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ORIGINAL RESEARCH

Patatin-Like Phospholipase Domain-Containing Protein 3 (*PNPLA3*) rs738409 Variant and Non-Alcoholic Fatty Liver Disease Risk in Vietnamese Working-Age Adults: A Case-Control Study with Metabolic Insights

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Background: Non-alcoholic fatty liver disease (NAFLD) is an increasing public health concern in Vietnam, particularly among working-age adults (18–60 years). The *PNPLA3* rs738409 variant (C>G) is a well-established risk factor for NAFLD globally; however, its impact on the Vietnamese population remains inadequately studied. This study investigates its association with NAFLD risk and its interaction with metabolic factors.

Methods: A case-control study was conducted with 135 NAFLD patients and 270 age- and sex-matched controls, collected from April to August 2023. Hepatic steatosis was evaluated via ultrasound, and NAFLD was diagnosed in cases without excessive alcohol consumption and other liver conditions. Data on demographics, clinical characteristics, and biochemical markers (eg, lipid profiles, liver enzymes) were collected. The rs738409 variant was genotyped using real-time PCR. Statistical methods included Hardy-Weinberg equilibrium testing, allele and genotype frequency comparisons, multivariable logistic regression adjusting for metabolic covariates, and ROC curve analysis to evaluate the predictive accuracy of rs738409.

Results: The frequency of the G allele was significantly higher in NAFLD patients (35.93%) compared to controls (28.15%, p = 0.024). Individuals with CG+GG genotypes exhibited an increased risk of NAFLD (OR = 1.433, p = 0.042), with a stronger association in those with low HDL-c (OR = 2.074, p = 0.009). However, multivariable logistic regression analysis indicated that the *PNPLA3* rs738409 variant was not an independent risk factor for NAFLD in this population, in contrast to obesity and high triglycerides. ROC analysis revealed rs738409 alone had limited predictive power for NAFLD (AUC = 0.5537) but predictive accuracy improved slightly when combined with metabolic factors such as BMI and triglyceride levels (AUC = 0.7840).

Conclusion: The *PNPLA3* rs738409 variant modestly increases NAFLD risk in Vietnamese working-age adults, particularly in those with dyslipidemia. However, metabolic factors, such as obesity and lipid disorders, play a more dominant role. This underscores the importance of lifestyle interventions and metabolic control in NAFLD management.

Keywords: hepatic steatosis, metabolic syndrome, obesity, high triglycerides

Introduction

Non-alcoholic fatty liver disease (NAFLD) is increasingly prevalent worldwide, affecting approximately 25.2% of the global population and up to 30% in Asia, driven by rapid lifestyle changes over the past two decades.[1–3] In 2023, the population of Vietnam was recorded to be more than one million inhabitants.[4] The country has witnessed a rapid

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urbanization, dietary shifts, and rising obesity have intensified the burden of NAFLD, particularly among working-age adults (18–60 years) - a group critical to economic productivity and social stability.[5,6] Although Vietnamese NAFLD prevalence data are not available, the prevalence of metabolic syndrome in the working-age group, a condition closely associated with NAFLD, was estimated to be 16%.[7] NAFLD is marked by excessive hepatic fat accumulation without significant alcohol use, poses a public health challenge due to its potential progression and lack of approved pharmacological treatments.[8,9] Recent global analyses suggest that metabolic dysfunction-associated steatotic liver disease (MASLD) - a broadened definition that links hepatic steatosis with at least one cardiometabolic risk factor – now affects about 38% of adults and projections indicate it could exceed 55% by 2040.[10–12] NAFLD/MASLD is increasingly recognized as a multisystem disorder, leading not only to liver complications like steatohepatitis, fibrosis, cirrhosis, and cancer but also to extra-hepatic conditions. Cardiovascular disease is the main cause of death, and patients are at higher risk for type 2 diabetes, CKD, sarcopenia, and certain cancers.[13] While many aspects of NAFLD pathogenesis have been well-documented, with severe outcomes like hepatocellular carcinoma occurring in some cases,[14–16] the genetic underpinnings in specific populations, such as Vietnamese adults, remain underexplored.

Genetic factors significantly influence NAFLD pathogenesis, interacting with environmental triggers to drive disease onset. The *PNPLA3* gene, encoding patatin-like phospholipase domain-containing protein 3, has emerged as a key player, with its rs738409 C>G variant (I148M) identified through genome-wide association studies (GWAS) as the most robust genetic risk factor for NAFLD.[17] This variant impairs triglyceride hydrolysis, promoting lipid accumulation in hepatocytes, with meta-analyses showing GG genotype carriers face a 4.0-fold higher risk (OR = 4.01, 95% CI: 2.93–5.49) and GC heterozygotes a 1.9-fold increase (OR = 1.88, 95% CI: 1.58–2.24) compared to CC carriers. The G allele also correlates with advanced disease states, underscoring its clinical relevance.[18] However, this association varies across ethnic groups, with extensive data from Caucasian, Chinese, and Japanese cohorts but limited evidence from Southeast Asian populations, including Vietnam. These differences, likely due to variations in allele frequency and metabolic profiles, highlight the need for region-specific studies.[19] The absence of such data hampers early screening and personalized approaches, often delaying diagnosis and intervention.[20]

In Vietnam, NAFLD's genetic basis remains poorly characterized, particularly among working-age adults who face distinct environmental pressures - such as sedentary lifestyles, high-calorie diets, and occupational stress - that may amplify genetic risks. Despite the established role of *PNPLA3* rs738409 in other populations, no studies have examined its contribution to NAFLD in this demographic, leaving a critical gap in understanding its relevance in Vietnam's unique genetic and epidemiological context, which differ from well-characterized East Asian and Western cohorts.[3,6] This omission is significant, as early identification of genetic risk factors in this group could yield substantial health and economic benefits. This case-control study, conducted from April to August 2023, investigates the association between *PNPLA3* rs738409 and NAFLD risk among Vietnamese working-age adults, exploring its interplay with anthropometric and clinical factors. By addressing this knowledge gap, our findings aim to inform ethnicity-specific risk stratification and enhance NAFLD prevention strategies in Vietnam. Moreover, this research contributes to the global understanding of genetic heterogeneity in NAFLD, offering insights for future studies across diverse populations. Integrating *PNPLA3* rs738409 into public health and clinical programs could improve early screening and intervention efforts tailored to Vietnam's working population.

Patients, Materials and Methods

Patients and Study Design

A matched case-control study was conducted on Vietnamese patients under 60 years old with NAFLD, diagnosed according to the 2018 American Association for the Study of Liver Diseases (AASLD) criteria,[9] recruited consecutively from the outpatient clinic of Nguyen Tri Phuong Hospital between April and August 2023. NAFLD diagnosis was based on ultrasound evidence of hepatic steatosis, defined as increased liver echogenicity relative to the renal cortex, assessed by experienced radiologists, following AASLD guidelines, in the absence of excessive alcohol consumption (>20 g/day for women, >30 g/day for men),[21] viral hepatitis (HBsAg, Anti-HCV negative), or other chronic liver diseases. The severity was then graded based on the degree of sound attenuation: Grade 1 (Mild) allowed clear visualization of the

diaphragm and intrahepatic vasculature; Grade 2 (Moderate) caused partial obscuration of these structures; and Grade 3 (Severe) resulted in their poor or non-visualization, including the posterior right hepatic lobe. Exclusion criteria included individuals under 18 years of age, pregnant women, and those with malignancies. Participants with insufficient blood sample volume and quality after health screening for molecular biological testing were also excluded. The control group comprised healthy Vietnamese volunteers from Pham Ngoc Thach University of Medicine, consisted of age- and sex-matched individuals without evidence of hepatic steatosis on ultrasound, no history of liver disease, normal liver enzyme levels, and no metabolic or liver disorders, confirmed through structured interviews and clinical examinations by a trained physician. Sample size was determined for a matched case-control study with an unequal group ratio. Using the Kelsey formula[22] and a meta-analysis of 21 studies by Dai et al[18] which reported an Odds Ratio (OR) of 1.88 for the association between the G allele of SNP rs738409 in the *PNPLA3* gene and NAFLD, we used the G allele frequency in the control group ($p_1 = 0.360$) from our pilot study in the Vietnamese population. With a control-to-case ratio (r) of 2:1, a type I error (α) of 0.05, and a type II error (β) of 0.2, the minimum sample size was calculated. After adjusting for a 10% attrition rate, the final estimated sample size was 134 controls and 268 cases. Informed consent was obtained from all participants. At study completion, a total of 135 NAFLD patients and 270 age- and sex-matched controls were enrolled, with all participant data deemed eligible for analysis and reporting.

Patients' Data Collection

Data was collected from each patient using a standardized, pre-tested questionnaire administered by trained research staff to ensure consistency and minimize interviewer bias. All cases underwent a physical examination, and their medical history, anthropometric parameters, and medication use were obtained from questionnaires and patient records. Historical and anthropometric data included a history of hypertension and diabetes, as well as weight and height measurements. Blood pressure was measured using a calibrated automated sphygmomanometer (Omron HEM-7121) after 5 minutes of rest, with the average of two readings recorded. Medication and alcohol use histories were also documented. Biochemical parameters recorded during health screening included lipid profiles (LDL-c, HDL-c, triglycerides, and total cholesterol), liver enzymes (AST, ALT), and fasting blood sugar (FBS). Fasting blood samples were collected after an overnight fast of ≥ 8 hours, stored at 4°C, and analyzed within 24 hours. BMI was calculated and classified according to the WHO expert consultation for Asian populations.[23] Biochemical parameters and standard sample acceptance criteria (HBsAg, Anti-HBs, and Anti-HCV) were measured using the Architect I2000SR immunoassay system (Abbott Laboratories, USA). Metabolic syndrome was diagnosed according to the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) criteria.[24] It was defined as the presence of three or more of the following five criteria: (1) waist circumference > 90 cm (men), > 80 cm (women); (2) blood pressure >130/85 mmHg; (3) fasting triglycerides >150 mg/dL; (4) fasting high-density lipoprotein (HDL) cholesterol <40 mg/dL (men) or <50 mg/dL (women); and (5) fasting blood sugar >100 mg/dL.

PNPLA3 rs738409 Genotyping

Genomic DNA was extracted from whole blood (Toppure blood extraction kit, ABT) and quality-controlled (A260/A280 ratio 1.8–2.0) prior to analysis. The *PNPLA3* rs738409 polymorphism was genotyped using an allele-specific real-time PCR assay with SYBR Green chemistry on a QuantStudio 12K Flex system (Applied Biosystems). The primers used were: Forward C (5'-CCTTGGTATGTTCCTGCTTCATC-3'), Forward G (5'-CCTTGGTATGTTCCTGCTTCATG-3'), and Reverse (5'-CACACTTCAGAGGCCCCC-3'). Reactions were performed with 2.5 ng of DNA under standard thermal cycling conditions. Genotypes were assigned based on the cycle threshold difference (ΔCt) between the two allele-specific reactions: samples were classified as heterozygous (GC) if $\Delta Ct < 5$, wild-type (CC) if $\Delta Ct \geq 5$ and the C-allele reaction had a lower Ct, or homozygous mutant (GG) if $\Delta Ct \geq 5$ and the G-allele reaction had a lower Ct. The assay's accuracy was confirmed by 100% concordance with Sanger sequencing in a subset of 147 samples (36%), and a 100% final call rate was achieved for the entire cohort.[25]

Statistical Analysis

Statistical analysis was performed using Stata (version 14). Continuous variables were assessed for normality (Shapiro–Wilk test) and are presented as median (interquartile range, IQR); categorical variables are presented as n (%). Hardy-Weinberg equilibrium in controls was evaluated using a χ^2-test. Differences in allele and genotype frequencies between cases and controls were compared using χ^2 or Fisher's exact tests. Conditional multivariable logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for the association between the rs738409 genotype and NAFLD, adjusting for age, sex, BMI, and metabolic syndrome components. Effect modification by metabolic syndrome was assessed using the Wald test for an interaction term. The predictive performance of models was evaluated with Receiver Operating Characteristic (ROC) curve analysis. Outliers identified by the 1.5×IQR method were excluded only if deemed non-physiological measurement errors. A sensitivity analysis was conducted by re-running models without these outliers. A p-value <0.05 was considered statistically significant. Data visualizations were generated using Flourish Studio (https://flourish.studio/) and Biorender (https://www.biorender.com/).

Ethical Approval

This study was approved by the Ethical Committee of Pham Ngoc Thach University of Medicine (Decision No. 859/TĐHYKPNT-HĐĐĐ, April 20, 2023) and the Ethical Committee of Nguyen Tri Phuong Hospital (Decision No. 778/NTP-HĐĐĐ, April 27, 2023). It was conducted in accordance with the Declaration of Helsinki and ICH-GCP guidelines. Written informed consent was obtained from all participants before enrollment. Personal data were anonymized using unique identification codes, stored in a password-protected database accessible only to authorized personnel, and will be retained for five years post-study before secure deletion. Biological samples, labeled with corresponding codes, were stored at −20°C in a secure biobank and will be destroyed after five years per institutional policy. Participants were informed of their right to withdraw at any time without consequence and did not receive financial compensation.

Results

Demographic Data and Clinical Characteristics

A comprehensive overview of the characteristics of the study population, including the patient group (NAFLD group) (N =135) and the control group (N = 270), is presented in Table 1. The patient and control groups were matched for age and sex, with a nearly equivalent male-to-female ratio. Both groups exhibited a median age of 38 years, and the majority of participants were within the working-age range, with the 31–40-year age group being predominant (37.04%: data not shown). Consistent with the established pathophysiology of non-alcoholic fatty liver disease, the baseline metabolic and biochemical profiles of the NAFLD and control groups were, as anticipated, significantly different. The NAFLD cohort was characterized by a higher prevalence of obesity (BMI ≥ 25 kg/m², $p < 0.001$), metabolic syndrome (p <0.001), and elevated blood pressure ($p = 0.044$). Furthermore, this group exhibited significant alterations in blood parameters, including elevated liver transaminases (AST, ALT), fasting glucose, and a pro-atherogenic lipid profile with increased triglycerides, total cholesterol, LDL-c, and decreased HDL-c (all $p < 0.05$). These well-defined phenotypic differences confirm the appropriate classification of our study populations and provide the necessary clinical context for the primary analysis of the *PNPLA3* rs738409 variant's role.

PNPLA3 rs738409 Genotypes

In the study population, the C allele of the *PNPLA3* rs738409 variant exhibited a higher frequency in both the NAFLD (64.07%) and control (71.85%) groups, while the G allele showed a lower frequency (NAFLD: 35.93%; control: 28.15%), resulting in a statistically significant difference ($p = 0.024$). The genotype distribution of this variant was consistent with Hardy-Weinberg equilibrium in the NAFLD group ($p = 0.767$), the control group ($p = 0.482$), and the overall study population ($p = 0.361$) (Figure 1). This conformity suggests the absence of significant deviations from expected genotype frequencies, indicating that the study population is representative of the Vietnamese working-age adult population. These findings enhance the validity of subsequent genetic analyses by minimizing potential bias due to population stratification.

Table 1 Epidemiological and Clinical Characteristics of the Study Populations

Characteristics		NAFLD (N=135)	Controls (N=270)	*p*-value
Age (y.o) median (IQC)		38 (31–47)	38 (31–47)	1.0
Gender (Female) n, (%)		70 (51.85)	140 (51.85)	1.0
BMI ≥ **25** kg/m^2 n, (%)		63 (46.67)	39 (14.44)	**<0.001***
Metabolic Syndrome$^{\#}$ n, (%)		42 (31.11)	24 (8.89)	**<0.001***
Elevated blood pressure n, (%)		15 (11.11)	15 (5.56)	**0.048***
Diabetes mellitus n, (%)		5 (3.70)	3 (1.11)	0.077*
Fasting glucose (mmol/L) median (IQC)		5.14 (4.86–5.63)	5.09 (4.68–5.48)	**0.038****
Elevated fasting glucose n, (%)		36 (26.67)	58 (21.48)	0.240*
AST (U/L) median (IQC)		22.35 (18.01–27.47)	20.58 (17.80–26.44)	**0.041****
ALT (U/L) median (IQC)		23.79 (16.64–39.18)	18.29 (13.69–25.15)	**<0.001****
Triglyceride (mmol/L) median (IQC)		1.54 (1.16–2.37)	1.03 (0.68–1.67)	**<0.001****
Elevated triglyceride n, (%)		58 (42.96)	66 (24.44)	**<0.001****
Total cholesterol (mmol/L) median (IQC)		4.86 (4.10–5.40)	4.42 (3.76–5.11)	**0.001****
LDL-c (mmol/L) median (IQC)		2.94 (2.37–3.37)	2.57 (2.11–3.14)	**<0.001****
HDL-c (mmol/L) median (IQC)		1.08 (0.94–1.24)	1.17 (1.00–1.36)	**<0.001****
Reduced HDL-c n, (%)		91 (67.41)	132 (28.89)	**<0.001***
rs738409	**CC** n, (%)	58 (43.0)	145 (53.7)	0.096*
	CG n, (%)	57 (42.2)	98 (36.3)	
	GG n, (%)	20 (14.8)	27 (10.0)	

Notes: *Chi-square and Exact Fisher test; **Mann–Whitney U-test; $^{\#}$: ≥3 components by the NCEP ATP III criteria. Elevated blood pressure: ≥ 130 mmHg systolic blood pressure or ≥ 85 mmHg diastolic blood pressure or on treatment. Elevated fasting glucose: ≥ 100 mg/dL or on treatment. Elevated triglyceride: ≥ 150 mg/dL or on treatment. Reduced HDL-c: < 40 mg/dL in men, < 50 mg/dL in women or on treatment). Upper Limit of Normal (ULN): AST:40 U/L; ALT:45 U/L (Male), 33 U/L (Female) p < 0.05 is considered significant and is shown in bold.

Abbreviations: IQR, interquartile range; y.o: years old; BMI, Body Mass Index; AST, Aspartate transferase; ALT, Alanine transferase; HDL-c, cholesterol in High Density Lipoprotein.

To elucidate the association between the *PNPLA3* rs738409 variant genotype and NAFLD susceptibility, we compared genotype frequencies across various genetic models between the NAFLD and control groups (Figure 2). The G allele exhibited a significantly higher frequency in the NAFLD group (35.93%) compared to the control group (28.15%, $p = 0.024$), indicating that the G allele is associated with an increased risk of NAFLD. In the dominant model (CC vs CG+GG), the CG+GG genotype frequency was also significantly higher in the NAFLD group (57.04%) than in the control group (46.3%, $p = 0.042$), suggesting that the presence of at least one G allele confers an elevated risk of NAFLD. No statistically significant differences were observed in the recessive or additive genetic models. These findings demonstrate that the G allele of *PNPLA3* rs738409 is associated with NAFLD risk under the dominant model, which aligns with previous studies in other populations.

PNPLA3 rs738409 Variant and Its Potential for Predicting the Risk of NAFLD

To investigate the interaction between the *PNPLA3* rs738409 variant and phenotypic risk factors for NAFLD, univariate analysis under the dominant model (Table 2) revealed the following: In individuals without hypertriglyceridemia (normal triglyceride levels), the CG+GG genotype was associated with an OR of 1.746 ($p = 0.038$), demonstrating that the

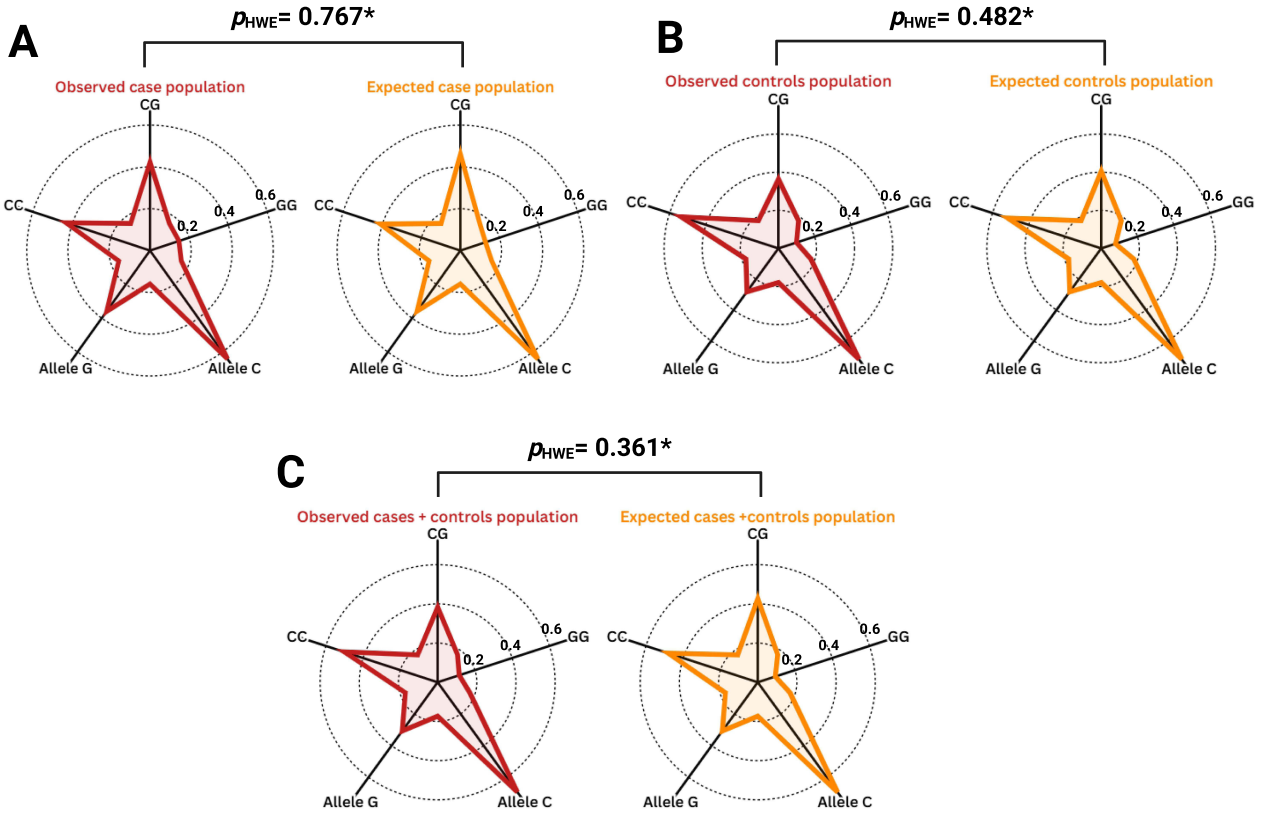

Figure 1 Hardy-Weinberg equilibrium of *PNPLA3* rs738409 in the study populations. Illustrates the frequency and distribution patterns of alleles and genotypes of the *PNPLA3* rs738409 variant in the observed and expected populations within three groups: (**A**) the NAFLD group, (**B**) the control group, and (**C**) the overall study population (NAFLD and control groups combined). The close alignment of the observed (red lines) and expected (yellow lines) distributions, as assessed by the Chi-square test, demonstrates that each group is in Hardy-Weinberg equilibrium (all p > 0.05) (all p_{HWE} > 0.05). *: Chi-square test.
Abbreviation: HWE, Hardy-Weinberg equilibrium.

G allele significantly increases the risk of NAFLD, even in the absence of elevated triglycerides. In individuals with low high-density lipoprotein cholesterol (HDL-c) levels, the CG+GG genotype exhibited an OR of 2.074 (p = 0.009), indicating a pronounced effect of the G allele in the context of dyslipidemia. No statistically significant associations were observed in individuals with elevated fasting glucose, elevated blood pressure, or obesity. Therefore, the G allele appears to increase NAFLD risk, particularly in individuals with dyslipidemia, especially those with low HDL-c levels. This suggests that the effect of the *PNPLA3* rs738409 variant may be modulated by lipid metabolism.

Conditional multivariate regression analysis, conducted on the entire study population (n = 405), with NAFLD as the dependent variable and various clinical and metabolic factors as independent variables, yielded the results presented in Figure 3. After adjusting for these covariates, the dominant genotype (CG+GG) of the *PNPLA3* rs738409 variant was not identified as an independent predictor of NAFLD (OR = 1.485, p = 0.088). Independent predictors of NAFLD included hypertriglyceridemia, and obesity. Elevated fasting glucose, low HDL-c and elevated blood pressure did not achieve statistical significance. Elevated blood pressure showed a significant difference in univariate analysis but did not remain significant in the multivariate model, possibly due to its weaker impact compared to other metabolic factors. These findings suggest that in the presence of metabolic risk factors, the association between the *PNPLA3* rs738409 variant and NAFLD is attenuated. Obesity and dyslipidemia appear to be the primary drivers of NAFLD in this population, indicating that environmental and clinical factors may exert a stronger influence than genetic predisposition.

To quantify the predictive accuracy of the *PNPLA3* rs738409 variant for NAFLD, ROC curve analysis was conducted using the dominant genetic model, with results presented in Figure 4. The model employing only the dominant genotype yielded an area under the curve (AUC) of 0.5537, indicating poor discrimination between NAFLD and control groups. In

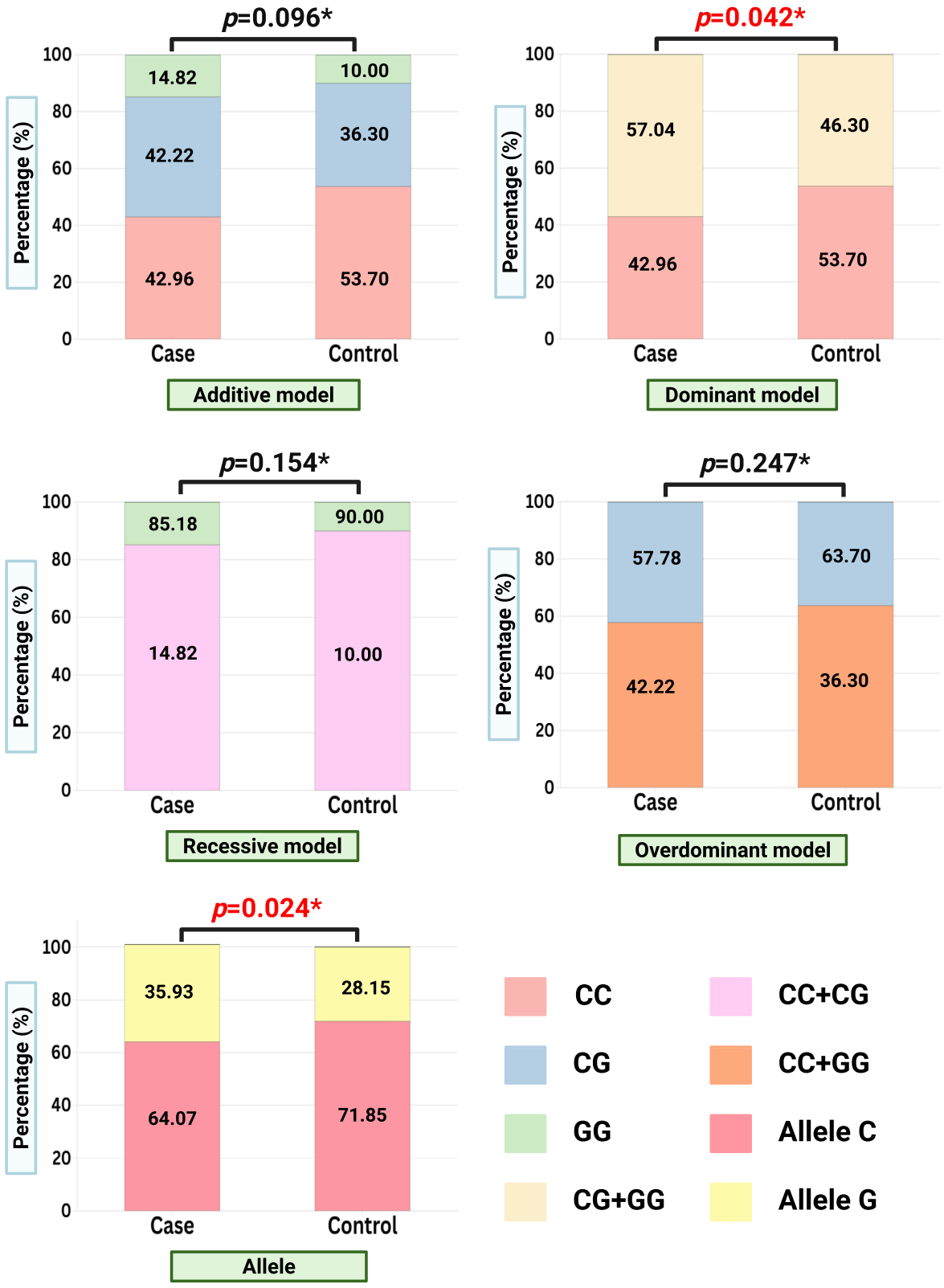

Figure 2 Distribution of *PNPLA3* rs738409 genotypes and allele frequencies in NAFLD patients and controls. Illustrates the comparative analysis of genotype and allele frequencies between the case and control populations under five different genetic inheritance models: additive, dominant, recessive, overdominant, and allelic. Statistically significant differences between the groups were identified in the dominant model ($p = 0.042$) and the allele model ($p = 0.024$). The other models did not show a significant association. An asterisk (*) indicates the p-value was calculated using the chi-square test; p-values shown in red indicate statistical significance ($p < 0.05$).

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Table 2 Association of *PNPLA3* rs738409 Variant and Clinical Risk Factors of NAFLD in a Dominant Genetic Model

Subgroup (NAFLD vs ctrl.)	Dominant Model (CC vs CG+GG)		
	OR	95% CI	p-value*
EFG+	1.435	0.6222–3.310	0.396
EFG-	1.578	0.976–2.555	0.062
EBP+	4.125	0.883–19.273	0.063
EBP-	1.396	0.903–2.159	0.132
ETG+	1.158	0.571–2.351	0.684
ETG-	1.746	1.027–2.969	**0.038**
RHDL+	2.074	1.202–3.581	**0.009**
RHDL-	0.909	0.460–1.796	0.784
OB+	1.498	0.671–3.346	0.324
OB-	1.474	0.866–2.509	0.153

Notes: EFG: Elevated fasting plasma glucose (≥ 100 mg/dL); EBP: Elevated blood pressure (≥ 130 mmHg systolic blood pressure or ≥ 85 mmHg diastolic blood pressure or on treatment.); ETG: Elevated plasma Triglyceride (≥ 150 mg/dL); RHDL: Reduced plasma HDL-c (< 40 mg/dL in men, < 50 mg/dL in women); OB: obesity class is BMI ≥25.0 kg/m^2 (with the Asia-Pacific classification); "+": with; "-": no; Ctrl: control group; OR: Odds ratio; CI: confident interval; *Chi-square test; $p < 0.05$ is considered significant and is shown in bold.

contrast, the model utilizing phenotypic factors (BMI and TG) achieved an AUC of 0.7795, demonstrating substantial predictive performance. The combined model, incorporating both genotype and phenotype, resulted in an AUC of 0.7840, representing a marginal increase in predictive accuracy compared to the phenotype-only model. These findings suggest that the addition of the *PNPLA3* rs738409 variant does not significantly improve the predictive capacity for

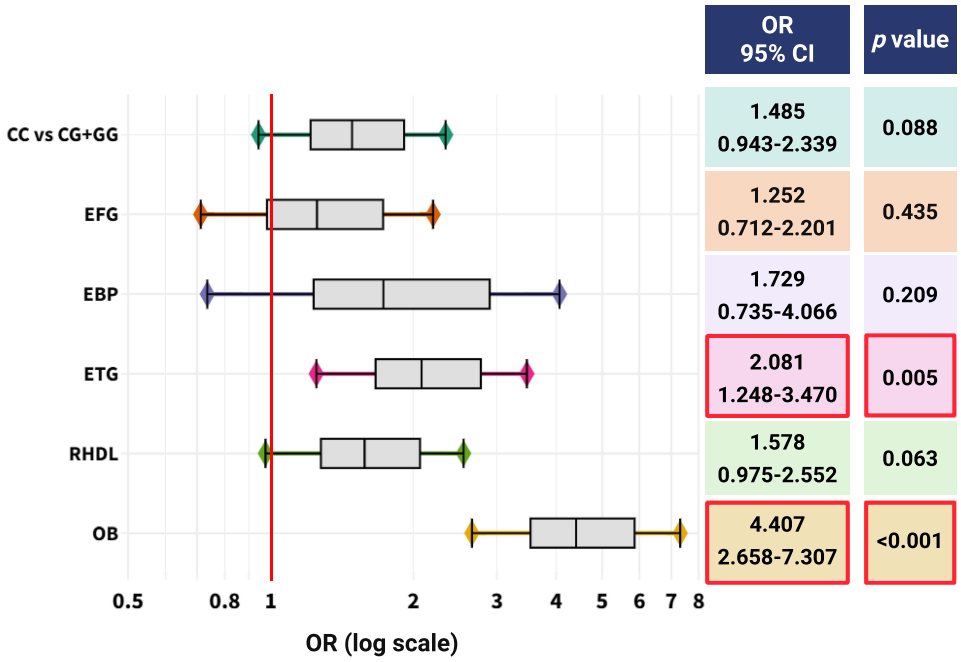

Figure 3 Multivariable logistic regression Analysis: Predictors of NAFLD. EFG: Elevated fasting plasma glucose (≥ 100 mg/dL); EBP: Elevated blood pressure (≥ 130 mmHg systolic blood pressure or ≥ 85 mmHg diastolic blood pressure or on treatment.); ETG: Elevated plasma Triglyceride (≥ 150 mg/dL); RHDL: Reduced plasma HDL-c (< 40 mg/dL in men, < 50 mg/dL in women); OB: obesity class is BMI ≥25.0 kg/m^2 (with the Asia-Pacific classification); OR: Odds ratio; CI: confident interval; *Wald test; $p < 0.05$ is considered significant and is shown in red box.

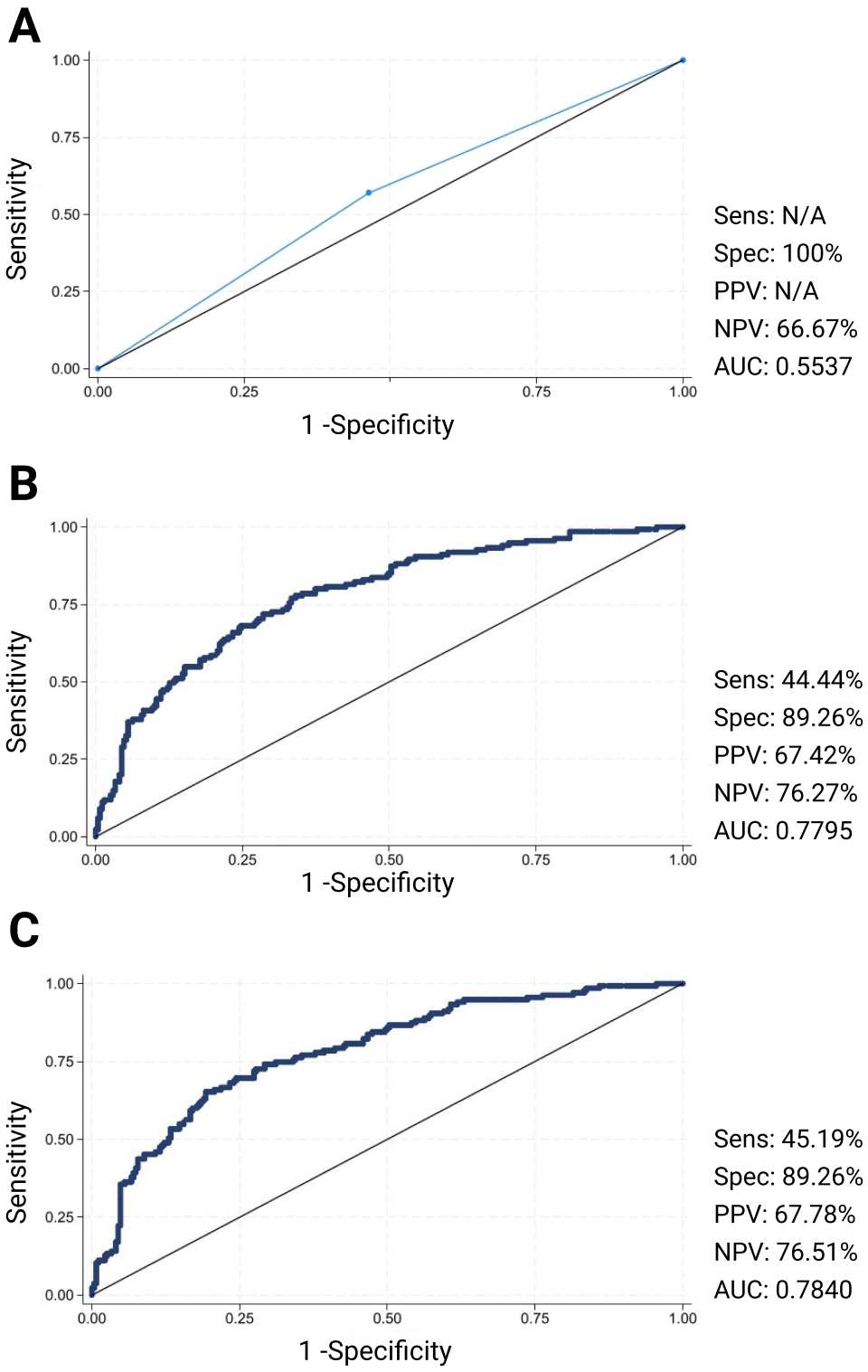

Figure 4 ROC curve analysis for NAFLD prediction models (**A**) Dominant model (CC vs CG+GG) only. (**B**) Phenotypes (BMI value + TG level) only. (**C**) Dominant model (CC vs CG+GG) combined with phenotypes (BMI value + TG level). HDL-c (< 40 mg/dL in men, < 50 mg/dL in women).

Abbreviations: ROC, Receiver operating characteristic; BMI, Body mass index; TG, Triglyceride; RHDL, Reduced plasma; AUC, Area Under the Curve; Sens, sensitivity; Spec, specificity; PPV, positive predictive value; NPV, negative predictive value; N/A, not applicable.

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NAFLD in this population. Consequently, the *PNPLA3* rs738409 variant exhibits limited independent predictive utility and provides minimal enhancement when integrated with clinical indicators.

To assess the robustness of our findings, a sensitivity analysis was performed using a bootstrap procedure with 1000 replications on our conditional logistic regression model. In each iteration, 90% of the matched pairs were resampled to calculate the Bias-Corrected (BC) 95% confidence intervals (CIs) for the odds ratios (ORs). The analysis confirmed that associations for high triglycerides (OR = 4.14, 95% BC CI: 2.63–33.37) and BMI over 25 (OR = 4.57, 95% BC CI: 1.28–13.02) remained statistically significant. Interestingly, the dominant genotype model also emerged as significant in this sensitivity analysis (OR = 2.22, 95% BC CI: 1.19–25.36). However, its exceptionally wide confidence interval with a lower bound close to 1.0 suggests that while a genetic contribution is present, its role may not be strong, and its effect is highly variable.

Discussion

Non-alcoholic fatty liver disease constitutes a growing global health burden, characterized by increasing incidence and severe complications, including cirrhosis, hepatocellular carcinoma, and a substantial demand for liver transplantation. The intricate pathogenesis, which remains only partially understood, is widely acknowledged to involve an interaction between genetic and environmental factors. In clinical practice, the diagnosis of NAFLD relies on imaging or biopsy evidence of hepatic steatosis, along with ruling out alcohol consumption and other concomitant chronic liver diseases.[9] The paucity of individualized genetic information has created a gap between the mechanistic understanding of pathogenesis and the phenotype-based approach to screening and diagnosis. Consequently, patients are often diagnosed at a late stage, with limited data available for developing treatment and prognostic strategies in the era of personalized medicine.[20]

Multiple genetic variants in the *PNPLA3, TM6SF2, MBOAT7, SAMM50*, and *GCKR* genes, which encode proteins regulating hepatic lipid metabolism, have been implicated in NAFLD progression.[26,27] The G allele of the rs738409 C>G (I148M) variant in the *PNPLA3* gene is not only highly prevalent but is also recognized as a "risk allele" linked to hepatic fat content and adverse NAFLD outcomes, correlating with all stages of disease progression.[18] Consequently, genotyping this variant has been incorporated into several recent practice guidelines for the diagnostic and therapeutic management of NAFLD.[28–30]

The link between *PNPLA3* rs738409 and NAFLD can be attributed to its biological function. A C-to-G substitution at nucleotide position 43928847 in the *PNPLA3* gene leads to the replacement of isoleucine with methionine at amino acid position 148, giving rise to the *PNPLA3* I148M variant. This loss-of-function variant disrupts the processing, remodeling, and secretion of very low-density lipoprotein (VLDL), resulting in the accumulation of unsaturated fatty acids in diacylglycerols and triglycerides within hepatocytes and ultimately triggering hepatic steatosis.[31,32] Unlike the wild-type PNPLA3 protein, which undergoes rapid degradation, the *PNPLA3* I148M variant - lacking lipase activity - accumulates in the cytoplasm, disrupting lipid droplet remodeling and de novo PNPLA3 synthesis. This effect is particularly pronounced in individuals with reduced HDL-c levels, given HDL-c's role in reverse lipid transport from the liver to peripheral tissues. A reduction in HDL-c impairs the elimination of excess fat, further exacerbating hepatic fat accumulation in G allele carriers. This mechanism elucidates the heightened NAFLD risk in individuals with dyslipidemia.[33,34]

This study demonstrates an association between the *PNPLA3* rs738409 variant, particularly the G allele, and susceptibility to NAFLD in a Vietnamese working-age adult population (18–60 years). The G allele was significantly more prevalent in the NAFLD group (35.93%) than in the control group (28.15%, $p = 0.024$), and in the dominant model (CC vs CG+GG), individuals with CG+GG genotypes exhibited an increased NAFLD risk (OR = 1.433, $p = 0.042$). Notably, this association was more pronounced in those with reduced HDL-c (OR = 2.074, $p = 0.009$), highlighting a potential metabolic context dependency. These findings not only reinforce previous studies in other populations but also highlight the significance of rs738409 in NAFLD pathogenesis within a Southeast Asian cohort.

According to allele frequency data compiled by the National Center for Biotechnology Information (NCBI) from dbGap, a marked difference in the allele distribution of the rs738409 variant is observed across ethnic groups. In African, European, and Latin American populations, the G allele is less frequent than the C allele, with G allele frequencies of

0.09, 0.21, and 0.27, respectively. In contrast, Asian populations exhibit a higher G allele frequency than other continents, averaging 0.36 (ranging from 0.20 to 0.40).[26,35] In this study, the G allele frequencies were 0.36 and 0.28 in the patient and control groups, respectively. When compared to other Asian populations, the G allele frequencies in the Vietnamese patient and control groups appear lower.[36–38] This difference may reflect unique genetic characteristics of the Vietnamese population, particularly in younger age groups, combined with environmental factors such as urbanized lifestyles and energy-dense diets, which may amplify the impact of rs738409 relative to neighboring Asian countries. Although previous studies have consistently reported a strong association between rs738409 and NAFLD,[18,36–38] findings from Vietnam suggest that while this variant plays a significant role, its effect is not predominant compared to metabolic factors. As discussed further below, the effect of rs738409 becomes more pronounced in subgroups with specific clinical characteristics. These findings suggest that while the genetic risk conferred by rs738409 in Vietnamese individuals may be moderate, the high prevalence of adverse environmental factors in the working-age population may amplify its impact. These observations underscore the importance of population-specific studies on rs738409 to refine our understanding of NAFLD risk.

A key observation is that rs738409 did not remain an independent risk factor in multivariable analysis (OR = 1.485, $p = 0.088$), in contrast to the univariate results (OR = 1.433, $p = 0.042$). Several factors may explain this finding. First, the strong impact of metabolic factors such as obesity (OR = 4.407, $p < 0.001$) and elevated triglycerides (OR = 2.081, $p = 0.005$) may have masked the effect of the genetic variant, as these are primary risk factors in the pathogenesis of NAFLD, directly contributing to hepatic lipid accumulation. Although the rs738409 variant influences triglyceride hydrolysis, its effect appears to be overshadowed by these metabolic factors in the multivariable model, suggesting that its influence may be more context-dependent rather than a direct determinant. Second, the relatively small sample size (N = 405) may limit the statistical power to detect complex interactions or subtle effects of the rs738409 variant, particularly in conditional multivariate analyses,[39] especially in a population with a moderate G allele frequency (28.15% in the control group). Furthermore, other potential confounding factors (eg, smoking, stress levels, and comorbidities) were not accounted for, potentially influencing the accuracy of the association between rs738409 and NAFLD.[40] These findings suggest that this variant may play a supporting role, dependent on the metabolic context, rather than serving as an independent determinant in the Vietnamese population.

ROC curve analysis revealed that the *PNPLA3* rs738409 variant alone had limited predictive accuracy for NAFLD (AUC = 0.5537), indicating poor discriminatory power as a standalone marker. However, when combined with metabolic factors such as BMI, triglycerides, and HDL-c, the AUC improved a little to 0.7840, suggesting a moderate predictive ability. This aligns with the multivariable findings, where metabolic factors overshadowed rs738409's independent effect, reinforcing its role as a supportive rather than primary risk factor.[18] The enhanced performance in the combined model highlights the potential of integrating genetic and metabolic data for risk stratification in Vietnamese working-age adults.[20]

Several recent studies further contextualise our findings. Using NHANES 2017–2020 and 2021–2023 data, Bril and colleagues reported that US MASLD prevalence declined from 37.6% to 32.5%, but rates of clinically significant fibrosis and cirrhosis increased, especially among individuals with diabetes (27.4% had significant fibrosis and 10.2% cirrhosis).[41] Globally, MASLD prevalence increased from 25% to 38% between 1990–2006 and 2016–2019; among people with diabetes, prevalence rose from 56% to 69%.[11,42] These trends highlight that metabolic dysfunction drives progression to advanced liver disease even when overall MASLD prevalence plateaus. In our study, participants with elevated triglycerides or obesity had odds ratios exceeding 2.0 and 4.0, respectively, supporting the dominant influence of metabolic factors.

In the working-age population, prevalent environmental factors such as high-fat diets, sedentary lifestyles, and chronic stress may interact with the G allele, thereby increasing NAFLD risk through metabolic and inflammatory pathways.[40,43] Individuals carrying the CG or GG genotype are more prone to hepatic fat accumulation, particularly in the presence of unhealthy lifestyle factors. These research findings support the aforementioned theories. Therefore, disease management strategies for this population should prioritize controlling environmental and metabolic factors rather than relying solely on genetic risk, highlighting the importance of personalized prevention measures. To enhance immediate applicability, targeted approaches could prioritize high-risk groups, such as working-age adults with elevated

BMI (>25 kg/m²), who exhibit a higher NAFLD prevalence (46.67% in our cohort). Practically, rs738409 genotyping could be incorporated into routine health check-up programs for employees in urban settings, enabling early identification and tailored lifestyle interventions. Such strategies, combining genetic screening with metabolic risk assessment, could optimize resource allocation and improve outcomes, particularly in Vietnam's workforce.

This study possesses several noteworthy strengths. First, investigating Vietnamese adults (18–60 years) carries significant clinical and epidemiological implications, contributing to the development of NAFLD prevention and treatment strategies for the core working population, ultimately reducing disease burden and healthcare costs. This study is among the first to evaluate the role of rs738409 in NAFLD among Vietnamese individuals, contributing valuable insights into genetic diversity in Southeast Asia - a region with limited data compared to Western populations and other East Asian countries, such as China and Japan. Second, the age- and sex-matched case-control design mitigates bias from demographic confounding factors. Third, genotyping using real-time PCR and Sanger sequencing ensures high accuracy. Fourth, this study examined various genetic models (additive, dominant, recessive, overdominant) to evaluate the association between rs738409 and NAFLD, offering a comprehensive perspective on the variant's impact. Furthermore, this study explored interactions between genotype and clinical factors, such as dyslipidemia, elucidating the role of rs738409 in various metabolic contexts. These analyses are supported by the observation that the rs738409 variant conforms to Hardy-Weinberg equilibrium in both the patient and control groups, as well as the overall study population, suggesting minimal genetic bias and a representative sample.

Despite possessing strengths, important limitations deserve emphasis. First, the case–control design precludes assessment of causal relationships or temporal progression to MASLD complications. Second, our sample size may have limited power to detect gene–environment interactions or modest effect sizes, as indicated by the wide confidence intervals for genotype associations. Third, environmental and lifestyle variables (diet, physical activity, socioeconomic status) were not comprehensively measured, potentially confounding the association between genetics and NAFLD. Finally, hepatic and extra-hepatic outcomes such as fibrosis stage, cardiovascular disease or kidney function were not evaluated; therefore, the clinical implications of rs738409 for disease progression remain undetermined.

Future studies should increase the sample size to at least 1,000 participants, recruited from multiple medical centers to reduce selection bias and enhance population representativeness. A long-term cohort study should also be conducted to determine the predictive role of rs738409 and assess its impact over time in the Vietnamese population while assessing genotype-environment interactions, including diet, physical activity, and stress, in the working-age population.

Conclusion

The rs738409 variant of the *PNPLA3* gene contributes to an increased risk of NAFLD in the Vietnamese working-age population, particularly in individuals with dyslipidemia, such as low HDL-c. However, its impact is not strong enough to serve as an independent predictor, and metabolic factors, including obesity and elevated plasma triglycerides, remain the primary determinants of the disease. This study lays the groundwork for personalized strategies in the prevention and treatment of NAFLD, while also underscoring the need to integrate MASLD/MAFLD definitions and multisystem outcomes in future research. In clinical practice, these results reinforce current guidelines that prioritise assessment and management of metabolic risk factors and caution against routine genetic testing, providing a basis for further investigations into the genetic determinants of NAFLD within this population.

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