ORIGINAL RESEARCH

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PNPLA3 Polymorphisms and Liver Aminotransferase Levels in a Mexican American Population

Abstract

Purpose: This study examined genetic associations of patatin-like phospholipase domain containing 3 gene (*PNPLA3*) polymorphisms and liver aminotransferases in an extensively documented, randomly recruited Mexican American population at high risk of liver disease.

Methods: Two single nucleotide polymorphisms (SNP) in the *PNPLA3* gene (i.e., rs738409 and rs2281135) were genotyped in 1532 individuals. Population stratification was corrected by the genotyping of 103 ancestry informative markers (AIMs) for Mexican Americans.

Results: Both *PNPLA3* SNPs showed highly significant association with alanine aminotransferase (ALT) levels, but was also, in males, associated with aspartate aminotransferase (AST) levels. Haplotypic association test of the two SNPs suggested stronger genetic association with rs738409 than rs2281135. Obvious sex effects were observed: rs738409-sex interaction in ALT levels $P=8.37 \times 10^{-4}$; rs738409-sex interaction in AST levels $P=5.03 \times 10^{-3}$.

Conclusions: This population study highlights a sex-specific association of *PNPLA3* polymorphisms and elevated liver enzymes in a population-based study, independent of common pathological factors of the metabolic syndrome. The strong genetic association found in women ≤ 50 years old, but not in women > 50 years old, suggests that sex hormones may mediate the sex effect.

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The Hispanic population, and Mexican Americans in particular, have high rates of obesity, diabetes, metabolic syndrome (MetS) and liver disease, particularly non-alcoholic fatty liver disease (NAFLD) [1-5]. The patatin-like phospholipase domain containing 3 gene (*PNPLA3*) at Chr22q13.31 was originally identified in adipose tissue by Baulande et al. and named adiponutrin [6]. This gene is specifically expressed in adipose tissue and is under tight nutritional regulation [6]. What implicates *PNPLA3* in liver disease is that, in 2008, Romeo et al. identified an association between an amino acid substitution I[Ile]148M[Met] (NCBI dbSNP ID: rs738409) in the *PNPLA3* gene and genetic susceptibility for NAFLD [7]. This observation was confirmed in a number of subsequent reports [8-13]. Recent functional experiments showed that the I148M substitution from the single nucleotide polymorphism (SNP) rs738409 caused a loss of enzymatic activity [14]. Studies have also showed an association of *PNPLA3* variations with increased plasma levels of liver enzymes [15,16]. The strongest signal of the quantitative trait association was tagged by the SNP rs2281135. Genetic association of a *PNPLA3* variant with alcoholic liver disease was also proposed [17]. Together these interesting observations suggest that *PNPLA3* might encode a key molecule mediating the pathological processes of liver injury in metabolic disorders.

Given the high rate of NAFLD in Hispanics, the genetic association of *PNPLA3* SNPs and liver enzymes was examined in a well-studied cohort of Mexican Americans: the Cameron County Hispanic Cohort (CCHC) from the Lower Rio Grande Valley (LRGV) in south Texas, USA [18]. The participants of this cohort were randomly recruited in the city of Brownsville, Cameron County, based on the 2000 census tract data. In this population, a 49.5% prevalence of obesity, a 30.7% prevalence of diabetes and a high incidence of abnormal serum liver enzymes were observed [3,19,20]. Assessing the genetic

effect of *PNPLA3* variants in this population may lead to further understanding of genetic drivers of liver injury in this underserved minority population.

Materials and Methods

Subjects

This study investigated 1532 individuals from the CCHC. The characteristics of this cohort have been extensively described in previous reports [18,19,21]. The participants were randomly selected from this population and no disease criteria were applied in their selection; therefore, the participants are representative of the general population of the town. Blood samples were obtained at recruitment. Informed consent was obtained from each individual, and the Committee for the Protection of Human Subjects of the University of Texas Health Science Center at Houston (UTHealth) approved this study.

Genotyping

The genotyping for this study was done using the Sequenom iPLEX assay (Sequenom, Cambridge, MA). Two single nucleotide polymorphisms (SNP) in the *PNPLA3* gene (i.e., rs738409 and rs2281135) were genotyped. rs738409 was the SNP I148M that was originally identified as having genetic association with NAFLD [7]. The other SNP, rs2281135, was identified as the strongest association in this genetic region with elevated ALT by a genome-wide association study in a European population [15]. As is shown by HapMap data, rs738409 and rs2281135 are in tight linkage disequilibrium (LD) in three major human ethnic groups: African, Caucasian and East Asian (Table 1). The genotyping call rates of both SNPs were 100%. The Mexican Americans in our study are an admixed population of predominantly European and Native American ancestry. To address the potential bias of population

TABLE 1. Linkage disequilibrium of the two *PNPLA3* SNPs associated with ALT/AST levels

Population*	rs738409 (minor allele, frequency)	rs2281135 (minor allele, frequency)	LD of rs738409-rs2281135 (D', r ²)
CEU	G, 0.233	T, 0.208	0.839, 0.609
YRI	G, 0.125	T, 0.158	0.920, 0.643
CHB+JPT	G, 0.388	T, 0.388	0.976, 0.953
CCHC	G, 0.495	T, 0.468	0.983, 0.832

* The abbreviations represent: YRI, Yoruba in Ibadan, Nigeria; CEU, Utah residents with Northern and Western European ancestry from the CEPH collection; JPT, Japanese in Tokyo, Japan; CHB, Han Chinese in Beijing, China. These reference populations were genotyped in the HapMap study (<http://www.hapmap.org>).

TABLE 2. Quantitative trait association test between *PNPLA3* polymorphisms and ALT/AST levels

Trait	SNP (reference allele)	Males (n=496)			Females (n=1036)		
		Regression coefficient (β , U/L)	Standard error (SE)	<i>P</i>	Regression coefficient (β , U/L)	Standard error (SE)	<i>P</i>
ALT	rs738409 (C)	7.517	1.591	2.99 x10 ⁻⁶	2.970	0.658	7.10 x10 ⁻⁶
ALT	rs2281135 (T)	7.202	1.635	1.30 x10 ⁻⁵	2.542	0.658	1.18x10 ⁻⁴
AST	rs738409 (C)	4.589	1.208	1.64 x10 ⁻⁴	0.987	0.663	0.137
AST	rs2281135 (T)	3.996	1.243	1.40 x10 ⁻³	0.833	0.661	0.208

stratification, 103 continental ancestry informative markers (AIMs) for Mexican Americans, identified by a previous study [22], were genotyped. All these AIMs are on auto-chromosomes. The genotyping call rates of the 103 AIM SNPs were between 95.4% and 100%, with a median of 99.9%. For the purpose of quality control, 93 DNA samples were genotyped in duplicate and the concordance rate of each duplicate was 100%.

Statistical methods

The population structure of the Mexican American population was analyzed by principal component analysis using the Eigensoft version 2.0 software [19]. Quantitative trait association tests were performed using PLINK software (<http://pngu.mgh.harvard.edu/~purcell/plink/>). The additive genetic model was used in the association test. All association test results were adjusted for age. CCHC participants showed the predominant one-dimensional character of an admixture of European and American Indian ancestries. The first principal component (PC1) explained the majority of population structure; therefore, all association test results in this study were corrected for PC1.

Results

Sex difference in liver injury was observed in our previous epidemiological analyses of this population [3,19]. Previous studies have also suggested a possibility of sex difference in the genetic association of *PNPLA3* and liver enzymes [23]. In our study, significant sex-SNP interaction was observed: rs738409-sex interaction in ALT levels had $P=8.37 \times 10^{-4}$; rs738409-sex interaction in AST levels had $P=5.03 \times 10^{-3}$; therefore, genetic effects were assessed separately in males and females. By the quantitative trait association test, *PNPLA3* SNPs showed highly significant association with ALT levels in both males and females (Table 2). Association between the *PNPLA3*

SNPs and AST levels was also statistically significant in males; however, the genetic association between the *PNPLA3* SNPs and AST levels was not seen in females. The sex difference in AST association has a statistical significance of $p=0.024$ for rs738409, and $p=0.055$ for rs2281135.

Table 1 shows that two *PNPLA3* SNPs, rs738409 and rs2281135, are in tight linkage disequilibrium (LD) in these Mexican Americans - similar to that seen in three other major human ethnic groups: African, Caucasian and East Asian. The genetic association was replicated in haplotypic association tests (Table 3). As shown by the haplotypic association tests, the direction of the genetic effect of two haplotypes carrying the same C allele of rs738409 is the same, but this is not the case for the two haplotypes carrying the same C allele of rs2281135. The ALT associations of the rs738409-rs2281135 haplotypes C-C vs. G-C are heterogeneous with highly statistical significances ($p=2.97 \times 10^{-5}$ in males, and $p=5.89 \times 10^{-6}$ in females). The AST associations of the two haplotypes were also heterogeneous with statistical significances ($p=2.36 \times 10^{-5}$ in males, and $p=0.076$ in females).

To further examine the effect of sex on ALT association, step-wise logistic regression analyses were performed by taking a series of cutoffs of ALT or AST values for classification (Table 4). Corrected for age and population structure, the logistic regression analysis also suggested a sex effect. Interestingly, using the traditional cutoff of over 40U/L as denoting elevated ALT, the association of rs738409 or rs2281135 with elevated ALT was not significant in males ($p=0.187$ for rs738409, $p=0.164$ for rs2281135), but was highly significant in females ($p=1.72 \times 10^{-5}$ for rs738409, $p=1.30 \times 10^{-5}$ for rs2281135). When a higher cutoff value was used, a high statistical significance was also seen in the logistic regression in males. The *PNPLA3* SNP rs738409 has a stronger association with considerably more elevated ALT levels in males in comparison with females. A similar trend towards much higher elevation in males was also observed in the association with AST levels.

TABLE 3. Haplotypic association test between *PNPLA3* variant and ALT/AST levels

Trait	Haplotype (rs738409- rs2281135)	Males(n=496)			Females (n=1036)		
		Frequency	Regression coefficient (β , U/L)	<i>P</i>	Frequency	Regression coefficient (β , U/L)	<i>P</i>
ALT	C-T	0.444	6.820	2.74×10^{-5}	0.473	2.490	1.54×10^{-4}
ALT	C-C	0.045	4.460	0.255	0.040	3.100	0.064
ALT	G-C	0.506	-7.710	2.02×10^{-6}	0.483	-3.020	5.51×10^{-6}
AST	C-T	0.444	3.580	3.63×10^{-3}	0.473	0.741	0.260
AST	C-C	0.045	5.980	0.043	0.040	1.500	0.367
AST	G-C	0.506	-4.840	8.10×10^{-5}	0.483	-1.070	0.108

As we have already shown in this Mexican American population, obesity, insulin resistance, and the metabolic syndrome (MetS) are common [3,19]. We have also shown that in this population the ratio between plasma levels of two key adipokines, adiponectin and leptin is critical in MetS[21]. To determine how these metabolic disorders might be related to the genetic association of *PNPLA3* polymorphisms and liver enzymes, the association among the *PNPLA3* SNPs and Body Mass Index (BMI), the homeostasis model assessment-estimated insulin resistance (HOMA-IR), adiponectin, leptin, and Log(leptin/adiponectin) were tested. After correcting for age and population structure, no genetic association was identified between *PNPLA3* polymorphisms and BMI, HOMA-IR, adiponectin and leptin (Table 5).

The genetic association of rs738409 and ALT in women ≤ 50 years old versus women > 50 years old were then compared, to test the theory that sex hormone levels may be responsible for gender differences. Although not measured in these subjects, it is assumed that there are significant differences in estrogen levels in these two age groups. A strong genetic association was found in women ≤ 50 years old ($\beta=0.202$, $P=1.69 \times 10^{-7}$), but not in women > 50 years old ($\beta=0.060$, $P=0.231$).

Discussion

This study shows that the association between *PNPLA3* polymorphisms and elevated plasma liver enzymes is highly statistically significant in a Mexican American population and that that population structure of this admixed sample has no obvious effect on this association. Furthermore, the haplotypic association test suggests the genetic association tagged by the two *PNPLA3* SNPs represents a single genetic effect. Interestingly

a sex effect of *PNPLA3* polymorphisms was also observed, which was further characterized in detail in this Mexican American population.

Phospholipase A2 (PLA2) catalyzes the hydrolysis of the sn-2 position of membrane glycerophospholipids to yield arachidonic acid (AA) and lysophospholipids[24]. AA is a precursor of eicosanoids, including prostaglandins (PGs) and leukotrienes (LTs). Lysophospholipids are potent biologically active lipid mediators that exert a wide range of cellular effects through specific G protein-coupled receptors [25]. In the PLA2 enzyme superfamily, six families of PLA2, with different action mechanisms and biological activities, have been identified [26]. *PNPLA3* encodes a member of the Calcium-Independent PLA2 (iPLA2) family and the iPLA2 enzymes have been known to play a major role in membrane phospholipid remodeling [27]. *PNPLA3* was originally identified in adipose tissue as Adiponutrin [6], where its expression is under tight nutritional regulation. In the fasting status, *PNPLA3* mRNA is almost undetectable, but increases dramatically after a high carbohydrate challenge [6]. Studies have shown triacylglycerol (TAG) lipase activity and acylglycerol-transacylase activity of the *PNPLA3* protein [28]. Important insight into *PNPLA3* involvement in liver lipid metabolism comes from the recent genetic studies: the derived allele of SNP rs738409 in *PNPLA3* was found to be associated with increased liver fat content [7,13], NAFLD [7-13] and increased plasma levels of liver enzymes [15,16]. Functional experiments showed that the I148M substitution from SNP rs738409 caused a loss of enzyme activity[14].

Prior to our study, a meta-analysis by Sookoian and Pirola suggested the possibility of sex difference in the genetic association of *PNPLA3* polymorphisms and liver enzymes; that is, a negative association between the effect of rs738409 on liver fat

TABLE 4. Discrete trait association test between PNPLA3 polymorphisms and ALT/AST levels

cutoff	ALT and rs738409-C in males (n=496)				ALT and rs2281135-T in males (n=496)				ALT and rs2281135-T in females (n=1036)				AST and rs738409-C in males (n=496)				AST and rs2281135-T in females (n=1036)				AST and rs2281135-T in females (n=1036)								
	>cutoff (n)	P	OR (95%CI)	>cutoff (n)	P	OR (95%CI)	>cutoff (n)	P	OR (95%CI)	>cutoff (n)	P	OR (95%CI)	>cutoff (n)	P	OR (95%CI)	>cutoff (n)	P	OR (95%CI)	>cutoff (n)	P	OR (95%CI)	>cutoff (n)	P	OR (95%CI)	>cutoff (n)	P	OR (95%CI)		
30	443	0.926 (0.620, 1.382)	0.705	791	1.355 (1.095, 1.677)	0.521E-03	443	0.901 (0.596, 1.363)	0.622	791	1.322 (1.066, 1.638)	0.011	295	1.283 (0.979, 1.681)	0.070	532	1.083 (0.907, 1.294)	0.379	295	1.241 (0.942, 1.634)	0.125	532	1.083 (0.907, 1.294)	0.377	532	1.241 (0.942, 1.634)	0.125	532	1.083 (0.907, 1.294)
31	435	0.925 (0.624, 1.371)	0.697	717	1.298 (1.067, 1.579)	0.03E-03	435	0.929 (0.615, 1.402)	0.725	717	1.316 (1.081, 1.602)	0.012	278	1.243 (0.950, 1.627)	0.112	507	1.018 (0.852, 1.217)	0.846	278	1.170 (0.890, 1.538)	0.261	507	1.013 (0.848, 1.210)	0.888	507	1.170 (0.890, 1.538)	0.261	507	1.013 (0.848, 1.210)
32	416	1.109 (0.779, 1.580)	0.565	659	1.359 (1.127, 1.639)	0.003E-03	416	1.092 (0.748, 1.596)	0.649	659	1.336 (1.108, 1.612)	0.013	269	1.331 (1.019, 1.740)	0.036	489	1.022 (0.855, 1.222)	0.812	269	1.236 (0.941, 1.623)	0.127	489	1.026 (0.859, 1.226)	0.777	489	1.236 (0.941, 1.623)	0.127	489	1.026 (0.859, 1.226)
33	399	1.146 (0.823, 1.595)	0.421	605	1.449 (1.206, 1.740)	0.001E-03	399	1.116 (0.787, 1.582)	0.540	605	1.398 (1.165, 1.678)	0.011	254	1.415 (1.082, 1.851)	0.011	464	1.030 (0.860, 1.234)	0.748	254	1.343 (1.022, 1.765)	0.034	464	1.049 (0.877, 1.255)	0.598	464	1.343 (1.022, 1.765)	0.034	464	1.049 (0.877, 1.255)
34	381	1.113 (0.812, 1.525)	0.507	546	1.504 (1.255, 1.802)	0.007E-03	381	1.090 (0.780, 1.522)	0.614	546	1.422 (1.188, 1.703)	0.012	248	1.385 (1.061, 1.809)	0.017	437	1.074 (0.896, 1.289)	0.439	248	1.341 (1.021, 1.761)	0.035	437	1.091 (0.911, 1.307)	0.346	437	1.341 (1.021, 1.761)	0.035	437	1.091 (0.911, 1.307)
35	356	1.165 (0.863, 1.572)	0.319	491	1.605 (1.337, 1.926)	0.003E-03	356	1.150 (0.840, 1.573)	0.383	491	1.524 (1.273, 1.826)	0.013	235	1.284 (0.986, 1.672)	0.064	401	1.035 (0.861, 1.245)	0.714	235	1.213 (0.926, 1.588)	0.160	401	1.073 (0.894, 1.288)	0.448	401	1.213 (0.926, 1.588)	0.160	401	1.073 (0.894, 1.288)
36	345	1.160 (0.865, 1.556)	0.321	436	1.586 (1.318, 1.908)	0.007E-03	345	1.156 (0.849, 1.574)	0.357	436	1.534 (1.278, 1.840)	0.013	221	1.305 (1.002, 1.700)	0.048	367	1.074 (0.889, 1.297)	0.460	221	1.244 (0.950, 1.629)	0.112	367	1.123 (0.932, 1.353)	0.222	367	1.244 (0.950, 1.629)	0.112	367	1.123 (0.932, 1.353)
37	325	1.121 (0.846, 1.487)	0.427	390	1.601 (1.325, 1.936)	0.001E-03	325	1.108 (0.827, 1.484)	0.491	390	1.522 (1.264, 1.832)	0.012	209	1.341 (1.028, 1.749)	0.030	337	1.107 (0.914, 1.341)	0.299	209	1.260 (0.962, 1.652)	0.093	337	1.164 (0.963, 1.407)	0.116	337	1.260 (0.962, 1.652)	0.093	337	1.164 (0.963, 1.407)
38	307	1.211 (0.920, 1.595)	0.173	356	1.561 (1.288, 1.891)	0.006E-03	307	1.185 (0.892, 1.573)	0.241	356	1.498 (1.241, 1.808)	0.013	194	1.372 (1.050, 1.794)	0.021	306	1.086 (0.893, 1.321)	0.408	194	1.290 (0.982, 1.695)	0.067	306	1.146 (0.944, 1.391)	0.169	306	1.290 (0.982, 1.695)	0.067	306	1.146 (0.944, 1.391)
39	291	1.166 (0.889, 1.530)	0.267	316	1.552 (1.275, 1.889)	0.007E-03	291	1.195 (0.904, 1.581)	0.210	316	1.490 (1.229, 1.807)	0.013	182	1.387 (1.057, 1.819)	0.018	270	1.098 (0.898, 1.342)	0.361	182	1.312 (0.997, 1.728)	0.053	270	1.153 (0.946, 1.406)	0.160	270	1.312 (0.997, 1.728)	0.053	270	1.153 (0.946, 1.406)
40	275	1.200 (0.915, 1.574)	0.187	283	1.538 (1.273, 1.908)	0.002E-03	275	1.218 (0.923, 1.608)	0.164	283	1.537 (1.276, 1.900)	0.012	174	1.390 (1.058, 1.826)	0.018	248	1.132 (0.920, 1.393)	0.241	174	1.358 (1.031, 1.789)	0.029	248	1.180 (0.963, 1.447)	0.111	248	1.358 (1.031, 1.789)	0.029	248	1.180 (0.963, 1.447)
41	266	1.261 (0.962, 1.652)	0.093	253	1.526 (1.236, 1.883)	0.003E-03	266	1.290 (0.978, 1.702)	0.071	253	1.480 (1.205, 1.817)	0.013	162	1.492 (1.129, 1.972)	0.021	226	1.133 (0.915, 1.403)	0.252	162	1.451 (1.096, 1.920)	0.049	226	1.186 (0.961, 1.464)	0.112	226	1.451 (1.096, 1.920)	0.049	226	1.186 (0.961, 1.464)
42	257	1.293 (0.988, 1.692)	0.061	230	1.487 (1.196, 1.848)	0.004E-03	257	1.301 (0.988, 1.713)	0.061	230	1.457 (1.179, 1.800)	0.013	153	1.540 (1.159, 2.046)	0.013	199	1.079 (0.864, 1.348)	0.305	153	1.499 (1.127, 1.993)	0.03	199	1.109 (0.890, 1.381)	0.338	199	1.499 (1.127, 1.993)	0.03	199	1.109 (0.890, 1.381)
43	241	1.407 (1.074, 1.841)	0.013	206	1.529 (1.216, 1.922)	0.002E-03	241	1.430 (1.085, 1.884)	0.011	206	1.494 (1.197, 1.864)	0.012	144	1.612 (1.206, 2.156)	0.018	177	1.071 (0.847, 1.355)	0.366	144	1.542 (1.154, 2.059)	0.03	177	1.075 (0.854, 1.355)	0.538	177	1.542 (1.154, 2.059)	0.03	177	1.075 (0.854, 1.355)
44	226	1.434 (1.094, 1.879)	0.003	189	1.456 (1.150, 1.843)	0.003E-03	226	1.408 (1.069, 1.853)	0.015	189	1.411 (1.124, 1.772)	0.013	134	1.491 (1.106, 2.010)	0.021	149	1.156 (0.898, 1.488)	0.260	134	1.347 (1.002, 1.811)	0.049	149	1.160 (0.907, 1.483)	0.236	149	1.347 (1.002, 1.811)	0.049	149	1.160 (0.907, 1.483)
45	212	1.470 (1.119, 1.932)	0.003	174	1.471 (1.153, 1.878)	0.001E-03	212	1.446 (1.096, 1.908)	0.013	174	1.420 (1.124, 1.793)	0.013	127	1.559 (1.145, 2.122)	0.013	136	1.145 (0.885, 1.480)	0.304	127	1.403 (1.034, 1.904)	0.030	136	1.139 (0.887, 1.464)	0.308	136	1.403 (1.034, 1.904)	0.030	136	1.139 (0.887, 1.464)
46	197	1.506 (1.142, 1.988)	0.003	162	1.489 (1.159, 1.912)	0.003E-03	197	1.357 (1.017, 1.795)	0.011	162	1.436 (1.132, 1.822)	0.012	115	1.835 (1.316, 2.558)	0.013	125	1.150 (0.880, 1.504)	0.306	115	1.573 (1.143, 2.164)	0.03	125	1.103 (0.849, 1.434)	0.462	125	1.573 (1.143, 2.164)	0.03	125	1.103 (0.849, 1.434)
47	183	1.573 (1.186, 2.085)	0.003	147	1.613 (1.235, 2.107)	0.003E-03	183	1.633 (1.227, 2.175)	0.003	147	1.480 (1.156, 1.895)	0.013	103	1.674 (1.183, 2.369)	0.013	110	1.241 (0.931, 1.652)	0.141	103	1.479 (1.066, 2.051)	0.019	110	1.176 (0.890, 1.553)	0.254	110	1.479 (1.066, 2.051)	0.019	110	1.176 (0.890, 1.553)
48	167	1.451 (1.090, 1.931)	0.011	131	1.743 (1.300, 2.336)	0.004E-03	167	1.498 (1.120, 2.004)	0.013	131	1.594 (1.219, 2.085)	0.013	97	1.644 (1.150, 2.351)	0.013	102	1.277 (0.954, 1.711)	0.100	97	1.464 (1.042, 2.055)	0.028	102	1.203 (0.907, 1.596)	0.200	102	1.464 (1.042, 2.055)	0.028	102	1.203 (0.907, 1.596)
49	150	1.652 (1.224, 2.230)	0.003	120	1.810 (1.331, 2.462)	0.003E-03	150	1.697 (1.255, 2.296)	0.003	120	1.662 (1.254, 2.245)	0.012	90	1.627 (1.130, 2.398)	0.013	93	1.311 (0.962, 1.786)	0.086	90	1.468 (1.034, 2.085)	0.032	93	1.230 (0.911, 1.659)	0.177	93	1.468 (1.034, 2.085)	0.032	93	1.230 (0.911, 1.659)
50	141	1.635 (1.209, 2.210)	0.003	110	1.739 (1.275, 2.373)	0.003E-03	141	1.665 (1.229, 2.254)	0.003	110	1.587 (1.193, 2.112)	0.012	83	1.646 (1.130, 2.398)	0.013	83	1.350 (0.958, 1.904)	0.087	83	1.463 (1.024, 2.090)	0.037	78	1.232 (0.888, 1.708)	0.211	78	1.463 (1.024, 2.090)	0.037	78	1.232 (0.888, 1.708)
51	136	1.710 (1.259, 2.323)	0.003	105	1.776 (1.291, 2.442)	0.003E-03	136	1.727 (1.271, 2.346)	0.003	105	1.648 (1.227, 2.213)	0.012	78	1.832 (1.231, 2.726)	0.013	72	1.448 (1.010, 2.076)	0.044	78	1.579 (1.093, 2.281)	0.015	72	1.335 (0.948, 1.879)	0.098	72	1.579 (1.093, 2.281)	0.015	72	1.335 (0.948, 1.879)
52	129	1.719 (1.254, 2.356)	0.003	98	1.813 (1.306, 2.516)	0.003E-03	129	1.700 (1.242, 2.328)	0.003	98	1.661 (1.229, 2.245)	0.012	69	1.753 (1.173, 2.620)	0.013	66	1.423 (0.990, 2.046)	0.057	69	1.557 (1.065, 2.277)	0.022	66	1.340 (0.951, 1.887)	0.094	66	1.557 (1.065, 2.277)	0.022	66	1.340 (0.951, 1.887)
53	124	1.778 (1.293, 2.445)	0.003	92	1.784 (1.270, 2.506)	0.003E-03	124	1.752 (1.276, 2.404)	0.003	92																			

TABLE 5. Quantitative trait association test between PNPLA3 polymorphisms and metabolic criteria

Trait	SNP (reference allele)	Males			Females		
		Regression co-efficient (β)	Standard error (SE)	<i>P</i>	Regression co-efficient (β)	Standard error (SE)	<i>P</i>
BMI	rs738409 (C)	0.263	0.401	0.512	0.030	0.331	0.927
BMI	rs2281135 (T)	0.165	0.411	0.689	-0.124	0.330	0.708
HOMA-IR	rs738409 (C)	0.139	0.286	0.629	-0.241	0.172	0.162
HOMA-IR	rs2281135 (T)	0.221	0.293	0.452	-0.331	0.172	0.054
Adiponectin*	rs738409 (C)	-1.082 (pg/ml)	1.561	0.493	1.213 (pg/ml)	1.484	0.415
adiponectin*	rs2281135 (T)	-1.893 (pg/ml)	1.511	0.219	0.769 (pg/ml)	1.526	0.615
Leptin*	rs738409 (C)	-3009 (ng/ml)	2768	0.285	2284 (ng/ml)	4304	0.597
Leptin*	rs2281135 (T)	-446 (ng/ml)	2767	0.873	2144 (ng/ml)	4428	0.629
Log(leptin/adiponectin) *	rs738409 (C)	-0.232	0.186	0.220	0.029	0.061	0.635
Log(leptin/adiponectin) *	rs2281135 (T)	0.035	0.181	0.848	0.051	0.063	0.418

*The quantitative trait association test of adiponectin, leptin, and Log(leptin/adiponectin) was based on the measurement of adiponectin and leptin at protein levels in 153 individuals randomly selected from the subjects in this study[21].

content and male sex using meta-regression analysis [23]. Our study confirms and advances this observation by showing that the *PNPLA3* SNPs have a stronger association with more highly elevated ALT levels in males, but are not associated with moderately elevated ALT in males. A recent report by Huang et al. showed that, in mice, nutritional control of *PNPLA3* expression in liver was regulated by a feed-forward loop that couples transcriptional and posttranslational controls. The transcription factor SREBP-1c plays key roles by directly activating *PNPLA3* transcription and indirectly inhibiting *PNPLA3* degradation through the stimulation of fatty acid synthesis [29]. Transcription of *PNPLA3* is regulated by the transcription factor, sterol regulatory element binding protein 1c (SREBP-1c)[29]. SREBP-1c is a direct target gene of the liver X receptor (LXR) /retinoid X receptor (RXR) heterodimer [30]. Transcriptional regulation of *PNPLA3* by fasting and refeeding requires both SREBP-1c and LXR/RXR [29]. Estrogen can cause down-regulation of expression of both LXR and SREBP-1c [31]. This sex hormone mediated mechanism may explain the interesting sex effect characterized in this study.

In human adipose tissue, *PNPLA3* mRNA expression is also known to be regulated by energy intake. *PNPLA3* mRNA levels have not been found to be different between non-obese and obese women, and do not correlate with adiposity indexes

or leptin or adiponectin mRNAs [32]. Our data provides further evidence that obesity, insulin resistance and adipose disorder are not involved in this genetic association of liver enzymes. Although MetS has a high prevalence and is the major common factor in liver injury in our population [3,21], our data suggest *PNPLA3*-mediated liver enzyme change is independent of these common pathological factors of MetS.

To our knowledge, this is the first study of the genetic association of *PNPLA3* polymorphisms and liver enzymes using material from a community cohort in a small USA city with a high minority population and a relatively homogenous culture and life-style. This homogeneity makes this population a unique resource for biomarker studies [19,21]. Besides the genetic effect of *PNPLA3* polymorphism, a number of other factors were found to be involved in elevated liver enzymes in this population, including plasma insulin level and insulin resistance, BMI and body weight, serum triglycerides and non-HDL cholesterol and hypertension. After correction for these factors, the genetic effect from *PNPLA3* polymorphism was found to be independent (e.g. rs738409 and ALT levels $P=2.00 \times 10^{-7}$)[33].

Our study had a number of limitations. 1. Diet is a risk factor of NAFLD; However, there was no robust method to measure diet in this community population. 2. We did not identify the association of the *PNPLA3* variant and AST in

females. 3. Plasma AST level lacks specificity for liver injury, as AST is found widely in multiple tissues: red blood cells, liver, heart, muscle tissue, pancreas and kidneys. 4. Although an interesting sex-specific effect of the *PNPLA3* variant, underlying mechanisms could not be characterized further with existing data. Sex hormone may mediate the sex effect, however, data concerning plasma sex hormone levels in our cohort participants was not available. Trying to partially compensate for this, the genetic association of rs738409 and ALT were compared in women ≤ 50 years old versus women > 50 years old, suggests that, if sex hormones are involved in liver injury, endogenous estrogen is not protective in liver injury in women.

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Author Contributions

Quan Li researched data, reviewed/edited the manuscript. Hui-Qi Qu conceived the study, researched the data, and wrote the manuscript. Megan L Grove and Yang Lu performed the experiments. Anne R Rentfro, Shaper Mirza, Craig L Hanis, and Eric Boerwinkle, reviewed/edited the manuscript. Susan P Fisher-Hoch and Joseph B McCormick conceived the study and wrote the manuscript.

List of Abbreviations

ALT	alanine aminotransferase
AIMs	ancestry informative markers
AST	aspartate aminotransferase

BMI	Body Mass Index
CCHC	Cameron County Hispanic Cohort
HOMA-IR	homeostasis model assessment-estimated insulin resistance
LD	linkage disequilibrium
MetS	metabolic syndrome
NAFLD	non-alcoholic fatty liver disease
<i>PNPLA3</i>	patatin-like phospholipase domain containing 3 gene
SNP	single nucleotide polymorphisms

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