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ASSOCIATION OF *PNPLA3* SNP RS738409 WITH LIVER DENSITY IN AFRICAN AMERICANS WITH TYPE 2 DIABETES MELLITUS

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Abstract

Aim—Non-alcoholic fatty liver disease (NAFLD) is commonly diagnosed in patients with obesity and type 2 diabetes mellitus (T2DM), and has been associated with the single nucleotide polymorphism (SNP) rs738409 in the *PNPLA3* gene. This association remains to be investigated in African Americans with T2DM, a group at lower risk for hepatic steatosis relative to European Americans with T2DM.

Methods—We examined 422 African Americans with T2DM (40.3% male; age: 56.4 ± 9.6 years; BMI: 35.2 ± 8.2 kg/m^2), all with measures of liver density reflecting hepatic fat content on abdominal computed tomography, and blood glucose and lipid profiles. Associations between rs738409 and phenotypes of interest were determined using SOLAR, assuming an additive model of inheritance with covariates age, sex, BMI and use of lipid-lowering medications.

Results—Mean ± SD liver density was 55.4 ± 10.2 Hounsfield Units. SNP rs738409 in *PNPLA3* was significantly associated with liver density (P=0.0075) and hepatic steatosis (P=0.0350), but not with blood glucose, HbA_{1c}, total cholesterol, triglycerides, high-density or low-density lipoprotein levels or liver function tests (P=0.15–0.96).

Conclusion—These findings provide evidence that the *PNPLA3* SNP rs738409 contributes to risk for increased liver fat content in African Americans with T2DM, an effect that appears to be independent from serum lipids. Although African Americans are less susceptible to fatty liver than European Americans, *PNPLA3* appears to be a risk locus for hepatic steatosis in diabetic African Americans.

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Keywords

type 2 diabetes; genetics; fatty liver disease; African American

Introduction

The rates of both obesity and type 2 diabetes mellitus (T2DM) have dramatically increased over the past two decades. Increased liver fat infiltration in the form of non-alcoholic fatty liver disease (NAFLD) is commonly seen in patients with T2DM and obesity, with prevalence as high as 75% in subjects with diabetes [1]. Potential complications of NAFLD include liver cirrhosis with possible need for liver transplantation. As such, understanding risk for NAFLD is important, particularly in individuals with T2DM. The patatin-like phospholipase domain containing 3 gene (*PNPLA3*) which functions as a triacylglycerol lipase, is of interest in this context [2]. The non-synonomous SNP rs738409 in the third exon of *PNPLA3* (resulting in an isoleucine to methionine amino acid substitution) has been associated with liver fat content in a range of ethnic groups, including Hispanics, African Americans and European-ancestry populations [3–6].

PNPLA3 expression has been shown to be increased in obese relative to lean individuals [7], with expression levels associated with liver fat content among those with obesity [8]. However, whether expression of the gene also differs in T2DM patients is not clear. It is also unknown whether the association between rs738409 and liver fat content exists in African Americans with overt T2DM, where blood lipid profiles are known to differ from population-based references ranges. *PNPLA3* SNP rs738409 was found to be associated with liver fat content, but not body mass index (BMI) or visceral fat area, in a single report evaluating Europeans with diabetes [9]. This suggests that the risk of NAFLD associated with rs738409 may be independent of underlying metabolic disturbance [9]. Similarly, studies involving Europeans [10] and Hispanics/African Americans [5] with varying degrees of insulin resistance (but not overt T2DM) also report the association of rs738409 with liver fat content to be independent of insulin resistance. The aim of the current study was to examine the association of the *PNPLA3* SNP rs738409 with liver fat content in African Americans with T2DM enrolled in the African American-Diabetes Heart Study (AA-DHS).

Methods

Subject ascertainment and study designs for the AA-DHS have been described previously [11]. All protocols were approved by the Institutional Review Board of Wake Forest University School of Medicine, and all participants provided written informed consent prior to participation. Individuals with serious health conditions including known liver disease and renal insufficiency were excluded from participation. All African Americans with T2DM and measures of liver density (n=442) were evaluated.

Liver density on computed tomography (CT) is an accurate indirect measure of hepatic fat infiltration, lower density scores reflect increased fat infiltration [12]. Liver density was recorded in Hounsfield Units (HU) from abdominal CT scans imaging infra-renal aorta calcified atherosclerotic plaque, as described previously [5]. Hepatic steatosis was defined as liver density <40 HU [12]. Fasting serum glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglyceride concentrations, alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) were measured at Laboratory Corporation of America (Burlington, NC; methodologies at www.labcorp.com).

DNA was extracted from whole blood using a PUREGENE DNA isolation kit (Gentra, Inc.; Minneapolis, MN). Genotyping for the *PNPLA3* SNP rs738409 was completed using the MassARRAY SNP Genotyping System (Sequenom Inc.; San Diego, CA) with SNP-specific primers for PCR amplification and extension reactions designed using the MassARRAY Assay Design Software (Sequenom Inc. San Francisco, CA).

Descriptive statistics are expressed as mean ± standard deviation (SD). Liver density was transformed using the square transformation to best approximate normality. Association analysis was undertaken using SOLAR (Southwest Foundation for Biomedical Research; San Antonio, TX) to account for any family structure. In keeping with prior reports, an additive model of inheritance was assumed [5,6,10]. Age, sex, BMI and use of lipid lowering medications were included as covariates in the models. Statistical significance was accepted at $P<0.05$.

Results

The sample of 442 African Americans were 40.3% male, with mean age 56.4 ± 9.6 years; BMI 35.2 ± 8.2 kg/m^2; and diabetes duration 10.7 ± 8.2 years; 49.5% were using lipid lowering medications. Mean liver density was 55.4 ± 10.2 HU, with 7.2% of the sample considered to have liver density scores suggestive of hepatic steatosis. Fasting blood glucose was 8.65 ± 3.95 mmol.L^{-1}; HbA_{1c} 8.2 ± 2.1% (66 ± 1 mmol.mol^{-1}) ; total cholesterol 4.76 ± 1.22 mmol.L^{-1}; HDL 1.24 ± 0.36 mmol.L^{-1}; LDL 2.85 ± 1.01 mmol.L^{-1}; triglycerides 1.51 ± 1.58 mmol.L^{-1}; ALT 25.5 ± 17.9 IU.L^{-1}; AST 25.0 ± 19.8 IU.L^{-1}; ALP 88.8 ± 78.5 IU.L^{-1}. For rs738409, the minor (G) allele frequency (MAF) was 0.137 and the genotype frequencies were CC 0.742; CG 0.273; and GG 0.015. Genotype frequencies were consistent with Hardy-Weinberg proportions (P=0.65). Under an additive model of inheritance, rs738409 was significantly associated with liver density (P=0.0075) and hepatic steatosis (P=0.0350), but not with blood glucose, HbA_{1c}, total cholesterol, HDL, LDL, triglyceride concentrations, ALT, AST or ALP (Table 1). The association of rs738409 with liver density was also evident under dominant (P=0.02) and recessive (P=0.05) models of inheritance.

Discussion

Findings from this study confirm an association of the *PNPLA3* SNP rs738409 with liver fat content in African Americans with overt T2DM. In keeping with prior studies examining the relationship between rs738409 and hepatic steatosis, we found the minor allele to confer risk for increased fat deposition in the liver [4,8,10]. The frequency of the minor (G) allele was lower in this sample of African Americans from the southeastern USA relative to those reported in European (~0.30) [8,9] and Hispanic American cohorts (~0.40) [4,5], but is consistent with investigations in non-diabetic African Americans (~0.15) [4,5]. Observed differences in the MAF for rs738409 between different racial groups are in keeping with differences in documented risk for hepatic steatosis; African Americans are known to experiences a lower incidence [13,14] and less progressive NAFLD [15] than individuals of European ancestry. Associations of rs738409 with measures of liver function have been reported less consistently [2,4,10], and we did not observe association with ALT, AST or ALP in this sample of African Americans with T2DM.

Although other studies have examined rs738409 and risk of NAFLD in African Americans, few have evaluated cohorts limited to those with type 2 diabetes. While we report that the association between rs738409 and liver density in African Americans with overt T2DM is maintained, we acknowledge that this CT-derived phenotype is a surrogate measure and does not directly assess incident NAFLD. Further, given the low absolute number of minor allele homozygotes in this sample, these findings require replication in larger independent

diabetic populations. It is likely that factors in addition to rs738409 also influence progression from increased liver fat deposition to NAFLD in African Americans with T2DM; these have yet to be identified. The rs738409 association with hepatic fat deposition does not appear to be related to high blood glucose or lipids, suggesting that other pathways contribute to liver fat accumulation. The possibility that the association between rs738409 and liver fat content in African Americans with T2DM is independent of other metabolic disturbances, as proposed by investigations in European-ancestry groups, requires further evaluation. In conclusion, this report provides evidence that the *PNPLA3* SNP rs738409 contributes to risk for increased liver fat content in African Americans with T2DM, an effect independent from blood lipids and blood glucose. Further work is required to clarify whether this association is independent from other underlying metabolic disturbance in African Americans with T2DM.

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Table 1

Association analysis results for *PNPLA3* SNP rs738409 assuming an additive model of inheritance with covariates: age, sex, BMI, lipid-lowering medications.

	Mean ± SD or % (n)			Association Results	
Phenotype	**CC**	**CG**	**GG**	***P*-value**	**β ± SE**
Liver density (HU)	56.0 ± 9.7 (327)	54.5 ± 9.8(108)	44.6 ± 23.4(7)	**0.0075**	−265.8 ± 99.0
Hepatic steatosis (%)	6.1% (20)	9.3% (10)	28.6% (2)	**0.0350**	−0.378 ± 0.177
Fasting blood glucose (mmol.L^{-1})	8.82 ± 4.05 (327)	8.27 ± 3.61(108)	6.84 ± 3.93(7)	0.1526	−10.45 ± 6.91
HbA_{1c} (%)	8.35 ± 2.14(324)	8.33 ± 2.21(108)	8.21 ± 1.76 (7)	0.9394	0.016 ± 0.211
Total cholesterol (mmol.L^{-1})	4.72 ± 1.23(325)	4.88 ± 1.21(108)	4.33 ± 0.93 (7)	0.3904	0.020 ± 0.023
HDL cholesterol (mmol.L^{-1})	1.24 ± 0.36(325)	1.23 ± 0.32(108)	1.37 ± 0.56(7)	0.9239	−0.009 ± 0.090
LDL cholesterol (mmol.L^{-1})	2.81 ± 1.02(317)	3.00 ± 0.97(106)	2.55 ± 0.70(7)	0.2077	4.29 ± 3.41
Triglycerides (mmol.L^{-1})	1.52 ± 1.41(325)	1.52 ± 2.05(108)	0.91 ± 0.46(7)	0.4200	−0.043 ± 0.048
AST (IU.L^{-1})	25.7 ± 19.0 (326)	24.7 ± 14.6 (106)	26.9 ± 15.3(7)	0.9648	−0.002 ± 0.042
ALT (IU.L^{-1})	25.3 ± 21.5(324)	24.3 ± 14.0 (106)	23.1 ± 7.8(7)	0.6230	0.025 ± 0.050
ALP (IU.L^{-1})	89.9 88.2 (326)	85.0 39.4 (106)	90.4 21.8(7)	0.7994	−0.010 ± 0.041