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PNPLA3 gene-by-visceral adipose tissue volume interaction and the pathogenesis of fatty liver disease: The NHLBI Family Heart Study

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Abstract

Background—Fatty liver disease (FLD) is characterized by increased intrahepatic triglyceride content with or without inflammation and is associated with obesity, and features of the metabolic syndrome. Several recent GWAS have reported an association between SNP rs738409 in the *PNPLA3* gene and FLD. Liver attenuation (Hounsfield Units, HU) by computed tomography is a non-invasive measure of liver fat, with lower values of HU indicating higher liver fat content. Clinically, a liver attenuation (LA) value of ≤ 40 HU indicates moderate-to-severe hepatic steatosis.

Objective—We investigated whether missense rs738409 *PNPLA3* interacted with abdominal visceral adipose tissue volume (cm^3) to reduce liver attenuation (i.e. increased liver fat) in 1,019 European American men and 1,238 European American women from the Family Heart Study.

Methods—We used linear regression to test the additive effect of genotype, abdominal visceral adipose tissue (VAT), and their multiplicative interaction on LA adjusted for age, BMI, HDL-cholesterol, insulin resistance, serum triglycerides, abdominal subcutaneous adipose tissue, and alcohol intake.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Results—In men and women combined, the interaction between each copy of the rs738409 variant allele (MAF 0.23) and 100cm^3/150mm slice VAT decreased LA by 2.68±0.35 HU ($p < 0.01$). The interaction of 100cm^3 VAT and the variant allele was associated with a greater decrease in LA in women than men (−4.8±0.6 and −2.2±0.5 HU, respectively).

Conclusions—The interaction between genotype and VAT volume suggest key differences in the role of *PNPLA3* genotype in conjunction with abdominal VAT in liver fat accrual. The stronger association of the PNPLA3 genotype and liver fat in women suggests that women may be more sensitive to liver fat accumulation in the setting of increased visceral fat, compared to men. The presence of the *PNPLA3* variant genotype, particularly in the context of high visceral adipose tissue content may play an important role in FLD.

Keywords

Fatty liver disease; *PNPLA3* locus; abdominal obesity; visceral obesity

INTRODUCTION

Fatty liver disease (FLD) is characterized by increased intrahepatic triglyceride content, with or without inflammation, and is associated with obesity and features of the metabolic syndrome (1–9). The global increase in obesity prevalence has foreshadowed a subsequent rise in associated FLD (5, 9). FLD can lead to liver failure and is accompanied by substantial morbidity and mortality, thus it is a major public health concern.

Several recent genome wide association studies (GWAS) reported an association of the minor (G) allele of rs738409, a non-synonymous SNP in the patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) gene (rs738409: C>G, NP_079501.2:p.I148M), encoding an isoleucine to methionine amino acid change, with fatty liver and alcoholic liver disease (10–13). The association is seen across ethnicities yet is stronger in Hispanic and European Americans than in African Americans and correlates well with differences in the allele frequencies and disparities in the prevalence of FLD across these populations (10, 12). However, disparities in liver density persisted after adjustment for the *PNPLA3* variant, suggesting that other unidentified factors also influence the disparities of risk of FLD across these different ethnic populations (10, 12–14).

The *PNPLA3* variant could influence liver fat content through different mechanisms. Studies in mice illustrate that the variant advances triglyceride accumulation by limiting triglyceride hydrolysis, thereby promoting hepatic steatosis (15). Also, a western-type diet fed to mice stimulated *PNPLA3* expression levels 23-fold, suggesting that PNPLA3 is an important player in the metabolism of liver fat under high lipid exposure (16). Limited studies have explored the epidemiological context of the *PNPLA3* rs738409 effects. One exception is the recent interrogation of the effect of *PNPLA3* by dietary fat interaction on the pathogenesis of liver disease in the IRAS family study, although no statistically significant interaction was noted (12). A second notable exception is the positive association between liver fat and the interaction of the *PNPLA3* variant with a high simple carbohydrate diet in overweight Hispanic youth (17).

Increasing evidence suggests that excess abdominal visceral adipose tissue (VAT) is positively associated with increased liver fat and perhaps the most important factor for the development of hepatic steatosis. Studies in obese and even relatively 'lean' participants with non-alcoholic steatohepatitis have observed higher abdominal obesity in these individuals (18). These observations are independent of total adipose mass, and subcutaneous fat storage appears to be protective (18–20). Moreover, in the participants of the Family Heart study, we have recently demonstrated an important influence of VAT on the distribution of liver attenuation (LA) and the prevalence of FLD (38). The purpose of this study was to investigate whether the effect of the G allele in rs738409 *PNPLA3* interacted with VAT to influence the distribution of LA or the prevalence of FLD.

MATERIALS and METHODS

Study population

The Family Heart Study (FHS) is a multicenter, population-based, family study designed to investigate the determinants of cardiovascular disease (21). Families in the FHS were selected at random (588 families) or ascertained for family history of CHD (656 families) using information collected in the parent studies—Framingham Heart Study (Framingham, MA, USA), the Utah Health Family Tree Study (Salt Lake City, UT, USA) or the Atherosclerosis Risk in Communities Study (Minneapolis Suburbs, MN, USA and Forsyth County, NC, USA). Between 2002 and 2003 about two-thirds of the families (largest families available who also had genome-wide anonymous markers typed by the Mammalian Genotyping Service (MGS) of the FHS) were invited to participate in a follow-up clinical examination that included measurement of the liver and abdomen with cardiac computed tomography (CT) using standardized procedures and quality control methods developed in NHLBI's MESA and CARDIA studies (22). Informed consent was obtained from all participants, and this project was approved by the Institutional Review Boards of all participating institutions.

We began our analysis with 1,080 and 1,335 non-diabetic European American men and women, respectively, with genotype information for SNP rs738409. We selected non-diabetic individuals as a means to focus on associations of VAT and the variant allele without confounding from known risk factors of a diabetic blood profile that are suggested to exacerbate accrual of liver fat (1, 23). Of these individuals, we excluded the following: 24 men and 21 women who were missing information for VAT (cm^3); 3 women and 1 man missing measures of LA; 63 women and 30 men missing information on alcohol consumption; and 2 men and 8 women missing measured fasting glucose, fasting insulin or high density lipoprotein cholesterol (HDL-cholesterol).

Among the remaining 1,021 men and 1,240 women with complete data, we further excluded two women and two men who reported *excessive alcohol* consumption (i.e. > 420g/week for men and >280 g/wk for women) who may have a liver that is impacted by alcoholic liver disease (24). We also ran models that excluded an additional 110 men and 126 women who reported consumption above the *recommended alcohol* level, i.e. > 140 g/week for men and >70 g/week for women (24). The parameter estimates for all explanatory variables except alcohol intake did not differ between models with either alcohol exclusion criteria in the

combined or gender stratified analyses. We chose to keep all men and women who consumed alcohol below the excessive level since alcohol consumption had little influence on other explanatory variables. Thus, our analytical sample included to 1019 men and 1238 women.

Compared to women with a complete set of data for analyses, women missing measures for alcohol intake had lower HDL-cholesterol, 49.9(11.6) versus 55.2(15.0) mg/dl, respectively, (p=0.003). Otherwise, we found no differences in outcome measures of liver fat, main predictors or covariates for men and women missing data as compared to those with a complete set of data

CT Scan Related Phenotypes

Participants completed a CT exam as part of the Family Heart Study using a standardized protocol originally designed for measuring coronary artery and abdominal aortoiliac calcified plaque (22). Technical parameters for the coronary artery calcium scan were 120 KVp, 4x 2.5 mm slices, 35 cm display field of view, prospective ECG gating and mAs comparable across the four CT centers based on CT equipment and participant weight. Technical parameters for the limited helical scan of the abdomen were 120 KVp, 150 mAs, 5 mm slices and 50 cm display field of view. The coronary calcium scan images were used to measure liver attenuation and the limited abdomen scan to measure abdominal fat in the current study. The effective radiation exposure for the average participant of each coronary scan was 1.5 mSv for men and 1.9 mSv for women and for the total CT exam (two coronary scans plus a limited CT of the lower abdomen) was less than 7 mSv. CT images from all study centers were sent electronically to the central CT reading center located at Wake Forest School of Medicine (Winston Salem, NC, USA).

Liver attenuation (LA)—Two cardiac gated images of the thorax were obtained which include the upper third of the liver. LA was measured by placing three round regions of interest (ROI's) in the superior right lobe of the liver. The first scan was used unless the breath-hold depth or other artifact required use of the second scan. The 6 ROI values are then averaged as a mean LA in HU, an estimate of the average lipid content of the liver parenchyma. CT analysts were trained and experienced in placement of the ROI's to avoid vascular structures and common cystic lesions of liver. In a pilot study, we demonstrated high intra-class correlation (>0.95) of ROI's throughout all lobes of the liver with the exceptions of the regions of the *porta hepatis* and caudate lobe. In addition, intra-reader correlation (>0.95) between repeat analyses of the same liver regions were demonstrated. As part of the FHS-SCAN CT protocol all participants were imaged with standardized phantom that contains material that simulates water (HU=0) as well as increasing densities of calcium (50, 100 and 200 mg of calcium). These participant specific calibrations were used to adjust and standardize the LA measurements. More specifically, once we obtained an average of the fat measures in HU, we regressed this average measure on an average of several measures of the phantom standard to account for any variations in the CT calibration. Lower values of LA indicate higher fat content. A LA value of ≤ 40 HU, as found by non-contrast enhanced CT, has been shown to correlate best with a pathologic fat content of ≥ 30%, indicating at least moderate hepatic steatosis (25).

Adiposity Depots in the Abdomen (VAT and SAT)—CT scans of the abdomen were reconstructed into 5 mm slices with the maximum 50 cm field-of-view to include the whole abdomen for body composition. Total and adipose tissues were measured volumetrically from two 5 mm contiguous slices located at the level of the lumbar disk between the 4th and 5th vertebra so as to be comparable to a single 10 mm slice used historically. Tissues with attenuation between −190 to −30 Hounsfield units was define as adipose tissue (26). The Medical Image Processing, Analysis, and Visualization (MIPAV) application (27) was used by experienced analysts to segment the images based on anatomic boundaries (skin, subcutaneous fat-muscle interface and peritoneum) into the entire abdomen, abdominal wall and intra-abdominal compartments (28). In each compartment the total volume and fat volumes were determined allowing calculation of the total abdominal adipose tissue, subcutaneous adipose tissue and visceral adipose tissue contained with the 10 mm slice located at L4-5.

Other covariate phenotypes

Biochemical measures—Total cholesterol and triglyceride levels were measured by a Roche COBAS FARA centrifugal analyzer (Böhringer Mannheim Corp., Indianapolis, IN 46250-0457). High density lipoprotein cholesterol (HDL-C) was determined after precipitation of other lipoprotein fraction by dextran sulfate (29). Fasting serum glucose was measured on a clinicalchemistry slide (EKTACHEM; Eastman Kodak Co, Rochester, NY). Insulin was measured by the coated-tube RIA method distributed by Diagnostic Products Corporation (Los Angeles, CA 90045). In this solid-phase RIA, insulin specific antibody is immobilized on the wall of a polypropylene tube. Radio-labeled antigen and antigen from the sample compete for a limited number of antibody binding sites. The amount of labeled antigen bound to antibody is inversely proportional to the amount of insulin in the sample. We calculated the HOMA index as a measure of insulin resistance (HOMA-IR). It can be calculated simply as the product of fasting insulin (in microunits per milliliter) and fasting glucose (in milligrams per deciliter) divided by 405 (Mathews et al. 1985). Body mass index (BMI) was calculated as weight (in kilograms) divided by the square of height (in meters). Participants self-reported the number of 1.5-oz cocktails, 12-oz glasses (or cans) of beer, and 4-oz glasses of wine they consumed by day and in 1 week. Total alcohol intake in grams per day was computed from the reported intakes of beer, wine, and spirits. All Individuals that reported excessive alcohol consumption (> 1000 grams/day) were excluded because of the unique pathology of alcoholic liver disease.

Genotypes in FHS

Data for the *PNPLA3* rs738409 polymorphism was derived from a genome-wide Illumina array (30). In the FHS cohort, the Illumina genotyping platform (974 subjects were genotyped with HumanHap550v1.1 chip, 249 with Human610-Quadv1 and 1,472 with a Human1M-Duov3 chip). Quality control filters excluded SNPs with call rates < 98%, with HWE p-value < 10-6, or with MAF< 1%. Mendelian errors were evaluated with LOKI (31) and two individuals with an unacceptable number of Mendelian errors were removed. As a final familial quality control check, we used GRR software (32) to check familial relationships based on identity by state (IBS). We additionally assessed data completeness (>95%) and confirmed Hardy-Weinberg equilibrium ($p > 0.05$) for rs738409. We also

considered population stratification but none of the principal components were associated with the outcome.

Statistical Methods

After adjusting for phantom and center, LA residuals were used as the dependent variable in the models. Using linear mixed models (to control for family relatedness), we regressed LA residuals, on VAT and *PNPLA3* (rs738409) genotype. We analyzed models with and without interactions between *PNPLA3* genotypes and VAT. We adjusted models minimally for age, sex, and BMI (Minimal adjusted model). We also ran full models that adjusted for age, sex, and BMI, as well as measures for subcutaneous adipose tissue volume (SAT), alcohol consumption, triglyceride concentration, insulin resistance, and HDL-cholesterol (Full adjusted model). Because women and men vary in the amount of VAT they carry, we considered 3-way interactions for sex, variant allele, and VAT to evaluate any differences between men and women in their response to the combined influence of the allele variant and VAT on liver attenuation. We calculated the 2- and 3-way interactions for our models using 2 or more of the following: sex as a dichotomous variable (men=1, women=0), *PNPLA3* genotype using the continuous imputed value (ranging from 0 to 2), and VAT in cm^3. To provide a more realistic effect estimate for VAT, we multiplied the beta for VAT and all interaction betas that included VAT by 100. All models were adjusted for genotype batch effects. We also considered population stratification but none of the principal components were associated with the outcome.

RESULTS

Descriptive statistics of the study participants and differences in means by gender for each predictor variable are provided in Table 1. Mean LA varied significantly between women and men (unadjusted 61.0 ± 10.5 HU, 57.7 ± 11.0 HU; adjusted 1.5 ±10.1 HU, −0.9 ±10.6 HU). Moderate-to-severe hepatic steatosis, as defined by LA ≤ 40 HU, was found in 5% of women and 8% of men. Men had more VAT but less SAT than women (VAT: 197.7 cm^3 versus 131.4 cm^3; and SAT: 244.2 cm^3 versus 311.7 cm^3, respectively). Women had higher levels of HDL-cholesterol than men, 55.2 mg/dl versus 42.5 mg/dl, respectively. Men had higher levels of triglycerides and HOMA-IR than women (respectively, 152.4 versus 132.3mg/dl and 2.9 versus 2.3). MAF for *PNPLA3* rs738409 was similar in women (0.23) and men (0.22).

Models for the quantitative outcome of LA (HU) are provided in Table 2. Parameter estimates for minimal and fully adjusted models in Table 2 are shown for each copy of the variant rs738409 allele and 100 cm^3 of VAT, as an easy, more realistic estimate of VAT contribution (as opposed to 1 cm^3 VAT). A complete list of parameter estimates for all main independent variables and covariates are provided in Supplement Table 1.

Without the interaction of PNPLA3 genotype and VAT and after accounting for age, sex, and BMI, each copy of the rs738409 variant was associated with a decrease in LA of 4.66±0.34 HU, and each 100cm^3 of VAT was associated with a decrease in LA of 3.74 ±0.34 HU. Adjusting for additional covariates in a full model had little influence on the association of the variant and LA, but resulted in a smaller, −2.04 ±0.35 HU, yet significant

association of each 100cm^3 of VAT with LA. In the minimal model, including the interaction between 100cm^3 of VAT and each copy of the rs738409 variant contributed to a decrease in LA of 7.94±0.66 HU. Furthermore, the contribution of 100cm^3 VAT alone to LA strengthened to −27.93±1.07, while the contribution of the variant rs738409 (−0.61±0.68 HU) on LA decreased thereby having an effect mainly through its interaction with VAT. Once we adjusted for other covariates (full model), the association of LA with the interaction between the rs738409 variant and 100cm^3 of VAT was attenuated but remained significant (−2.68±0.35 HU). Furthermore, the relative size of the associations changed and the variant rs738409 only had an effect through the interaction with VAT, similar to the minimal model.

To illustrate our results, we compared the estimated population marginal means of LA across *PNPLA3* genotypes for men and women with higher volumes of VAT (≥80th percentile) or lower volumes of VAT (≤20th percentile). The individuals with VAT within the ≤ 20th percentile included women with ≤ 60cm^3 VAT and men with ≤ 105cm^3 VAT. The individuals with VAT within the ≥ 80th percentile included women with ≥ 200cm^3 VAT and men with ≥ 290cm^3 VAT. We estimated marginal means using LS means in SAS and compared genotypes using the Tukey-Kramer method.

We illustrated the interaction of VAT and *PNPLA3* genotype on LA (Figure 1) by comparing the estimated population marginal means of LA across *PNPLA3* genotypes for men and women with higher volumes of VAT (≥80th percentile) or lower volumes of VAT (≤20th percentile). At lower VAT volume, there was no significant difference in LA across genotypes. On the other hand, at higher volumes of VAT the presence of each additional variant allele was associated with a significantly lower LA (i.e., higher liver fat) ($p<0.001$).

We further found a 3-way interaction between VAT, rs738409 allele, and sex ($p<0.001$, see Supplement Table 2), indicating a difference between men and women in how the liver responds to the combined influence of the rs738409 allele and VAT content. Therefore, we stratified models by sex to see how the interaction differed between men and women (see Table 3 and Supplement Table 3). The pattern of influence from the interaction of 100cm^3 VAT and each copy of the rs738409 variant allele was similar in men and women, but the associated decrease was stronger in women than men (−4.8 ± 0.6 and −2.2 ± 0.5 HU, respectively). In the interaction model, the independent contribution of 100cm^3 VAT to LA strengthened in both women (−10.65 ± 1.07) and men (−4.50 ± 0.91), while the contribution of the variant rs738409 on LA appeared mainly through its interaction with VAT. In gender stratified models, the interaction results were further demonstrated. Figure 2 illustrates the interaction of VAT and *PNPLA3* genotype on LA stratified by gender. Among men and women with lower VAT volume (≤20th percentile), no significant genotype effect was noted ($p>0.05$). In contrast, in men and women with high levels of VAT (≥80th percentile) a significant genotype was noted.

DISCUSSION

Liver attenuation, as measured from CT scans, is a validated measure of liver fat content and is a noninvasive surrogate of histologically diagnosed FLD or hepatic steatosis (25).

Previous studies have demonstrated independent associations between the *PNPLA3* gene, known to hydrolyze fat, and VAT, with fatty liver (10–13). Given the obvious connection between VAT and the hydrolysis of fat, we were interested in their possible interaction among European American participants of the NHLBI Family Heart Study.

Similar to previous studies, we detected a significant univariate association between VAT and the G variant of rs738409 on LA in men and women. We also detected a novel interaction between the G allele of rs738409 and VAT on LA, illustrating that the presence of the variant genotype in an environment of high VAT can potentially increase the accumulation of liver fat more drastically than in an environment with low VAT. Further, in an environment with low VAT, presence of the G variant of rs738409 had minimal influence on LA. The *PNPLA3* gene codes for a triacylglycerol lipase protein, also called adiponutrin, which mediates triacylglycerol hydrolysis in adipocytes. Thus, there are several proposed mechanisms by which adiponutrin expression may impact the accumulation of fat in the liver. In vitro animal studies have shown that the rs738409 G variant promotes triglyceride accumulation by limiting triglyceride hydrolysis, thereby promoting hepatic steatosis (16). Moreover, recent studies in mice have demonstrated an important effect of the rs738409 polymorphism in the context of a high fat high calorie diet (16). Thus, it is biologically plausible that excess visceral tissue, particularly in the abdominal area, and circulating triglycerides may influence the function of *PNPLA3* protein and the accumulation of fat in the liver.

While none of the metabolic traits such as triglyceride levels, HDL-cholesterol, HOMA-IR, abdominal subcutaneous adipose tissue volume, and BMI can ever be fully isolated, we controlled for these measures as a means to more fully understand the independent influence of *PNPLA3* genotype and VAT content (see specific parameter estimates in Supplement tables 1 and 3). In particular, given the high correlation between VAT and HOMA-IR, we wanted to make sure that HOMA-IR was not confounding the apparent interaction between genotype, VAT and LA. Thus, we also considered an independent interaction of *PNPLA3* genotype with HOMA-IR because *PNPLA3* is regulated by insulin through SREBP1 to stimulate triglyceride lipogenesis (33, 34). In the presence of central obesity and insulin resistance (increased VAT and/or HOMA-IR), *PNPLA3* comes into play to increase hepatic fat and injury in obese individuals with insulin resistance (10, 35). We detected an interaction between *PNPLA3* genotype and HOMA-IR such that the presence of the variant allele ($p<0.001$) decreased LA by −1.12±0.15 HU per unit of HOMA-IR. When we evaluated the interaction between each VAT and HOMA-IR with *PNPLA3* genotype together in the same model we found that both interactions, while attenuated, were still significant: *PNPLA3* variant*VAT and *PNPLA3* variant*HOMA-IR decreased LA by −1.72±0.15 HU per 100 cm^3 VAT and −0.73±0.17 HU per unit HOMA-IR. This appears to illustrate the potential influence of *PNPLA3* genotype on the accumulation of liver fat through both VAT and HOMA-IR, although the effect with VAT was notable stronger.

Triglyceride levels were also inversely associated with LA. We evaluated the interaction between triglycerides and the variant genotype with LA and found a similar association, beta ± SE=−1.54±0.37 per 100mg/dl of triglycerides, as seen between VAT and the variant genotype. Since triglyceride levels are strongly correlated with VAT, associations with

triglyceride level may be reflective of the amount of VAT present. On the other hand, a previous study found that the positive relationship between VAT and triglycerides was present in normal weight men, but not overweight men (36). Thus, the interaction of VAT, as opposed to triglycerides, and *PNPLA3* variant allele may be a better predictor for liver fat.

We hypothesized that the association between fatty liver and *PNPLA3* genotype may be exacerbated by increases in VAT tissue. The ability to expand subcutaneous adipose tissue in response to increased caloric intake may also be important (37), as it possibly draws fatty acids away from the organ tissues to areas below the skin where they are less metabolically active. In this regard, women may have more ability to expand the lower extremity subcutaneous fat. In fact, we found a positive association between LA and SAT (indicative of lower liver fat) in models for men and women combined and ($p=0.02$) in women only ($p=0.02$). In men, there was no association between LA and SAT ($p=0.4$).

Recently, we illustrated a stronger inverse association of VAT with liver fat (quantified as LA) in women compared to men of the FHS (38). In light of findings from this prior study, in the current study we ran a model (Supplement Table 2) that included a 3-way interaction between sex, *PNPLA3* genotype, and VAT. We found a significant three way interaction between sex, VAT, and the *PNPLA3* genotype on liver attenuation. Indeed, models stratified by sex that included the 2-way interaction between *PNPLA3* genotype, and VAT, also suggest a stronger association of VAT and LA in women, that may be further exacerbated by *PNPLA3* genotype. While VAT is associated with poor liver health (lower liver attenuation) in men and women, the association is of a stronger magnitude in women compared to men. This suggests that liver health in women may be more sensitive to the volume of visceral fat that is carried, compared to men.

This study has several important strengths. First, we used a non-invasive and well validated measure of liver attenuation from CT scans with a standardized phantom on a large number of subjects in a population-based study. In addition, these study participants were extensively characterized for multiple metabolic phenotypes of interest, particularly with CT measured adipose tissue volumes as well as parameters of lipid and glucose metabolism. This study was also able to characterize liver attenuation and thus fatty liver disease in a population based family study, early in the course of disease and prior to the onset of symptoms.

The most notable limitation of our study is the possibility of ascertainment bias, as some of the families were recruited because of a familial excess of coronary artery disease. However, the distribution of LA by family ascertainment status (for CHD versus random) was not significantly different in European American families (all $P > 0.46$). In addition, we cannot generalize our findings beyond European descent populations or to younger age groups (i.e. <35 yrs). We are also limited because we only were able to assess abdominal adipose tissue. If increased ability to expand subcutaneous adipose tissue draws fatty acids away from the organ tissues (37), then other measures of subcutaneous adipose tissue, particularly in the buttocks and thigh areas, may have more influence in women than men on the presence of liver fat. The location of sample measures used to predict total VAT volume may be gender-specific (39, 40), thus influencing the stronger inverse association of VAT with LA that we

found in women compared to men. The location of sample measures used to estimate VAT volume may vary by age, race (39), and disease phenotype (41). Given our sample size and the strength of the association, these kinds of differences are more likely to make our results more or less precise rather than change the effect estimate or significance. Nevertheless, these kinds of group differences in patterning of VAT could influence our results. Future studies should interrogate some of these limitations and hypotheses directly.

In conclusion, the presence of high levels of visceral adipose tissue appears to exacerbate the established effect of the rs738409 G variant on LA and FLD. Our study findings need to be replicated in other population-based studies, and further study is needed on the mechanisms by which the *PNPLA3* gene acts in the context of visceral adipose tissue in the pathogenesis of fatty liver disease. Further exploring the influences of interactions between adipose tissue and genotype on liver fat accrual may lead to improved therapeutic strategies for the prevention and treatment of fatty liver disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Kotronen A, Johansson LE, Johansson LM, Roos C, Westerbacka J, Hamsten A, Bergholm R, Arkkila P, Arola J, Kiviluoto T, et al. A common variant in PNPLA3, which encodes adiponutrin, is associated with liver fat content in humans. Diabetologia. 2009; 52:1056–60. [PubMed: 19224197]
2. Kotronen A, Vehkavaara S, Seppala-Lindroos A, Bergholm R, Yki-Jarvinen H. Effect of liver fat on insulin clearance. Am J Physiol Endocrinol Metab. 2007; 293:E1709–15. [PubMed: 17895288]
3. Brunt EM. Pathology of nonalcoholic fatty liver disease. Nat Rev Gastroenterol Hepatol. 2010; 7:195–203. [PubMed: 20195271]
4. Byrne CD. Fatty liver: role of inflammation and fatty acid nutrition. Prostaglandins Leukot Essent Fatty Acids. 2010; 82:265–71. [PubMed: 20189787]
5. Lewis JR, Mohanty SR. Nonalcoholic fatty liver disease: a review and update. Dig Dis Sci. 2010; 55:560–78. [PubMed: 20101463]
6. Marchesini G, Brizi M, Morselli-Labate AM, Bianchi G, Bugianesi E, McCullough AJ, Forlani G, Melchionda N. Association of nonalcoholic fatty liver disease with insulin resistance. Am J Med. 1999; 107:450–5. [PubMed: 10569299]
7. Sanyal AJ. Nonalcoholic fatty liver disease in the Indian subcontinent: a medical consequence of globalization? Indian J Gastroenterol. 2001; 20:215–6. [PubMed: 11817771]
8. Seppala-Lindroos A, Vehkavaara S, Hakkinen AM, Goto T, Westerbacka J, Sovijarvi A, Halavaara J, Yki-Jarvinen H. Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. J Clin Endocrinol Metab. 2002; 87:3023–8. [PubMed: 12107194]
9. Tiniakos DG, Vos MB, Brunt EM. Nonalcoholic fatty liver disease: pathology and pathogenesis. Annu Rev Pathol. 2010; 5:145–71. [PubMed: 20078219]

10. Romeo S, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, Boerwinkle E, Cohen JC, Hobbs HH. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet. 2008; 40:1461–5. [PubMed: 18820647]
11. Stickel F, Buch S, Lau K, zu Schwabedissen HM, Berg T, Ridinger M, Rietschel M, Schafmayer C, Braun F, Hinrichsen H, et al. Genetic variation in the PNPLA3 gene is associated with alcoholic liver injury in caucasians. Hepatology. 2011; 53:86–95. [PubMed: 21254164]
12. Wagenknecht LE, Palmer ND, Bowden DW, Rotter JI, Norris JM, Ziegler J, Chen YD, Haffner S, Scherzinger A, Langefeld CD. Association of PNPLA3 with non-alcoholic fatty liver disease in a minority cohort: the Insulin Resistance Atherosclerosis Family Study. Liver Int. 2011; 31:412–6. [PubMed: 21281435]
13. Speliotes EK, Yerges-Armstrong LM, Wu J, Hernaez R, Kim LJ, Palmer CD, Gudnason V, Eiriksdottir G, Garcia ME, Launer LJ, et al. Genome-wide association analysis identifies variants associated with nonalcoholic Fatty liver disease that have distinct effects on metabolic traits. PLoS Genet. 2011; 7:e1001324. [PubMed: 21423719]
14. Guerrero R, Vega GL, Grundy SM, Browning JD. Ethnic differences in hepatic steatosis: an insulin resistance paradox? Hepatology. 2009; 49:791–801. [PubMed: 19105205]
15. He S, McPhaul C, Li JZ, Garuti R, Kinch L, Grishin NV, Cohen JC, Hobbs HH. A sequence variation (I148M) in PNPLA3 associated with nonalcoholic fatty liver disease disrupts triglyceride hydrolysis. J Biol Chem. 2010; 285:6706–15. [PubMed: 20034933]
16. Hoekstra M, Li Z, Kruijt JK, Van Eck M, Van Berkel TJ, Kuiper J. The expression level of non-alcoholic fatty liver disease-related gene PNPLA3 in hepatocytes is highly influenced by hepatic lipid status. J Hepatol. 2010; 52:244–51. [PubMed: 20015565]
17. Davis JN, Le KA, Walker RW, Vikman S, Spruijt-Metz D, Weigensberg MJ, Allayee H, Goran MI. Increased hepatic fat in overweight Hispanic youth influenced by interaction between genetic variation in PNPLA3 and high dietary carbohydrate and sugar consumption. Am J Clin Nutr. 2010; 92:1522–7. [PubMed: 20962157]
18. Fan JG, Saibara T, Chitturi S, Kim BI, Sung JJ, Chutaputti A. Asia-Pacific Working Party for N. What are the risk factors and settings for non-alcoholic fatty liver disease in Asia-Pacific? J Gastroenterol Hepatol. 2007; 22:794–800. [PubMed: 17498218]
19. Fan JG, Farrell GC. VAT fat is bad for the liver, SAT fat is not! J Gastroenterol Hepatol. 2008; 23:829–32. [PubMed: 18565017]
20. Hamdy O, Porramatikul S, Al-Ozairi E. Metabolic obesity: the paradox between visceral and subcutaneous fat. Curr Diabetes Rev. 2006; 2:367–73. [PubMed: 18220642]
21. Higgins M, Province M, Heiss G, Eckfeldt J, Ellison RC, Folsom AR, Rao DC, Sprafka JM, Williams R. NHLBI Family Heart Study: objectives and design. Am J Epidemiol. 1996; 143:1219–28. [PubMed: 8651220]
22. Carr JJ, Nelson JC, Wong ND, McNitt-Gray M, Arad Y, Jacobs DR Jr, Sidney S, Bild DE, Williams OD, Detrano RC. Calcified coronary artery plaque measurement with cardiac CT in population-based studies: standardized protocol of Multi-Ethnic Study of Atherosclerosis (MESA) and Coronary Artery Risk Development in Young Adults (CARDIA) study. Radiology. 2005; 234:35–43. [PubMed: 15618373]
23. Sookoian S, Castano GO, Burgueno AL, Gianotti TF, Rosselli MS, Pirola CJ. A nonsynonymous gene variant in the adiponutrin gene is associated with nonalcoholic fatty liver disease severity. J Lipid Res. 2009; 50:2111–6. [PubMed: 19738004]
24. Farrell GC, Chitturi S, Lau GK, Sollano JD. Asia-Pacific Working Party on N. Guidelines for the assessment and management of non-alcoholic fatty liver disease in the Asia-Pacific region: executive summary. J Gastroenterol Hepatol. 2007; 22:775–7. [PubMed: 17565629]
25. Kodama Y, Ng CS, Wu TT, Ayers GD, Curley SA, Abdalla EK, Vauthey JN, Charnsangavej C. Comparison of CT methods for determining the fat content of the liver. AJR Am J Roentgenol. 2007; 188:1307–12. [PubMed: 17449775]
26. Yoshizumi T, Nakamura T, Yamane M, Islam AH, Menju M, Yamasaki K, Arai T, Kotani K, Funahashi T, Yamashita S, et al. Abdominal fat: standardized technique for measurement at CT. Radiology. 1999; 211:283–6. [PubMed: 10189485]

27. Center for Information Technology, C. Health, N.I.o. MIPAV (Medical Image Processing, Analysis, and Visualization). Vol. 2011. National Institutes of Health; Bethesda: 2007.
28. Wheeler GL, Shi R, Beck SR, Langefeld CD, Lenchik L, Wagenknecht LE, Freedman BI, Rich SS, Bowden DW, Chen MY, et al. Pericardial and visceral adipose tissues measured volumetrically with computed tomography are highly associated in type 2 diabetic families. Invest Radiol. 2005; 40:97–101. [PubMed: 15654254]
29. Warnick GR, Mayfield C, Benderson J, Chen JS, Albers JJ. HDL cholesterol quantitation by phosphotungstate-Mg2+ and by dextran sulfate-Mn2+-polyethylene glycol precipitation, both with enzymic cholesterol assay compared with the lipid research method. Am J Clin Pathol. 1982; 78:718–23. [PubMed: 6182791]
30. Kraja AT, Hunt SC, Rao DC, Davila-Roman VG, Arnett DK, Province MA. Genetics of hypertension and cardiovascular disease and their interconnected pathways: lessons from large studies. Curr Hypertens Rep. 13:46–54. [PubMed: 21128019]
31. Heath SC. Markov chain Monte Carlo segregation and linkage analysis for oligogenic models. Am J Hum Genet. 1997; 61:748–60. [PubMed: 9326339]
32. Abecasis GR, Cherny SS, Cookson WO, Cardon LR. GRR: graphical representation of relationship errors. Bioinformatics. 2001; 17:742–3. [PubMed: 11524377]
33. Huang Y, He S, Li JZ, Seo YK, Osborne TF, Cohen JC, Hobbs HH. A feed-forward loop amplifies nutritional regulation of PNPLA3. Proc Natl Acad Sci U S A. 2010; 107:7892–7. [PubMed: 20385813]
34. Larter CZ, Chitturi S, Heydet D, Farrell GC. A fresh look at NASH pathogenesis. Part 1: the metabolic movers. J Gastroenterol Hepatol. 25:672–90. [PubMed: 20492324]
35. Farrell GC. PNPLeAse get the fats right: Does lipogenesis or lipolysis cause NASH? Hepatology. 2011; 52:819–821.
36. Gan SK, Kriketos AD, Poynten AM, Furler SM, Thompson CH, Kraegen EW, Campbell LV, Chisholm DJ. Insulin action, regional fat, and myocyte lipid: altered relationships with increased adiposity. Obes Res. 2003; 11:1295–305. [PubMed: 14627749]
37. Kim JY, van de Wall E, Laplante M, Azzara A, Trujillo ME, Hofmann SM, Schraw T, Durand JL, Li H, Li G, et al. Obesity-associated improvements in metabolic profile through expansion of adipose tissue. J Clin Invest. 2007; 117:2621–37. [PubMed: 17717599]
38. North KE, Graff M, Franceschini N, Reiner AP, Feitosa MF, Carr JJ, Gordon-Larsen P, Wojczynski MK, Borecki IB. Sex and race differences in the prevalence of fatty liver disease as measured by computed tomography liver attenuation in European American and African American participants of the NHLBI family heart study. Eur J Gastroenterol Hepatol. 24:9–16. [PubMed: 21900826]
39. Demerath EW, Sun SS, Rogers N, Lee M, Reed D, Choh AC, Couch W, Czerwinski SA, Chumlea WC, Siervogel RM, et al. Anatomical patterning of visceral adipose tissue: race, sex, and age variation. Obesity (Silver Spring). 2007; 15:2984–93. [PubMed: 18198307]
40. Shen W, Punyanitya M, Wang Z, Gallagher D, St-Onge MP, Albu J, Heymsfield SB, Heshka S. Visceral adipose tissue: relations between single-slice areas and total volume. Am J Clin Nutr. 2004; 80:271–8. [PubMed: 15277145]
41. Demerath EW, Reed D, Rogers N, Sun SS, Lee M, Choh AC, Couch W, Czerwinski SA, Chumlea WC, Siervogel RM, et al. Visceral adiposity and its anatomical distribution as predictors of the metabolic syndrome and cardiometabolic risk factor levels. Am J Clin Nutr. 2008; 88:1263–71. [PubMed: 18996861]

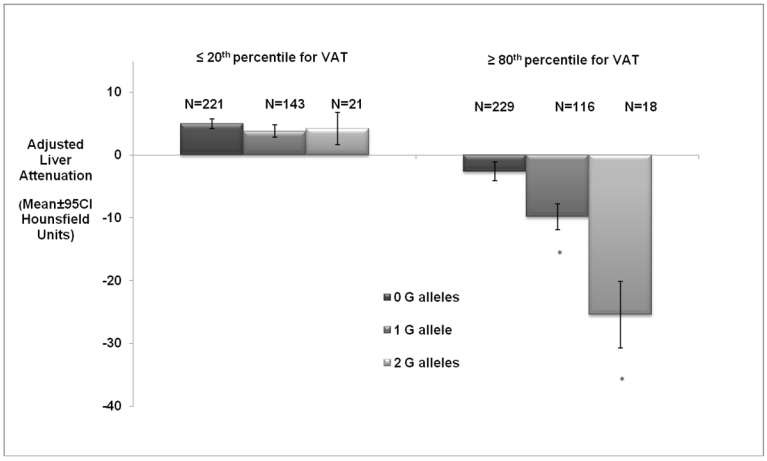

Figure 1.

Estimated Liver Attenuation (mean with 95% CIs) across the Number of *PNPLA3* (rs738409) G alleles for European American Men and Women, together, with ≤ 20^{th} percentile or ≥ 80^{th} percentile for Volume (cm^3) of Abdominal Visceral Adipose Tissue (VAT)[1]

[1] ≤ 20^{th} percentile VAT volume was equivalent to $105cm^3$ for men and $60cm^3$ for women; ≥ 80^{th} percentile VAT volume was equivalent to $290cm^3$ for men and $200cm^3$ for women.

*$p<0.05$ for decrease in liver attenuation for men and women with 0 versus 1, 0 versus 2, or 1 versus 2 *PNPLA3* (rs738409) G alleles.

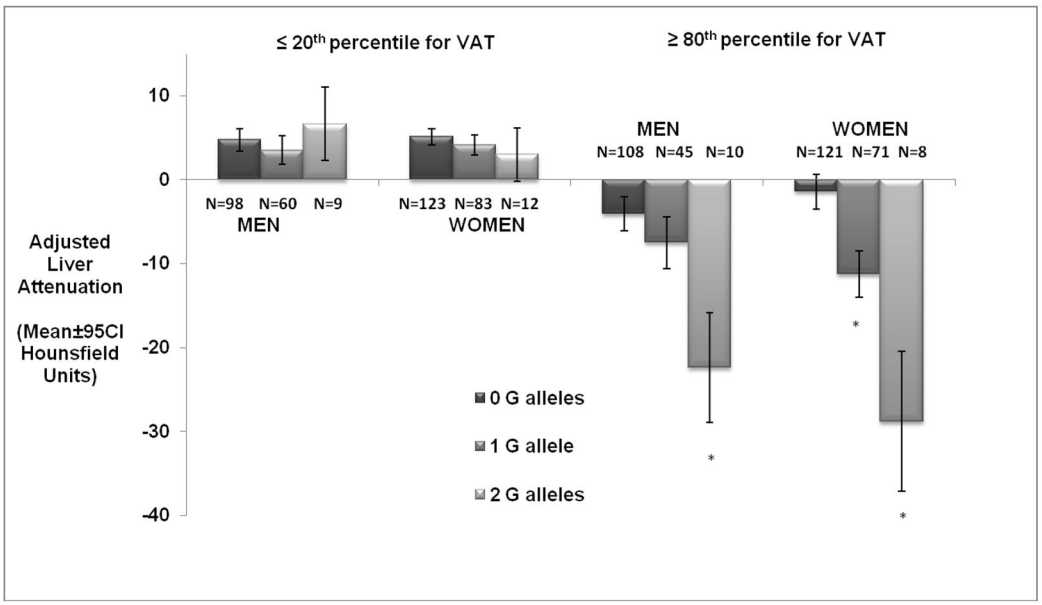

Figure 2.

Estimated Liver Attenuation (mean with 95% CIs) across the Number of *PNPLA3* (rs738409) G alleles for European American Men and Women, separately, with ≤ 20th percentile or ≥ 80th percentile for Volume (cm^3) of Abdominal Visceral Adipose Tissue (VAT)[1]

[1] ≤ 20th percentile VAT volume was equivalent to 105cm^3 for men and 60cm^3 for women; ≥ 80th percentile VAT volume was equivalent to 290cm^3 for men and 200cm^3 for women.

*$p<0.05$ for decrease in liver attenuation for men and women with 0 versus 2 or 1 versus 2 *PNPLA3* (rs738409) G alleles. † $p<0.05$ for decrease in liver attenuation for women with 0 versus 1 *PNPLA3* (rs738409) G alleles.

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Table 1

Descriptive Statistics of Non-diabetic European American Women and Men in the Family Heart Study cohort.

	All Men and Women, n=2257	Women, n=1238	Men, n=1019	Differences between men and women
	Mean (SD) or %	Mean (SD) or %	Mean (SD) or %	P-value
Liver attenuation, unadjusted (HU)	59.5 (10.9)	61.0 (10.5)	57.7 (11.0)	0.001
Liver attenuation[1] (HU)	0.38 (10.4)	1.5 (10.1)	−0.9 (10.6)	<0.0001
Liver attenuation below 40 HU[1] (% yes)	6.7	5.5	8.1	0.02
Age (yrs)	56.5 (13.3)	57.1 (13.1)	55.8 (13.5)	0.02
Alcohol (grams/wk)	31.6 (61.0)	19.2 (39.4)	46.4 (76.9)	0.0001
Drinks alcohol (% yes)	51.5	49.3	54.1	0.02
Body Mass Index (kg/m^2)	28.5 (5.4)	28.0 (6.0)	29.0 (4.6)	<0.0001
VAT[2] (cm^3)	161.4 (88.5)	131.4 (72.8)	197.7 (92.2)	<0.0001
SAT[3] (cm^3)	281.3 (130.8)	311.7 (140.3)	244.2 (106.5)	<0.0001
Fasting glucose (mg/dl)	96.1 (14.3)	93.7 (14.6)	99.0 (13.6)	<0.0001
Fasting insulin (mU/L)	10.2 (7.7)	9.4 (6.8)	11.2 (8.6)	<0.0001
HOMA-IR[4]	2.5 (2.3)	2.3 (1.9)	2.9 (2.6)	<0.0001
Triglycerides (mg/dl)	141.7 (93.1)	132.3 (79.5)	152.4 (104.2)	<0.0001
HDL-Cholesterol (mg/dl)	49.5 (14.6)	55.2 (15.0)	42.5 (10.6)	<0.0001
***PNPLA3* SNPrs738409 MAF (N)**	0.22 (1015)	0.23 (563)	0.22 (452)	0.8
1 variant allele %(N)	34.9 (787)	35.6 (441)	33.9 (346)	
2 variant alleles %(N)	5.1 (114)	4.9 (61)	5.2 (53)	

HU=Hounsfield Units,

[1]Adjusted for center and phantom

[2]VAT=abdominal visceral adipose tissue

[3]SAT=abdominal subcutaneous adipose tissue

[4]HOMA-IR (homeostasis model of insulin resistance)=[fasting serum glucose (mg/dl)*fasting serum insulin(mU/L)/405]

Table 2

Parameter Estimates for the Presence of each *PNPLA3* (SNP rs738409) allele, per 100cm^3 Abdominal Visceral Adipose Tissue (VAT) and their Interaction on Quantitative LA (HU) in European American Men and Women (Minimal and Full Adjusted Models).

	Minimal adjusted model[1] (N=2257)		**Fully adjusted model[2] (N=2257)**	
	Parameter Estimate (SE)		**Parameter Estimate (SE)**	
Independent variable	**No interaction**	**With interaction**	**No interaction**	**With interaction**
100cm^3 VAT	−3.74 (0.34)**	−27.93 (1.07)**	−2.04 (0.35)**	−6.17 (0.64)**
PNPLA3 SNP rs738409 variant allele	−4.66 (0.34)**	−0.61 (0.68)	−4.51 (0.32)**	−0.20 (0.65)
100cm^3 VAT × *PNPLA3 SNP rs738409* variant allele	--	−7.64 (0.66)**	--	−2.68 (0.35)**

LA=liver attenuation, HU=Hounsfield Units, VAT= abdominal visceral adipose tissue;

*p<0.05;

**p<0.001

[1]Models were adjusted for sex, age, and BMI. LA residual outcomes were adjusted for center and phantom.

[2]Models were adjusted for sex, age, BMI, alcohol intake, HDL-cholesterol, triglycerides, insulin resistance (HOMA-IR), and abdominal subcutaneous adipose tissue (see covariate effect estimates in Supplement Table 1). LA residual outcomes were adjusted for center and phantom.

Table 3

Parameter Estimates for *PNPLA3* Genotype (SNP rs738409), Abdominal Visceral Adipose Tissue Volume (cm^3) and their Interaction on Quantitative LA (HU) in European American Men and Women.

	WOMEN N=1238		MEN N=1019	
	Parameter Estimate[1] (SE)		Parameter Estimate[1] (SE)	
Independent variable	**No interaction**	**With interaction**	**No interaction**	**With interaction**
100 cm^3 VAT	−3.15 (0.58)**	−10.65 (1.07)**	−1.3 (0.45)*	−4.5 (0. 91)**
PNPLA3 SNP rs738409 variant allele	−4.50 (0.42)**	1.65 (0.85)	−4.54 (0.49)**	−0.36 (1.14)
100 cm^3 VAT × *PNPLA3 SNP rs738409* variant allele	--	−4.8 (0.58)**	--	−2.21 (0.51)**

LA=liver attenuation, HU=Hounsfield Units, VAT=abdominal visceral adipose tissue;

*p<0.05;

**p<0.001

HOMA-IR (homeostasis model of insulin resistance)= [fasting serum glucose (mg/L) * fasting serum insulin (mU/L)/405]

[1]Models were adjusted for sex, age, and BMI, alcohol intake, HDL-cholesterol, triglycerides, insulin resistance (HOMA-IR), and abdominal subcutaneous adipose tissue (see covariate effect estimates in Supplement Table 3). LA residual outcomes were adjusted for center and phantom.

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