Nutrition, Metabolism & Cardiovascular Diseases (2019) **29**, 965–971

Available online at www.sciencedirect.com

Nutrition, Metabolism & Cardiovascular Diseases

journal homepage: www.elsevier.com/locate/nmcd

PNPLA3 gene polymorphism in Brazilian patients with type 2 diabetes: A prognostic marker beyond liver disease?

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Received 25 February 2019; received in revised form 6 June 2019; accepted 6 June 2019
Handling Editor: A. Siani
Available online 14 June 2019

KEYWORDS
Nonalcoholic fatty liver disease;
PNPLA3 protein;
Transient elastography;
Type 2 diabetes

Abstract *Background & aims:* Genetic factors may impact nonalcoholic fatty liver disease (NAFLD) severity. We aimed to assess the prevalence of patatin-like phospholipase domain-containing 3 protein (*PNPLA3*) gene rs738409 C > G polymorphism in Brazilian individuals with type 2 diabetes and to investigate its association with liver disease severity, diabetic chronic degenerative complications, and metabolic control.
Methods and Results: PNPLA3 genotyping was performed and classified as CC, CG, and GG. Clinical and laboratory data were obtained, including chronic degenerative diabetes complications. Liver stiffness and steatosis were evaluated by transient hepatic elastography with CAP using FibroScan®. Multiple logistic regression was performed to investigate the association of *PNPLA3* G allele with clinical and laboratory variables and with hepatic fibrosis/steatosis. Three hundred three patients were included (118 male, mean age 59 ± 9.5 years). The G allele frequency was 32.5% (CC 47%, CG 41%, and GG 12%). Significant liver fibrosis and severe steatosis were diagnosed in 26% and 43% of patients, respectively. The variables independently associated with the G allele were coronary artery disease (OR: 2.25; 95% CI: 1.03–4.88; $p = 0.04$), better glycemic control (OR for having an $HbA_{1c} \geq 8\%$ [64 mmol/mol]: 0.53; 95% CI: 0.31–0.89; $p = 0.01$), and significant liver fibrosis (OR: 1.82; 95% CI: 1.04–3.17; $p = 0.03$).
Conclusion: In individuals with diabetes and NAFLD, *PNPLA3* gene rs738409 C > G polymorphism is a marker for the risk of significant liver fibrosis and cardiovascular disease and may be associated with better glycemic control.

Introduction

Type 2 diabetes is a major and recognized risk factor for cardiovascular disease and liver-related morbidity and mortality in prospective studies [1,2]. Several studies have shown that patients with type 2 diabetes have increased risk for nonalcoholic fatty liver disease (NAFLD) and its complications [3,4]. Diverse pathogenic mechanisms contribute to the development of both NAFLD and type 2 diabetes [5]. In this complex setting, it is possible that genetic factors interact with environmental and metabolic derangements to accelerate NAFLD progression in the presence of type 2 diabetes.

In a genome-wide association study, the most validated locus influencing NAFLD risk is the patatin-like

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https://doi.org/10.1016/j.numecd.2019.06.002
0939-4753/

phospholipase domain-containing 3 protein (*PNPLA3*) gene [6]. *PNPLA3* rs738409 C > G induces an adiponutrin protein variant (isoleucine-to-methionine substitution) with reduced activity on triglyceride hydrolysis, predisposing to an increase in liver fat content and progressive fibrosis without significant impact on glucose metabolism [7–11].

Few studies have investigated the prevalence of *PNPLA3* rs738409 C > G variant in subjects with type 2 diabetes [8,12–14] and described that the frequency of the G allele was not different from those reported in previous studies in the general population [6,14,15]. Thus far, only Petit et al. evaluated the association of *PNPLA3* rs738409 variant with NAFLD severity in individuals with diabetes and showed that liver fat content and moderate to severe liver fibrosis were significantly more frequent in carriers of the minor G allele; however, the association of this variant with chronic diabetes complications was not evaluated [7,11].

Therefore, the present study aimed to assess the prevalence of *PNPLA3* rs738409 C > G polymorphism in individuals with type 2 diabetes and NAFLD and investigate its association with liver steatosis and fibrosis, presence of diabetic chronic degenerative complications, and metabolic control.

Methods

This is a cross-sectional study nested within the Rio de Janeiro Type 2 Diabetes Cohort Study; details regarding this particular subgroup cohort have been previously reported [3,16]. Briefly, all included individuals were adults from the metropolitan area of the city of Rio de Janeiro in Southeast Brazil, with the diagnosis of type 2 diabetes up to 80 years old with either microvascular or macrovascular complication or with at least two other modifiable cardiovascular risk factors. Exclusion criteria for the entire cohort were BMI ≥40 kg/m^2, estimated glomerular filtration rate ≤30 ml/min/1.73 m^2 calculated using the CKD-EPI equation, and concomitant life-threatening diseases. Coronary heart disease was diagnosed using clinical and electrocardiographic criteria or by positive ischemic stress tests and cerebrovascular disease was diagnosed by history and physical examination. For the diagnosis of nephropathy, two albuminuria measurements of at least 30 mg/24 h or a confirmed reduction in the glomerular function rate (eGFR ≤60 ml/min/1.73 m^2 or serum creatinine >130 μmol/l) were required. Peripheral neuropathy was determined by clinical examination (ankle reflexes, light touch, vibration, pinprick, and temperature sensations), and neuropathic symptoms were assessed using a standard validated questionnaire [17]. For this study, exclusion criteria were current daily alcohol ingestion >30 g/day for men or >20 g/day for women, other etiologies of chronic liver disease, use of hepatotoxic drugs, aminotransferases higher than five times the upper limit of normality, cholestasis, ascites, and liver congestion due to their impact on the accuracy of transient hepatic elastography (THE). The local ethics committee approved the study protocol, and each participant signed a written informed consent.

All participants underwent a standard baseline protocol, which included clinical examination, laboratory evaluation, *PNPLA3* genotyping, and THE examination. Laboratory evaluations including fasting plasma glucose, glycated hemoglobin (HbA_{1c}), serum triglyceride, total cholesterol, HDL- and LDL-cholesterol, aminotransferases, and gammaglutamyl transferase were performed two to four times a year during follow-up [16]. The mean values of HbA_{1c}, lipids, and liver enzymes were obtained during the year in which THE was performed. The mean value of HbA_{1c} at the first year of follow-up was also assessed. An HbA_{1c} cutoff of 8% (64 mmol/mol) was chosen as a value for satisfactory glycemic control because this cohort comprised mainly elderly patients with high prevalence of degenerative complications [18].

Transient hepatic elastography

Liver stiffness evaluation and CAP measurement by THE were performed with FibroScan 502 equipment (Echosens, Paris, France). All measurements were acquired with either the 3.5 MHz standard M probe or the 2.5 MHz standard XL probe, whenever there were unsuccessful measurements with the M probe. THE was performed by two experienced operators blinded to patients' data. Measurements were performed on the right hepatic lobe through the intercostal spaces, with the patient lying in dorsal decubitus with the right arm in maximal abduction after at least 2 h fasting. Only procedures with at least ten valid measurements, a success rate of at least 60%, and an interquartile range (IQR)/median value of liver stiffness ≤ 0.3 for both THE and CAP were considered reliable. Significant liver fibrosis was defined when median value of liver stiffness was ≥7.9 kPa with M probe or ≥7.2 kPa with XL probe [19–21]. A CAP cutoff >296 dB/m was used to define severe liver steatosis for both probes [21].

Genetic analysis

Genomic DNA was extracted from EDTA whole-blood samples following standard procedures according to the manufacturer's directions (QIAamp DNA Mini Kit; Qiagen, Hilden, Germany) and stored at a temperature of −70 °C. Genotyping was performed by real-time polymerase chain reaction using allele-specific TaqMan® probes (Applied Biosystems, Foster City, USA) and CFX96 optical reaction module (Bio-Rad, Hercules, USA). Initial denaturation was performed at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 15 s and annealing/extension at 60 °C for 1 min. For control purposes, samples with previously known genotypes were used for each batch of reactions: a homozygous sample was used for the wild-type allele for the positive control, while a homozygous sample was used for the risk allele for the negative control. In the end, *PNPLA3* rs738409 polymorphism was expressed as CC, CG, and GG genotypes and distribution of their frequencies was according to Hardy–Weinberg equilibrium ($p = 0.29$; www.snpstats.net) in the studied sample.

Statistical analysis

Statistical analyses were performed with SPSS software 21.0 (Chicago, USA). For descriptive analyses, continuous variables were expressed as mean (standard deviation) for normally distributed data, medians (interquartile) for asymmetrically distributed data, or absolute numbers (proportions) for categorical data. Differences between wild-type homozygous (genotype CC) and G allele carriers (genotypes CG and GG) were examined by the unpaired *t*-test and Mann–Whitney test for continuous variables and nonparametric variables, respectively, and by the chi-square test or Fisher exact test for categorical variables. Comparisons among the genotypes were performed by ANOVA or Kruskal–Wallis test.

Multiple logistic regressions were performed to investigate the independent correlates of the presence of the *PNPLA3* G allele. The candidate variables to enter the logistic modeling, based on biological plausibility, were the following: age, gender, body mass index (BMI), waist circumference, smoking status, diabetes duration, presence of hypertension, presence of each micro- and macrovascular complications, mean HbA_{1c}, serum lipids, aminotransferases, and gamma glutamyl transferase levels, liver stiffness, and CAP parameters. A backward procedure was used to select the independently associated covariates and a p-value <0.10 was the criterion to remain into the models. Separate logistic models were fitted using HbA1c and liver fibrosis as continuous and categorical variables ($<8\%$ *vs.* $\geq 8\%$ (64 mmol/mol); ≥ 7.9 kPa (with M probe) or ≥ 7.2 kPa (with XL probe), respectively). Age and gender were forced into all logistic models. We also performed a multiple logistic regression model to investigate the association of coronary artery disease and significant liver fibrosis with *PNPLA3* G allele. Results were presented as odds ratios with their 95% confidence intervals, and a 2-tailed p-value <0.05 was regarded as significant.

Results

Among 303 patients, PNPLA3 genotyping was obtained in 302 who compounded the study. Baseline characteristics of all participants categorized according to the presence of the G allele are shown in Table 1. Most patients were females (age 58.7 $\pm$ 9.5 years, BMI 30.8 $\pm$ 5.5 kg/m^2), with median diabetes duration of 17 years (2.0–47.0 years). Two hundred fifty-five individuals (84.4%) had the diagnosis of arterial hypertension, 270 (89.4%) had dyslipidemia, 152 (50.3%) had microvascular complications, and 79 (26.1%) had macrovascular diabetic complications. Among patients with macrovascular complications, 37 had the diagnosis of coronary artery disease. Most subjects (90.7%) used metformin, while 137 (45.4%) were using insulin. According to THE and CAP, 25.2% presented significant fibrosis by THE and severe steatosis by CAP was present in 57.9% of patients.

Genotyping of *PNPLA3* rs738409 polymorphism was identified as CC in 47% of the individuals, CG in 41%, and GG in 12% of them. The frequency of the mutant G allele in the population was 32.5%. Carriers of the G allele had a higher prevalence of coronary artery disease and a borderline higher prevalence of overall cardiovascular complications than the CC homozygous carriers but a similar prevalence of microvascular complications. Carriers of the G allele had lower HbA_{1c} levels, both during the first-year of follow-up and during the year in which THE was performed. There was a higher prevalence of good glycemic control (HbA_{1c} $<8.0\%$, 64 mmol/mol) in G allele carriers than in CC genotype individuals. Otherwise, the G allele carriers had a higher prevalence of significant liver fibrosis (29.3% *vs.* 20.4%; $p = 0.05$), than the CC homozygous individuals but equal liver steatosis prevalence. Table 2 shows selected clinical and laboratory characteristics among the 3 genotypes. A higher prevalence of coronary artery disease was already observed in the CG heterozygous individuals than in CC homozygous individuals, whereas an increased prevalence of liver fibrosis, higher aminotransferase levels, and a better glycemic control were particularly observed in the GG homozygous individuals.

Table 3 shows the association of independent variables with the presence of the *PNPLA3* G allele (CG and GG). On multivariate logistic regression, the presence of G allele was associated with greater odds of having coronary artery disease (OR: 2.25; 95% CI: 1.03–4.88; $p = 0.04$) and with significant liver fibrosis on THE (OR: 1.82; 95% CI: 1.04–3.17; $p = 0.03$). In addition, patients with G allele presented less risk of having mean $HbA_{1c} \geq 8\%$ (64 mmol/mol) (OR: 0.53; 95% CI: 0.31–0.89; $p = 0.01$). When considering liver stiffness on THE and HbA_{1c} values as continuous variables, their associations with G allele remained significant.

On logistic multivariate regression, G allele was associated with the presence of coronary artery disease after adjustment for age, gender, BMI, smoking, presence of arterial hypertension, presence of dyslipidemia, and HbA1c values (age, gender: OR: 2.12; 95% CI: 0.99–4.52 $p = 0.051$; after multivariate adjustment, OR: 2.20; 95% CI 1.01–4.78; $p = 0.046$). G allele was also independently associated with significant liver fibrosis after adjustment for age, gender, BMI, smoking, presence of arterial hypertension, presence of dyslipidemia, HbA1c values, aminotransferases, and gamma glutamyl transferase (age, gender: OR: 1.79; 95% CI 1.01–3.16, $p = 0.043$; after multivariate adjustment, OR: 2.17; 95% CI: 1.14–4.16; $p = 0.019$).

Discussion

This study shows that in Brazilian individuals with type 2 diabetes, the mutant G allele of the *PNPLA3* rs738409 C > G polymorphism is associated with a more severe stage of liver fibrosis by THE and with the presence of coronary artery disease, although it was also associated with a better glycemic control. The association with coronary artery disease in patients with diabetes suggests that, beyond advanced liver fibrosis, the G allele might be a marker for the risk of atherosclerotic coronary disease.

Table 1 Baseline clinical characteristics, laboratory variables, and liver stiffness measurements of all subjects with type 2 diabetes divided according to the presence of the *PNPLA3* G allele.

Variables	All participants (n = 302)	CC (n = 142)	CG + GG (n = 160)	p-value
Female gender	185 (61.2%)	86 (60.6%)	99 (61.9%)	0.81
Age (years)	58.7 (9.5)	57.7 (9.0)	59.5 (9.8)	0.10
Body mass index (kg/m^2)	30.8 (5.5)	30.8 (5.8)	30.8 (5.2)	0.97
Waist circumference (cm)	104.9 (11.8)	104.1 (12.9)	105.7 (10.7)	0.27
Diabetes duration (years)	17 (2–47)	17 (4–46)	17 (2–47)	0.82
Arterial hypertension	255 (84.4%)	118 (83.0%)	137 (85.6%)	0.54
Dyslipidemia	270 (89.4%)	123 (86.6%)	147 (91.9%)	0.14
Microvascular complications	152 (50.3%)	68 (47.9%)	84 (52.5%)	0.42
Retinopathy	83 (27.5%)	34 (23.9%)	49 (30.6%)	0.19
Nephropathy	78 (25.8%)	35 (24.6%)	43 (26.9%)	0.65
Neuropathy	64 (21.2%)	29 (20.4%)	35 (22%)	0.73
Macrovascular complications	79 (26.1%)	30 (21.1%)	49 (30.6%)	0.06
Coronary artery disease	37 (12.2%)	11 (7.7%)	26 (16.3%)	0.02
Cerebrovascular disease	19 (6.3%)	6 (4.2%)	13 (8.1%)	0.16
Peripheral arterial disease	42 (13.9%)	18 (12.7%)	24 (15%)	0.56
Diabetes treatment				
Metformin	274 (90.7%)	130 (91.5%)	144 (90.0%)	0.76
Sulfonylureas	132 (43.7%)	65 (45.8%)	67 (41.8%)	0.52
Insulin	137 (45.4%)	65 (45.8%)	72 (45.0%)	0.93
Statins	231 (76.5%)	108 (76.0%)	123 (76.8%)	0.79
Laboratory variables				
Fasting glucose (mmol/L)	7.8 (3.1)	8.1 (3.3)	7.6 (3.0)	0.14
Mean HbA_{1c}[a] (%)	7.6 (1.3)	7.8 (1.4)	7.4 (1.2)	0.02
(mmol/mol)	60 (14.2)	62 (15.3)	57 (13.1)	0.02
Mean first-year HbA_{1c} (%)	7.5 (1.4)	7.7 (1.4)	7.3 (1.3)	0.04
(mmol/mol)	58 (15.3)	61 (15.3)	56 (14.2)	0.04
HbA_{1c} < 8% (64 mmol/mol)	196 (64.9%)	84 (59.1%)	112 (70.0%)	0.02
Total cholesterol (mmol/L)	4.2 (1.1)	4.3 (1.2)	4.1 (1.1)	0.15
HDL-cholesterol (mmol/L)	1.1 (0.4)	1.1 (0.4)	1.1 (0.4)	0.32
LDL-cholesterol (mmol/L)	2.4 (1.2)	2.4 (0.9)	2.3 (0.9)	0.11
Triglycerides (mmol/L)	1.4 (0.9)	1.3 (0.9)	1.5 (0.8)	0.10
Alanine aminotransferase (U/L)	39 (16)	39 (15)	38 (17)	0.74
Aspartate aminotransferase (U/L)	24 (11)	23 (10)	24 (10)	0.50
γ-Glutamyl transpeptidase (U/L)	42 (36)	43 (38)	41 (34)	0.88
Transient Elastography				
Liver stiffness (kPa)	5.9 (3.5)	5.6 (3.2)	5.9 (4.2)	0.17
Significant fibrosis[b]	76 (25.2%)	29 (20.4%)	47 (29.3%)	0.05
CAP (dB/m)	279.6 (57.5)	281.2 (60.4)	282.0 (55.1)	0.90
Severe steatosis[c]	119 (39.4)	55 (38.7)	64 (40.0)	0.92

Values are presented as mean (standard deviation), median (interquartile range), or absolute number (proportion).
Abbreviation: CAP, controlled attenuation parameter.
[a] Mean HbA_{1c} during the year in which liver transient elastography was performed.
[b] Defined as liver stiffness ≥7.9 kPa (with M probe) or ≥7.2 kPa (with XL probe).
[c] Defined as CAP >296 dB/m.

Previous investigations showed that I148M substitution, robustly associated with hepatic steatosis, leads to a gain of lipogenic function of adiponutrin, which is the most reliable theory about triglyceride accumulation in hepatocytes [22–24]. In our study, despite a similar distribution of PNPLA3 genotypes as those previously described, there was no association between the G allele and severe steatosis [6,8]. Forty percent of the patients included in the present study had severe steatosis, but it was not associated with the G allele. Thus far, few studies evaluated the correlation between *PNPLA3* rs738409 C > G polymorphism and liver steatosis in patients with diabetes mellitus [11,13]. Petit et al. evaluated steatosis in patients with type 2 diabetes by MRI and found an association between the G allele with liver fat content, independently of total and visceral adipose tissue [11]. Cox et al. also showed an association of G allele with liver steatosis in African Americans, diagnosed by tomography [13]. However, neither of these investigations considered the relation of the G allele with the severity of steatosis [11,13]. It is important to emphasize that in our study, which comprised an exclusive population of patients with diabetes mellitus, the association of advanced steatosis was neither with the G allele nor with the values of CAP as a continuous variable. It is possible that in patients with type 2 diabetes, other mechanisms are involved in the pathogenesis of liver steatosis, and the PNPLA3 polymorphism has no impact on its severity. The relation of

Table 2 Comparisons of selected patients' characteristics among different *PNPLA3* genotypes.

Selected characteristics	*PNPLA3* genotypes			p-value
	CC (n = 142)	CG (n = 124)	GG (n = 36)	
Age	57.7 (9.0)	59.5 (9.8)	59.4 (9.9)	0.26
Female gender	86 (60.6%)	79 (63.7%)	20 (55.6%)	0.65
BMI	30.8 (5.8)	30.7 (4.9)	31.2 (6.1)	0.90
Waist circumference	104.1 (12.9)	105.7 (9.7)	105.4 (13.7)	0.54
Arterial Hypertension	118 (83.0%)	107 (86.2%)	30 (83.3%)	0.75
Coronary artery disease	11 (7.7%)	20 (16.1%)	6 (16.6%)	0.07
Mean HbA_{1c} (%)	7.8 (1.4)	7.6 (1.2)	7.1 (1.4)	0.01
(mmol/mol)	62 (15.3)	60 (13.1)	54 (15.3)	0.01
HbA_{1c} < 8% (64 mmol/mol)	84 (59.1%)	86 (73%)	26 (76.5%)	0.06
Liver stiffness (kPa)	5.6 (3.2)	5.6 (3.7)	7.9 (5.7)	0.002
Significant fibrosis[a]	29 (20.4%)	30 (24.1%)	17 (47.2%)	0.002
Alanine aminotransferase (U/L)	39 (15)	37 (15)	47 (28)	0.037
Aspartate aminotransferase (U/L)	23 (10)	23 (9)	29 (16)	0.017

Values are presented as mean (standard deviation), median (interquartile range), or absolute number (proportion).
[a] Defined as liver stiffness ≥7.9 kPa (with M probe) or ≥7.2 kPa (with XL probe).

Table 3 Results of multivariate logistic regression for the variables independently associated with the presence of the *PNPLA3* G allele (CG and GG genotypes).

Dependent Variable: *PNPLA3* CG + GG	Odds ratio	95% CI	p-value
Independent covariates *			
Model 1			
HbA_{1c} <8% *vs.* ≥8% (64 mmol/mol)	0.53	0.31–0.89	0.01
Significant liver fibrosis[a] (present *vs.* absent)	1.82	1.04–3.17	0.03
Coronary artery disease (present *vs.* absent)	2.25	1.03–4.88	0.04
Model 2			
HbA_{1c} (increment of 1%, 10.9 mmol/mol)	0.65	0.44–0.97	0.03
Liver stiffness (increment of 1 kPa)	1.26	1.01–1.55	0.03
Coronary artery disease (present *vs.* absent)	6.19	1.05–36.4	0.04

* The candidate variables to enter the logistic modeling were the following: age, gender, body mass index (BMI), waist circumference, smoking status, diabetes duration, presence of hypertension, presence of each micro- and macrovascular complication, mean HbA_{1c}, serum lipids, aminotransferases, and gammaglutamyl transferase levels, liver stiffness, and CAP parameters. Model 1: HbA1c and liver stiffness were entered as categorized variables; Model 2: HbA1c and liver stiffness were entered as continuous variables.
Abbreviations: CI, confidence interval.
[a] Defined as liver stiffness ≥7.9 kPa (with M probe) or ≥7.2 kPa (with XL probe).

steatosis' severity and the G allele has been previously shown by Sookian et al. [25]. However, in that case–control study, the association between the degree of steatosis and G allele was not analyzed in the specific group of patients with diabetes.

The association of the G allele and also the GG genotype with the severest stages of liver fibrosis has already been shown in NAFLD [6]. Our study indicated that this association also occurs on a population of patients with diabetes, in which the risk of significant fibrosis was 1.82 times higher for G allele carriers. Petit et al. showed that the *PNPLA3* polymorphism was associated with moderate to advanced liver fibrosis evaluated by FibroTest® but only for the population above the age of 60 years [7].

This study is one of the first showing that *PNPLA3* rs738409 C > G is associated with diabetic complications. The independent association of cardiovascular disease with the G allele is rather unexpected and might point to *PNPLA3* polymorphism as a surrogate marker of cardiovascular disease in this population. In contrast to our study, few studies have demonstrated a lower risk of cardiovascular disease associated with this allele or association between *PNPLA*3 and liver fat content but without relation to coronary artery disease [26–29]. Evidence links NAFLD with an increased risk of cardiovascular disease and other cardiac complications in patients with type 1 or type 2 diabetes [16,30]. However, a genetic marker in patients with diabetes that was associated both with NAFLD worse prognosis and with cardiovascular disease has not been identified yet. New longitudinal studies are needed to better investigate the association with the G allele of PNPLA3 polymorphism and to define its importance on cardiovascular outcomes on type 2 diabetes populations, but our results suggest that the *PNPLA3* gene is a potential prognostic marker of both liver and cardiovascular disease in this setting.

The finding that lower levels of HBA1c are related to the G allele might be unexpected. Currently, there is a debate whether the mean HBA1c of the first year of diagnosis of diabetes might have any impact on further complications

and prognosis of the disease [31]. Remarkably, in our study, since the first year of diagnosis of diabetes mellitus, patients with G allele presented lower levels of HBA1c than those with the homozygous C allele. It seems that the PNPLA3 gene in human hepatocytes is upregulated by glucose, but it might be possible that even lower levels of glucose in subjects with diabetes would be enough to overexpress the I148M gene [32]. Although we do not have data to confirm this hypothesis, this should also be evaluated in the future in the G allele group compared to the homozygous C allele group. In addition, as this is a cross-sectional study, we do not know whether these patients with lower levels of HBA1c were treated more carefully justifying a better glycemic control.

Considering the model obtained after multivariate logistic regression analysis and the variables that were independently associated with the G allele, we also performed a comparative analysis among the three PNPLA3 genotypes to achieve a better understanding of the impact of the G allele as homozygous or heterozygous on the independent variables. Thus, we demonstrated that for liver fibrosis, the high risk is related only to the GG genotype, as well as for HBA1c, whereas for cardiovascular disease, both CG and GG genotypes are implicated in high-risk outcomes.

This study has some limitations: First, it has a cross-sectional design, and thus, it does not allow any inference between the G allele and the outcomes. Second, we cannot exclude that the rather unexpected association between PNLA3 allele polymorphism and coronary artery disease might be due to casual association concerning the relatively small number of individuals included in the study and also to differences between methods and studied populations. Third, liver fibrosis was assessed by vibration-controlled elastography by FibroScan® and not by the gold standard, liver biopsy. However, because of the efficient performance of THE and the severity of the clinical profile of patients with diabetes included in this study, liver stiffness assessed by a noninvasive test like THE would be an useful parameter and might reflect the stage of liver fibrosis with desirable accuracy as demonstrated by Wong et al. [19,33].

In conclusion, the *PNPLA3* rs738409 C > G polymorphism (I148M variant) in individuals with type 2 diabetes and NAFLD may be a useful marker for the risk of liver fibrosis and cardiovascular disease. However, future prospective studies are needed to determine whether the association between a lower HBA1c and this polymorphism may confer a reduction in the risk of liver and coronary heart disease outcomes in high-risk diabetic populations.

Statement of financial support

This study was supported by grants from Conselho Brasileiro de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Brazil.

Conflicts of interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.numecd.2019.06.002.

References

[1] Morrish NJ, Wang SL, Stevens LK, et al. Mortality and causes of death in the WHO multinational study of vascular disease in diabetes. Diabetologia 2001;44(Suppl 2):S14–21.
[2] Younossi ZM, Gramlich T, Matteoni CA, et al. Nonalcoholic fatty liver disease in patients with type 2 diabetes. Clin Gastroenterol Hepatol: Off Clin Pract J Am Gastroenterol Assoc 2004;2:262–5.
[3] Leite NC, Villela-Nogueira CA, Pannain VL, et al. Histopathological stages of nonalcoholic fatty liver disease in type 2 diabetes: prevalences and correlated factors. Liver Int: Off J Int Assoc Study Liver 2011;31:700–6.
[4] Targher G, Bertolini L, Padovani R, et al. Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. Diabetes Care 2007;30:1212–8.
[5] Williams KH, Shackel NA, Gorrell MD, et al. Diabetes and nonalcoholic Fatty liver disease: a pathogenic duo. Endocr Rev 2013;34:84–129.
[6] Romeo S, Kozlitina J, Xing C, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 2008;40:1461–5.
[7] Petit JM, Guiu B, Masson D, et al. PNPLA3 polymorphism influences liver fibrosis in unselected patients with type 2 diabetes. Liver Int 2011;31:1332–6.
[8] Sookoian S, Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of nonalcoholic fatty liver disease. Hepatology 2011;53:1883–94.
[9] Valenti L, Al-Serri A, Daly AK, et al. Homozygosity for the patatin-like phospholipase-3/adiponutrin i148m polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease. Hepatology 2010;51:1209–17.
[10] Rotman Y, Koh C, Zmuda JM, et al. The association of genetic variability in patatin-like phospholipase domain-containing protein 3 (PNPLA3) with histological severity of nonalcoholic fatty liver disease. Hepatology 2010;52:894–903.
[11] Petit JM, Guiu B, Masson D, et al. Specifically PNPLA3-mediated accumulation of liver fat in obese patients with type 2 diabetes. J Clin Endocrinol Metab 2010;95:430–6.
[12] Posadas-Sanchez R, Lopez-Uribe AR, Posadas-Romero C, et al. Association of the I148M/PNPLA3 (rs738409) polymorphism with premature coronary artery disease, fatty liver, and insulin resistance in type 2 diabetic patients and healthy controls. The GEA study. Immunobiology 2016. Epub 2016 Sep. 13.
[13] Cox AJ, Wing MR, Carr JJ, et al. Association of PNPLA3 SNP rs738409 with liver density in African Americans with type 2 diabetes mellitus. Diabetes Metab 2011;37:452–5.
[14] Kantartzis K, Peter A, Machicao F, et al. Dissociation between fatty liver and insulin resistance in humans carrying a variant of the patatin-like phospholipase 3 gene. Diabetes 2009;58:2616–23.
[15] Kotronen A, Johansson LE, Johansson LM, et al. A common variant in PNPLA3, which encodes adiponutrin, is associated with liver fat content in humans. Diabetologia 2009;52:1056–60.
[16] Leite NC, Villela-Nogueira CA, Ferreira MT, et al. Increasing aortic stiffness is predictive of advanced liver fibrosis in patients with type 2 diabetes: the Rio-T2DM cohort study. Liver Int 2016;36:977–85.
[17] Cardoso CR, Leite NC, Ferreira MT, et al. Prognostic importance of baseline and serial glycated hemoglobin levels in high-risk patients with type 2 diabetes: the Rio de Janeiro Type 2 Diabetes Cohort Study. Acta Diabetol 2015;52:21–9.
[18] Care D. American diabetes association's standards of medical care in diabetes. Diabetes Care 2018;2018(41 Suppl1):S1–2.

[19] Wong VW, Vergniol J, Wong GL, et al. Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. Hepatology 2010;51:454–62. Epub 2010 Jan 27.
[20] Roulot D, Roudot-Thoraval F, NK G, et al. Concomitant screening for liver fibrosis and steatosis in French type 2 diabetic patients using Fibroscan. Liver Int: Off J Int Assoc Study Liver 2017;37:1897–906.
[21] de Ledinghen V, Vergniol J, Foucher J, et al. Non-invasive diagnosis of liver steatosis using controlled attenuation parameter (CAP) and transient elastography. Liver Int: Off J Int Assoc Study Liver 2012; 32:911–8. Epub 2012 Jun 08.
[22] Speliotes EK, Yerges-Armstrong LM, Wu J, 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. https://doi.org/10.1371/journal.pgen.1001324.
[23] Kumari M, Schoiswohl G, Chitraju C, et al. Adiponutrin functions as a nutritionally regulated lysophosphatidic acid acyltransferase. Cell Metabol 2012;15:691–702.
[24] Sookoian S, Pirola CJ. PNPLA3, the triacylglycerol synthesis/hydrolysis/storage dilemma, and nonalcoholic fatty liver disease. World J Gastroenterol 2012;18:6018–26.
[25] Sookoian S, Castano GO, Burgueno AL, et al. A nonsynonymous gene variant in the adiponutrin gene is associated with nonalcoholic fatty liver disease severity. J Lipid Res 2009;50:2111–6.
[26] Stefan N, Haring HU, Cusi K. Non-alcoholic fatty liver disease: causes, diagnosis, cardiometabolic consequences, and treatment strategies. The Lancet Diabetes Endocrinol 2018. https://doi.org/10.1016/S2213-8587(18)30154-2.
[27] Liu DJ, Peloso GM, Yu H, et al. Exome-wide association study of plasma lipids in >300,000 individuals. Nat Genet 2017;49: 1758–66.
[28] Lauridsen BK, Stender S, Kristensen TS, et al. Liver fat content, non-alcoholic fatty liver disease, and ischaemic heart disease: mendelian randomization and meta-analysis of 279 013 individuals. Eur Heart J 2018;39:385–93.
[29] Simons N, Isaacs A, Koek GH, Kuč S, Schaper NC, Brouwers MCGJ. PNPLA3, TM6SF2, and MBOAT7 genotypes and coronary artery disease. Gastroenterology 2017;152:912–3.
[30] Targher G, Bertolini L, Poli F, et al. Nonalcoholic fatty liver disease and risk of future cardiovascular events among type 2 diabetic patients. Diabetes 2005;54:3541–6.
[31] Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. N Engl J Med 2008;359:1577–89.
[32] Perttila J, Huaman-Samanez C, Caron S, et al. PNPLA3 is regulated by glucose in human hepatocytes, and its I148M mutant slows down triglyceride hydrolysis. Am J Physiol Endocrinol Metab 2012;302:1063–9.
[33] Wong VW, Vergniol J, Wong GL, et al. Liver stiffness measurement using XL probe in patients with nonalcoholic fatty liver disease. Am J Gastroenterol 2012;107:1862–71.