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Genetic determinants of circulating lipoproteins in nonalcoholic fatty liver disease

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Abstract

Background—Recent genome-wide association studies have identified two genetic polymorphisms in association with nonalcoholic fatty liver disease (NAFLD): PNPLA3 and TM6SF2, both of which appear to influence the production of very low density lipoprotein (VLDL). The impact of these genetic variations on lipoprotein metabolism in the setting of nonalcoholic steatohepatitis (NASH) and liver fibrosis are not fully characterized.

Methods—We measured comprehensive lipoprotein profiles by nuclear magnetic resonance among 170 serially recruited patients in an NAFLD registry, and determined their relationships with PNPLA3 and TM6SF2 genotypes.

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Conflict of Interest

Authors declare no additional conflict of interest.

Authorship Statement

Guarantor of the article: Z. Gordon Jiang

ZGJ and ML were involved in the primary study design and data analysis; MAC measured the comprehensive lipoprotein profiles; MK, SK and MH determined the PNPLA3, TM6SF2 genotypes; EUY assisted in the review of liver biopsy histology; all authors participated in the preparation of this manuscript.

All authors have approved the final version of the manuscript

Results—In this cohort, 72% patients had at least one allele of either PNPLA3 I148M or TM6SF2 E167K, and 30% carried two alleles. In multivariate models adjusting for histological features of NASH and liver fibrosis, PNPLA3 I148M is associated with a decrease in VLDL particle size. Both PNPLA3 I148M and TM6SF2 E167K genotypes were associated with increases in the size of low density lipoprotein (LDL) and high density lipoprotein particles, phenotypes considered atheroprotective. When adjusted for both genotypes, NAS score, in particular the degree of hepatic steatosis was strongly associated with increases in the size of VLDL particles, the concentration of LDL, especially small LDL particles, and a decrease in the size of HDL particles, all of which are linked with a proatherogenic phenotype.

Conclusions—PNPLA3 and TM6SF2 are common genetic variants among NAFLD patients and impact lipoprotein profiles in slightly different ways. The interactions between genotypes, hepatic steatosis and lipoprotein metabolism shed lights on the pathophysiology of NAFLD, and provide opportunities for personalized treatment in the era of emerging NAFLD therapeutics.

Keywords

Nonalcoholic fatty liver disease (NAFLD); nonalcoholic steatohepatitis (NASH); liver fibrosis; lipoprotein; very low density lipoprotein (VLDL); low density lipoprotein (LDL); nuclear magnetic resonance (NMR)

INTRODUCTION

Recent studies have revealed the heritability of nonalcoholic fatty liver disease (NAFLD), a common form of chronic liver disease affecting up to one third of the US population.[1–5] To date, two major genetic polymorphisms have been found that impact the susceptibility and progression of NAFLD: an I148M mutation in patatin-like phospholipase domain containing 3 (PNPLA3) and an E167K mutation in Transmembrane 6 Superfamily Member 2 (TM6SF2).[6–8] Although the function of either protein is not fully understood, both genotypes appear to alter lipoprotein metabolism, particularly decreases the production of apolipoprotein B (apoB) and very low density lipoprotein (VLDL).[9, 10]

These advances underscore the pivotal link between lipoprotein metabolism and the pathogenesis of NAFLD. Dyslipidemia, a major modifiable risk factor for atherosclerosis, is intricately related to NAFLD.[11] On the one hand, metabolic syndrome and hepatic steatosis are associated with a proatherogenic lipid profile characterized by increases in the concentration of VLDL and small dense low density lipoprotein (sdLDL).[12–15] Inflammation associated with nonalcoholic steatohepatitis (NASH) and advanced fibrosis may negatively influence the production of both apoB-containing lipoproteins (VLDL and LDL) and high density lipoproteins (HDL). It has been shown that that NASH and fibrosis can be associated with impaired production of apoB/VLDL derived lipoproteins.[16–18] We have demonstrated that NASH is associated with an increase in the size of VLDL particles whereas the presence of stage 2 or above liver fibrosis is associated with a decrease in the concentration of small VLDL particles.[19] These relationships are entangled by the causal link between genetic variants in lipoprotein metabolism and the development of NAFLD. In population studies, PNPLA3 I148M does not seem to impact serum lipids.[8] However, it has been shown that PNPLA3 I148M impairs VLDL production from the liver with a

paradoxical decrease in *de novo* hepatic lipid synthesis.[9, 20, 21] In comparison, TM6SF2 E167K is associated with impaired production of VLDL and LDL cholesterol (LDL-C), and lower incidence of cardiovascular events despite its association with NAFLD.[10]

The relationship between PNPLA3, TM6SF2 genotypes and lipoprotein metabolism has not been examined in depth among NAFLD patients. This question is important as cardiovascular disease is the leading cause of death among NAFLD patients.[22] This is of particular interest in the era of emerging therapeutics for NASH and NASH fibrosis, where circulating lipids and lipoprotein profile are important clinical endpoints to determine whether the treatment will offer overall benefit to the patient.

Herein, we aim to define the relationship between PNPLA3, TM6SF2 genotypes and lipoprotein metabolism in the setting of NASH and NASH fibrosis in an NAFLD registry. We examined the relations with conventional lipid profiles, as well as lipoprotein profiles measured by nuclear magnetic resonance (NMR), a comprehensive lipoprotein analysis widely used in cardiovascular research.[23]

MATERIAL AND METHODS

Patient population

Study subjects were prospectively enrolled in an NAFLD registry from a tertiary referral fatty liver clinic at Beth Israel Deaconess Medical Center (BIDMC) between 2009 and 2015. Patients with other forms of chronic liver diseases or daily consumption of greater than 20 grams of alcohol were excluded from the registry. We collected data on patient demographics, medical, social history, physical exam and laboratory testing at study enrollment. Serum samples were collected at enrollment and stored at -80°C for future analysis. Within three months of the index visit, liver biopsy was performed on each study subject. Patients with biopsy proven nonalcoholic fatty liver disease were included. At the time of this study, 170 subjects in the registry had completed all tests required for complete data analysis. Patients with competing etiologies for liver disease or incomplete data for analysis were excluded. All patients signed written consent upon enrollment into the registry. This study has been approved by the BIDMC institutional review board.

Clinical biochemistry and measurements of lipoprotein profiles

Routine blood tests including complete blood count, chemistry, liver function test, and lipid panel were processed at the BIDMC clinical laboratory. Conventional lipid panel, including triglycerides, total cholesterol, LDL-C and HDL cholesterol (HDL-C), was only performed on fasting patients as the calculation of LDL-C relies on triglyceride levels, a parameter influenced by post-prandial states.

Comprehensive lipoprotein profiles were measured with nuclear magnetic resonance (NMR) spectroscopy at LipoScience, Laboratory Corporation of America® Holdings (Labcorp, Raleigh, NC), which was blinded to clinical data. Frozen samples were thawed, aliquoted (500 μl), and shipped with ice packs for NMR measurements. Lipoprotein particle concentrations and sizes were calculated from the measured amplitudes of the spectroscopically-distinct signals from different-size lipoprotein subclasses using the

LipoProfile-3 algorithm.[24] Diameter range estimates for the reported subclasses are as follows: large VLDL, >60 nm; medium VLDL, 42–60 nm; small VLDL, 29–42 nm; intermediate-density lipoprotein (IDL), 23–29 nm; large LDL, 20.5–23 nm; small LDL, 18–20.5 nm; large HDL, 9.4–14 nm; medium HDL, 8.2–9.4 nm; small HDL, 7.3–8.2 nm. Weighted-average lipoprotein particle sizes were derived from the sum of the diameter of each subclass multiplied by its relative mass percentage and reported in nanometer (nm) units. We measured comprehensive lipoprotein profiles on both fasting and non-fasting samples as these lipoprotein particles are directly measured and only the VLDL, especially large VLDL particles are likely influenced by post-prandial chylomicrons, which are typically much larger.

To facilitate comparison, we calculated standardized lipid/lipoprotein concentrations and lipoprotein sizes by the Z-score of each value. The Z-score was defined by the difference to the mean divided by the standard deviation of each variable.

Liver biopsy

Ultrasound guided liver biopsy was performed within three months of enrollment. Biopsy results were processed locally and interpreted by staff pathologists who specialize in hepatopathology. All liver biopsies were assessed and reported in a standardized fashion using a scoring system described by Kleiner *et al.*, including fibrosis stages (0–4) and NAFLD activity score (NAS, 0–8) calculated based on the degrees of steatosis (0–3), lobular inflammation (0–3) and balloon degeneration (0–2).[25] NASH was diagnosed by the overall histological impression including the degree of steatosis and presence of balloon degeneration.

Genotyping

Genomic DNA was extracted from frozen human whole blood. Briefly, 0.4 ml of whole blood was mixed with 0.6 ml of red blood cell lysis solution (1 mM EDTA). White blood cells were isolated by centrifugation at 2,000 g for 10 min at room temperature after removal of supernatants. The pellet was re-suspended in 0.1 ml of NaCl (1M) and lysed by 0.6 ml of cell lysis buffer (10 mM Tris-HCL, 26 mM EDTA, 0.5% SDS) and 5 µl of RNase A solution (4 mg/ml). The cell lysate was incubated at 37 °C overnight. Genomic DNA was extracted using phenol chloroform. Ethanol-precipitated DNA was resuspended in double distilled water. Single nucleotide polymorphism (SNP) for PNPLA3 I148M (rs738409) and TM6SF2 E167K (rs58542926) were genotyped by TaqMan allelic discrimination, using predesigned TaqMan SNP genotyping assays (Applied Biosystems).

Statistical analysis

We first compared patients among 7 groups based on the combination of PNPLA3 I148M (rs738409) and TM6SF2 E167K (rs58542926) genotypes, and examined their background characteristics, conventional lipid profile and comprehensive lipoprotein profile determined by NMR, including total and subclass lipoprotein concentrations as well as mean lipoprotein particle sizes.

We then constructed multivariate linear regression models to determine the relationship between PNPLA3, TM6SF2 genotypes and the conventional lipid profiles, including triglyceride, total cholesterol, LDL-C, HDL-C, as well as comprehensive lipoprotein profiles, including total and subclass concentrations of major circulating lipoproteins: VLDL, IDL/LDL and HDL, and their mean particle sizes. PNPLA3 I148M GC and GG genotypes were compared with CC genotype, whereas combined CT/TT genotypes were compared with CC due to the minimal number of individuals with TT genotype. We also calculated p value for trend across three PNPLA3 I148 genotypes, using CC, GC, and GG genotypes as a continuous variable. To facilitate comparison, we used standardized lipid/lipoprotein measurements using the Z-score in the unit of standard deviation. Each regression model was adjusted for the component scores of NASH disease activity (hepatic steatosis, lobular inflammation and balloon degeneration) and the stage of liver fibrosis. We also performed sensitivity tests using the degree of NASH activity measured by NAS score (0–8) and the presence of stage 2 or above liver fibrosis in binary variables. The selection of covariates were based on biological relevance to lipoprotein phenotypes. Each regression was adjusted for age, BMI in continuous variables, and gender, the history of diabetes, the use of statin in binary variables. All 170 individuals were included in the regression analyses for the comprehensive lipoprotein profile. Only individuals with fasting samples were included in the analysis of conventional lipid profiles. Sensitivity test was performed for VLDL particle concentration and size using only fasting samples using the same covariates, as post-prandial chylomicron from the small intestine may confound such analyses. We also performed same analysis for the concentration of apoB-containing lipoproteins, calculated by the sum of total VLDL and IDL/LDL particles. We did not perform Bonferroni correction for multiple analyses as neither outcomes nor exposures were independent in this study. All analyses were performed in Stata13.0 (Stata corp., Texas).

RESULTS

Patient characteristics

The clinical characteristics of 170 serially recruited subjects in the NAFLD registry are summarized in Table 1. The average age of the cohort was 50.5, among whom 40% were female and 13.5% were Hispanics. The average BMI of the cohort was 34, with 29% carrying a diagnosis of diabetes at enrollment. The prevalence of PNPLA3 I148M genotypes was 42% GC and 21% GG. In comparison, the prevalence of TM6SF2 E167K was lower with 19% CT and 1% TT. NASH patients (83%) constituted the majority of the cohort, with a mean NAS score of 4.5. Among NASH cases, 23 (13.5%) patients had rare balloon degeneration and no liver fibrosis that could be characterized as borderline NASH. Approximately half of the patients (47%) had stage 2 or above fibrosis and 8% had cirrhosis.

Patient characteristics associated with PNPLA3 and TM6SF2 genotypes

We compared patient characteristics among groups categorized by the possible combinations of PNPLA3 I148M and TM6SF2 E167K genotypes (Table 2). Among 170 NAFLD patients, 122 patients (72%) had at least one allele of PNPLA3 I148M or TM6SF2 E167K, and 51 patients (30%) had two or more alleles. Consistent with prior reports, Hispanics were more likely to carry PNPLA3 I148M, but not TM6SF2. Known comorbidities of NAFLD, such as

obesity, diabetes, hypertension and hyperlipidemia were prevalent in all groups despite genotypes.

We then examined the conventional lipid profile, lipoprotein concentrations and sizes (Table 3). Groups 3 – 5, patient with homozygous PNPLA3 I48M or heterozygous TM6SF2 E167K had lower concentrations of total VLDL particles, whereas individuals with TM6SF2 E167K had lower total IDL/LDL particles. These differences however were not significant by ANOVA test.

Multivariate analysis of the association between genotypes and lipoprotein profiles

We then evaluated the associations between PNPLA3, TM6SF2 genotypes and lipid, lipoprotein profiles in multivariate models adjusting for NAFLD disease severity characterized by NAS score and the presence of stage 2–4 fibrosis. In multivariate analyses, we did not find significant associations between PNPLA3, TM6SF2 genotypes and fasting lipid levels (Supplemental Table 1). It was noted that histological score of hepatic steatosis correlated with the level of fasting triglyceride.

The associations between PNPLA3, TM6SF2 genotypes and lipoprotein profiles were more prominent, especially with the size of lipoprotein particles (Table 4, Supplemental Table 2). PNPLA3 I48M and hepatic steatosis appeared to have associations with VLDL particle size in opposite directions. The homozygous genotype of I48M was associated with a decrease of 0.85 *s.d.* (95% CI –1.34 to –0.36, $p = 0.001$) in VLDL size, whereas I48M heterozygous was associated with a decrease of 0.39 *s.d.* (95% CI –0.79 to 0.01, $p = 0.06$), with a p value for trend of 0.003 across three PNPLA3 genotypes. In contrast, each point of hepatic steatosis on biopsy was associated with an increase of 0.53 *s.d.* (95% CI 0.24 to 0.82, $p < 0.001$) in VLDL particle size and 0.50 *s.d.* (95% CI 0.26 to 0.74, $p < 0.001$) in the concentration of large VLDL particles (Table 4, Supplemental Table 3). No significant associations were seen with PNPLA3 genotype and total VLDL particle concentration.

TM6SF2 E167K and NASH were associated with IDL/LDL particle concentration and size in opposite directions. TM6SF2 E167K was associated with a lower IDL/LDL concentration that appeared to be driven largely by a lower concentration of small LDL particles (–0.59 *s.d.*, 95% CI –0.95 to –0.22, $p = 0.002$) (Table 4, Supplemental Table 3). In fact, TM6SF2 E167K was associated with a 0.49 *s.d.* decrease (95% CI –0.87 to –0.10, $p = 0.01$) in the total concentration of apoB-containing lipoproteins, the sum of VLDL and IDL/LDL concentrations. NASH demonstrated an opposite association. This is however mostly driven by hepatic steatosis with each point of histology score of steatosis associated with an increase of 0.37 *s.d.* (95% CI 0.13 to 0.61, $p = 0.003$) in total IDL/LDL particle concentration, 0.54 *s.d.* (95% CI 0.30 to 0.52, $p < 0.001$) in the concentration of small LDL particles. Both PNPLA3 I48M homozygous and TM6SF2 E167K were associated with increases of ~0.5 – 0.6 *s.d.* in the size of IDL/LDL particles, with a p value for trend of 0.02 across PNPLA3 genotypes, whereas hepatic steatosis was associated with smaller IDL/LDL particle sizes (Table 4, Supplemental Table 3).

Both PNPLA3 and TM6SF2 genotypes were associated with HDL particle size, but not total HDL concentration. PNPLA3 I48M homozygous and TM6SF2 E167K carriers were

associated with similar increases in the size of HDL particles by approximately half a standard deviation. Similarly, a positive p value for trend of 0.004 across PNPLA genotypes was observed. One point increase in hepatic steatosis score was associated with a decrease of 0.36 *s.d.* (95% CI -0.59 to -0.14 , $p = 0.002$) in the size of HDL particles (Table 4). These relationships with HDL particle size were explained by the differential associations with the concentration of subclass HDL particles, positive between PNPLA3 GG, TM6SF2 CT/TT, and liver fibrosis with large HDL particles, but negative with NAS (Supplemental Table 3).

We also performed sensitivity analysis using NAS score in place of components of NASH. Each point of NAS was associated 0.27 *s.d.* increase in VLDL particle size (95% CI 0.15 to 0.39, $p < 0.001$), 0.20 *s.d.* decrease in the IDL/LDL particle size (95% CI -0.33 to -0.78 , $p = 0.002$) and 0.12 *s.d.* decrease in the HDL particle size (95% CI -0.23 to 0.0003, $p = 0.05$), a trend in keep with the score of hepatic steatosis. In these models, the associations with PNPLA3 and TM6SF2 genotypes remained the same.

DISCUSSION

Dyslipidemia, the most important modifiable risk factor in cardiovascular disease, is common among NAFLD patients. As the vast majority of patients with NAFLD will not experience liver-related morbidities, rather they are at increased risk of cardiovascular disease, even among those with advanced fibrosis.[22, 26] Unfortunately, conventional lipid profiles fail to fully capture the changes in lipoprotein metabolism among our patients. This distinction is critically important with respect to both our conduct of clinical trials and our evolving understanding NAFLD pathophysiology. For instance, the recent FLINT trial observed an increase in LDL-C in the group treated with obeticholic acid.[27] These data from our prospectively defined cohort of American patients with biopsy proven NAFLD demonstrate that beyond conventional lipid concentration, the lipoprotein profile is associated with advanced NAFLD histology, and both are associated with genetic polymorphisms that predispose to NAFLD. Further, we show that lipoprotein particle size, rather than the quantity, defines the lipoprotein phenotype associated with NASH and NAFLD genetics, that may underlie our patients' cardiovascular risk.

Our study extends a growing body of literature on the genetics and lipoprotein metabolism in NAFLD in several ways. First of all, our study suggested a strong genetic component of NAFLD. Estimates for heritable traits of hepatic steatosis is 26–27% by GWAS study and 52% suggested by the recent twin study.[1, 4] PNPLA3 I148M is a common genetic variant especially among Hispanics with a homozygous frequency reaching approximately 25%, in comparison to 4% in European whites and 2% among African Americans.[8] TM6SF2 E167K is rarer, with carrier frequency (heterozygous) of 14% among European whites, and 6–8% among African Americans and Hispanics.[6] In our cohort with patients identified by clinical suspicion of NAFLD and only 14% Hispanics, 72% patients carried at least one allele of either variant, and 30% carried two alleles. The prevalence of PNPLA3 I148M and TM6SF2 E167K were much higher than the general population.

Secondly, our study demonstrated a strong association between lipoprotein profiles and PNPLA3 I48M, TM6SF2 E167K (Table 5). The simultaneous associations of key genotypes

with both NAFLD and lipoprotein profiles underpins the link between the pathophysiology of NAFLD and lipoprotein metabolism. Consistent with previous work, both genotypes appear to impact VLDL profiles.[9, 10, 21, 28] The use of a comprehensive lipoprotein profile allowed us to gain a deeper insight into these relationships. Interestingly, PNPLA3 I148M is associated with smaller size of VLDL particles, whereas TM6SF2 E167K is associated with lower concentration of IDL/LDL particles and total apoB-containing lipoproteins. Furthermore, we found that both PNPLA3 and TM6SF2 were associated with larger sizes in LDL and HDL particles, opposite to the direction of associations with NAS score and hepatic steatosis, after the adjustment for BMI and diabetes. Small dense LDL is a well-established risk factor for cardiovascular disease in addition to LDL-C, whereas larger sizes of HDL particles are thought to be atheroprotective.[29–31] These associations with PNPLA3 and TM6SF2 genotypes are sizable, accounting for 0.4–0.5 standard deviations. How PNPLA3 and TM6SF2 genotypes impact LDL and HDL profiles is not entirely clear. The prevalence of metabolic comorbidities, such as obesity, diabetes, hypertension, are similar among groups categorized by genotypes, suggesting this may not be fully explained by lower levels of insulin resistance among individuals with increased genetic susceptibility for NAFLD. Animal studies suggested that TM6SF2 is likely involved in the lipidation of VLDL.[32] Multiple genetic coding variants in TM6SF2 were associated with lipid levels.[33] Individuals with TM6SF2 E167K have been shown to have decreased risk of cardiovascular outcomes.[10, 34] Our observations appear to support the concept raised by Kahali *et al.* that genotypes associated with NAFLD may render some protection against atherosclerosis.[35] Interestingly, national survey data from NHANES III have failed to detect a positive link between hepatic steatosis identified by liver ultrasound and overall or cardiovascular mortality.[36] On the other hand, PNPLA3 I148M has been shown to be associated with worse carotid atherosclerosis and premature coronary artery disease.[37, 38] The mechanism that links PNPLA3 I148M genotype and a rather broad range of phenotypes, including hepatic steatosis, rapid progression of steatohepatitis and fibrosis may implicate its role in both hepatocytes and hepatic stellate cells.[39]

Third, we demonstrated a strong association between a proatherogenic lipoprotein profile and histological diagnosis of NASH as well as NAS, characterized by increases in concentration of large VLDL particles and small LDL particles, resulting in large VLDL particle size, but small LDL particle size (Table 5). This finding is different from a study by Siddiqui *et al.* where they did not see significant differences in lipoprotein profiles between NASH and simple steatosis.[12] This discrepancy could be both due to a larger sample size in the current study and methodological, as Siddiqui and his coworkers used a different platform for lipoprotein analysis. However, among three components of NAS, hepatic steatosis was the major determinant in association with the lipoprotein profile. This association is likely driven by the fact that patients with NASH tend to have more hepatic steatosis. We did not observe association between NAS and total cholesterol, LDL or HDL cholesterol, suggesting conventional lipid profiles may not provide enough granularity to capture the changes in lipoprotein metabolism in association with NASH.

The data presented here must be interpreted in the context of the study design. The sample size was modest. In particular, we had only two patients with TM6SF2 E167K homozygous genotype, limiting the characterization of the impact of TT genotype. However, E167K

appears to have a codominant impact with significant phenotype even among heterozygous carriers. In addition, some modest associations need to be examined with caution in light of multiple comparisons. The study subjects derived from a tertiary referral center, with a cohort under-represented by individuals with simple steatosis. Several newly identified genetic variants associated with NAFLD are not studied here, including a glucokinase regulator (GCKR) gene variant that enhance de novo hepatic lipogenesis and membrane bound O-acyltransferase domain-containing 7 (MBOAT7) gene variant that alters the remodeling of phosphatidylinositol acyl chain.[4, 40] Further investigations are need to validate these findings, and determine the impact of genotypes and lipoprotein metabolism on patient outcome.

In summary, we found that NAS score, in particular the score of hepatic steatosis, is associated with a proatherogenic lipoprotein phenotype characterized by large VLDL particles and higher concentrations of small LDL particles, whereas PNPLA3 I148M and TM6SF2 E167K, two major genotypes linked to NAFLD, are associated with an atheroprotective lipoprotein phenotype in all three classes of lipoproteins. These insights may help to explain the heterogeneity in hepatic versus cardiovascular outcomes among NAFLD patients, and provide opportunities for personalized risk stratification and treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Disclosure

MAC is an employee at the Laboratory Corporation of America® Holdings (LabCorp). LabCorp employees were responsible for sample testing, but blinded to any patient level information. Only investigators at BIDMC were involved for data analysis.

Abbreviations

ApoB	apolipoprotein B
IDL	intermediate density lipoprotein
HDL	high density lipoprotein
LDL	low density lipoprotein
NAFLD	nonalcoholic fatty liver disease
nm	nanometer
NAS	NAFLD activity score
NASH	nonalcoholic steatohepatitis

NMR	nuclear magnetic resonance
nonHDL-C	non-HDL cholesterol
PNPLA3	patatin-like phospholipase domain-containing 3
TM6SF2	transmembrane 6 superfamily member 2
sdLDL	small dense LDL
VLDL	very low density lipoprotein

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Table 1

Baseline characteristics of study participants

NAFLD registry (n = 170)	
Age (mean $\pm$ sd)	50.5 $\pm$ 12.6
Gender (%Female)	40%
Hispanics (%)	14%
Medical history (n, %)	
Diabetes	49 (29%)
Hypertension	79 (46%)
Dyslipidemia	72 (42%)
BMI (kg/m ²)	34.3 $\pm$ 6.5
Waist Circumference (cm)	109 $\pm$ 14
Statin use (n, %)	51 (30%)
Laboratory (mean $\pm$ sd)	
ALT (IU/dL)	75 $\pm$ 51
AST (IU/dL)	50 $\pm$ 32
Albumin (g/dL)	4.6 $\pm$ 0.4
Platelet count ($\times 10^3$ / μ L)	249 $\pm$ 74
NASH (n, %)	
Simple steatosis	29 (17%)
NASH	141 (83%)
NAS Score (mean $\pm$ sd)	4.5 $\pm$ 1.4
Stage of Fibrosis (n, %)	
0	53 (31%)
1	38 (22%)
2	49 (29%)
3	17 (10%)
4	13 (8%)
rs738409 PNPLA3 I148M (n, %)	
CC	63 (37%)
GC	71 (42%)
GG	36 (21%)
rs58542926 TM6SF2 E167K (n, %)	
CC	135 (79%)
CT	33 (19%)
TT	2 (1%)

Table 2

Baseline characteristics by PNPLA3 and TM6SF2 genotypes

Genotype Group	1	2	3	4	5	6	7
PNPLA3 I148M (rs738409)	-/- (CC)	-/+ (CG)	+/+ (GG)	-/- (CC)	-/+ (CG)	+/+ (GG)	-/+ (CG)
TM6SF2 E167K (rs58542926)	-/- (CC)	-/- (CC)	-/- (CC)	-/+ (CT)	-/+ (CT)	-/+ (CT)	+/+ (TT)
Num (n, %)	48 (28%)	56 (32%)	31 (19%)	15 (9%)	13 (7%)	5 (3%)	2 (1%)
Age	50.8 ± 12.2	50.5 ± 13.7	48.5 ± 11.1	55.2 ± 10.6	47.5 ± 14.5	56.3 ± 13.3	37.0 ± 7.9
Female (%)	42%	41%	32%	40%	38%	60%	50%
Hispanics (%)	6%	11%	35%	0%	15%	0%	50%
Diabetes (%)	29%	29%	16%	40%	38%	60%	0%
Hypertension (%)	48%	45%	35%	60%	54%	60%	50%
Dyslipidemia (%)	50%	36%	35%	47%	54%	60%	0%
BMI (kg/m ²)	35.1 ± 6.2	34.6 ± 7.1	33.6 ± 5.9	33.0 ± 7.1	34.8 ± 5.6	30.7 ± 7.1	30.7 ± 7.1
Waist Circumference (cm)	111 ± 15	110 ± 15	108 ± 13	110 ± 18	108 ± 10.0	105 ± 15	108

Table 3

Lipid and lipoprotein profiles by PNPLA3 and TM6SF2 genotypes

Genotype group	1	2	3	4	5	6	7
PNPLA3 T148M (rs738409)	-/- (CC)	-/+ (CG)	+/+ (GG)	-/- (CC)	-/+ (CG)	+/+ (GG)	-/+ (CG)
TM6SF2 E167K (rs58542926)	-/- (CC)	-/- (CC)	-/- (CC)	-/+ (CT)	-/+ (CT)	-/+ (CT)	+/+ (TT)
n (%)	48 (28%)	56 (32%)	31 (19%)	15 (9%)	13 (7%)	5 (3%)	2 (1%)
Conventional lipid profile ¹ (mg/dL)							
Triglyceride	205 ± 162	210 ± 102	185 ± 121	177 ± 85	142 ± 65	208 ± 156	558
Total cholesterol	192 ± 56	197 ± 34	202 ± 47	187 ± 53	168 ± 45	168 ± 42	195
LDL-C	108 ± 45	117 ± 29	118 ± 40	104 ± 51	97 ± 37	85 ± 53	98
HDL-C	48 ± 17	43 ± 11	47 ± 16	48 ± 10	42 ± 13	48 ± 9	26
Comprehensive lipoprotein profile VLDL concentration (nmol/L)							
Total	81 ± 54	87 ± 47	72 ± 46	58 ± 28	61 ± 36	99 ± 70	92 ± 78
Large VLDL	11 ± 11	10 ± 7	9 ± 11	9 ± 8	8 ± 6	7 ± 7	19 ± 17
Medium VLDL	41 ± 28	45 ± 21	37 ± 24	28 ± 16	29 ± 16	50 ± 41	43 ± 38
Small VLDL	29 ± 25	32 ± 29	26 ± 20	21 ± 14	24 ± 18	43 ± 24	30 ± 22
IDL & LDL concentration (nmol/L)							
Total	1487 ± 541	1497 ± 503	1448 ± 451	1335 ± 717	1355 ± 543	1062 ± 432	1294 ± 707
IDL	88 ± 81	110 ± 89	80 ± 55	93 ± 62	99 ± 57	72 ± 31	14 ± 1
Large LDL	403 ± 279	432 ± 309	517 ± 285	538 ± 359	439 ± 347	353 ± 261	77 ± 15
Small LDL	996 ± 478	955 ± 453	850 ± 388	704 ± 545	817 ± 393	637 ± 335	1204 ± 691
HDL concentration (μmol/L)							
Total	36.6 ± 8.3	36.7 ± 6.7	36.5 ± 8.6	38.4 ± 5.8	36.2 ± 10.2	41.6 ± 10.1	30.1 ± 6.2
Large HDL	3.5 ± 2.4	3.6 ± 2.8	4.5 ± 4.8	4.7 ± 4.2	5.3 ± 3.8	6.9 ± 1.5	2.8 ± 3.2
Medium HDL	11.5 ± 5.7	12.0 ± 5.2	13.2 ± 5.5	11.2 ± 5.0	10.4 ± 7.2	14.5 ± 5.9	9.4 ± 5.4
Small HDL	8.9 ± 0.4	9.0 ± 0.5	9.1 ± 0.5	9.2 ± 0.5	9.2 ± 0.7	9.5 ± 0.3	8.7 ± 0.7
Lipoprotein particle size (nm)							
VLDL ³	56.4 ± 6.9	55.4 ± 6.9	54.2 ± 7.3	56.9 ± 10.1	56.5 ± 7.4	48.9 ± 4.3	64.4 ± 4.0
IDL and LDL	20.6 ± 0.9	20.7 ± 0.8	21.0 ± 0.7	21.3 ± 0.9	20.8 ± 1.0	21.1 ± 0.8	19.7 ± 0.1
HDL	8.9 ± 0.4	9.0 ± 0.5	9.1 ± 0.5	9.2 ± 0.5	9.2 ± 0.7	9.5 ± 0.3	8.7 ± 0.7

Only subjects had measurements on conventional profile

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Table 4

Multivariate models for standardized lipoprotein particle concentration and size

		Standardized VLDL conc. (s.d.) ¹				Standardized VLDL size (s.d.)			
		β^2	95% CI	p value	β	95% CI	p value		
PNPLA3 ³	GC	0.29	-0.15 to 0.72	0.2	-0.39	-0.79 to 0.01	0.06		
	GG	-0.04	-0.56 to 0.49	0.9	-0.85	-1.34 to -0.36	0.001		
	CT/TT	-0.31	-0.79 to 0.18	0.2	-0.17	-0.61 to 0.28	0.5		
TM6SF2 ⁴	Stage of fibrosis	-0.13	-0.35 to 0.09	0.2	-0.002	-0.20 to 0.20	1.0		
	Steatosis	0.25	-0.07 to 0.56	0.1	0.53	0.24 to 0.82	<0.001		
	Lobular inflammation	-0.18	-0.55 to 0.20	0.4	0.16	-0.18 to 0.50	0.3		
	Balloon degeneration	-0.10	-0.41 to 0.21	0.5	0.16	-0.12 to 0.45	0.3		
		Standardized IDL/LDL conc. (s.d.)				Standardized IDL/LDL size (s.d.)			
		β	95% CI	p value	β	95% CI	p value		
PNPLA3	GC	-0.10	-0.44 to 0.25	0.6	0.06	-0.28 to 0.41	0.7		
	GG	-0.30	-0.72 to 0.13	0.2	0.57	0.15 to 0.99	0.008		
	CT/TT	-0.46	-0.84 to -0.08	0.02	0.53	0.15 to 0.99	0.007		
TM6SF2	Stage of fibrosis	-0.03	-0.19 to 0.12	0.7	-0.09	-0.25 to 0.08	0.3		
	Steatosis	0.37	0.13 to 0.61	0.003	-0.54	-0.77 to -0.30	<0.001		
	Lobular inflammation	0.28	-0.03 to 0.58	0.07	0.04	-0.26 to 0.34	0.8		
	Balloon degeneration	-0.15	-0.40 to 0.10	0.2	-0.06	-0.31 to 0.18	0.6		
		Standardized HDL conc. (s.d.)				Standardized HDL size (s.d.)			
		β	95% CI	p value	β	95% CI	p value		
PNPLA3	GC	-0.01	-0.36 to 0.34	1.0	0.14	-0.18 to 0.46	0.4		
	GG	0.10	-0.33 to 0.53	0.6	0.58	0.19 to 0.97	0.004		
	CT/TT	0.12	-0.26 to 0.51	0.5	0.53	0.18 to 0.88	0.003		

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Stage of fibrosis	-0.12	-0.28 to 0.04	0.2	0.14	-0.01 to 0.29	0.07
Steatosis	0.02	-0.23 to 0.27	0.9	-0.36	-0.59 to -0.14	0.002
Lobular inflammation	-0.04	-0.35 to 0.27	0.8	0.04	-0.24 to 0.32	0.8
Balloon degeneration	0.05	-0.20 to 0.30	0.7	0.003	-0.23 to 0.23	1.0

¹ Standardized lipoprotein particle concentration and size were calculated by Z-scores in units of standard deviation.

² β coefficient calculated from linear regression models with independent variables including PNPLA3, TM6SF2 genotypes, the stage of liver fibrosis,, component scores of NAS (steatosis, lobular inflammation and balloon degeneration), as well as potential confounders including age, gender, BMI, history of diabetes and the use of statin.

³ PNPLA3 GC- and GG genotypes were compared with CC genotype.

⁴ Combined TM6SF2 CT/TT genotypes were compared with CC genotype due to the limited number of patients with TT genotype.

Summary of changes in lipoprotein profiles associated with genotypes, NASH activity and liver fibrosis

Table 5

	VLDL		LDL		HDL	
	Concentration	Size	Concentration	Size	Concentration	Size
PNPLA3 I148M	-	↓↓↑	-	↑ ²	-	↑↑
TM6SF2 E167K	-	-	↓	↑	-	↑↑
Hepatic steatosis	-	↑↑	↑↑	↓↓	-	↓↓

¹. Double arrows represent associations with p value < 0.005.

². Single arrow represents associations with p value between 0.005 – 0.05.