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An association study of the *PNPLA3* I148M polymorphism (rs738409) with serum lipids in patients with dyslipidemia

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Abstract

Aim: One single nucleotide polymorphism (SNP) rs738409 in the patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) gene has been considered a major genetic risk factor of nonalcoholic fatty liver disease (NAFLD). Data have indicated that NAFLD is related to insulin resistance and dyslipidemia, but whether rs738409 is associated with circulating lipid and lipoproteins is not fully elucidated. The main aim of this study was to assess the association of rs738409 with lipid and lipoprotein levels in patients with dyslipidemia.

Methods: This was a post-hoc analysis of a study in patients with dyslipidemia recruited on an outpatient basis. Morning blood samples were collected after a 12-h fast. Genomic DNA was extracted from whole-blood samples.

Results: One hundred seventy-five patients with dyslipidemia were included (97 women). Lipid levels [total cholesterol (TC), triglycerides (TGs), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C)] or glycosylated hemoglobin (HbA_{1c}) were not associated with the SNP, even after adjustment for gender, body mass index (BMI) and type 2 diabetes mellitus (T2DM), using either the additive (CC *vs.* CG *vs.* GG) or the dominant (CC *vs.* GG + CG) inheritance model. When data were stratified for obesity, significant associations between the variant and TC (P = 0.014) or LDL-C levels (P = 0.046) in the non-obese were observed. Pairwise comparison revealed significant changes only in TC between CC and CG genotypes (P = 0.012).

Conclusions: No association was shown between rs738409 SNP and lipid/lipoprotein levels in patients with dyslipidemia. In subgroup analysis, TC was higher in non-obese, but not in obese, patients with CC, compared to CG carriers.

Keywords

Dyslipidemia, nonalcoholic fatty liver disease, rs738409, patatin-like phospholipase domain-containing protein 3

Introduction

Adiponutrin, also known as patatin-like phospholipase domain-containing protein 3 (*PNPLA3*), is expressed in many tissues, including the liver, where it is primarily localized in lipid droplets [1]. PNPLA3 exhibits hydrolase activity on monoacyl-, diacyl- and triacylglycerols/triglycerides (TGs) and lysophosphatidic acid acyltransferase activity, thus participating in the hepatic metabolism of TGs and phospholipids (PLs), while some authors suggest that it may be secondarily associated with glucose homeostasis [2–4].

The single nucleotide polymorphism (SNP) rs738409 in the *PNPLA3* gene has been strongly associated with the development and progression of nonalcoholic fatty liver disease (NAFLD) [5, 6]; indeed, this is the most validated SNP linked to the full spectrum of NAFLD [7]. rs738409 (c444C>G) leads to the substitution of methionine for isoleucine in the active center of the protein; this alteration affects access, substrate, and, consequently, enzymatic activity [8], leading to reduced hydrolytic removal of fatty acids and increased accumulation of TGs in hepatocytes and retinol retention in hepatic stellate cells, thus contributing to hepatic steatosis and fibrosis [8, 9]. The risk of nonalcoholic steatohepatitis (NASH) in patients with this variant is maximized when coexists with high adiposity, illustrating the additive effects of genetic and environmental contributors to this disease [10].

While NAFLD is closely associated with insulin resistance, dyslipidemia, and hyperglycemia, the association of rs738409 to lipid and glucose metabolism remains unclear. The main aim of this study was the investigation of the association of rs738409 with serum lipids in patients with dyslipidemia. The secondary aim was the association of SNP with glycosylated hemoglobin (HbA_{1c}).

Materials and methods

This was a post-hoc analysis of a study in patients with dyslipidemia recruited in an outpatient basis in the dyslipidemia clinic, between 2009 and 2012. All the participants provided informed consent and the study was approved by the Bioethics Committee of the Medical School of Aristotle University of Thessaloniki. The inclusion criteria were: 1) dyslipidemia defined as morning fasting total cholesterol (TC) > 240 mg/dL and/or low-density lipoprotein cholesterol (LDL-C) > 160 mg/dL and/or TGs > 200 mg/dL in at least two measurements with an interval > 1 week; 2) lack of any hypolipidemic therapy or discontinuation of any hypolipidemic agent for at least 3 months.

The patients' personal and family medical history, anthropometric characteristics [gender, age, and body mass index (BMI)], cigarette smoking, and alcohol consumption were recorded. Morning blood samples were collected after a 12-h fast. Lipid tests were performed with standard methods using a biochemical analyzer (Hitachi 912, Roche, Ramsey, MN, USA). LDL-C was calculated with the Friedewald equation [11]. HbA_{1c} measurement was determined by high performance liquid chromatography (HA-8121, Menarini, Firenze, Italy). Genomic DNA was extracted from whole-blood samples by means of the Ron's Blood DNA minikit (BIORON GmbH, Ludwigshafen, Germany) according to the manufacturer's instruction. Genotyping for rs738409 was accomplished using a previously reported polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method [12].

Statistical analysis

To check the normality of the distributions of continuous variables, the Kolmogorov-Smirnov test was used. Continuous data were presented as mean ± standard deviation (SD) or median and interquartile range (IQR), depending on the normality of the distribution. Categorical data were presented as frequencies. One-way analysis of variance (ANOVA) or Kruskal-Wallis, and Chi-square or Fischer exact test were used for the comparisons between groups, in case of continuous and categorical variables, respectively. Independent

sample *t* test or Mann-Whitney test were used for the comparisons of continuous variables between groups, in case of two compared groups. The association of the SNP with serum parameters was tested with one-way analysis of co-variance (ANCOVA) after adjustment for potential covariates, followed by Bonferroni correction, when deemed appropriate. Significance was set at $P < 0.05$ or $P < 0.017$ in multiple pairwise comparisons (Bonferroni, three groups). All statistical analyses were performed with SPSS 23.0 (IBM Corp., Armonk, NY).

Results

One hundred seventy-five patients with dyslipidemia were included [97 women, aged 59 (13), BMI 29 (4), type 2 diabetes mellitus (T2DM) 43%, hypertension 69.7%] (Table 1). Genotypic for rs738409 was successfully performed in 165 patients. Genotype distribution did not deviate from Hardy-Weinberg equilibrium ($P = 0.810$). Gender, age, and BMI did not differ significantly between genotypes (CC *vs.* CG *vs.* GG), contrary to smoking, T2DM, and coronary artery disease. Circulating lipids [TC, TGs, LDL-C, high-density lipoprotein cholesterol (HDL-C)] or HbA_{1c} were not associated by the polymorphism following adjustment for gender, BMI, and T2DM diagnosis, using either the additive or the dominant model of inheritance of the minor allele (Table 2).

Table 1. Baseline data of the overall study population

Parameter	Data
Women	97/175 (55.4%)
Age (years)	59 (13)
BMI (kg/m^2)	29 (4)
Cigarette smoking (any)	25 (14.3%)
Alcohol use (any)	30 (17.1%)
Coronary artery disease	25 (14.3%)
Hypertension	122 (69.7%)
T2DM	75 (42.9%)
Stroke	12 (6.9%)
TC	247 ± 47.4
HDL-C	40 (12)
LDL-C	158.6 ± 48.6
TGs	230 (126)
HbA_{1c}	5.7 (1.5)

Data are presented as mean ± SD when they are normally distributed or as median [interquartile range (IQR)] when they are not. Qualitative variables are reported as frequencies (%)

Table 2. Data on serum lipids, lipoproteins, and HbA_{1c} levels in different groups of rs738409 SNP of the *PNPLA*3 gene

Parameter	CC	CG	GG	*P* value*	*P* value†
TC (mg/dL)	250.5 ± 54.52	239.5 ± 39.41	250.3 ± 35.76	0.254	0.203
TGs (mg/dL)	258.8 ± 170.73	238.3 ± 122.89	323.6 ± 225.33	0.475	0.868
LDL-C (mg/dL)	163.6 ± 54.21	151.2 ± 42.92	160.3 ± 41.74	0.250	0.149
HDL-C (mg/dL)	40.8 ± 9.43	41.1 ± 10.77	39.3 ± 8.33	0.778	0.974
HbA_{1c} (%)	6.2 ± 1.17	5.9 ± 1.06	5.5 ± 0.26	0.226	0.068

Data are presented as mean ± SD. Gender, BMI, and T2DM were included as covariates. * Additive genetic model (CC *vs.* CG *vs.* GG); † Dominant model (CC *vs.* GG + CG)

Interestingly, upon comparison of genotype distributions following stratification of patients according to T2DM, we detected no diabetic patients homozygous for the G allele, whereas, in the non-diabetic group, 12.4% had the GG genotype ($P = 0.005$); no significant association was found between rs738409 and serum lipids/lipoproteins or HbA_{1c} in the subgroup analysis. When stratifying according to obesity (BMI cut-off 30 kg/m^2), no significant difference was observed in genotype distribution. However, subgroup analysis produced a significant association between rs738409 and TC ($P = 0.014$) and LDL-C ($P = 0.046$) in

the non-obese, with the lowest levels observed in carriers of the CG genotype (Table 3). A post-hoc, multiple pairwise comparison showed significant difference in TC between carriers of the CC and CG genotypes (P = 0.012) but no specific difference in LDL-C following the Bonferroni correction. No such difference was detected in the obese subgroup. The design of this study cannot allow safe conclusions on these different associations observed between non-obese and obese. Although residual confounding may have affected these results (e.g., differences between obese and non-obese in dietary and exercise habits, hepatic and renal function, percentage of NAFLD, etc.), further studies are needed to validate or not these differences and to investigate their pathophysiological and clinical implications.

Table 3. Data on serum lipids, lipoproteins, and HbA_{1c} levels in different groups of rs738409 SNP of the *PNPLA3* gene after subgroup analysis according to obesity

Parameter	Obese (*n* = 78)					Non-obese (*n* = 87)				
	PNPLA3 genotype									
	CC (*n* = 38)	CG (*n* = 34)	GG (*n* = 6)	*P* value*	*P* value†	CC (*n* = 44)	CG (*n* = 37)	GG (*n* = 6)	*P* value*	*P* value†
TC (mg/dL)	226 ± 44	238 ± 41	233 ± 24	0.470	0.230	271 ± 54	240 ± 37	267 ± 39	0.014	0.009
TGs (mg/dL)	266 ± 186	249 ± 127	371 ± 291	0.288	0.772	252 ± 157	227 ± 119	275 ± 144	0.634	0.953
LDL-C (mg/dL)	142 ± 42	148 ± 44	139 ± 32	0.797	0.625	181 ± 56	153 ± 41	178 ± 42	0.046	0.027
HDL-C (mg/dL)	40.3 ± 8.7	39.7 ± 10.5	34.6 ± 6.8	0.400	0.541	41.2 ± 10	42.3 ± 11	44 ± 7.3	0.778	0.541
HbA_{1c} (%)	6.5 ± 1.13	6.1 ± 1.21	5.4 ± 0.23	0.062	0.088	5.9 ± 1.12	5.7 ± 0.86	5.4 ± 0.30	0.934	0.334

Data are presented as mean ± SD. *Additive genetic model (CC *vs.* CG *vs.* GG); † Dominant model (CC *vs.* GG + CG)

Discussion

In this study, no association was found between rs738409 SNP and circulating lipids/lipoproteins or HbA_{1c} in patients with dyslipidemia. In subgroup analysis, TC levels were significantly higher in non-obese, but not in obese, dyslipidemic patients with CC, compared to CG carriers.

A meta-analysis confirming the association between rs738409 and NAFLD did not find any association between the polymorphism and circulating lipids/lipoproteins [13]. Some authors have reported that obese individuals carrying the G allele did not show any difference in serum lipids or glucose compared to non-carriers [14, 15], similar to our findings; however, others showed that individuals carrying the G allele presented with either lower [16] or higher [17] TG levels. Still, a more recent report indicated that homozygotes for GG had elevated TG, but not other lipids/lipoproteins, glucose, or HbA_{1c} [18]. Finally, in contrast to our results, in a study with obese NAFLD patients, it was shown that those with the CG or GG genotype presented with a 9.6-fold higher risk of glucose disorders [19]. In that study, a higher rate of CG carriers was observed in the group of dyslipidemic patients [19]. Importantly, a recent meta-analysis, which included 63 studies, showed significant associations of rs738409 polymorphism of *PNPLA3* with TG and TC, but not with LDL-C and HDL-C. The rs738409 polymorphism was associated with lower TG and TC levels [20]. However, we should highlight that: 1) the overall magnitude of the associations of this SNP with TG and TC was small; 2) the associations of this SNP with TG and TC were not significant in all subpopulations, possibly implying population differences; and 3) most of the studies that were included in this meta-analysis had not separately shown associations between rs738409 polymorphism of *PNPLA3* and lipid levels [20], similarly to our study. While we have no convincing explanation for the discrepancies between studies, they may be, at least partly, attributed to underlying population and methodological differences (e.g., ethnicity, dietary habits, obesity, percentage of NAFLD patients).

The main strength of this study was that it may be the first to evaluate the rs738409 SNP in patients with dyslipidemia; in previous studies, dyslipidemia had been evaluated in the setting of patients with NAFLD and/or T2DM. Its limitations include: 1) the relatively small sample size and the lack of an a priori

power calculation; however, the sample was sufficient to provide significant difference in TC in the subgroup of the non-obese; 2) the cross-sectional design, which does not allow establishing causal relationships; 3) the lack of a validation set, in spite of the largely negative results; 4) the lack of data on NAFLD; 5) the lack of data on metabolic syndrome, kidney function, and concomitant medications; 6) the lack of recorded plasma glucose levels, since glycemic parameters were not the primary aim of this study.

In conclusion, this study showed no association between rs738409 SNP of *PNPLA3* gene and serum lipids/lipoproteins or HbA_{1c} in patients with dyslipidemia. An association between this SNP and TC in non-obese patients needs validation by other independent studies. This study largely confirms that the rs738409 SNP is not associated with lipid and glycemic profile, contrary to its reported high association with the development and severity of NAFLD.

Abbreviations

BMI: body mass index

HbA_{1c}: glycosylated hemoglobin

HDL-C: high-density lipoprotein cholesterol

LDL-C: low-density lipoprotein cholesterol

NAFLD: nonalcoholic fatty liver disease

PNPLA3: patatin-like phospholipase domain-containing protein 3

SD: standard deviation

SNP: single nucleotide polymorphism

T2DM: type 2 diabetes mellitus

TC: total cholesterol

TGs: triacylglycerols/triglycerides

Declarations

Author contributions

DI: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing—review & editing. ESM: Data curation, Formal analysis, Visualization, Writing—original draft, Writing—review & editing. SAP: Data curation, Formal analysis, Validation, Visualization, Writing—original draft, Writing—review & editing. CN: Data curation, Investigation, Writing—review & editing. DA: Data curation, Writing—review & editing. GG: Data curation, Writing—review & editing. AG: Conceptualization, Supervision, Formal Analysis, Investigation, Validation, Methodology, Writing—review & editing.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the Declaration of Helsinki 1964 and its later amendments or comparable ethical standards. The study was approved by the Bioethics Committee of the Medical School of Aristotle University of Thessaloniki.

Consent to participate

Informed consent was obtained from all the participants included in the study.

Consent to publication

Not applicable.

Availability of data and materials

The data associated with the paper are not publicly available but are available from the corresponding author on reasonable request.

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