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NAFLD Susceptibility Genes and their Association with Type 2 Diabetes and Obesity in a New Mexico Population

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Abstract

Objective: Genome-wide association studies have identified single-nucleotide polymorphisms (SNPs) that increase the risk of developing non-alcoholic fatty liver disease (NAFLD). One purpose of this study was to determine the frequencies of NAFLD susceptibility SNPs in a non-Hispanic white and Hispanic population who attended a clinic in northeast Albuquerque, NM. Another goal was to determine associations with selected indicators in this New Mexican population.

Methods: This cohort study involving 168 volunteer subjects in the NM population (88 non-Hispanic whites, 63 Hispanics, 4 Native Americans, 11 Asian Americans, 2 unreported ethnicity).Eight SNPs within 6 NAFLD susceptibility genes including PNPLA3 (rs738409), LYPLAL1 (rs12137855), APOC3 (rs2854116, rs2854117), GCKR (rs780094, rs741038), FABP2 (rs1799883), PEMT (rs7946) were analyzed by genotyping using the TaqMan genotyping assay (Applied Biosystems, Foster City, CA). Statistical analyses were carried out using statistical package SAS 9.3.

Results: The NAFLD allele frequencies were similar in non-Hispanic whites and Hispanics except for PNPLA3 (rs738409), FABP2 (rs1799883), and PEMT (rs7946). Eight SNPs in 5 NAFLD susceptibility genes were significantly associated OR marginally associated with selected indicators for NAFLD, metabolic syndrome, overweight, obesity, insulin resistance, type 2 diabetes, hypertension, dyslipidemia. No SNPs were significantly associated with the same indicator in both the non-Hispanic white and Hispanic groups.

Conclusions: In this population of non-Hispanic whites and Hispanics, there were only heterozygotes for the APOC3 derived alle le whereas for all other genes tested, both heterozygotes and homozygotes were found. Associations of alleles with indicators of chronic disease were different in non-Hispanic whites compared to Hispanics.

Keywords: Type 2 diabetes; NAFLD; Obesity; Overweight; Polymorphisms; Derived allele

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is defined as the presence of fat deposits in > 5% of hepatocytes as diagnosed by a liver biopsy or magnetic resonance imaging without an association to alcohol intake or medication[1]. NAFLD affects 20-30% of the adult population in the United States[2]. The major risk factors for NAFLD include overweight, obesity, insulin resistance, type 2 diabetes, hypertension, hypertriglyceridemia, metabolic syndrome, and visceral adiposity[3-5]. Evidence suggests that NAFLD precedes the development of type 2 diabetes[6,7]. Furthermore, when individuals had both type 2 diabetes and obesity, advanced fibrosis was evident in 66% of patients with NAFLD[4].

Data from the United States (U.S.) National Center for Health Statistics identified chronic liver disease (alcoholic and non-alcoholic) as the sixth most common cause of death in the U.S. Hispanic population and the twelfth highest cause of death in non-Hispanic whites[8]. Hepatic steatosis was found to have a prevalence of 45% in the Hispanic population and 33% in the non-Hispanic white population in Texas (TX) (5). The prevalence of hepatic steatosis among the ethnic groups was found to be unrelated to differences in ethanol intake (P = 0.459) (5). In California (CA),

the prevalence of newly diagnosed NAFLD was 44.7% of the non-Hispanic white population and 28.3% in the Hispanic population[9]. Prevalence data is not available for hepatic steatosis due to either nonalcoholic or alcoholic causes in non-Hispanic whites and Hispanics in New Mexico (NM), Arizona, and Nevada. NAFLD susceptibility single nucleotide polymorphisms (SNPs) may contribute to this epidemic of chronic liver disease. The frequency of NAFLD susceptibility SNPs is unknown in the adult NM population. Hispanics in NM have substantial Native American ancestry because many Native Americans settled in this region before the influx of Hispanics occurred[10]. Hispanics with Native American genes may have a dissimilar risk of developing NAFLD compared to other Hispanic populations with other genetic ancestry. Therefore, because of the social and genetic admixture between Native American genes in the NM Hispanics and non-Hispanic whites, the data regarding NAFLD for the Albuquerque, NM population may be different from the known published data from CA and TX. There are 32 NAFLD susceptibility genes and 52 SNPs that have been identified by GWAS. The GWAS genes found in the Hispanic and non-Hispanic white populations that interact with different diets include PNPLA3, LYPLAL1, APOC3, GCKR, FABP2, and PEMT. Table 1 summarizes the functions of the genes when they are homozygous for the ancestral allele. Table 1 also summarizes changes in function when the derived allele is present. The derived allele is underlined to indicate a risk allele for NAFLD. Only PNPLA3, GCKR, and FABP2 have been evaluated in relation to type 2 diabetes or obesity, which are known to be associated with NAFLD[11-18].

Table 1: NAFLD Gene function and changes with polymorphisms

Gene/SNP	Function and Changes
PNPLA3 rs738409 (C > G; I148M)	- Breakdown of TG in liver[4,13] - Polymorphism increases hepatic steatosis by preventing TG breakdown[4,13]
LYPLAL1 rs12137855 (T>C)	The functions for LYPLAL1 are hypotheses with variable support. 1) Codes for an acyl thioesterase that may act as a TG lipase particularly in subcutaneous adipose tissue of obese individuals[31,34] 2) Other potential function is the control of depalmitoylation of calcium-activated potassium (BK) channels, slowing exit from the Golgi so there are fewer functioning BK channels in the cell surface membrane. A splice variant of LYPLAL1 cannot depalmitoylate efficiently, either because it is nonfunctional or because it only targets some channels resulting in even fewer BK channels in the cell membrane[34] - Polymorphism acts with PNPLA3 protein, adiponutrin, in the breakdown of TG in fat cells to mobilize free fatty acids[31]
APOC3 rs2854116 (T > C) rs2854117 (C>T)	- Dampens lipoprotein lipase, hepatic lipase, and uptake of TG rich particles causing hypertriglyceridemia[22] - Under lipid rich conditions, APOC3 aids assembly and secretion of very low-density lipoprotein (VLDL)[22] - Both polymorphisms inhibit this assembly and secretion of VLDL particles resulting in TG remaining in the liver causing NAFLD[22]
GCKR rs780094 (C>T), rs741038 (T>C)	- Glucokinase Regulatory Protein (GCKR) inhibits glucokinase and down-regulates hepatic glucose uptake and liver fat storage[16] - Polymorphism decreases the inhibitory effect of GCK regulatory protein on glucokinase activity and this polymorphism results in higher de novo lipogenesis and liver TG accumulation in humans[16] - Lowers glucokinase regulatory protein and provides more substrate for de novo lipogenesis and upregulates glucokinase activity[16] - Inherited together more often than expected by chance with rs1260326, encoding for the P446L protein variant, which downregulates the ability of GCKR to inhibit glucokinase when fructose-6-phosphate is high[30] - Cause elevated hepatic glucokinase activity resulting in higher glucose uptake by the liver[30] - No information available on changes in these functions with this polymorphism
PEMT rs7946 (G > A; V175M)	- Required for phosphatidylcholine synthesis which is important for VLDL synthesis[35] - Polymorphism interferes with efficient synthesis of VLDL with the consequence being lipid remaining in the liver causing NAFLD[35]
FABP2 rs1799883 (A>G; Ala54Thr)	- Participate in the uptake, intracellular metabolism and/or transport of long-chain fatty acids (LCFA)[20] - Polymorphism of codon 54 in FABP2 causes a substitution of threonine (Thr) for the common alanine (Ala)[18,20] -Thr-containing protein has twice the affinity for LCFA compared to the Ala-containing protein[18,20] - This may cause increased absorption and then re-esterification or oxidation of fatty acids by peripheral tissues[18,20] - Individuals with the polymorphism may absorb extra LCFA or they may be acquired at a quicker rate[18,20]

-Underline indicates the risk allele for NAFLD

In different studies sugar sweetened beverages, carbohydrate intake, linoleic acid, dietary fat, whole grains, choline deficient diet, vegetables, niacin, Mediterranean diet, and omega-3 polyunsaturated fatty acids (PUFA) have been evaluated with regards to a relationship to these genes. Sugar sweetened beverages, carbohydrate intake, linoleic acid, dietary fat, whole grains, and a choline deficient diet exacerbate expressions of detrimental gene polymorphisms[14,19-24]. In contrast, a high vegetable intake and a Mediterranean diet diminish expression of these polymorphic genes as opposed to a standard Western diet[14,25]. In mice, the expression of the polymorphic APOC3 was lowered by niacin[26]. The same dietary component may interact differently with dissimilar genes. A higher dietary intake of omega-3 polyunsaturated fatty acids (PUFA) was associated with a detrimental increase in plasma APOC3 in those individuals with the CC polymorphism APOC3 (rs2854116)[27]. However, fish oil supplementation was found to lower triglyceride (TG) levels in human subjects homozygous for GCKR (rs741038)[28]. Genetic polymorphisms exacerbated by dietary factors may play a role in the pathogenesis of NAFLD in all populations or perhaps only individuals at risk for obesity or type 2 diabetes.

It has been reported that polymorphisms in these 6 genes (PNPLA3, APOC3, GCKR, FABP2, PEMT, LYPLAL1) change important functions and predispose individuals to NAFLD. Moreover PNPLA3, LYPLAL1, APOC3, GCKR, and FABP2 have been evaluated in relation to type 2 diabetes or obesity, which are known to be associated with NAFLD (See Supplemental Table 1 for references).

Supplemental Table 1: NAFLD gene associations with Type 2 diabetes and obesity

Gene	Function and Changes	Our Study: Non-Hispanic Whites	Our Study: Hispanics
PNPLA3 rs738409	-Association with Type 2 Diabetes and obesity[a] -Association with increased BMI[b]	-Marginal association in the ancestral allele for high HbA1c	-Marginal association for higher BMI in the ancestral allele
LYPLAL1 rs12137855	-Association with Type 2 Diabetes[c] -Elevated serum triglycerides and fasting insulin[d]	-Significant association with ancestral allele for higher LDL levels	
APOC3 rs2854116	Association with Type 2 Diabetes[e]	-Significant association with the ancestral allele for higher BMI	
APOC3 rs2854117	Association with Type 2 Diabetes[e]	-Significant association with elevated DBP with the derived allele -Marginal association with low MAP in the derived allele	-Significant association was observed for high LDL in the derived allele
GCK-Rrs780094	-Higher triglycerides and post-OGTT glucose[f] - Increased risk of Type 2 diabetes[g] -Increased insulin levels[h] -Studies are divided and very little is known about Hispanics and Native Americans -Most studies have been conducted in Caucasians and in one study the protection against diabetes was not found in blacks	-Low HDL was marginally associated with the derived allele	-Derived allele significantly associated with elevations in ALT and marginally associated with elevations in AST -Significant association was observed for high LDL in the derived allele
GCKR rs741038	-No association of obesity or Type 2 diabetes with this SNP	-Low HDL was significantly associated with the ancestral allele -Marginal association for higher triglycerides was observed with the ancestral allele -Significant association for elevated HbA1c in the ancestral allele	
PEMT rs7946	-No data associating this gene with Type 2 diabetes or obesity in previous studies		-Marginal association with derived allele for higher BMI
FABP2 rs1799883	-Shown to cause altered postprandial lipemia[i] - Association with insulin resistance, increased insulin concentration, and elevated fasting oxidation of fatty acids in Pima Indian population[j] -Increased fasting insulin concentration, fasting fatty acid oxidation and reduced glucose uptake in a Chilean population[k]		

a) Palmer, C.N., Maglio, C., Pirazzi, C., et al. Paradoxical lower serum triglyceride levels and higher type 2 diabetes mellitus susceptibility in obese individuals with the PNPLA3 148M variant. (2012) PLoS One 7(6): 39362.

b) Dunn, W., Zeng, Z., O'Neil, M., et al. The interaction of rs738409, obesity, and alcohol: a population-based autopsy study. (2012) Am J Gastroenterol 107(11): 1668-1674.

c) Yeung, E., Qi, L., Hu, F.B., et al. Novel abdominal adiposity genes and the risk of type 2 diabetes: findings from two prospective cohorts. (2011) Int J Mol Epidemiol Genet 2(2):138-144.

d) Heid, I.M., Jackson, A.U., Randall, J.C., et al. Meta-analysis identifies 13 new loci associated with waist-hip ratio and reveals sexual dimorphism in the genetic basis of fat distribution. (2010) Nat Genet 42(11): 949-960.

e) van Hoek, M., van Herpt, T.W., Dehghan, A., et al. Association of an APOC3 promoter variant with type 2 diabetes risk and need for insulin treatment in lean persons. (2011) Diabetologia 54(6): 1360-1367.

f) Petta, S., Miele, L., Bugianesi, E., et al. Glucokinase regulatory protein gene polymorphism affects liver fibrosis in non-alcoholic fatty liver disease. (2014) PLoS One 9(2): 87523.

g) Li, H., Xu, R., Peng, X., et al. Association of glucokinase regulatory protein polymorphism with type 2 diabetes and fasting plasma glucose: a meta-analysis. (2013) Mol Bio Rep 40(6): 3935-3942.

h) Bi, M., Kao, W., Boerwinkle, E., et al. Association of rs780094 in GCKR with metabolic traits and incident diabetes and cardiovascular disease: the ARIC study. (2010) PLoS One 5(7): 11690.

i) Agren, J.J., Valve, R., Vidgren, H., et al. Postprandial lipemic response is modified by the polymorphism at codon 54 of the fatty acid-binding protein 2 gene. (1998) Arterioscler Thromb Vasc Biol 18(10): 1606-1610.

j) Morcillo, S., Rojo-Martinez, G., Cardona, F., et al. Effect of the interaction between the fatty acid-binding protein 2 gene Ala54Thr polymorphism and dietary fatty acids on peripheral insulin sensitivity: a cross-sectional study. (2007) Am J Clin Nutr 86(4): 1232-1237.

k) Albala, B.C., Jiménez, R.B., Pérez, B.F., et al. Fatty acid binding protein 2 (FABP-2) polymorphism, obesity and insulin resistance. (2006) Rev Med Chil 134(3): 372-379.

Hepatic insulin resistance may be an important indicator for the predisposition to NAFLD as well as type 2 diabetes. Type 2 diabetes and dyslipidemia are known risk factors usually associated with cardiovascular and liver-related deaths in NAFLD. In a study with 2,322 French patients (52% Male, 48% Female; age 52; BMI 26 [15-64]) followed for 12 years, the authors discovered that type 2 diabetes patients had the highest occurrence of steatosis, advanced fibrosis, and liver-related mortalities compared to those with isolated hyperlipidemia[29]. Evidence suggests that the development of liver damage is influenced by hepatic insulin resistance in nonalcoholic steatohepatitis (NASH)[30]. Improving insulin resistance may help slow down the development of NASH[30]. NAFLD should be regarded as an independent risk factor for type 2 diabetes.

The objective of this research was to determine the frequencies of NAFLD susceptibility SNPs in a non-Hispanic white and Hispanic population who attended a clinic located in a northeast section of Albuquerque, NM. We focused on 8 SNPs in 6 genes for which published feed studies were available[14,19-28]. A second objective was to determine the associations of these

genes with selected indicators for NAFLD (AST, ALT), for three indicators of metabolic syndrome which were overweight/obesity (BMI), insulin resistance (high triglyceride plus low HDL levels) and hypertension (SBP, DBP, MAP), for type 2 diabetes (HbA1c) and for cardiovascular disease (higher than optimal levels of LDL and total cholesterol) in a specific NM population.

Materials and Methods

The protocol was approved by the Human Subject Research Review Committee of the University of New Mexico Health Sciences Center. Informed written consent was obtained from all volunteers. Volunteers between 30 and 80 years of age were recruited between 2012 to 2014 from established patients attending a primary care clinic of the University of New Mexico Health Sciences Center, Albuquerque, NM after reviewing the clinic medical charts of individuals who had previously visited the clinic. The cohort encompasses 168 adult samples obtained from the University of New Mexico Family Health Clinic located in the northeast section of Albuquerque, NM (88 non-Hispanic whites, 63 Hispanics, 4 Native Americans, 11 Asian Americans, 2 unreported ethnicity). Ethnicity was self-reported as being either non-Hispanic white, Hispanic, Native American, or Asian American. The group called "other" in Table 2 consisted of Native Americans, Asian Americans, or unreported ethnicity. The associations of the polymorphisms in both non-Hispanic white and Hispanic ethnicities for gender were determined.

Table 2: Demographics of NE clinic patients (n = 168)

Demographics	NE Clinic Non-Hispanic White + Hispanic + Other *(n=168) Mean ± SD (Range)
Age (years) BMI (kg/m2)	54.8 ± 12.1 (25.0 - 92.0) 30.9 ± 7.7 (19.5 - 66.0)
Gender female male	 109 (64.9%) 59 (35.1%)
AST optimal (6-58) abnormal	 153 (91.1%) 15 (8.9%)
ALT optimal (14-67) abnormal	 156 (92.9%) 12 (7.1%)
Triglyceride optimal (<150) abnormal	 71 (42.3%) 97 (57.7%)
Cholesterol optimal (<200) abnormal	 119 (70.8%) 49 (29.2%)
HDL optimal (>40) abnormal	 101 (60.1%) 67 (39.9%)
LDL missing optimal (<100) abnormal	 11 (6.5%) 95 (56.5%) 62 (36.9%)
HbA1c values(%) optimal (<5.7) PreD(5.7-6.4) Diab(6.5+)	 37 (22.0%) 74 (44.0%) 57 (33.9%)
SBP optimal (90-140) abnormal	 156 (92.9%) 12 (7.1%)
DBP optimal (60-90) abnormal	 154 (91.7%) 14 (8.3%)
MAP (MAP=[(2 x diast)+syst]/3) missing optimal (70-105) abnormal	 0 (0.0%) 157 (93.5%) 11 (6.5%)

*Other refers to Native American (n=4), Asian American (n=11), unreported ethnicity (n=2)

Abbreviations: BMI: Body Mass Index; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; AST: Aspartate Aminotransferase; ALT: Alanine Transaminase; Trig: Triglycerides; Chol: Cholesterol; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; HbA1c: Glycated Hemoglobin; MAP: Mean Arterial Pressure.

Information regarding pertinent medical history, demographics, current medications, and alcohol and tobacco use was obtained by means of a questionnaire administered by a member of the research team. A blood sample was obtained from each subject by venipuncture and collected in a serum-separating tube for the determination of HbA1C, glucose, creatinine, albumin, total protein, AST, ALT, uric acid, and lipids. The subjects also provided a urine sample for the measurement of creatinine and microalbumin. Height, weight and blood pressure were also measured. Clinical chemistry measurements were performed at the Tricore Reference Laboratories, Albuquerque, NM using clinical diagnostic assays certified by the Clinical Laboratory Improvement Amendments (CLIA) of the Centers for Medicare and Medicaid Services. Obesity was determined using the BMI data recorded in the patients' electronic medical database calculated from measured height and weight at the time blood samples were taken.

Only data from non-Hispanic whites and Hispanics were used for genotype frequency of SNPs with alleles and associations with indicators. The genomic DNA was extracted from peripheral blood leukocytes using the Puregene Blood Core Kit A (Germantown, Maryland).

Genotyping

Genotyping of SNPs was carried out using the TaqMan genotyping assay (Applied Biosystems, Foster City, CA). The GTxpress Master Mix was used to detect the SNP present. The Master Mix included 10x mango buffer, nucleotides dNTPs, MgCl2, Taq polymerase, Primer-F, Primer-R wt 400bbt (+), Primer-R ins 194b (-), and H2O (Applied Biosystems)[31-35]. The assay part number for the TaqMan probes was 4331349 for PNPLA3 (rs738409, rs6006460), for APOC3 (rs2854116, rs2854117), for GCKR (rs780094, rs741038), for FABP2 (rs1799883), for PEMT (rs7946, rs12325817), and for LYPLAL1 (rs12137855).

The total experimental volume for each sample was 10.05 µl per reaction, which was composed of 4.8 µl of the GTxpress Master Mix, 0.25 µl of the TaqMan genotyping assay, 3 µl molecular biology reagents (Sigma Life Science), and 2 µl DNA. Amplification was carried out by the 7500 Fast Real-Time PCR Machine under the following conditions: 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for1 min until completion.

Statistical analysis

The biometric and genotype data were recorded using statistical package SAS 9.3. Pearson's Chi square test was used to examine differences in the distribution of the SNP genotype frequencies between ethnic groups. Logistic regression models were used to investigate the relationship between specific alleles and metabolic disease phenotypes. Odds Ratio (ORs) and 95% Confidence Intervals (CI) were calculated to estimate the association. Linear regression models were performed to evaluate the relationship between the SNPs and BMI. An additive genetic model (examining 0, 1, or 2 copies of the specific alleles) was performed to determine the per-allele effect of each SNP in ethnicity/ethnic groups and total population. This genetic model was used to test for log-additive effects of the previously reported NAFLD risk alleles for obesity and type 2 diabetes, where by one risk allele was assessed to increase predisposition by r-fold for heterozygous alleles (1/2) and r^2-fold for homozygous alleles (2/2). Although other genetic models exist, the additive genetic model is the most commonly used approach to assess the association of genetic determinants with metabolic disease phenotypes (obesity and type 2 diabetes) in current genome-wide association studies. A two-sided P-value < 0.05 was considered to be statistically significant.

The genomic DNA was extracted and isolated from blood samples using a Gentra Puregene Blood Core Kit (Quiagen) that uses an isopropanol precipitation reaction. To perform the precipitation reaction 65-100 µl of blood was obtained, and the following reagents were added: 20 µl of 10 mM EDTA and 200 µl Lysis buffer. The tube was microfuged so as to pellet the nuclei. The supernatant was discarded and the steps repeated until there was no more hemoglobin. The nuclear pellet was re-suspended in 100 µl PBND with 60 µl/mL proteinase K and incubated at 55°C for 60 min. Samples were then heated to 97°C for 10 min to inactivate proteinase K, and 1-5 µl of DNA solution was added for a 25 µl PCR reaction.

To perform genotype analysis and allelic discrimination (AD) assay was used along with the use of quantitative polymerase chain reaction (qPCR) methodology. This AD assay design allows for SNP determination for both alleles using 1-20 ng of template genomic DNA. First, the reaction mix and the genomic DNA were loaded into wells and a pre-read run is performed to record the background fluorescence of each well of the 96-well plate. Second, the amplification run was performed to amplify the target sequences and provide fluorescence of the resulting PCR products. Third, the pre-read run fluorescence was subtracted from the post-read run fluorescence to account for pre-amplification background fluorescence, thereby ensuring accurate results. After the post-read run the Sequence Detection System (SDS) software was used to i) analyze the raw data, ii) produce a scatter plot for the two alleles (allele X and allele Y), iii) perform "allele calling" for identification of alleles that are present in the samples, and iv) generate a final report for samples.

Results

Demographics of patients in relation to ethnicity/ethnic group

A total of 168 subjects were consented and enrolled for this study. The results indicated that the mean age for the total population was 54.8 years (age range 25-92). There were 59 (35.1%) males and 109 (64.9%) females. The sample included 37 (22%) without type 2 diabetes, 74 (44%) with prediabetes, and 57 (33.9%) with type 1 or type 2 diabetes as indicated by HbA1c levels. The mean BMI was30.9 kg/m^2 (range 19.5 to 66.0kg/m^2) and the median BMI was 29.3kg/m^2. For all ethnic groups (n = 168), an elevated HbA1c (> 6.5 %) was present in 74 patients (44.0%), while 57 patients (33.9%) had preexisting type 2 diabetes and 37 (22.0%) had normal HbA1c (<5.7) values (Table 2). However, due to the relatively low number of Native Americans (n=4), Asian Americans (n=11) and ethnicity unreported (n=2), data from these subjects were excluded to prevent confounding results due to population stratification in the determination of risk associated with NAFLD genes. Therefore, a total of 151 patients that self-reported as non-Hispanic white or Hispanic were used for analysis in this study in all tables except for Table 2. The percentage of individuals within each ethnicity/ethnic group was 58% non-Hispanic white and 42% Hispanic.

Frequency of SNP genotypes in relation to ethnicity/ethnic group

Results for frequencies of alleles by ethnicity group are listed in Table 3. The non-Hispanic whites had a significantly higher percentage (P = 0.0001) of one or more of the derived alleles (91.5%) for PNPLA3 (rs738409) than Hispanics (87.1%). The non-Hispanic whites had a marginally significantly lower percentage (P = 0.097) of one or more of the derived alleles (52.3%) for FABP2 (rs1799883) than the Hispanics (61.3%). In non-Hispanic whites, the derived allele was significantly higher (P = < 0.0001) in frequency for PEMT (rs7946) (97.7%) compared to Hispanics (77.8%). There was a greater percentage of the derived allele in non-Hispanic whites than in Hispanics for PNPLA3 (rs738409) and PEMT (rs7946) but not for FABP2 (rs1799883). No other genes examined in this study were significantly different in frequency between non-Hispanic whites and Hispanics (P > 0.05). For both populations, the major allele was the derived allele in both non-Hispanic whites and Hispanics for PNPLA3 (rs738409), GCKR (rs741038), and PEMT (rs7946). Furthermore, the ancestral allele was the major allele for all of the following genes: APOC3 (rs2854116; rs2854117), GCKR (rs780094), FABP2 (rs1799883), and LYPLAL1 (rs12137855). For these 5 SNPs in four genes, the ancestral allele was more frequent compared to the derived allele in both ethnic groups.

Table 3: Genotype Frequency of SNPs and Alleles by Ethnic Groups

Variable	White[SEP](N=88)	Hispanic[SEP](N=63)	Total	P-Value
PNPLA3(1)_C				
0	7 (8.4%)	8 (12.9%)	15 (10.3%)	0.0001[2]
1	27 (32.5%)	39 (62.9%)	66 (45.5%)	
2	49 (59.0%)	15 (24.2%)	64 (44.1%)	
Total	83	62	145	
PNPLA3(1)_G				
0	49 (59.0%)	15 (24.2%)	64 (44.1%)	0.0001[2]
1	27 (32.5%)	39 (62.9%)	66 (45.5%)	
2	7 (8.4%)	8 (12.9%)	15 (10.3%)	
Total	83	62	145	
APOC3 (1)_T				

1	77 (88.5%)	58 (92.1%)	135(90.0%)	0.47[2]
2	10 (11.5%)	5 (7.9%)	15 (10.0%)	
Total	87	63	150	
APOC3 (1)_C				
0	10 (11.5%)	5 (7.9%)	15 (10.0%)	0.47[2]
1	77 (88.5%)	58 (92.1%)	135(90.0%)	
Total	87	63	150	
APOC3(2)_C				
0	6 (6.8%)	4 (6.3%)	10 (6.6%)	0.78[2]
1	26 (29.5%)	22 (34.9%)	48 (31.8%)	
2	56 (63.6%)	37 (58.7%)	93 (61.6%)	
Total	88	63	151	
APOC3(2)_T				
0	56 (63.6%)	37 (58.7%)	93 (61.6%)	0.78[2]
1	26 (29.5%)	22 (34.9%)	48 (31.8%)	
2	6 (6.8%)	4 (6.3%)	10 (6.6%)	
Total	88	63	151	
GCKR(1)_A				
0	28 (31.8%)	27 (43.5%)	55 (36.7%)	0.31[2]
1	47 (53.4%)	26 (41.9%)	73 (48.7%)	
2	13 (14.8%)	9 (14.5%)	22 (14.7%)	
Total	88	62	150	
GCKR(1)_G				
0	13 (14.8%)	9 (14.5%)	22 (14.7%)	0.31[2]
1	47 (53.4%)	26 (41.9%)	73 (48.7%)	
2	28 (31.8%)	27 (43.5%)	55 (36.7%)	
Total	88	62	150	
GCKR(2)_G				
0	40 (45.5%)	27 (42.9%)	67 (44.4%)	0.60[2]
1	39 (44.3%)	32 (50.8%)	71 (47.0%)	
2	9 (10.2%)	4 (6.3%)	13 (8.6%)	
Total	88	63	151	
GCKR(2)_A				
0	9 (10.2%)	4 (6.3%)	13 (8.6%)	0.60[2]
1	39 (44.3%)	32 (50.8%)	71 (47.0%)	
2	40 (45.5%)	27 (42.9%)	67 (44.4%)	
Total	88	63	151	
FABP2_A				
0	42 (47.7%)	24 (38.7%)	66 (44.0%)	0.097[2]
1	41 (46.6%)	28 (45.2%)	69 (46.0%)	
2	5 (5.7%)	10 (16.1%)	15 (10.0%)	
Total	88	62	150	
FABP2_G				
0	5 (5.7%)	10 (16.1%)	15 (10.0%)	0.097[2]
1	41 (46.6%)	28 (45.2%)	69 (46.0%)	
2	42 (47.7%)	24 (38.7%)	66 (44.0%)	
Total	88	62	150	
PEMT_G				
0	49 (55.7%)	19 (30.2%)	68 (45.0%)	<.0001[2]
1	37 (42.0%)	30 (47.6%)	67 (44.4%)	
2	2 (2.3%)	14 (22.2%)	16 (10.6%)	
Total	88	63	151	
PEMT_A				
0	2 (2.3%)	14 (22.2%)	16 (10.6%)	<.0001[2]
1	37 (42.0%)	30 (47.6%)	67 (44.4%)	
2	49 (55.7%)	19 (30.2%)	68 (45.0%)	
Total	88	63	151	
LYPLA1_C				
0	3 (3.4%)	1 (1.6%)	4 (2.6%)	0.56[3]
1	24 (27.3%)	22 (34.9%)	46 (30.5%)	
2	61 (69.3%)	40 (63.5%)	101(66.9%)	
Total	88	63	151	
LYPLA1_T				
0	61 (69.3%)	40 (63.5%)	101(66.9%)	0.56[3]
1	24 (27.3%)	22 (34.9%)	46 (30.5%)	
2	3 (3.4%)	1 (1.6%)	4 (2.6%)	
Total	88	63	151	

[2] based on Chi-square test
[3] based on Fisher's exact test

Association of alleles of NAFLD susceptibility genes with metabolic disease phenotypes in relation to ethnicity/ethnic group

Logistic or linear regression analysis was performed to determine the association of alleles in NAFLD susceptibility genes with metabolic disease phenotypes in relation to ethnicity/ethnic groups. All results are provided in Tables 4- Table11. Significant and marginally significant results are provided in the following narrative.

Elevated ALT levels were not associated with the NAFLD susceptibility genes in the non-Hispanic white population whereas in the Hispanic group the derived allele of GCKR (rs780094) was associated (OR 3.974, 95% CI 1.094-14.433, P = 0.036) with abnormally high ALT (Table 4). Furthermore, AST was not associated with the NAFLD susceptibility SNPs in non-Hispanic whites but in the Hispanics the derived allele of GCKR (rs780094) was marginally associated (OR 2.495, 95% CI 0.973-6.399, P = 0.057) with higher AST (Table 5). The ancestral allele of GCKR (rs741038) was associated (OR 3.384, 95% CI 1.243-9.212, P = 0.017) with elevated HbA1c (>6.5mmol/mol) in the non-Hispanic white group, but not in the Hispanic group (Table 6). Additionally, the ancestral allele ofPNPLA3 (rs738409) was marginally associated (OR 2.362, 95% CI 0.866-6.438, P = 0.093) with elevated HbA1c (>6.5) in the non-Hispanic white group, but not in Hispanics (Table 6).The genes associated with increased BMI were different in non-Hispanic whites compared to Hispanics. The ancestral allele of APOC3 (rs2854116) was significantly associated (Estimate 5.883, 95% CI 1.165-10.601, P = 0.015) with increased BMI in the non-Hispanic whites, but not in the Hispanic individuals (Table 7). The ancestral allele of PNPLA3 (rs738409) and the derived allele of PEMT (rs7946) were marginally associated (Estimate 2.723, CI -0.547-5.992, P = 0.101; Estimate 2.529, CI -0.131-5.189, P = 0.062; respectively) with increased BMI in the Hispanic population, but not in the non-Hispanic whites (Table 7). Elevated TG were marginally associated (OR 1.788, 95% CI 0.913-3.503, P = 0.090) with the ancestral allele of GCKR (rs741038) in the non-Hispanic white population but not in the Hispanics (Table 8). In the non-Hispanic white population, but not in Hispanics the ancestral allele of GCKR (rs741038) was

associated (OR 2.124, 95% CI 1.074-4.201, P = 0.030) with low HDL and the derived allele of GCKR (rs780094) was marginally associated (OR 1.893, 95% CI 0.960-3.734, P = 0.065) with low HDL(Table 9). The genes associated with elevations in LDL were different in non-Hispanic whites compared to Hispanics. The ancestral allele of LYPLAL1 (rs12137855) was found to be associated (OR 2.262, 95% CI 1.109-4.615, P = 0.025) with high LDL levels in the non-Hispanic whites, but not in Hispanics (Table 10). In the Hispanic group, but not in non-Hispanic whites the derived allele of APOC3 (rs2854117) and GCKR (rs780094) were associated (OR 2.115, CI 1.029-4.349, P = 0.042; OR 1.953, CI 1.039-3.672, P = 0.038; respectively) with higher LDL (Table 10). Elevated DBP was found to be associated (OR 3.553, 95% CI 1.108-11.392, P = 0.033) with the derived allele of APOC3 (rs2854117) in non-Hispanic whites, but not in Hispanics (Table 11). Lastly, in non-Hispanic whites for the derived allele of APOC3 (rs2854117), there was a marginal association (OR 3.454, 95% CI 0.988-12.067, P = 0.052) with abnormal MAP whereas an association was not found in Hispanics.

Table 4: Risk of elevated ALT with alleles associated with NAFLD

Non-Hispanic Whites					
Gene	**SNP**	**Allele**	**OR**	**95% CI**	**P value**
PNPLA3	rs738409	C derived	1.293	0.286-5.853	0.739
	rs738409	G ancestral	0.773	0.171-3.501	0.739
APOC3	rs2854116	T ancestral	NA*		
	rs2854116	C derived	NA*		
APOC3	rs2854117	C ancestral	2.371	0.312-18.051	0.404
	rs2854117	T derived	0.422	0.055-3.210	0.404
GCKR	rs780094	A derived	0.930	0.236-3.669	0.918
	rs780094	G ancestral	1.075	0.273-4.238	0.918
GCKR	rs741038	G ancestral	1.429	0.382-5.341	0.596
	rs741038	A derived	0.700	0.187-2.617	0.596
PEMT	rs7946	G ancestral	1.583	0.322-7.783	0.572
	rs7946	A derived	0.632	0.128-3.105	0.572
LYPLAL1	rs12137855	C ancestral	NA		
	rs12137855	T derived	NA		
Hispanics					
Gene	**SNP**	**Allele**	**OR**	**95% CI**	**P value**
PNPLA3	rs738409	C derived	1.181	0.288-4.846	0.817
	rs738409	G ancestral	0.846	0.206-3.473	0.817
APOC3	rs2854116	T ancestral	2.651	0.246-28.505	0.421
	rs2854116	C derived	0.377	0.035-4.057	0.421
APOC3	rs2854117	C ancestral	0.602	0.170-2.123	0.430
	rs2854117	T derived	1.662	0.471-5.865	0.430
GCKR	rs780094	A derived	3.974	1.094-14.433	0.036
	rs780094	G ancestral	0.252	0.069-0.914	0.036
GCKR	rs741038	G ancestral	1.102	0.273-4.441	0.891
	rs741038	A derived	0.908	0.225-3.657	0.891
PEMT	rs7946	G ancestral	2.504	0.709-8.844	0.154
	rs7946	A derived	0.399	0.113-1.410	0.154
LYPLAL1	rs12137855	C ancestral	3.105	0.354-27.268	0.307
	rs12137855	T derived	0.322	0.037-2.828	0.307

*Assay not done

Table 5: Risk of elevated AST with alleles associated with NAFLD

Non-Hispanic Whites					
Gene	**SNP**	**Allele**	**OR**	**95% CI**	**P value**
PNPLA3	rs738409	C derived	0.569	0.142-2.277	0.426
	rs738409	G ancestral	1.757	0.439-7.030	0.426
APOC3	rs2854116	T ancestral	NA*		
	rs2854116	C derived	NA*		
APOC3	rs2854117	C ancestral	0.837	0.179-3.909	0.821
	rs2854117	T derived	1.194	0.256-5.573	0.821
GCKR	rs780094	A derived	1.496	0.333-6.733	0.599
	rs780094	G ancestral	0.668	0.149-3.007	0.599
GCKR	rs741038	G ancestral	2.206	0.517-9.413	0.285
	rs741038	A derived	0.453	0.106-1.935	0.285
PEMT	rs7946	G ancestral	1.127	0.182-6.961	0.898
	rs7946	A derived	0.887	0.144-5.481	0.898
LYPLAL1	rs12137855	C ancestral	1.444	0.176-11.854	0.732
	rs12137855	T derived	0.693	0.084-5.687	0.732
Hispanics					
Gene	**SNP**	**Allele**	**OR**	**95% CI**	**P value**
PNPLA3	rs738409	C derived	1.739	0.568-5.327	0.332
	rs738409	G ancestral	0.575	0.188-1.761	0.332
APOC3	rs2854116	T ancestral	1.200	0.121-11.907	0.876
	rs2854116	C derived	0.833	0.084-8.268	0.876
APOC3	rs2854117	C ancestral	1.073	0.366-3.143	0.898
	rs2854117	T derived	0.932	0.318-2.729	0.898
GCKR	rs780094	A derived	2.495	0.973-6.399	0.057
	rs780094	G ancestral	0.401	0.156-1.028	0.057
GCKR	rs741038	G ancestral	1.837	0.623-5.414	0.270
	rs741038	A derived	0.544	0.185-1.605	0.270
PEMT	rs7946	G ancestral	1.861	0.731-4.737	0.192
	rs7946	A derived	0.537	0.211-1.367	0.192
LYPLAL1	rs12137855	C ancestral	0.499	0.151-1.646	0.254
	rs12137855	T derived	2.005	0.608-6.618	0.254

*Assay not done

Table 6: Risk of elevated HbA1c (>6.5 mmol/mol) with alleles associated with NAFLD

Non-Hispanic Whites					
Gene	**SNP**	**Allele**	**OR**	**95% CI**	**P value**
PNPLA3	rs738409	C derived	0.423	0.155-1.154	0.093
	rs738409	G ancestral	2.362	0.866-6.438	0.093
APOC3	rs2854116	T ancestral	3.426	0.284-41.266	0.332
	rs2854116	C derived	0.292	0.024-3.516	0.332
APOC3	rs2854117	C ancestral	0.979	0.386-2.484	0.964
	rs2854117	T derived	1.022	0.403-2.593	0.964
GCKR	rs780094	A derived	0.666	0.257-1.725	0.402
	rs780094	G ancestral	1.502	0.580-3.889	0.402
GCKR	rs741038	G ancestral	3.384	1.243-9.212	0.017
	rs741038	A derived	0.295	0.109-0.804	0.017
PEMT	rs7946	G ancestral	1.244	0.413-3.743	0.698
	rs7946	A derived	0.804	0.267-2.419	0.698
LYPLAL1	rs12137855	C ancestral	2.536	0.644-9.994	0.183
	rs12137855	T derived	0.394	0.100- 1.554	0.183

Hispanics					
Gene	**SNP**	**Allele**	**OR**	**95% CI**	**P value**
PNPLA3	rs738409	C derived	0.504	0.153-1.665	0.261
	rs738409	G ancestral	1.983	0.601-6.547	0.261
APOC3	rs2854116	T ancestral	4959 826.09	0.000-1	0.994
	rs2854116	C derived	0.000	0.000-1	0.994
APOC3	rs2854117	C ancestral	1.398	0.463-4.217	0.552
	rs2854117	T derived	0.715	0.237-2.158	0.552
GCKR	rs780094	A derived	1.505	0.553- 4.100	0.424
	rs780094	G ancestral	0.664	0.244-1.809	0.424
GCKR	rs741038	G ancestral	1.474	0.458-4.749	0.515
	rs741038	A derived	0.678	0.211-2.184	0.515
PEMT	rs7946	G ancestral	0.830	0.319- 2.158	0.702
	rs7946	A derived	1.205	0.463-3.135	0.702
LYPLAL1	rs12137855	C ancestral	0.801	0.183-3.499	0.768
	rs12137855	T derived	1.249	0.286- 5.457	0.768

Table 7: The association between BMI with alleles associated with NAFLD

Non-Hispanic Whites					
Gene	**SNP**	**Allele**	**Estimate**	**95% CI**	**P value**
PNPLA3	rs738409	C derived	-0.389	-2.873-2.095	0.756
	rs738409	G ancestral	0.389	-2.095-2.873	0.756
APOC3	rs2854116	T ancestral	5.883	1.165-10.601	0.015
	rs2854116	C derived	-5.883	-10.601-1.165	0.015
APOC3	rs2854117	C ancestral	-1.166	-3.730-1.397	0.368
	rs2854117	T derived	1.166	-1.397-3.730	0.368
GCKR	rs780094	A derived	0.717	-1.686- 3.119	0.555
	rs780094	G ancestral	-0.717	-3.119-1.686	0.555
GCKR	rs741038	G ancestral	1.128	-1.278-3.535	0.354
	rs741038	A derived	-1.128	-3.535-1.278	0.354
PEMT	rs7946	G ancestral	0.274	-2.659-3.207	0.853
	rs7946	A derived	-0.274	-3.207-2.659	0.853
LYPLAL1	rs12137855	C ancestral	1.479	-1.445-4.402	0.317
	rs12137855	T derived	-1.479	-4.402-1.445	0.317
Hispanics					
Gene	**SNP**	**Allele**	**Estimate**	**95% CI**	**P value**
PNPLA3	rs738409	C derived	-2.723	-5.992-0.547	0.101
	rs738409	G ancestral	2.723	-0.547-5.992	0.101
APOC3	rs2854116	T ancestral	2.445	-4.815- 9.705	0.503
	rs2854116	C derived	-2.445	-9.705-4.815	0.503
APOC3	rs2854117	C ancestral	-2.012	-5.181-1.156	0.209
	rs2854117	T derived	2.012	-1.156-5.181	0.209
GCKR	rs780094	A derived	1.052	-1.772-3.876	0.459
	rs780094	G ancestral	-1.052	-3.876-1.772	0.459
GCKR	rs741038	G ancestral	-1.189	-4.463-2.086	0.471
	rs741038	A derived	1.189	-2.086- 4.463	0.471
PEMT	rs7946	G ancestral	-2.529	-5.189-0.131	0.062
	rs7946	A derived	2.529	-0.131-5.189	0.062
LYPLAL1	rs12137855	C ancestral	3.339	-0.372-7.049	0.077
	rs12137855	T derived	-3.339	-7.049-0.372	0.077

Table 8: Risk of elevated triglycerides with alleles associated with NAFLD

Non-Hispanic Whites					
Gene	**SNP**	**Allele**	**OR**	**95% CI**	**P value**
PNPLA3	rs738409	C derived	0.553	0.269-1.137	0.107
	rs738409	G ancestral	1.809	0.880-3.719	0.107
APOC3	rs2854116	T ancestral	1.186	0.310-4.542	0.803
	rs2854116	C derived	0.843	0.220-3.229	0.803
APOC3	rs2854117	C ancestral	0.903	0.455-1.791	0.770
	rs2854117	T derived	1.107	0.558-2.196	0.770
GCKR	rs780094	A derived	0.756	0.398-1.435	0.392
	rs780094	G ancestral	1.324	0.697-2.513	0.392
GCKR	rs741038	G ancestral	1.788	0.913-3.503	0.090
	rs741038	A derived	0.559	0.285-1.096	0.090
PEMT	rs7946	G ancestral	1.202	0.551-2.622	0.644
	rs7946	A derived	0.832	0.381-1.814	0.644
LYPLAL1	rs12137855	C ancestral	0.814	0.370-1.789	0.608
	rs12137855	T derived	1.229	0.559-2.703	0.608
Hispanics					
Gene	**SNP**	**Allele**	**OR**	**95% CI**	**P value**
PNPLA3	rs738409	C derived	0.903	0.378-2.157	0.819
	rs738409	G ancestral	1.107	0.464-2.644	0.819
APOC3	rs2854116	T ancestral	2.270	0.238-21.665	0.476
	rs2854116	C derived	0.440	0.046-4.203	0.476
APOC3	rs2854117	C ancestral	1.101	0.476-2.547	0.822
	rs2854117	T derived	0.908	0.393-2.100	0.822
GCKR	rs780094	A derived	0.854	0.406-1.794	0.676
	rs780094	G ancestral	1.171	0.557-2.461	0.676
GCKR	rs741038	G ancestral	1.480	0.609-3.597	0.387
	rs741038	A derived	0.676	0.278-1.643	0.387
PEMT	rs7946	G ancestral	0.790	0.383-1.627	0.522
	rs7946	A derived	1.266	0.615-2.609	0.522
LYPLAL1	rs12137855	C ancestral	1.174	0.435-3.169	0.752
	rs12137855	T derived	0.852	0.316-23.00	0.752

Table 9: Risk of low HDL with alleles associated with NAFLD

Non-Hispanic Whites					
Gene	**SNP**	**Allele**	**OR**	**95% CI**	**P value**
PNPLA3	rs738409	C derived	0.722	0.366-1.426	0.349
	rs738409	G ancestral	1.385	0.701-2.735	0.349
APOC3	rs2854116	T ancestral	0.671	0.161-2.800	0.585
	rs2854116	C derived	1.489	0.357-6.210	0.585
APOC3	rs2854117	C ancestral	1.032	0.512-2.080	0.929
	rs2854117	T derived	0.969	0.481-1.952	0.929
GCKR	rs780094	A derived	1.893	0.960-3.734	0.065
	rs780094	G ancestral	0.528	0.268-1.042	0.065
GCKR	rs741038	G ancestral	2.124	1.074-4.201	0.030
	rs741038	A derived	0.471	0.238-0.931	0.030
PEMT	rs7946	G ancestral	0.561	0.244-1.291	0.174
	rs7946	A derived	1.781	0.775-4.095	0.174
LYPLAL1	rs12137855	C ancestral	0.752	0.343-1.652	0.478
	rs12137855	T derived	1.329	0.605-2.918	0.478

Hispanics					
Gene	**SNP**	**Allele**	**OR**	**95% CI**	**P value**
PNPLA3	rs738409	C derived	1.435	0.612-3.366	0.406
	rs738409	G ancestral	0.697	0.297-1.634	0.406
APOC3	rs2854116	T ancestral	0.880	0.137-5.671	0.893
	rs2854116	C derived	1.136	0.176-7.323	0.893
APOC3	rs2854117	C ancestral	0.976	0.433-2.199	0.953
	rs2854117	T derived	1.025	0.455-2.310	0.953
GCKR	rs780094	A derived	1.458	0.709-2.994	0.305
	rs780094	G ancestral	0.686	0.334-1.409	0.305
GCKR	rs741038	G ancestral	0.812	0.351-1.881	0.628
	rs741038	A derived	1.231	0.532-2.849	0.628
PEMT	rs7946	G ancestral	1.018	0.509-2.037	0.960
	rs7946	A derived	0.982	0.491-1.965	0.960
LYPLAL1	rs12137855	C ancestral	1.072	0.407-2.819	0.888
	rs12137855	T derived	0.933	0.355-2.454	0.888

Table 10: Risk of elevated LDL with alleles associated with NAFLD

Non-Hispanic Whites					
Gene	**SNP**	**Allele**	**OR**	**95% CI**	**P value**
PNPLA3	rs738409	C derived	1.256	0.691-2.284	0.455
	rs738409	G ancestral	0.796	0.438-1.448	0.455
APOC3	rs2854116	T ancestral	1.447	0.433-4.836	0.549
	rs2854116	C derived	0.691	0.207-2.310	0.549
APOC3	rs2854117	C ancestral	0.815	0.443-1.498	0.510
	rs2854117	T derived	1.228	0.667-2.258	0.510
GCKR	rs780094	A derived	0.874	0.494-1.546	0.643
	rs780094	G ancestral	1.144	0.647-2.025	0.643
GCKR	rs741038	G ancestral	1.517	0.831-2.769	0.174
	rs741038	A derived	0.659	0.361-1.203	0.174
PEMT	rs7946	G ancestral	0.957	0.475-1.929	0.903
	rs7946	A derived	1.045	0.519-2.105	0.903
LYPLAL1	rs12137855	C ancestral	2.262	1.109-4.615	0.025
	rs12137855	T derived	0.442	0.217-0.902	0.025
Hispanics					
Gene	**SNP**	**Allele**	**OR**	**95% CI**	**P value**
PNPLA3	rs738409	C derived	0.816	0.391-1.703	0.589
	rs738409	G ancestral	1.225	0.587-2.556	0.589
APOC3	rs2854116	T ancestral	4.869	0.954-24.838	0.057
	rs2854116	C derived	0.205	0.040-1.048	0.057
APOC3	rs2854117	C ancestral	0.473	0.230-0.972	0.042
	rs2854117	T derived	2.115	1.029-4.349	0.042
GCKR	rs780094	A derived	1.953	1.039-3.672	0.038
	rs780094	G ancestral	0.512	0.272-0.963	0.038
GCKR	rs741038	G ancestral	0.833	0.405-1.714	0.619
	rs741038	A derived	1.201	0.583-2.472	0.619
PEMT	rs7946	G ancestral	0.674	0.369-1.230	0.198
	rs7946	A derived	1.484	0.813-2.707	0.198
LYPLAL1	rs12137855	C ancestral	1.583	0.685-3.657	0.282
	rs12137855	T derived	0.632	0.273-1.459	0.282

Table 11: Risk of elevated DBP with alleles associated with NAFLD

Non-Hispanic Whites					
Gene	**SNP**	**Allele**	**OR**	**95% CI**	**P value**
PNPLA3	rs738409	C derived	NA*		
	rs738409	G ancestral	NA*		
APOC3	rs2854116	T ancestral	NA*		
	rs2854116	C derived	NA*		
APOC3	rs2854117	C ancestral	0.281	0.088-0.902	0.033
	rs2854117	T derived	3.553	1.108-11.392	0.033
GCKR	rs780094	A derived	1.009	0.288-3.535	0.988
	rs780094	G ancestral	0.991	0.283-3.470	0.988
GCKR	rs741038	G ancestral	0.676	0.174-2.624	0.571
	rs741038	A derived	1.480	0.381-5.749	0.571
PEMT	rs7946	G ancestral	0.237	0.027-2.052	0.191
	rs7946	A derived	4.213	0.487-36.416	0.191
LYPLAL1	rs12137855	C ancestral	1.028	0.219-4.819	0.972
	rs12137855	T derived	0.973	0.208-4.558	0.972
Hispanics					
Gene	**SNP**	**Allele**	**OR**	**95% CI**	**P value**
PNPLA3	rs738409	C derived	2.475	0.494-12.388	0.270
	rs738409	G ancestral	0.404	0.081-2.023	0.270
APOC3	rs2854116	T ancestral	NA*		
	rs2854116	C derived	NA*		
APOC3	rs2854117	C ancestral	NA*		
	rs2854117	T derived	NA*		
GCKR	rs780094	A derived	1.213	0.341-4.318	0.765
	rs780094	G ancestral	0.824	0.232-2.933	0.765
GCKR	rs741038	G ancestral	1.624	0.366-7.205	0.524
	rs741038	A derived	0.616	0.139-2.732	0.524
PEMT	rs7946	G ancestral	0.773	0.212-2.818	0.697
	rs7946	A derived	1.293	0.355-4.711	0.697
LYPLAL1	rs12137855	C ancestral	2.439	0.270-22.058	0.427
	rs12137855	T derived	0.410	0.045-3.708	0.427

*Assay not done

In summary, the derived allele of GCKR (rs780094) was associated with elevations of the liver enzymes ALT and AST that may be indicators of liver damage in Hispanics. NAFLD susceptibility genes were not associated with HbA1c, TG, HDL, DBP, and MAP in Hispanics, but they were associated with these indicators in non-Hispanic whites. For the NAFLD susceptibility SNPs measured in this study there was no single SNP that was associated with indicators in both the Hispanic and non-Hispanic white population. In both the non-Hispanic white and Hispanic groups, two indicators were associated with different SNPs. Increased BMI was associated with one SNP (APOC3, rs2854116, ancestral) in the non-Hispanic whites and with two different SNPs (PNPLA3, rs738409, ancestral; PEMT, rs7946, derived) in Hispanics. An elevated LDL was significantly associated with one SNP in the non-Hispanic whites (LYPLAL1, rs12137855, ancestral) and two SNPs in Hispanics (APOC3 rs2854117, derived; GCKR rs780094, derived).

Discussion

In this study, we found 8 SNPs in 5 NAFLD susceptibility genes that were significantly or marginally associated with selected indicators for NAFLD, metabolic syndrome, overweight, obesity, insulin resistance, type 2 diabetes, hypertension, or dyslipidemia. No one SNP was associated with the same indicator in both the non-Hispanic white and Hispanic groups in this population.ALT and AST are often used to screen for NAFLD, however they are neither sensitive nor specific enough to use for routine diagnosis of NAFLD because elevated liver enzymes are also associated with other liver diseases including hepatitis and cirrhosis. We found that the derived allele of GCKR (rs780094) was significantly associated with elevations in ALT and marginally associated with elevations in AST in the Hispanic group. Thus, the derived allele may increase the risk of liver damage as evidenced by elevations in ALT and AST. However, none of these gene polymorphisms were associated with elevated levels of ALT and AST in the non-Hispanic whites.

Metabolic syndrome is considered a risk factor for NAFLD and evidence suggests that NAFLD is a manifestation of metabolic syndrome[3,30,31]. Three indicators of metabolic syndrome were examined in our study; BMI to estimate overweight/obesity, high TG and low HDL levels which, when taken together as a ratio, are indicators of insulin resistance, and BP as a clinical measure of hypertension (SBP, DBP, MAP). Body fat distribution data for this study was unavailable and constitutes a limitation of this study because abdominal obesity as measured by waist circumference has been shown to be strongly associated with NAFLD[30]. A high BMI may also be seen in muscular individuals who are not over fat and therefore not necessarily at risk for NAFLD.

A significant association with increased BMI and the ancestral allele of APOC3 (rs2854116) was found in non-Hispanic whites. Interestingly, 100% of both non-Hispanic whites and Hispanics had one or more of the ancestral alleles for APOC3 and no one in this population had two of the derived alleles (See Table 3). We found a marginal association with increased BMI and the derived allele of PEMT (rs7946) in Hispanics only. The derived allele of PEMT (rs7946) was significantly more frequent in the non-Hispanic whites in our population (98% vs. 78% respectively). Moreover, a marginal association was found in the Hispanic group for increased BMI with the ancestral allele of PNPLA3 (rs738409). In our population, Hispanics had a significantly higher percentage (75.8%) of one or more of the ancestral alleles of PNPLA3 (rs738409) than non-Hispanic whites (40.9%). The derived allele of APOC3 (rs2854116) and PNPLA3 (rs738409) may protect against overweight and obesity whereas the derived allele of PEMT (rs7946) may be associated with increased BMI. This data may suggest that Hispanics in NM may be more susceptible to NAFLD because obesity is strongly associated with hepatic steatosis[11]. Obesity, insulin resistance, increased serum levels of ALT, and lower serum TG have been linked to an increased risk of having the derived allele of PNPLA3 (rs738409) (I148M; GG) in a cohort of 18,921 obese European individuals from Scotland and Sweden[11]. This PNPLA3 SNP reduces function and causes impaired hepatic TG efflux thus lowering serum TG and increasing liver fat retention[11]. A 1.9-fold increase of the NAFLD activity score, which evaluates histological changes by summing the scores for steatosis, hepatocellular ballooning and lobular inflammation, has also been associated with the derived allele of PNPLA3 (rs738409) in autopsies conducted in adult victims (n=405; Caucasian 326, Hispanic 30, Black 38, Other 11) of automobile accidents in Kansas and Missouri[32]. Petit et al demonstrated that liver fibrosis was associated with this PNPLA3 I148M carriage in individuals with type 2 diabetes[12]. There may be an increased risk of the progression of liver disease in individuals with the derived GG alleles because patients who needed a liver transplant were twice as likely to have this polymorphism[13]. The carriage of I148M is associated with the development and severity of NAFLD likely by the mechanism of reduced TG catabolism[14].

An elevated TG to HDL ratio ≥ 3 is used as an indicator of insulin resistance[33-35]. In the non-Hispanic white group only, low HDL was found to be related to two different SNPs in the GCKR gene. Low HDL was significantly associated with the ancestral allele of GCKR (rs741038) and marginally associated with the derived allele of GCKR (rs780094). The derived allele may be protective in GCKR (rs741038) and detrimental in GCKR (rs780094) for lowering HDL. An association of low HDL and elevated blood TG was not observed in this Hispanic population. However, a marginal association in the non-Hispanic whites for higher TG was observed with the ancestral allele of GCKR (rs741038). These data support the possibility that in non-Hispanic whites in NM, the GCKR ancestral gene may be associated with a higher risk of insulin resistance. The derived allele of GCKR (rs741038) may be protective. However, the derived allele of GCKR (rs780094) is associated with high insulin levels[23].

Limited data suggests that food may interact with GCKR (rs780094). In a recent meta-analysis, a higher number of reported whole grain servings compared to a lower number of reported servings was associated with lower fasting insulin levels in all individuals (n=33,784 European adults). However, the benefit was less in those carrying the GCKR (rs780094) derived allele compared with those carrying the ancestral allele[23]. In other studies, the derived allele of GCKR (rs780094) has been found to be associated with insulin resistance[15,17]. Each additional copy of the T-allele resulting from the C to T mutation of GCKR (rs780094) was associated with higher blood TG, CRP, post-OGTT glucose levels, higher prevalence of the metabolic syndrome, and with lower fasting glucose, fasting insulin and HOMA-IR in 10,929 non-Hispanic white but not 3,960 African American adult participants of the Atherosclerosis Risk In Communities (ARIC) Study[15,16]. Additionally, in a meta-analysis including 99,702 cases and 199,275 controls (70% Caucasian, 30% Asian; adults), a significant association was found between an elevated risk of type 2 diabetes and the GCKR (rs780094) derived allele[17]. In summary, the studies are divided and most studies have been conducted in non-Hispanic whites. It is currently unclear what the actual association with type 2 diabetes is for the GCKR (rs780094) derived allele. In a study of the other SNP, GCKR (rs741038), for which the ancestral allele was associated with both hypertriglyceridemia and low HDL in our study, other researchers showed the greatest decrease in TG in over weight subjects (Canadians; n= 208; 96 male, 112 Female; age 30.82 ± 8.66; BMI 27.84 ± 3.73) with the derived allele of GCKR (rs741038) after fish oil supplementation when on a high carbohydrate diet[28]. These data suggest that those with the ancestral allele may not benefit from n-3 fish oil as much as those

with the derived allele. Approximately 90% of both non-Hispanic whites and Hispanics in the New Mexican population carried the derived allele of GCKR (rs741038). Thus, an increase in n-3 fatty acid intake may be very important for this New Mexican population for lowering insulin resistance.

No association was found in either population for systolic blood pressure in APOC3 (rs2854117) although a significant association for elevated diastolic blood pressure with the derived allele was found in the non-Hispanic white group. Additionally, in the non-Hispanic whites, there was a marginal association with low MAP in the same derived allele of APOC3 (rs2854117). The derived allele may contribute to the vulnerability of abnormal blood pressure. No association with any of the NAFLD genes was found with these indicators of hypertension in the Hispanic population.

Type 2 diabetes may be preceded by metabolic syndrome including the previously discussed insulin resistance, dyslipidemia, and hypertension. In our study, we used HbA1c as a biomarker for type 2 diabetes. A significant association for elevated HbA1c was seen in the non-Hispanic white population for the ancestral allele of GCKR (rs741038). A marginal association was evident for the ancestral allele of PNPLA3 (rs738409) with high HbA1c. The derived allele of GCKR (rs741038) and PNPLA3 (rs738409) may have a protective effect against the development of type 2 diabetes. Associations were not found in the Hispanic group for HbA1c for any SNP.

Individuals with metabolic syndrome have a higher risk for developing cardiovascular disease. Elevated LDL and total cholesterol were the indicators used to evaluate risk factors for cardiovascular disease that were measured in our study. No NAFLD susceptibility genes were associated with total cholesterol levels. However, 3 genes were associated with LDL. Associations were found in both ethnicities for elevated LDL but with different genes in each ethnicity. A significant association with higher LDL levels was seen in the non-Hispanic white group for LYPLAL1 (rs12137855) and those with the ancestral gene had higher odds of having elevated LDL, suggesting the derived allele may be associated with lower LDL levels. Moreover, in the Hispanic group, a significant association was observed between high LDL and the derived allele for both APOC3 (rs2854117) and GCKR (rs780094). In both of these SNPs (APOC3 rs2854117; GCKR rs780094), the derived allele may be associated with an increased susceptibility for higher LDL levels.

In this study, there were different genes associated with each indicator in non-Hispanic whites compared to Hispanics. There was not a single SNP associated with the same indicator in both the non-Hispanic white or Hispanic groups. In different ethnicities, it may be possible that one SNP could mask another SNP. There may be other genes that were not tested in this study that may have an effect on the SNPs we tested. Gene-gene interactions are still not fully understood. The genes evaluated in this study have been associated with different responses to specific foods or nutrients in feeding studies. Further research is needed to determine the responses to foods on gene polymorphisms.

Conclusion

In this population of non-Hispanic whites and Hispanics, who attended a clinic in northeast Albuquerque, NM, both the derived and ancestral allele was found for all of the NAFLD susceptibility genes. For APOC3 there were only heterozygotes for the derived allele whereas for all other genes tested, both heterozygotes and homozygotes were found. The percentages of NAFLD allele frequencies in this cohort were similar in non-Hispanic whites and Hispanics except forPNPLA3 (rs738409), FABP2 (rs1799883), and PEMT (rs7946) and for each of these genes the major allele was more frequent for non-Hispanic whites whereas in Hispanics the major and minor alleles had similar frequencies. The derived allele was the major allele of PNPLA3 (rs738409), GCKR (rs741038), and PEMT (rs7946) and the minor allele of APOC3 (rs2854116; rs2854117), GCKR (rs780094), FABP2 (rs1799883), and LYPLAL1 (rs12137855) for non-Hispanic whites and Hispanics alike. Indicators of chronic disease were found to be associated with specific alleles in these NAFLD susceptibility SNPs that were never the same in the non-Hispanic white and Hispanic groups. The derived allele of GCKR (rs780094) was associated in Hispanics with elevated liver enzymes ALT and AST that are indicators of liver damage. In non-Hispanic whites but not in Hispanics, NAFLD genes were associated with elevated HbA1c, higher TG, lower HDL, increased DBP, and abnormal MAP. Two indicators were associated with different SNPs in both the non-Hispanic white and Hispanic groups.BMI was positively associated with ancestral APOC3 (rs2854116) in the non-Hispanic whites, while in Hispanics both ancestral PNPLA3 (rs738409) and derived PEMT (rs7946) were associated with increased BMI. In the non-Hispanic whites, LDL was significantly associated with ancestral LYPLAL1 (rs12137855) and in Hispanics with both derived APOC3 (rs2854117) and derived GCKR (rs780094).Further research is needed to determine the clinical significance of these relationships of chronic disease indicators with NAFLD susceptibility genes in these two ethnic groups. Early genetic screening in children may be a cost effective method for detecting the risk for NAFLD. A referral to a dietitian or other specialist for educational counseling targeted towards weight control, reduced consumption of sugar sweetened beverages, and increased physical activity are crucial strategies for preventing and potentially reversing NAFLD. In addition, more research regarding the interaction of nutrition with these genes that may impact the development of NAFLD, obesity and type 2 diabetes will facilitate the formulation of specific dietary guidelines for prevention and management in the non-Hispanic white and Hispanic population in NM.

Conflict of Interest: The authors declare no conflict of interest.

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