle factors and the development of chronic diseases (Denova-Gutiérrez et al., 2016).

A baseline assessment was conducted from 2004 to 2006 (Wave 1) and approximately 4,000 participants enrolled in the MHWCS. A second assessment from 2010 to 2013 served as a follow-up for the original participants and provided baseline information for new subjects who were enrolled in the MHWCS. For the follow-up phase, 2,500 MHWCS participants who were initially enrolled were invited to participate, and 1,855 (74%) took part in the second evaluation (Denova-Gutiérrez et al., 2016). Study participants completed several self-reported questionnaires that collected information about demographics, overall health status, and behavioral factors (eg. diet, physical activity, and alcohol consumption), at each follow-up period. They also underwent a complete physical examination and blood tests following an overnight fast, including transaminase levels (ALT and AST), cholesterol (total, HDL and LDL), triglycerides, glucose, proportion of body fat (DEXA), etc. at each follow-up phase. During Wave 2, the participants also provided a blood sample for genetic testing, after an

overnight fast. The clinical procedures, data coding, entry, and participant follow-up practices have been standardized and validated (Denova-Gutiérrez et al., 2011; Morales et al., 2014).

A total of 632 MHWCS participants aged 18 to 85 years were selected for this case-control study. The controls included 454 participants who had at least two consecutive normal alanine aminotransferase (ALT <40 IU/L) results in both Wave 1 (2004–2006) and Wave 2 (2011–2013). The 178 cases of NAFLD (ALT ≥40 IU/L) were confirmed by ultrasound to identify the accumulation of fat in the liver. Participants who self-reported as heavy or binge drinkers, (Jiles et al., 2005) were infected with HBV or HCV, or had a prior liver disease diagnosis were excluded from this study. The Ethics Committee of IMSS and all participating institutions approved the study and the MHWCS subjects provided written informed consent prior to initiating any study activities. The study was performed according to the principles of the 1975 Declaration of Helsinki.

Clinical and anthropometric measurements

Trained nurses used standardized procedures to obtain the anthropometric measures of all MHWCS participants. Weight (kg) and height squared (m^2) were used to calculate body mass index (BMI, kg/m^2). The World Health Organization (WHO) guidelines were used to classify study subjects as): normal weight (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2), or obese (≥30.0 kg/m^2), based on their BMI (WHO, 2000). Biochemical parameters were measured after 8–10 hours of overnight fasting. The following clinical measures were determined using commercial tests: serum glucose, triglycerides, cholesterol (total, HDL and LDL), ALT and AST.

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. Based on prior GWAS and candidate gene studies, we compiled a list of 288 SNPs in 60 genes that are associated with the development NAFLD, elevated transaminase levels, liver disease severity and metabolic comorbidities, which were included in a GoldenGate BeadArray (Illumina). Because the Mexican-Mestizo population is admixed, ancestry informative markers (AIMs) also were incorporated in the study design to assess whether any association could be confounded by population stratification. A panel of 96 AIMs distributed across the genome was selected from previous reports to mainly distinguish between three continental populations (American, European and African) (Flores et al., 2016; Kosoy et al., 2009).

Quality control

After genotype calling, the following quality control criteria were applied using the PLINK software (Purcell et al., 2007): (1) subject and SNP genotyping success rate ≥95 %; (2) minor allele frequency ≥0.05 %; and (3) departure from Hardy–Weinberg equilibrium (HWE) at P value ≥0.001. A total of 314 SNPs including the 96 AIMs, met the quality control criteria and were further analyzed.

Construction of the polygenic risk score

To evaluate the combined effect of the SNPs that were significantly associated with higher ALT and AST levels, we constructed a polygenic risk score (PRS) (Dudbridge, 2013) for each individual, which included six SNPs: *ADIPOQ* rs17366743, *COL13A1* (rs7101190 and rs1227756), *PNPLA3* (rs3810622 and rs738409) and *SAMM50* rs2143571. The PRS was constructed by summing the number of risk alleles from these six SNPs for each individual. *PNPLA3* rs12483959 and rs2896019 were excluded in the construction of the PRS because rs738409 was selected as a tag SNP in the haplotype block 1. The weighted PRS was calculated by multiplying the number of alleles for each SNP by the estimated effect (beta) obtained from the association analysis for ALT or AST levels. Genotypes for each SNP were scored using an additive model (0 for homozygous for the non-risk allele, 1 for heterozygous, and 2 for homozygous for the risk allele).

Statistical analyses

Ancestry from principal component analysis (PCA) was estimated using the smartpca program in the Eigensoft 3.0 package (Price, 2006), and ancestry estimates were included as confounding factors to correct for population stratification. The association between each SNP and elevated ALT or AST levels (≥40 IU/L) was tested using logistic regression analysis, adjusting for age, sex, BMI, principal components of ancestry, and number of tests performed. All associations were tested for additive, dominant, and recessive inheritance models, with the most significant model being reported. Multiple linear regression analysis was performed to test the independent effect of each risk allele or PRS on biochemical parameters. Because most of the SNPs we analyzed are well-validated variants, a *P*-value threshold of 0.05 was used to determine a significant association. Haploview software was used to construct haplotype blocks with strong linkage disequilibrium (LD) for which the one-sided upper 95% confidence bound on D' was >0.98. All statistical analyses were performed using SPSS (version 16.0; Chicago, IL).

Results

Characteristics of the study population

The demographic and clinical characteristics of the study population are described in Table 1. A total of 632 study participants were included, of which 454 controls (71.8%) had persistently normal ALT levels (<40 IU/L) and 178 cases (28.2%) had at least two consecutive elevated ALT levels (≥40 IU/L). The mean age of the study subjects was 46.8 ± 11.2 and 49.5 ± 13.3 years, 42.7% of the cases and 19.4% of the controls were males, and BMI was 29.4 ± 4.5 and 26.2 ± 4.5 kg/m^2 among the cases and controls, respectively. No significant differences were found between cases and controls in terms of hypertension, total cholesterol and LDL cholesterol (P>0.05). Significant differences were observed in age, sex, education level and certain clinical measures between the cases and controls. As expected, cases had a significantly higher mean BMI, glucose, triglycerides, ALT and AST levels, as compared to controls (P<0.01). Mean HDL-C levels were significantly lower among the cases (P<0.001).

Genetic association with elevated ALT levels

Of the 218 SNPs tested, 24 were significantly associated with elevated ALT levels (≥40 IU/L) (P<0.02, after adjusting for age, sex, BMI, and principal components of ancestry) (Supplementary Table 1). However, after correcting for number of tests performed, only *COL13A1* rs1227756 remained significantly associated with a higher risk of elevated ALT levels, as did *PNPLA3* (rs12483959 and rs2896019) with a lower risk of having elevated ALT levels (P=0.022, 0.004 and 0.018, respectively). The association of the *PNPLA3* SNPs (rs3810622 and rs738409) with risk of elevated ALT levels was found to have a borderline statistical significance (P=0.052 and 0.070, respectively) (Table 2). After stratifying by sex, the associations of the four *PNPLA3* variants (rs3810622, rs12483959, rs2896019 and rs738409) with elevated ALT levels were only significant among females (OR=3.01, 95% CI 1.81–5.00, P=0.005; OR=0.36, 95% CI 0.22–0.59, P=0.010; OR=0.36, 95% CI 0.22–0.58, P=0.009 and OR=2.57, 95% CI 1.59–4.16, P=0.028, respectively; adjusted for age, sex, BMI, principal components of ancestry, and number of tests).

Genetic association with ALT and AST levels

A significant association was observed between 16 variants and higher ALT or AST levels, and three SNPs were found to be associated with higher AST levels (P<0.05 after adjusting for age, sex, BMI, and principal components of ancestry) (Supplementary Table 2). However, after additionally controlling for number of tests, only two variants in *PNPLA3* (rs3810622 and rs738409) and *COL13A* rs1227756 remained significantly associated with higher ALT or AST levels, while *SAMM50* rs2143571 was only associated with higher AST levels (P<0.05). *PNPLA3* rs3810622 showed the strongest and most significant effect, an increase of 8.0 and 5.7 IU/L in ALT and AST levels, respectively (P≤0.001) (Table 3).

After adjusting for age, sex, BMI, and principal components of ancestry; twelve SNPs were significantly associated with lower ALT and AST levels (P<0.05) (Supplementary Table 2). As shown in Table 3, two *PNPLA3* SNPs (rs12483959 and rs2896019) were associated with lower ALT and AST levels (P<0.01), with *PNPLA3* rs12483959 having the most significant effect on decreased ALT and AST levels by 8.6 and 6.2 IU/L, respectively (P≤0.002). In addition, *COL13A1* rs7101190 and *ADIPOQ* rs17366743 also remained significantly associated with lower ALT levels (P<0.05). Interestingly, *ADIPOQ* rs17366743 has the strongest effect, decreasing ALT levels by 10.9 IU/L (P=0.014) (Table 3).

Genetic association with other metabolic parameters

Since the variants we analyzed have also been linked to other metabolic traits, we decide to evaluate their association with clinical variables that have been associated with NAFLD. We found a significant association between *SLC2A1* (rs841848 and rs841858) and higher serum glucose concentrations (P<0.001, after adjusting for age, sex, BMI and principal components of ancestry) (Supplementary Table 3), but only *SLC2A1* rs841848 remained significantly associated after correcting for number of tests (P=0.048). Of the all-biological candidate variants, six were significantly associated with BMI, four with triglycerides, and four with HDL-C levels (P<0.05, after adjusting for age, sex, BMI, and principal components of ancestry) (Supplementary Table 3). After adjusting for number of tests, only the association

of *TGM5* rs748404 with triglyceride levels remained significant, for each copy of the T risk allele, triglyceride levels increased 26.7 mg/ml (P=0.026).

Association between *PNPLA3* haplotype and risk of elevated ALT levels

The LD pattern of the *PNPLA3* polymorphisms was evaluated to determine haplotype blocks. Two independents blocks were detected, block 1 contained rs12483959, rs2896019 and rs738409, while block 2 contained rs3810622 (Supplementary Figure 1). The haplotype GGG in block 1 was significantly associated with risk of elevated ALT (≥40 IU/L) (P=0.035). The SNP rs738409 was selected as a haplotype-tag SNP.

Association of the PRS with elevated ALT levels

As the PRS increases, ALT and AST levels also rise as a function of the number of risk alleles (P<0.0001, respectively, adjusted for age, sex, BMI, and principal components of ancestry) Figure 1. The analysis using the weighted PRS showed similar results (P<0.0001, respectively). The PRS was also strongly associated with elevated ALT levels (OR=1.70, 95% CI 1.41–2.05, P<0.0001) (Figure 2).

Discussion

In this case-control study, we analyzed 218 SNPs that were associated with the development of NAFLD, elevated transaminase levels, liver disease severity, and metabolic comorbidities in previous studies (Macaluso et al., 2015; Speliotes et al., 2011). After adjusting for number of tests performed, *COL13A1* rs1227756 was significantly associated with a higher risk of elevated ALT levels, while individuals with the *PNPLA3* variants rs12483959 and rs2896019 had a lower risk of elevated ALT levels. Two *PNPLA3* SNPs (rs3810622 and rs738409) were also associated with a greater risk of elevated ALT levels, but only in females. Our results support the association between *PNPLA3* variant rs738409 (I148M) and elevated levels of ALT that has been observed in other studies with Mexican-Mestizo populations (Flores et al., 2016; Larrieta-Carrasco et al., 2014, 2013). The lack of significance among males could be due to the lower number of male participants in the MHWCS, as compared to females (70% vs. 30%, respectively) (Denova-Gutiérrez et al., 2016). Our results also support previous findings regarding sexual dimorphism in the genetic association between *PNPLA3* rs738409 and liver transaminases (Larrieta-Carrasco et al., 2014; Li et al., 2012; Sookoian and Pirola, 2011).

To the best of our knowledge, this is the first study to identify the association of the *PNPLA3* rs3810622 and *COL13A1* rs1227756 variants with a higher risk of elevated ALT levels, and *PNPLA3* rs2896019 and rs12483959 with a lower risk of elevated ALT levels in an admixed sample of Mexican adults. Previously, rs12483959 has been associated with obesity and insulin resistance in children (Johansson et al., 2009), while rs2896019 and rs3810622 have been linked to steatosis grade (Kitamoto et al., 2013), decreased serum triglycerides, hepatocyte ballooning and NAFLD activity score (NAS) (Kitamoto et al., 2013). The presence of *COL13A1* rs1227756 has been associated with lobular inflammation in NAFLD patients (Chalasani et al., 2010). Furthermore, we also found that ALT and AST levels were significantly higher among the carriers of *PNPLA3* variants rs3810622 and

rs738409, and rs1227756 in *COL13A1*, while rs2143571 in *SAMM50* was only associated with increased levels of AST. The link between *PNPLA3* rs738409 and higher ALT levels has been consistently replicated in different populations including Mexican adults, children, and Indigenous groups (Flores et al., 2016; Larrieta-Carrasco et al., 2014, 2013; León-Mimila et al., 2015). The effect of *PNPLA3* rs3810622 on transaminase levels observed in our study is similar to the findings of GWAS studies with Japanese (Kitamoto et al., 2013) and Chinese populations (Song et al., 2016), although our results indicate a stronger and more significant association.

This is the first study to report an association between rs1227756 *COL13A1* and risk of increased ALT and AST levels. Prior to our study, this variant had only been associated with lobular inflammation in NAFLD patients (Chalasani et al., 2010). The collagen type XIII, α 1 (*COL13A1*) gene encodes the chain of a non fibrillar collagen and it has been linked with modifying the inflammatory response genes in the mouse intestine (Tuomisto et al., 2008). Since *COL13A1* has been associated with the intestinal inflammatory response, it may also play a role in liver inflammation that has yet to be evaluated. Recently, a study of monozygotic twins with NASH-related cirrhosis found rs1227756 to be a contributing factor in disease progression (Grove et al., 2016). Additionally, we observed an association between rs1227756 *COL13A* and higher triglyceride levels, which supports the findings of a study conducted with NAFLD patients in India (Ravi Kanth et al., 2014). Our results differ from those of a recent study that did not observe an association between the rs1227756 variant and the presence of NAFLD in Chinese children (Shang et al., 2015). This leads us to consider that the effect of this SNP among the Mexican-Mestizo population may be more significant than in Europeans or other populations. Future studies need to examine the extent of the association between these variants and NAFLD in other populations.

The *SAMM50* gene is located in the same genetic region as *PNPLA3*, and polymorphisms of this gene, including rs2143571, have been associated with NAFLD among Japanese, Chinese, and Indian populations (Chen et al., 2015; Kitamoto et al., 2013; Ravi Kanth et al., 2014). We found that the variant rs2143571 *SAMM50* was associated with higher levels of AST in our sample of Mexican adults. A study with a Chinese population found that the variant rs2143571 was associated with elevated levels of both ALT and AST, as well as higher triglyceride levels (Chen et al., 2015). Moreover, the rs2143571 “A” allele was associated with an increased risk of developing NAFLD, compared with the non-carriers (Chen et al., 2015). Kitamoto et al. (Kitamoto et al., 2013) found that four variants of *SAMM50,* including variant rs2143571, were associated with histological severity and proposed that *SAMM50* could be involved in necroinflamation and fibrosis. Mitochondrial abnormalities have been observed in the liver biopsies of NASH patients (Caldwell et al., 1999; Sanyal et al., 2001). In addition, several studies have recognized that mitochondrial dysfunction plays an important role in insulin resistance (Lowell and Shulman, 2005; Ma et al., 2012). These reports and our results suggest that the *SAMM50* gene could be a key factor in mitochondrial dysfunction, due in part to a defective removal of reactive oxygen species, as a pathophysiological contributor to the development and progression of NAFLD (Rector et al., 2011).

There are several risk factors associated with the progression from NAFLD to NASH, which include obesity, insulin resistance, hypertriglyceridemia, and low high-density lipoproteins (HDL) cholesterol (Fazel et al., 2016; Than and Newsome, 2015). To further investigate these pathways, we evaluated the relationship between certain genetic variants and specific metabolic measures. We found an association between *SLC2A1* (rs841848 and rs841858) and higher levels of glucose. *SLC2A1* is a facilitative glucose transporter responsible for constitutive or basal glucose uptake (Rhoads, 1994). In the context of fatty liver, a correct balance in glucose uptake and subsequent metabolism prevents the development of insulin resistance, thereby avoiding the accumulation of fat. Two SNPs in *SLC2A1* (rs4658 and rs841856) were found to be associated with NAFLD in a Spanish population. Interestingly, in the liver biopsies of patients with NAFLD, *SLC2A1* expression was down-regulated and homozygous carriers of the rs4658 G-allele had lower expression of *SLC2A1* messenger. In addition, silencing *SLCA2A1* in THLE2 cells leads to an increased presence of lipid droplets when oleic acid is added to culture medium (Vazquez-Chantada et al., 2013). *SLC2A1* variants could be important in the balance of glucose uptake and in fat accumulation.

We also found an association between *TGM5* (rs748404) and high triglyceride levels. The *TGM5* gene is a member of the transglutaminase family. Transglutaminases (TGs) are Ca(2+)-dependent enzymes that catalyze the formation of covalent bonds between glutamine and lysine residues, and contribute to fibrotic diseases via crosslinking-mediated stabilization of extracellular matrix (Eckert et al., 2014; Iismaa et al., 2009; Lorand and Graham, 2003). *TGM1*, *TGM3* and *TGM5* have been associated with mouse liver fibrosis and altered healing processes (De Koning et al., 2012; Tatsukawa et al., 2017), which is interesting since NAFLD can progress to cirrhosis. According to the tissue-specific pattern of mRNA expression profiles from 79 human tissues and cell types (http://biogps.org), TGM5 is highly expressed in the liver, although the precise role of TGM5 in NAFLD is not known.

Although the rs17366743 variant of *ADIPOQ* was not associated with elevated levels of ALT or AST in our study, this gene may also play a key role in the development of NAFLD. *ADIPOQ*, also known as adiponectin, is an important adipokine involved in the control of fat metabolism and insulin sensitivity, and its variant rs17366743 has been associated with diabetes (Hivert et al., 2008). Other variants of *ADIPOQ* located in regulatory regions have been associated with low levels of adiponectin (Heid et al., 2006; Hivert et al., 2008; Menzaghi et al., 2007), which is particularly relevant in the context of metabolic syndrome and diabetes since they are both important risk factors in the development of NAFLD.

Since NAFLD is a complex disease involving multiple factors, it is important to understand that a single SNP cannot be responsible for the development of the disease, and much less for its progression to NASH, cirrhosis, or hepatocarcinoma. GWAS studies have attempted to evaluate different combinations of SNPs that may help explain the various genetic risk factors for developing NAFLD, as well as its progression to more serious liver disease. In our study, we found that a PRS based on six SNPs in the *ADIPOQ*, *COL13A1*, *PNPLA3,* and *SAMM50* genes was associated with the greatest risk of elevated ALT levels. Individuals carrying 9–12 risk alleles had 65.8% and 48.5% higher ALT and AST levels,

respectively, than those with 1–4 risk alleles. The PRS explained the variance of ALT levels with a higher level of significance than the individual variants, though less strongly when compared with *PNPLA3* rs3810622.

Twin studies suggest that transaminase levels have a genetic heritability irrespective of anthropometric features and environmental factors (Bathum et al., 2001; Rahmioglu et al., 2009), which means that certain genes may regulate liver enzyme levels. Studies of genetic regions like *PNPLA3*, *SAMM50* and *PARVB* support this idea, since variants in these genes have been associated with NAFLD and elevated ALT and AST levels (Flores et al., 2016; Kitamoto et al., 2013; Ravi Kanth et al., 2014). Krawczyk et al. found that the combined effect of several SNPs was linked to the presence of elevated ALT levels, as well as NAFLD severity (Krawczyk et al., 2017). The SNPs included in the PRS we created are likely associated with higher levels of ALT/AST and fatty liver through different mechanisms, which account for the complexity of NAFLD. *PNPLA3* variants may explain the hepatic fat accumulation that is essential for the development of NAFLD (Romeo et al., 2008; Speliotes et al., 2011). Additionally, *SAMM50* and *COL13A1* variants may promote inflammation (Ravi Kanth et al., 2014; Tuomisto et al., 2008), which also contributes to the development of NAFLD. Elevated fat intake increases ALT and AST levels as a normal metabolic response (Kechagias et al., 2008). A genetic condition could mimic or aggravate fat intake and might help to explain the elevated transaminase levels observed among NAFLD patients. Our results suggest that the combined presence of these six SNPs could have a greater impact on the development of NAFLD and subsequent liver damage than a single polymorphism.

This study has some limitations that should be considered. First, the diagnosis of NAFLD was based on the presence of persistently elevated transaminase levels and ultrasonography results. Although performing a liver biopsy is the gold standard for the diagnosis of NAFLD, it is expensive and in some cases may result in morbidity or very rarely death. According to the American Association for the Study of Liver Disease (AASLD) practice guidelines, a liver biopsy should only be obtained from patients who would receive a clear and significant benefit from a definitive diagnosis, treatment, and improved prognosis (Chalasani et al., 2012). Second, this study was based on a cross-sectional design and the selected SNPs were chosen from previous investigations that were conducted in mostly European populations. Third, we used data from a sample of Mexican adults, so our findings may not be representative of other Latino populations because of the heterogeneity observed among Latino groups. Fourth, the MHWCS participants are mostly female (70%) and health workers, who are likely more educated and healthier than the general population of Mexico. While the MHWCS is not a population-based sample, the participants are predominantly middle-class, urban adults from central Mexico, who are employed in the formal sector of the economy, and are representative of approximately 34% of the population (*Secretaría de Economía (2014) Programa nacional de protección a los derechos del consumidor 2013–2018. Diario Oficial de la Federación, México.*, n.d.). Additionally, after adjusting our analyses for multiple testing, the only variants that remained significantly associated with elevated ALT levels were rs12483959 and rs2896019 in *PNPLA3* and rs1227756 in *COL13A1*, probably due to our limited sample size and the elevated number of SNPs that were tested.

In conclusion, this study suggests that variants in the *PNPLA3*, *SAMM50*, *COL13A1* and *ADIPOQ* genes are associated with the presence of elevated ALT levels, in a sample of admixed Mexican adults. This study represents the first high-density single nucleotide screening for variations in NAFLD carried out in a Mexican-Mestizo population. The combined effect of these SNPs is likely to have an impact on the development of NAFLD, but further studies are required to determine the magnitude of this association in other Latino populations. Our results independently support those of other studies that have identified loci associated with an increased risk of NAFLD, but more studies are needed to confirm these findings in diverse populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was supported by funding from the Instituto Nacional de Medicina Genomica (INMEGEN) grant #119-11/2012/I, the Consejo Nacional de Ciencia y Tecnología (CONACyT) grants #26267M and SALUD-2011-01-161930, and the Instituto Mexicano del Seguro Social (IMSS). Dr. Flores was supported by NIH/NCI 5K07CA197179. The authors express their gratitude to the Mexican Health Worker Cohort Study participants, their families, and the IMSS clinicians and staff. We also acknowledge the services provided by the Microarray Core Facility at the INMEGEN (MSc. Raul Mojica Espinosa).

Abbreviations

ALT	alanine aminotransferase
AST	aspartate aminotransferase
SNP	Single Nucleotide Polymorphism
PRS	polygenic risk score
NAFLD	Non-alcoholic fatty liver disease

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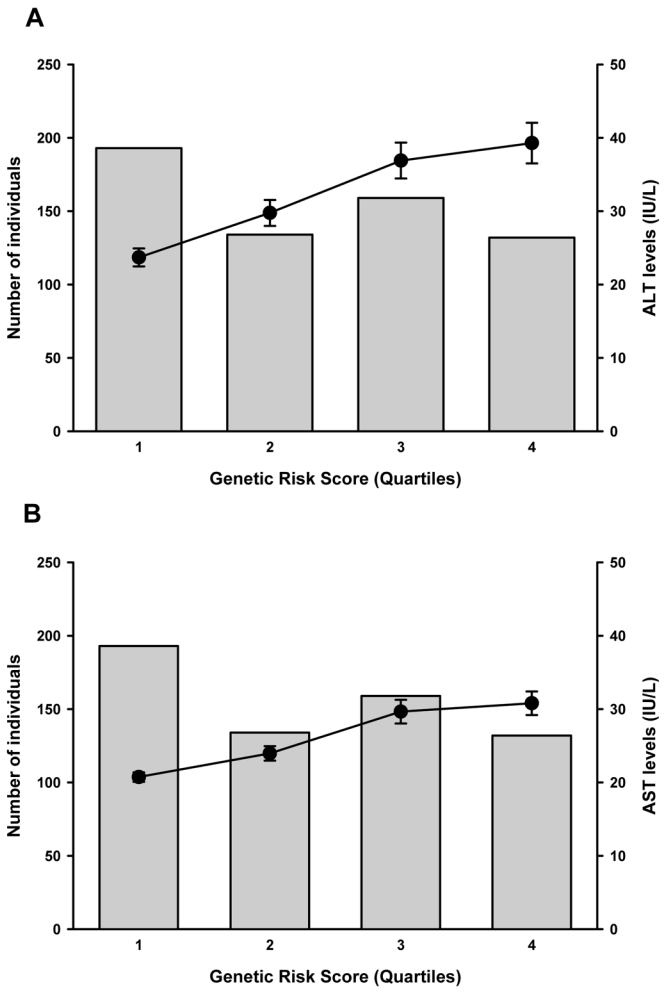

Fig. 1.

Distribution of the weighted genetic risk score and cumulative effects of the risk alleles from six SNPs on ALT and AST serum levels. Mean ALT (A) and AST (B) levels significantly increase as a function of the number of risk alleles (quartiles) (P=1.0×10^{-9} and 7.7×10^{-11}), respectively, adjusted for age, sex, BMI, and admixture.

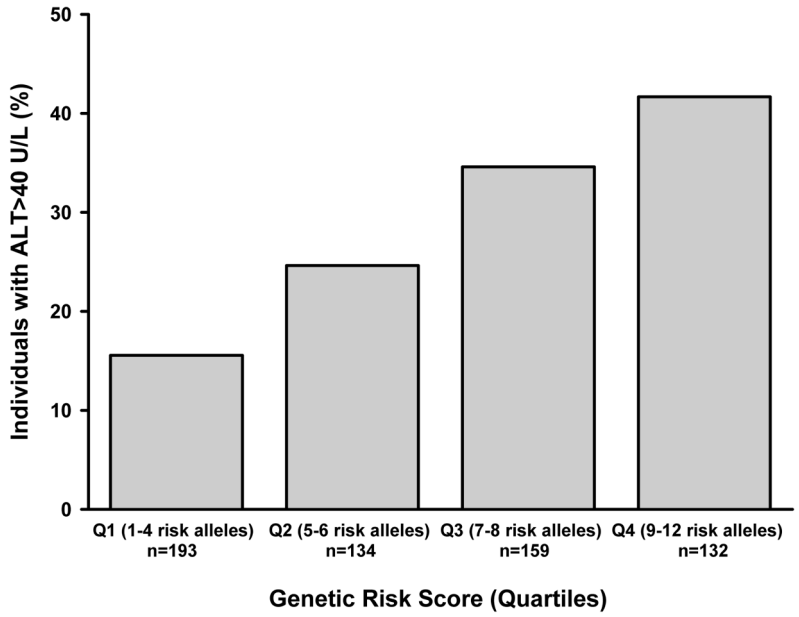

Fig. 2.

Association between the weighted genetic risk score from six SNPs and proportion of individuals with elevated ALT levels. The percentage of subjects with ALT ≥40 IU/L increased significantly as a function of the number of risk alleles (quartiles) (P<0.001 adjusted for age, sex, BMI, and admixture).

Table 1

Demographic and clinical characteristics of the study population

	All	Cases ALT ≥40 IU/L	Controls ALT <40 IU/L	*P*-value
N (%)	632	178 (28.2)	454 (71.8)	
Age (years)	48.7 ± 12.8	46.8 ± 11.2	49.5 ± 13.3	0.009
Sex (male %)	25.95	42.70	19.38	1.8×10^{-9}
Education Level				
≤ 6 years	17.09	11.80	19.16	0.041
≤ 12 years	39.08	42.70	37.67	
> 12 years	42.41	42.70	42.29	
Hypertension				
No	74.37	71.35	75.55	0.276
Yes	25.63	28.65	24.45	
BMI (kg/m^2)	27.1 ± 4.7	29.4 ± 4.5	26.2 ± 4.5	1.5×10^{-14}
Glucose (mg/dl)	99.1 ± 33.5	105.2 ± 33.5	96.8 ± 33.2	0.004
Total cholesterol (mg/dl)	200.3 ± 39.7	199.0 ± 36.2	200.8 ± 41.0	0.599
Triglycerides (mg/ml)	169.2 ± 119.1	200.0 ± 102.3	157.1 ± 123.1	8.5×10^{-10}
HDL-C (mg/ml)	38.7 ± 11.9	36.4 ± 9.6	39.5 ± 12.6	8.8×10^{-4}
LDL-C (mg/dl)	124.6 ± 36.9	123.6 ± 35.1	125.0 ± 37.5	0.666
ALT (IU/L)	31.9 ± 26.5	64.8 ± 29.5	19.0 ± 7.0	2.2×10^{-49}
AST (IU/L)	26.0 ± 16.1	44.6 ± 19.4	18.6 ± 5.1	9.9×10^{-42}

Results are presented as means ± standard deviations or n (%).

P values were obtained by comparing cases to controls.

Differences between proportions were performed using chi-square tests of homogeneity and differences between means were performed using t-test. Triglycerides were log-transformed before the analysis.

Abbreviations: BMI, body mass index; HDL-C, High Density Lipoprotein Cholesterol; HDL-C, Low Density Lipoprotein Cholesterol; ALT, alanine transaminase; AST, aspartate transaminase.

Table 2

Association between the *COL13A1 and PNPLA3* SNPs with elevated ALT levels, by sex. n (%)

	COL13A1				***PNPLA3***														
SNP	**rs1227756**				**rs3810622**				**rs12483959**				**rs2896019**				**rs738409**			
	AA	**AG**	**GG**	P_{rec}	**CC**	**CT**	**TT**	P_{rec}	**GG**	**GA**	**AA**	P_{dom}	**TT**	**TG**	**GG**	P_{dom}	**AA**	**AG**	**GG**	P_{rec}
Total (N=632)																			
Cases ALT ≥40 IU/L	20 (11.4)	66 (37.7)	89 (50.9)	**0.022**	8 (4.5)	54 (30.7)	114 (64.8)	0.052	79 (44.6)	71 (40.1)	27 (15.3)	**0.004**	75 (42.6)	74 (42.0)	27 (15.3)	**0.018**	21 (11.9)	73 (41.2)	83 (46.9)	0.070
Controls ALT <40 IU/L	65 (14.4)	232 (51.4)	154 (34.1)		30 (6.6)	194 (42.9)	228 (50.4)		125 (27.6)	232 (51.2	96 (21.1)		121 (26.7)	236 (52.1)	96 (21.2)		79 (17.5)	232 (51.4)	140 (31.0)	
Male (N=164)																			
Cases ALT ≥40 IU/L	11 (14.9)	28 (37.8)	35 (47.3)	NS	6 (8.0)	25 (33.3)	44 (58.7)	NS	32/42.7)	28 (37.3)	15 (20.0)	NS	29 (38.7)	31 (41.3)	15 (20.0)	NS	10 (13.2)	33 (43.3)	33 (43.4)	NS
Controls ALT <40 IU/L	14 (16.1)	44 (50.6)	29 (33.3)		4 (4.5)	30 (34.1)	54 (61.4)		32 (36.4)	42 (47.7)	14 (15.9)		32 (36.4)	42 (47.7)	14 (15.9)		12 (13.6)	39 (44.3)	37 (42.0)	
Female (N=468)																			
Cases ALT ≥40 IU/L	9 (8.9)	38 (37.6)	54 (53.5)	0.245	2 (2.0)	29 (28.7)	70 (69.3)	**0.005**	47 (46.1)	43 (42.2)	12 (11.8)	**0.010**	46 (45.5)	43 (42.6)	12 (11.9)	**0.009**	11 (10.9)	40 (39.6)	50 (49.5)	**0.028**
Controls ALT <40 IU/L	51 (14.0)	188 (51.6)	125 (34.3)		26 (7.1)	164 (45.1)	174 (47.8)		93 (25.5)	190 (52.1)	82 (22.5)		89 (24.4)	194 (53.2)	82 (22.5)		67 (18.5)	193 (53.2)	103 (28.4)	

P values were calculated by logistic regression analysis, and the statistically significant results are shown in bold.

All models were adjusted for age, BMI, ancestry and number of tests. The analyses with the total population were additionally adjusted for sex.

SNP, single nucleotide polymorphism; ALT, alanine transaminase; BMI, body mass index.

Table 3

Association between the *ADIPOQ, COL13A1, PNPLA3* and *SAMM50* SNPs with select clinical measures

Gene **SNP**	***ADIPOQ*** **rs17366743**		***COL13A1*** **rs7101190**		***COL13A1*** **rs1227756**		***PNPLA3*** **rs3810622**		***PNPLA3*** **rs12483959**		***PNPLA3*** **rs2896019**		***PNPLA3*** **rs738409**		***SAMM50*** **rs2143571**	
	Effect (SE)	P_{add}	**Effect (SE)**	P_{dom}	**Effect (SE)**	P_{rec}	**Effect (SE)**	P_{rec}	**Effect (SE)**	P_{dom}	**Effect (SE)**	P_{dom}	**Effect (SE)**	P_{rec}	**Effect (SE)**	P_{add}
BMI (kg/m^2)	−1.2 (0.6)	NS	−0.5 (0.4)	NS	0.3 (0.4)	NS	−0.8 (0.4)	NS	0.6 (0.4)	NS	0.4 (0.4)	NS	−0.2 (0.4)	NS	0.1 (0.3)	NS
TG (mg/dl)	−4.8 (13.9)	NS	−22.8 (9.3)	NS	22.9 (9.6)	NS	2.2 (9.4)	NS	−3.5 (10.1)	NS	−3.7 (10.2)	NS	−2.2 (9.9)	NS	−5.6 (6.9)	NS
HDL-C (mg/dl)	−1.2 (1.4)	NS	−0.9 (1.0)	NS	−0.6 (1.0)	NS	1.4 (1.0)	NS	−2.5 (1.0)	NS	−2.4 (1.0)	NS	2.4 (1.0)	NS	0.7 (0.7)	NS
Glucose (mg/dl)	−6.1 (3.9)	NS	−2.8 (2.6)	NS	3.8 (2.9)	NS	−3.1 (2.6)	NS	−0.1 (2.8)	NS	0.8 (2.9)	NS	2.3 (2.8)	NS	−1.3 (1.9)	NS
ALT (U/L)	−10.9 (2.8)	**0.014**	−6.6 (1.9)	**0.045**	7.3 (1.9)	**0.003**	8.0 (1.9)	**0.001**	−8.6 (2.1)	**0.002**	−7.6 (2.1)	**0.008**	6.8 (2.0)	**0.023**	5.1 (1.4)	0.077
AST (U/L)	−6.1 (1.8)	0.120	−4.1 (1.2)	0.074	4.8 (1.2)	**7.4×10^{-4}**	5.7 (1.2)	**8.2×10^{-5}**	−6.2 (1.3)	**1.6×10^{-4}**	−5.5 (1.3)	**0.001**	4.7 (1.2)	**0.015**	7.5 (1.8)	**0.032**

P values were calculated by linear regression analysis, and the statistically significant results are shown in bold.

All models were adjusted for, age, sex, BMI, ancestry and number of tests.

TG, ALT and AST were log transformed for the analysis.

SNP, single nucleotide polymorphism; SE, standard error; BMI, body mass index; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; ALT, alanine transaminase; AST, aspartate transaminase.