



MBOAT7 rs641738 variant in metabolic-dysfunction-associated fatty liver disease and cardiovascular risk

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Abstract

Introduction. Although metabolic-dysfunction-associated fatty liver disease (MAFLD) is associated with an increased cardiovascular risk, MAFLD predisposing genetic variants were not steadily related to cardiovascular events. Therefore, we aimed to assess whether membrane-bound O-acyltransferase domain-containing 7 (*MBOAT7*) rs641738 variant is associated with an increased cardiovascular risk in MAFLD patients.

Methods. We conducted an observational cross-sectional study including 77 subjects (38 MAFLD patients, 39 controls), between January-September 2020 using hepatic ultrasonography and SteatoTest™ to assess hepatic steatosis. Echocardiographic and Doppler ultrasound parameters were evaluated. Genomic DNA was extracted and rs641738 SNP was genotyped using TaqMan assays.

Results. The rs641738 variant was not significantly associated with MAFLD, with a p-value of 0.803, 0.5265, 0.9535, and 0.5751 for codominant, dominant, recessive, and overdominant genotypes, respectively. The rs641738 variant overdominant genotype significantly predicted atherosclerotic cardiovascular disease (ASCVD) risk algorithm in univariate analysis (-4.3 [95% CI -8.55 – -0.55, p-value= 0.048]), but lost significance after multivariate analysis (-3.98 [95% CI -7.9 – -0.05, p-value= 0.053]). The rs641738 variant recessive genotype significantly predicted ActiTest in univariate analysis (0.0963 [95% CI 0.0244 – 0.1681, p-value= 0.009]), but lost significance after multivariate analysis (0.0828 [95% CI -0.016 – 0.1816, p-value= 0.105]).

Conclusion. No significant association was observed between rs641738 variant and MAFLD in the studied population. The rs641738 variant was found to predict ASCVD risk score and ActiTest in univariate linear regression analysis. However, the significance of both associations was lost after performing multivariate analysis.

Keywords: metabolic associated fatty liver disease (MAFLD), hepatic steatosis, genes, *rs641738*, *MBOAT7*, cardiovascular disease

Introduction

Metabolic-dysfunction-associated fatty liver disease (MAFLD), previously known as nonalcoholic fatty liver disease (NAFLD), is defined by the presence of hepatic steatosis, in addition to overweight/obesity, type 2 diabetes mellitus, or confirmed metabolic risk dysregulation [1,2]. The manifestations, severity, and progression of MAFLD phenotypes are the outcome of cross-linked and complex environmental and genetic interactions [3]. Lately, the study of genetic factors contributing to fatty liver disease has gained increasing attention and interest in the literature, exerting an essential role in the pathogenesis of fatty liver disease, as well as its progression towards non-alcoholic steatohepatitis (NASH) and hepatocellular carcinoma [4]. This relationship has been proven in epidemiological studies (through familial aggregation, and twin studies), confirming the significant role exerted by genetics and its heritability [5,6].

Recently, the assessment of genetic single-nucleotide polymorphisms (SNPs) in fatty liver disease and cardiovascular (CV) outcomes has been evaluated [3,6,7]. The missense rs641738 C > T variant based in exon 1 of TMC4 (transmembrane channel-like 4) and mapped 500 bases downstream of *MBOAT7* (membrane-bound O-acyltransferase domain-containing 7) locus has been evaluated in fatty liver disease [8]. The rs641738C > T variant has been initially associated with an increased predisposition for developing alcohol-related cirrhosis [9]. Afterward, it was also reported to play a significant pathogenic role in several other hepatic pathologies, including fatty liver disease regardless of obesity [10], liver fibrosis in chronic hepatitis B and C viral infections [11-14], and hepatocellular carcinoma [15]. Conflicting results have been reported regarding the association between rs641738 variant and NAFLD. A recently published systematic review and a meta-analysis reported that rs641738 C>T variant is a risk factor for the presence and severity of NAFLD in subjects of European descent, with neutral effects in coronary artery disease (CAD) [16,17], while another meta-analysis found that rs641738 was not related to NAFLD risk [18].

Common pathogenic mechanisms have been suggested to play an important role in fatty liver disease-associated comorbidities, including type 2 diabetes mellitus, obesity, dyslipidemia, and cardiovascular disease (CVD), all of which have been showed to exert a substantial unfavorable effect on fatty liver disease's natural course and vice versa [5,19,20]. Although the fatty liver disease has been associated with increased CV risk, several genetic variants reported to predispose to fatty liver disease were not found to be linked with an increased CV risk [21]. Interestingly, a couple of fatty liver disease-related genetic variants were reported to exert cardioprotective effects [7,21].

Published studies assessed several CV outcomes in NAFLD patients [22,23]. However, limited data are

currently present in the literature evaluating CV outcomes in MAFLD patients [24,25]. Interestingly, a recent study reported that although MAFLD patients presented with increased risk of cardiovascular mortality and all-cause mortality, increased risk of all-cause deaths was not found in NAFLD patients after metabolic risk factors adjustment [24]. Thus, previously published data involving NAFLD patients, including genetic factors, might be different in patients with MAFLD due to significant clinical associated differences between the two terms and differences in diagnostic criteria [26,27].

Currently, no studies reported the effects of the rs641738 variant in MAFLD patients, using the recently defined diagnostic criteria. Moreover, the role of rs641738 variant near *MBOAT7* in MAFLD and cardiovascular disease (CVD) remains unclear [28,29]. Therefore, we conducted an observational cross-sectional study evaluating the role of rs641738 variant in MAFLD, in addition to its association with CV risk.

Methods

Study design, setting, and participants

The recruitment process, hepatic steatosis and echocardiographic assessment, as well as the laboratory tests were previously described in details [30,31]. In summary, we performed a cross-sectional observational study, enrolling participants using non-probability consecutive sampling between January 2020 - September 2020. Subjects between ≥ 18 and <65 years old were included in the study. Participants were divided into MAFLD group or controls. The MAFLD group included patients hospitalized in the Clinical Emergency County Hospital of Cluj-Napoca, Romania, who fulfilled the MAFLD diagnostic criteria [1]. Assessment of hepatic steatosis was performed using both hepatic ultrasonography and SteatoTest™ (BioPredictive) for all included subjects (MAFLD and controls) at inclusion, while MAFLD patients had to present evidence of hepatic steatosis using both methods, or else were excluded from this group. Regarding the controls, they were chiefly healthy hospital staff without hepatic steatosis.

The following exclusion criteria were used for both groups: BMI >40 kg/m², hepatic steatosis of other secondary causes, confirmed hepatitis B or C virus infection, coexistent liver disease, liver tumors of any etiology, acute hemolytic diseases, acute inflammatory diseases including deep venous thrombosis, ulcerative colitis or Crohn's disease, systemic lupus erythematosus, active malignancies, acute infections (COVID-19, dental, urinary, pulmonary, flu, etc.), active pulmonary exacerbations, not fasting for at least 12 hours prior to blood sampling, and participation refusal. We obtained a written informed consent for all subjects participating in this study. Conducting this observational study was approved by the local ethical and research committee of "Iuliu Hatieganu" University of Medicine and Pharmacy

Cluj-Napoca (no. 486/21.11.2019), according to the 1975 Helsinki Declaration guidelines and revised in 2013.

General definitions

The diagnosis of MAFLD was based on the international expert consensus statement published criteria [2]. We used the 2020 International Society of Hypertension Global Hypertension Practice Guidelines for classifying hypertension [32]. The American Diabetes Association recommendations - Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes – 2021 were used for identifying diabetes and prediabetes [33]. The National Cholesterol Education Program guidelines was used to establish dyslipidemia [34].

Hepatic ultrasonography

An experienced physician blinded to the study aims, patients' diagnosis, and labs assessed for the presence of hepatic steatosis using GE LOGIQ S7 Expert. Prior to the ultrasound assessment, subjects were asked to fast for at least 8 hours. The criteria for hepatic steatosis assessment were: liver and right kidney parenchyma ultrasonographic contrast, liver brightness; impaired visualizing of the diaphragm, and ultrasonographic deep attenuation penetration into the deep portion of the liver; and impaired intrahepatic vessels borders and narrowing of the lumen [35].

Echocardiography

A detailed echocardiographic evaluation was performed by a board-certified cardiologist using GE Vivid q Ultrasound Machine, and blinded to the study aims, patients' diagnosis, and labs. The echocardiographic assessment was independent of the genetic evaluation. We used the currently published guidelines and recommendations as a reference to interpret our obtained parameters such as M-mode, 2-dimensional, conventional color, and Doppler ultrasound [36-41]. To assess end-systolic volume (ESV) and end-diastolic volume (EDV), we calculated them through the 2- and 4-chamber apical views utilizing a dedicated software for automated calculation as well as left ventricular ejection fraction (LVEF). We verified and corrected the obtained automatically detected borders for precision.

Laboratory analysis

Participants were requested to fast for at least 12 hours prior to blood sampling that was collected through venipuncture in vacutainer tubes. The recommended protocols for the sampling of the blood and analysis of the obtained samples were followed.

1. Genetic variant analysis

For genetic testing, 3 ml of peripheral blood was collected on ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Genomic DNA was extracted using a commercially available extraction kit (Wizard® Genomic DNA Purification Kit, Promega) from blood leucocytes contained in a volume of 300µl. The single nucleotide polymorphism (rs641738) was genotyped by means of

TaqMan SNP Genotyping assays from Thermo Fisher Scientific according to the manufacturer's instructions.

2. FibroMax

We extracted the sera and stored them for a maximum of 1 day at 2°C – 8°C, after which the sera were assayed for the ten serum biomarkers included in the FibroMax score. The obtained result values were inputted into the BioPredictive network, where adjustments for age, gender, weight, and height were performed, along with computed algorithms. Afterward, the final score was obtained for each subject.

Nephelometry (BN ProSpec System from Siemens) was used to assess the serum haptoglobin, apolipoprotein A1, and α 2-macroglobulin levels. Spectrophotometry (Atellica from Siemens) was used to assess aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, gamma-glutamyltransferase (GGT), triglycerides, and total cholesterol levels. Furthermore, NaF/K2 oxalate spectrophotometry was used to measure plasma fasting glucose levels.

Statistical analysis

We used SNPassoc package for genetic analysis and R software environment for statistical computing and graphics version 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria) for completing other statistical analyses. Categorical data were described as frequencies and percentages, normally distributed continuous data were expressed as mean (standard deviation, SD), while non-normally distributed continuous data were described as median (interquartile range, IQR). The t-test was applied for independent samples of normally distributed data, evaluating the clinical characteristics of the study sample according to group categories. The Wilcoxon rank-sum test was applied for non-normally distributed data. Furthermore, the chi-square test and Fisher exact test were used to assess categorical data. We also performed Hardy Weinberg Equilibrium (HWE) evaluation to measure the strength of evidence against the null hypothesis for the rs641738 variant. For each variable of interest, we checked the relationship with rs641738 variant using different genetic models (dominant, recessive, overdominant, codominant, log-additive) and we chose the variables with the smallest Akaike information criterion (AIC) that were statistically significant. They were used in the multivariate analysis to control for other known confounders. Assessing the association between ActiTest according to rs641738 recessive variant, age, sex, hepatic steatosis, and dyslipidemia, as well as ASCVD risk algorithm according to rs641738 overdominant variant, group (MAFLD vs. controls), and BMI, we used univariate and multivariate linear regression models to control for confounding factors. We assessed the assumptions of residual normality by quantile-quantile plots, and homooskedasticity through a standardized residual vs. fitted values. then we checked for the existence of high leverage, high residuals, or high

influential points using standardized residuals vs. hat-values vs. Cook's distance plot, as well as linearity relation of continuous variables with the outcome by the use of component + residual plot for all linear models that were evaluated. Furthermore, we also assessed for the existence of multi-co-linearity in multivariate models by correlation coefficients and variance inflation factors. Our regression results were presented as model coefficients, 95% confidence interval (CI – computed with robust variance sandwich estimators in case of heteroskedasticity), and p-value. Finally we refitted all the multivariate models using multiple quantile regressions, to ensure deviations from model assumptions would be better accounted for. We performed two-sided statistical tests for all conducted analyses, where a p-value <0.05 was considered statistically significant.

Results

General characteristics and laboratory results

We screened a total of 252 individuals for eligibility, and 175 subjects were excluded for the following reasons: 99 subjects: >65 years old, 14 subjects: liver cirrhosis, 12 subjects: refused participation, 10 subjects: hepatitis C virus, 8 subjects: acute infections, 6 subjects: hepatitis B virus, 6 subjects: acute inflammatory conditions, 6 subjects: active cancer patients, 5 subjects: acute pulmonary disease, 3 subjects: liver tumors, 3 subjects: other coexistent liver

disease, 2 subjects: BMI > 40 kg/m², 1 subject: hepatic steatosis in control group. We were left with a total of 77 Caucasian participants who were included in our study's final analysis. We outline the participants' general characteristics in table I.

Subjects were divided into 2 groups, MAFLD patients and controls. The MAFLD group included 38 patients (49.35%) and the controls 39 subjects (50.65%). The mean age of all the included participants was 45 (ranging from 30 – 56). Gender distribution was 34 males (44.16%) and 43 females (55.84%), without a statistically significant difference (p value= 0.919). A higher BMI, larger waist circumference, presence of diabetes, and hypertension were observed in MAFLD patients compared to controls (p-value < 0.001). We found no significant difference regarding impaired fasting glucose, pulse, and smoking history. Measurements of blood pressure such as systolic blood pressure, diastolic blood pressure, mean arterial pressure, and pulse pressure were observed to have a statistically significant difference between both groups with a p-value of <0.001, <0.001, <0.001, and 0.029, respectively. Although levels of low-density lipoproteins (LDL) were significantly higher (p-value= 0.038) and high-density lipoproteins (HDL) were significantly lower (p-value <0.001) in MAFLD patients compared to controls, no significant difference was found in total cholesterol levels (p-value= 0.225).

Table I. General characteristics of included participants.

Characteristic	Total (n= 77)	Control (n= 39)	MAFLD (n= 38)	P-value
Age (years), median (IQR)	45 (30 - 56)	30 (27 - 42)	53.5 (48.25 - 59)	< 0.001
Gender (male), n (%)	34/77 (44.16)	17 (43.59)	17 (44.74)	0.919
BMI, median (IQR)	26.23 (22.22 - 30.39)	22.22 (20.16 - 24.92)	30.31 (28.05 - 33.27)	< 0.001
Waist circumference (cm), median (IQR)	96 (81 - 104)	82 (72 - 90.5)	104 (100 - 110.75)	< 0.001
Metabolic syndrome, n (%)	31/77 (40.26)	2 (5.13)	29 (76.32)	< 0.001
Diabetic, n (%)	15/77 (19.48)	0 (0)	15 (39.47)	< 0.001
Impaired fasting glucose, n (%)	5/77 (6.49)	2 (5.13)	3 (7.89)	0.675
Hypertensive, n (%)	36/77 (46.75)	6 (15.38)	30 (78.95)	< 0.001
SBP-mean (mmHg), median (IQR)	124 (116 - 133)	120.5 (112.5 - 125.75)	132.25 (122.12 - 148.5)	< 0.001
DBP-mean (mmHg), median (IQR)	78.5 (74 - 83.5)	75.5 (71 - 79)	82.25 (78.12 - 88.75)	< 0.001
MAP - mean (mmHg), median (IQR)	93.83 (89 - 100.5)	90.5 (84.33 - 93.92)	98.67 (92.04 - 108.75)	< 0.001
Pulse pressure - mean (mmHg), median (IQR)	45.5 (41 - 51)	44.5 (40 - 48.75)	48 (42.12 - 58.25)	0.029
Pulse - mean (bpm), median (IQR)	77.5 (71 - 84.5)	80 (74 - 84)	75.75 (67 - 84.5)	0.241
Smoking history, n (%)				
smoker:	15/77 (19.48)	8 (20.51)	9 (23.68)	0.974
ex-smoker:	18/77 (23.38)	9 (23.08)	22 (57.89)	
never smoked:	44/77 (57.14)	22 (56.41)	7 (18.42)	
LDL (mg/dL), median (IQR)	119 (91 - 160)	111 (84 - 138.5)	137.5 (102 - 168)	0.038
HDL (mg/dL), median (IQR)	48 (43 - 59)	54 (46.5 - 63)	44 (38.75 - 51.25)	< 0.001
Triglycerides (mg/dL), median (IQR)	110 (78 - 153)	82 (69 - 100.5)	147.5 (115.25 - 178.75)	< 0.001
Total cholesterol (mg/dL), median (IQR)	189 (152 - 219)	184 (152 - 212.5)	197.5 (156.25 - 232.75)	0.225
Fasting blood sugar (mg/dL), median (IQR)	91 (86 - 101)	87 (82.5 - 91.5)	98.5 (88.75 - 125.25)	< 0.001

MAFLD – metabolic associated fatty liver disease IQR – interquartile range; BMI – body mass index; DBP – diastolic blood pressure; HDL – high-density lipoproteins; LDL – low-density lipoproteins; MAP – mean arterial pressure; SBP – systolic blood pressure.

Evaluation of scores and indexes

Several cardiovascular and hepatic scores comparing MAFLD patients vs. controls are outlined in table II. The Framingham Risk Score, atherosclerotic cardiovascular disease (ASCVD) risk algorithm, total cholesterol:HDL ratio, TG:HDL ratio, atherogenic index of plasma (AIP), and visceral adiposity index (VAI) were significantly higher in MAFLD patients compared to controls ($p < 0.001$). However, no significant difference was observed in the direct bilirubin/total bilirubin ratio and AST:ALT ratio in both groups, with a p-value of 0.182 and 0.151, respectively.

Supplementary table I summarizes the presence of hepatic steatosis evaluated using ultrasonography as well as FibroMax scores (SteatoTest, FibroTest, ActiTest, NashTest, and AshTest) in the studied population.

Regarding FibroMax scores assessing hepatic steatosis, fibrosis, and inflammation, a significantly higher score was found in MAFLD patients compared to controls.

1.1. rs641738 Genetic Variant Genotypes and Alleles

In table III, the rs641738 genetic variant genotypes and alleles in the included subjects are outlined. Out of the 77 included participants, 15 (38.5%) presented the C/C genotype, 16 (41%) presented the C/T genotype, and 8 (20.5%) presented the T/T genotype, while 46 (59%) had the T allele and 32 (41%) had the C allele, with an HWE p-value of 0.48. Moreover, from the MAFLD group, 12 (31.6%) presented C/C genotype, 18 (47.4%) presented the C/T genotype, and 8 (21%) presented the T/T genotype, while 34 (44.7%) had the T allele and 42 (55.3%) had the C allele, with an HWE p-value of 0.75.

Table II. Cardiovascular, lipid, adiposity, and hepatic scores/indexes.

Characteristic	Total (n= 77)	Control (n= 39)	MAFLD (n= 38)	P-value
Framingham Risk Score (%), median (IQR)*	2.2 (0.9 - 5.85)	0.3 (0.1 - 1.7)	4.55 (2.1 - 9.2)	< 0.001
ASCVD risk algorithm (%), median (IQR)**	4.1 (2.45 - 9.55)	1.2 (0.5 - 2.7)	7.6 (3.45 - 12.15)	< 0.001
Total cholesterol:HDL ratio, median (IQR)	3.66 (3.26 - 4.54)	3.43 (2.71 - 3.79)	4.43 (3.61 - 5.05)	< 0.001
TG:HDL ratio, median (IQR)	2.48 (1.48 - 3.29)	1.5 (1.22 - 1.99)	3.15 (2.56 - 4.44)	< 0.001
Atherogenic Index of Plasma (AIP), median (IQR)	0.04 (-0.19 - 0.16)	-0.18 (-0.27 - -0.06)	0.14 (0.05 - 0.29)	< 0.001
Visceral adiposity index, median (IQR)	3.71 (2.45 - 5.76)	2.5 (1.78 - 3.2)	5.55 (4.38 - 7.39)	< 0.001
Direct bilirubin / total bilirubin ratio, median (IQR)	0.2 (0.17 - 0.26)	0.21 (0.18 - 0.26)	0.2 (0.16 - 0.26)	0.182
AST:ALT ratio, median (IQR)	0.95 (0.76 - 1.15)	1.06 (0.83 - 1.17)	0.82 (0.73 - 1.13)	0.151

*39 MAFLD patients and 21 controls were included due to the age requirement in the formula; **34 MAFLD patients and 21 controls were included due to the age requirement in the formula; MAFLD – metabolic associated fatty liver disease; ALT – alanine aminotransferase; ASCVD – atherosclerotic cardiovascular disease; AST – aspartate transaminase; HDL – high-density lipoproteins; TG – triglycerides.

Supplementary Table I. Evaluation of hepatic steatosis, fibrosis, and inflammation using ultrasonography and FibroMax.

Characteristic	Total (n= 77)	Control (n= 39)	MAFLD (n= 38)	P-value
Hepatic steatosis (Ultrasonography), n (%)	38/77 (49.35)	0 (0)	38 (100)	< 0.001
SteatoTest score, median (IQR)	0.36 (0.13 - 0.61)	0.13 (0.08 - 0.21)	0.62 (0.51 - 0.68)	< 0.001
FibroTest score, median (IQR)	0.14 (0.1 - 0.23)	0.12 (0.07 - 0.19)	0.17 (0.11 - 0.29)	0.008
ActiTest score, median (IQR)	0.08 (0.05 - 0.14)	0.06 (0.03 - 0.09)	0.11 (0.08 - 0.2)	< 0.001
NashTest score, median (IQR)	0.25 (0.25 - 0.5)	0.25 (0.25 - 0.25)	0.5 (0.5 - 0.5)	< 0.001
AshTest score, median (IQR)	0.01 (0.01 - 0.02)	0.01 (0 - 0.01)	0.02 (0.01 - 0.04)	< 0.001

Table III. rs641738 genetic variant genotypes and alleles of MAFLD patients and controls.

Characteristic	Total (n= 77)	Control (n= 39)	MAFLD (n= 38)
<i>Genotypes</i>			
C/C, n (%)	15 (38.5)	15 (38.5)	12 (31.6)
C/T, n (%)	16 (41)	16 (41)	18 (47.4)
T/T, n (%)	8 (20.5)	8 (20.5)	8 (21)
<i>Alleles</i>			
T, n (%)	46 (59)	46 (59)	34 (44.7)
C, n (%)	32 (41)	32 (41)	42 (55.3)
HWE (p-value)	0.48	0.34	0.75

MAFLD – metabolic associated fatty liver disease; HWE – Hardy Weinberg equilibrium

Moreover, as outlined in table IV, no significant relation was demonstrated between rs641738 variant and MAFLD patients vs. controls, with a p-value of 0.803, 0.5265, 0.9535, and 0.5751 for codominant, dominant, recessive, and overdominant genotypes, respectively.

1.2. rs641738 genetic variant and cardiovascular assessment

A summary of the obtained echocardiographic and Doppler ultrasound parameters is summarized in supplementary table II. MAFLD patients had a significantly

higher carotid intima-media thickness (CIMT), right and left ventricular diameter, interatrial and interventricular septal wall thickness, left ventricular end-systolic volume (LVESV), and left ventricular end diastolic volume (LVEDV), stroke volume, cardiac output, left ventricular posterior wall thickness (LVPWT), late diastolic peak velocity (A), and E/e' ratio compared to controls. On the other hand, controls had a significantly higher LVEF, early diastolic peak velocity (E), early diastolic velocity (e'), E/A ratio, and e'/a' ratio. However, no significant difference was observed in late diastolic velocity (a').

Table IV. rs641738 variant in MAFLD patients vs. controls.

	Controls	%	MAFLD	%	OR	lower	upper	p-value	AIC
Codominant									
C/C	15	38.5	12	31.6	1.00			0.803	112.3
C/T	16	41	18	47.4	1.41	0.51	3.88		
T/T	8	20.5	8	21.1	1.25	0.36	4.32		
Dominant									
C/C	15	38.5	12	31.6	1.00			0.5265	110.3
C/T-T/T	24	61.5	26	68.4	1.35	0.53	3.47		
Recessive									
C/C-C/T	31	79.5	30	78.9	1.00			0.9535	110.7
T/T	8	20.5	8	21.1	1.03	0.34	3.11		
Overdominant									
C/C-T/T	23	59	20	52.6	1.00			0.5751	110.4
C/T	16	41	18	47.4	1.29	0.53	3.19		
log-Additive									
0,1,2	39	50.6	38	49.4	1.15	0.62	2.11	0.6570	110.5

AIC – Akaike information criterion; MAFLD – metabolic associated fatty liver disease; OR – odds ratio.

Supplementary Table II. Echocardiographic and Doppler ultrasound cardiovascular parameters.

Characteristic	Total (n= 77)	Control (n= 39)	MAFLD (n= 38)	P-value
CIMT - right (mm), median (IQR)	9 (7 - 10)	7 (6 - 9)	9 (8 - 11)	< 0.001
CIMT - left (mm), median (IQR)	8 (7 - 10)	7 (6 - 8.5)	10 (8.25 - 11)	< 0.001
CIMT - mean (mm), median (IQR)	8.5 (7 - 10)	7 (6.5 - 8.5)	9.75 (8.5 - 11)	< 0.001
Left atrial diameter (mm), median (IQR)	31 (27 - 34)	29 (26 - 31)	34 (31 - 36.5)	< 0.001
Left ventricular diameter (mm), median (IQR)	44 (39 - 47)	42 (38.5 - 44)	45 (43 - 49)	0.003
Right ventricular diameter (mm), median (IQR)	23 (21 - 25)	22 (20.5 - 24)	24.5 (22 - 27)	0.004
Interventricular septal wall thickness (mm), median (IQR)	9 (8 - 10)	8 (8 - 9)	10 (9.25 - 11)	< 0.001
Interatrial septal wall thickness (mm), median (IQR)	6 (5 - 7)	5 (5 - 7)	6 (6 - 7)	0.023
LVEDV (ml), median (IQR)	95 (77 - 114)	84 (73.5 - 104)	102.5 (90 - 120.5)	0.002
LVESV (ml), median (IQR)	45 (37 - 56)	39 (33.5 - 47)	53 (43.25 - 61.5)	0.002
LVEF (%), median (IQR)	50 (46 - 56)	52 (48 - 57)	48.5 (46 - 53.5)	0.025
Stroke volume (ml), median (IQR)	50 (39 - 57)	44 (36.5 - 56)	52.5 (46.25 - 57)	0.035
Cardiac output, median (IQR)	3.5 (2.88 - 4.32)	3.13 (2.65 - 3.96)	3.76 (3.01 - 4.98)	0.038
LVPWT (mm), median (IQR)	9 (8 - 10)	8 (8 - 9)	10 (10 - 11)	< 0.001
Early diastolic peak velocity - E (m/s), median (IQR)	0.75 (0.62 - 0.9)	0.8 (0.72 - 0.96)	0.65 (0.57 - 0.78)	< 0.001
Late diastolic peak velocity - A (m/s), median (IQR)	0.51 (0.43 - 0.73)	0.48 (0.42 - 0.56)	0.71 (0.48 - 0.8)	< 0.001
Early diastolic velocity – e' (m/s), median (IQR)	0.13 (0.11 - 0.17)	0.17 (0.14 - 0.2)	0.11 (0.09 - 0.13)	< 0.001
Late diastolic velocity – a' (m/s), median (IQR)	0.09 (0.07 - 0.14)	0.09 (0.07 - 0.13)	0.09 (0.08 - 0.16)	0.252
E/A ratio, median (IQR)	1.41 (1 - 1.81)	1.73 (1.34 - 1.99)	1.12 (0.71 - 1.43)	< 0.001
e'/a' ratio, median (IQR)	1.5 (0.88 - 2.14)	1.67 (1.42 - 2.38)	1.02 (0.72 - 1.64)	< 0.001
E/e' ratio, median (IQR)	5.37 (4.47 - 6.67)	5.1 (4.16 - 5.64)	5.96 (4.93 - 7.32)	0.01

CIMT – carotid intima media thickness; LVEDV – left ventricular end diastolic volume; LVESV – left ventricular end systolic volume; LVPWT – left ventricular posterior wall thickness; MAFLD – metabolic associated fatty liver disease.

The overdominant rs641738 variant genotype was found to be significantly associated with ASCVD risk algorithm with a p-value of 0.047, while the codominant and overdominant rs641738 variant genotypes were found to be significantly associated with ActiTest with a p-value of 0.034 and 0.009, respectively, as shown in table V.

We proceeded by performing univariate and multivariate linear regression models, and multivariate quantile regression models in predicting ASCVD risk algorithm according to rs641738 variant overdominant

genotype (C/T vs. C/C-T/T), group (MAFLD vs. controls), and BMI as can be seen in table VI. In these analyses, we observed that rs641738 variant overdominant genotype significantly predicted ASCVD risk algorithm in univariate linear regression model with an unadjusted B of -4.3 (95% CI -8.55 – -0.55, p-value= 0.048). However, after multivariate linear regression (-3.98 [95% CI -7.9 – -0.05, p-value= 0.053]) and multivariate quantile regression models (-1.24 [95% CI -2.44 – 1.90, p-value= 0.611]), the significance was lost.

Table V. Association between rs641738 variant and ASCVD risk algorithm / ActiTest and using different genetic models.

rs641738 variant and ActiTest								
	n	mean	SE	Difference	95% CI lower	upper	p-value	AIC
<i>Codominant</i>								
C/C	18	8.828	1.9842	0.0000			0.08578	324.2
C/T	23	5.404	0.9887	-3.4234	-7.8816	1.0348		
T/T	6	12.317	4.4699	3.4889	-3.1893	10.1671		
<i>Dominant</i>								
C/C	18	8.828	1.9842	0.0000			0.38000	326.6
C/T-T/T	29	6.834	1.2757	-1.9933	-6.3997	2.4131		
<i>Recessive</i>								
C/C-C/T	41	6.907	1.0528	0.0000			0.09822	324.6
T/T	6	12.317	4.4699	5.4093	-0.8694	11.6881		
C/C-T/T	24	9.700	1.8351	0.0000			0.04770	323.3
C/T	23	5.404	0.9887	-4.2957	-8.4317	-0.1596		
<i>log-Additive</i>								
0,1,2				0.2949	-2.9412	3.5311	0.85904	327.4
rs641738 variant and ASCVD risk algorithm								
	n	me	se	dif	lower	upper	p-value	AIC
<i>Codominant</i>								
C/C	27	0.10148	0.014624	0.0000			0.034744	-91.60
C/T	34	0.09882	0.011850	-0.002658	-0.067966	1.0348		
T/T	16	0.19625	0.062208	0.094769	0.014837	0.17470		
<i>Dominant</i>								
C/C	27	0.10148	0.014624	0.0000			0.374476	-87.40
C/T-T/T	50	0.13000	0.022035	0.028519	-0.034043	0.09108		
<i>Recessive</i>								
C/C-C/T	61	0.10000	0.009171	0.0000			0.009328	-93.60
T/T	16	0.19625	0.062208	0.096250	0.025562	0.16694		
<i>Overdominant</i>								
C/C-T/T	43	0.13674	0.025443	0.0000			0.217973	-88.20
C/T	34	0.09882	0.011850	-0.037921	-0.097745	0.02190		
<i>log-Additive</i>								
0,1,2				0.041517	0.001696	0.08134	0.044520	-90.79

AIC – Akaike information criterion; ASCVD – atherosclerotic cardiovascular disease; CI – confidence interval; SE – standard error.

Table VI. Univariate and multivariate linear regression models, and multivariate quantile regression models predicting ASCVD risk algorithm according to rs641738 overdominant variant, group (MAFLD vs. controls), and BMI.

	B unadjusted	(95% CI)	P-value	R2	B Adjusted robust	(95% CI)	P-value	B Adjusted quantile	(95% CI)	P-value
rs641738 variant overdominant genotype (C/T vs. C/C-T/T)	-4.3	(-8.55 – -0.05)	0.048	0.084	-3.98	(-7.9 – -0.05)	0.053	-1.24	(-2.44 – 1.90)	0.611
Group (MAFLD vs. Control)	6.14	(1.53 – 10.75)	0.01	0.138	5.86	(0.28 – 11.44)	0.046	6.29	(3.72 – 9.52)	0.006
BMI	0.36	(-0.06 – 0.77)	0.091	0.062	0	(-0.5 – 0.51)	0.994	-0.05	(-0.34 – 0.17)	0.739

ASCVD – atherosclerotic cardiovascular disease; BMI – body mass index; CI – confidence interval; MAFLD – metabolic associated fatty liver disease; R2 – model's determination coefficient.

Table VII. Univariate and multivariate linear regression models, and multivariate quantile regression models predicting ActiTest according to *rs641738* recessive variant, age, sex, hepatic steatosis, and dyslipidemia

	B unadjusted	(95% CI)	P-value	R2	B Adjusted robust	(95% CI)	P-value	B Adjusted quantile	(95% CI)	P-value
<i>rs641738</i> variant recessive genotype (T/T vs. C/T+C/C)	0.0963	(0.0244 – 0.1681)	0.009	0.087	0.0828	(-0.016 – 0.1816)	0.105	-0.0011	(-0.0208 – 0.0673)	0.966
Age (years)	0.0006	(-0.0017 – 0.003)	0.585	0.004	-0.0015	(-0.0048 – 0.0018)	0.367	0.0011	(-0.0010 – 0.0020)	0.182
Sex (male vs. female)	0.0827	(0.0243 – 0.1411)	0.006	0.096	0.0702	(0.0207 – 0.1196)	0.007	0.0600	(0.0249 – 0.0892)	0.002
Hepatic steatosis (US)	0.0935	(0.0364 – 0.1506)	0.002	0.124	0.1228	(0.0295 – 0.2161)	0.012	0.0178	(0.0051 – 0.0878)	0.446
Dyslipidemia	0.0203	(-0.0406 – 0.0811)	0.509	0.006	-0.009	(-0.0809 – 0.063)	0.808	0.0000	(-0.0450 – 0.0188)	1

CI – confidence interval; R2 – model's determination coefficient.

Moreover, we performed univariate and multivariate linear regression models and multivariate quantile regression models in predicting ActiTest according to *rs641738* variant recessive genotype (T/T vs. C/T+C/C), age, sex, hepatic steatosis, and dyslipidemia as presented in table VII. In the conducted assessment, we found that *rs641738* recessive variant significantly predicted ActiTest in the univariate linear regression model with an unadjusted B of 0.096 (95% CI 0.024 – 0.168, p-value=0.009). Nevertheless, this significance was attenuated after conducting multivariate linear regression models (0.083 [95% CI -0.016 – 0.182, p-value=0.105]) and multivariate quantile regression models (-0.001 [95% CI -0.021 – 0.067, p-value=0.966]).

Discussion

Current literature includes several published studies among which systematic reviews and meta-analyses that investigate the association between *rs641738* variant near *MBOAT7* and NAFLD susceptibility, as well as cardiovascular outcomes, mainly coronary artery disease [16-18,28,29]. Nevertheless, to the best of our knowledge, there are no studies evaluating the association between this genetic variant as a predisposing gene in MAFLD and cardiovascular risk. Moreover, it was also not evaluated in relation to frequently used CV risk scores such as Framingham risk score and ASCVD risk algorithm, and multiple echocardiographic and Doppler ultrasound cardiovascular parameters. Therefore, in this cross-sectional observational study, we evaluated the effects of *rs641738* variant in MAFLD and several cardiovascular parameters, including ASCVD risk algorithm. We demonstrated that the *rs641738* variant was not associated with MAFLD in our study population, Caucasians of European descent. Although the *rs641738* variant overdominant genotype significantly predicted ASCVD risk algorithm in univariate analysis, this significance was attenuated to non-significant levels after performing multivariate linear and quantile regression analyses. Similarly, *rs641738* variant recessive genotype predicted ActiTest in univariate analysis, while

significance was lost after performing multivariate analyses.

In this observational study, hepatic steatosis was evaluated by hepatic ultrasonography, in addition to SteatoTest™ (Biopredictive). It was demonstrated that ultrasonography can detect hepatic steatosis only when exceeding 15-20%, with a sensitivity ranging between 60-94% and specificity between 88-95% [42,43]. Moreover, studies reported an AUROC of 0.81 (95% CI 0.79-0.83, P<0.0001) for SteatoTest™ (Biopredictive) in estimating hepatic steatosis, considering it as a simple and non-invasive quantitative estimation method for evaluating fat deposition [44, 45]. Histopathological assessment by liver biopsy remains the gold standard at present to identify and quantify hepatic steatosis and liver fibrosis. However, this procedure is considered invasive with possible risks and complications.

In our study, we found no significant association between *rs641738* variant and MAFLD vs. controls. Despite conflicting results, the *rs641738* variant has been evaluated in several studies, including recently published systematic reviews and meta-analyses, most of them concluding that it is associated with NAFLD in adult Caucasians, mainly from European background [16,17]. However, these findings were inconclusive, mainly in other populations such as Asians [17,18]. An important clinical consideration about the *rs641738* variant is the frequency of this variant in different backgrounds and ethnicities. It was reported that the T allele's mean allelic frequency could differ significantly, for example ranging between 0.24 in individuals from East Asia and up to 0.53 in individuals from South Asia, in addition to variations in the minor allele frequency [46]. Furthermore, variations in the genetic variant across different populations can also be possibly attributed to environmental factors.

Unlike NAFLD, the current literature is limited in data evaluating cardiovascular parameters in MAFLD patients [22,23]. Our results confirm the metabolic dysregulations present in MAFLD patients as defined in the published data [1,26]. Interestingly, an article published lately demonstrated a discrepancy in all-cause and

cardiovascular mortality between NAFLD and MAFLD [24]. The authors have reported that MAFLD patients are of increased risk of developing all-cause and cardiovascular mortality, unlike NAFLD that was not associated with increased all-cause mortality risk after adjusting for metabolic risk factors. Accordingly, cardiovascular risk factors, including genes that might lead to an increased cardiovascular risk in MAFLD patients, should be further evaluated in order to possibly clarify several unknown gaps in evidence, potentially leading to targeted therapies in such a complex disease.

Despite several studies associating fatty liver disease with increased cardiovascular risk [47,48], the causality of this relationship has been questioned recently, mainly due to several genetic variants that were found to predispose to fatty liver disease but were not demonstrated to also increase cardiovascular events, such as *PNPLA3* and *TM6SF2* that were actually reported to exert cardioprotective roles against coronary artery disease [7, 49]. On the contrary, *GCKR* variants were found to be associated with coronary artery disease [7]. In such cases, a frequently used technique to assess the causality assumption is by conducting Mendelian randomization analysis.

According to our evaluation, rs641738 variant was not associated with multiple evaluated structural and functional cardiovascular ultrasound parameters. However, although we demonstrated that the rs641738 variant overdominant genotype significantly predicted ASCVD risk algorithm in univariate analysis, the significance was lost after performing multivariate linear and quantile regression analyses. These findings are similar to other published studies evaluating the association between rs641738 variant and coronary artery disease. Simon et al. conducted a 48 genome-wide association study analysis, out of which 42 assessed the rs641738 T allele, concluding neutral effects on coronary artery disease [50]. Moreover, Brouwers et al. also concluded that rs641738 polymorphism was not found to cause coronary artery disease per se [51]. A possible interpretation for the neutral effects of rs641738 variant in MAFLD can be due to plasma lipids. Although conflicting evidence is reported regarding rs641738 variant and several metabolic risk factors, most studies report no significant relationship between this variant and lipid profile, waist circumference, and BMI [17].

Several limitations need to be addressed. Because of the observational study design, the causality of the evaluated associations cannot be confirmed or negated. As a result of the modest sample size of our study, we were not able to conduct subgroup analysis. Moreover, as this study involved only Caucasian subjects from European backgrounds, our results cannot be generalized without further confirmation in future studies. Lastly, the gold standard, namely liver biopsy with histopathological assessment for diagnosing hepatic steatosis, was not performed in our study. However, performing a biopsy entails health risks and performing this

procedure in healthy subjects raises additional problems.

On the other hand, our study also has several important strengths. We combined hepatic ultrasonography with SteatoTest™ (Biopredictive), thus improving the prediction accuracy for detecting hepatic steatosis. Moreover, we conducted a comprehensive cardiovascular assessment including cardiovascular risk scores, as well as multiple echocardiographic and Doppler ultrasound cardiovascular parameters in patients with the newly defined criteria for MAFLD, which identify fatty liver disease patients with higher risk for disease progression [26], and their association with rs641738 variant. To the best of our knowledge, this is the first study to evaluate the association between rs641738 variant and MAFLD using the new diagnosis criteria, in addition to multiple cardiovascular risk scores, echocardiographic, and Doppler ultrasound parameters.

Conclusions

We found no significant association between the rs641738 variant and MAFLD in the evaluated study population, Caucasian subjects of European descent. The rs641738 was not found to be associated with multiple cardiovascular parameters, including structural cardiac parameters, systolic and diastolic functions, as well as subclinical cardiovascular risk. Although the rs641738 variant significantly predicted ASCVD risk algorithm and ActiTest in univariate analysis, this significance was attenuated to non-significant levels after performing multivariate linear and quantile regression analyses.

Future studies are still necessary in order to assess the rs641738 and other genetic variants in multiple pathologies for possible therapeutic implications, especially in complex diseases such as MAFLD and CVD. Although genetic-driven drugs are still in their beginnings, they can possibly play a crucial role in the near future.

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