Original article

PNPLA3 and SAMM50 variants are associated with lean nonalcoholic fatty liver disease in Asian population

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ABSTRACT

Introduction and Objectives: Lean adults with nonalcoholic fatty liver disease (NAFLD) have a higher risk of metabolic syndrome than lean controls. The study aimed to investigate the clinical and genetic features of lean NAFLD which remain unclear in Asian populations.
Materials and Methods: This was a genetic cohort study conducted in the HAVO Health Exam Clinic in 2020 −2021 in Taiwan. Adults with a body mass index less than 24 kg/m^2 were enrolled. Fatty liver was defined by ultrasonography. The candidate gene approach was based on the library of the NHGRI-EBI website. After removing duplication and nonsignificant variants, rs738409 in the PNPLA3 gene and rs3761472 in the SAMM50 gene were chosen. Multiple logistic regression models and receiver operating characteristic (ROC) curves were used.
Results: A total of 1652 lean controls and 602 lean NAFLD patients were enrolled. The average age was 43.8 ± 11.5 years. Lean NAFLD subjects were older and had a higher percentage of metabolic syndrome (case vs. control: 10.5 % vs. 1.5 %). The GG genotypes of PNPLA3 rs12483959 (OR: 3.06; 95% CI: 2.15−4.37) and SAMM50 rs3761472 (OR: 2.90; 95% CI: 2.04−4.14) had a higher risk of fatty liver after adjusting for BMI and metabolic syndrome. The areas under the ROC curve for PNPLA3 rs738409 and SAMM50 rs3761472 in the detection of lean NAFLD were 0.859 (95%CI: 0.841, 0.877) and 0.860 (95%CI: 0.843, 0.877), respectively.
Conclusions: PNPLA3 rs738409 and SAMM50 rs3761472 gene polymorphisms are associated with a higher risk of fatty liver in lean individuals independent of BMI and metabolic syndrome in Asian populations.

1. Introduction

Non-Alcoholic Fatty Liver Disease (NAFLD) is a condition characterized by the accumulation of fat in the liver cells in individuals who consume little to no alcohol, often associated with metabolic syndrome, obesity, and insulin resistance. It ranges from simple steatosis to more severe forms like non-alcoholic steatohepatitis (NASH), which can lead to liver fibrosis, cirrhosis, and liver cancer. [1] Nonalcoholic fatty liver disease (NAFLD) is an increasing issue that affects approximately 25 % of the global population [2]. As the most common chronic liver disease in the world, NAFLD is highly related to obesity, metabolic syndrome, type 2 diabetes mellitus and cardiovascular disease [2]. However, approximately 10−20 % of patients with NAFLD have a normal body mass index (BMI), a noticeable subset we referred to as lean NAFLD [3]. Despite having a normal BMI, patients with lean NAFLD are considered metabolically unhealthy and have higher fasting glucose, insulin resistance, blood pressure, and dyslipidemia levels than lean controls [4]. Furthermore, lean NAFLD patients share a similar outcome with overweight-obese NAFLD patients in overall survival even if they do not eventually progress to overweight

or obese [5]. Therefore, growing evidence indicates that lean NAFLD is a unique pathophysiological entity, but the aspects of clinical, histological and genetic heterogeneity are still unclear [6,7].

There are numerous gene studies to recognize lean NAFLD. Genome-wide association studies have indicated that single nucleotide polymorphisms (SNPs) in patatin-like phospholipase domain-containing 3 (PNPLA3) are the most extensively elucidated SNPs related to lean NAFLD [8,9]. PNPLA3 encodes a protein known as adiponutrin that is expressed in adipocytes and hepatocytes. Moreover, this protein has lipolytic and lipogenic properties that play a critical role in lipid metabolism [10]. The PNPLA3 rs738409 variant seemed to be more common in Japanese, Hong Kong and Sri Lankan patients with lean NAFLD than in those with obese NAFLD, but there was no difference in Western countries [11−13]. In addition, the presence of the PNPLA3 rs738409G allele has been associated with an earlier presentation of NAFLD in Taiwanese children [14]. Therefore, we hypothesized that PNPLA3 variants were independently associated with NAFLD as a risk factor for lean adults in the Taiwanese population. Sorting and assembly machinery component 50 homolog (SAMM50) and the well-known PNPLA3 are both located on chromosome 22q13. The SAMM50 gene encodes a protein that resides in the outer membrane of mitochondria and is involved in mitochondrial dynamics and biogenesis. Polymorphisms in SAMM50 have been associated with various metabolic diseases, such as non-alcoholic fatty liver disease (NAFLD) and obesity [15]. In Asian studies, SAMM50 polymorphisms contributed to the occurrence and severity of fatty liver in the Chinese Han, Korean and Japanese populations [16,17]. To date, no study has demonstrated the association of SAMM50 in lean subjects with and without NAFLD.

Accordingly, a case−control study was conducted in healthy and lean adults with and without NAFLD who underwent health exams and genotyping. We aimed to examine the different risks for lean NAFLD with and without PNPLA3 and SAMM50 variants in lean NAFLD patients compared with lean controls.

2. Materials and Methods

2.1. Study population

This was a case–control study conducted in the HAVO Health Exam Clinic. The HAVO database included individuals aged more than 20 years who received a self-paid health check-up and SNP genotyping from Jan 2020 to the end of 2021. All subjects completed standardized questionnaires through individual interviews. The health survey questionnaire asked participants questions regarding socio-demographics, lifestyles and medical history. The health check-up included physical exams, blood analyses, and abdominal ultrasonography. Informed consent forms were signed. The study was approved by the Institutional Review Board of National Taiwan University Hospital (IRB NO. 202110005RIND).

For the lean NAFLD study population, the exclusion criteria were 1) body mass index $\geq$ 24 kg/m^2; 2) excessive alcohol use, which was defined as drinking more than 30 g of alcohol daily for men and 20 g for women; 3) chronic liver diseases, which included chronic hepatitis, autoimmune, drug-induced, vascular, and inherited hemochromatosis, or Wilson disease. 4) inability to undergo an abdominal ultrasound examination; and 5) incomplete SNP genotyping. The content of the physical examination included weight and height, which were measured by a standard electronic scale and stadiometer, and blood pressure (BP), which was measured by an electronic sphygmomanometer. Waist circumference was measured horizontally through the middle point between the upper border of the iliac bones and the lower border of the ribs. The content of the blood test included fasting glucose, triglycerides and high-density lipoprotein cholesterol (HDL-C). Abdominal ultrasonography was performed by trained physicians. Fatty liver was binarily defined by the presence or absence of liver-kidney contrast. The Ministry of Health and Welfare in Taiwan has established a BMI cut-off of 24 kg/m^2 for overweight and 27 kg/m^2 for obesity recently [18,19]. We have consistently applied the BMI cut-off of 24 kg/m^2 in Taiwan to define lean and overweight-obese individuals. [20]. Participants were considered to have metabolic syndrome if they met $\geq$3 of the following criteria: waist circumference $\geq$90 cm in men or $\geq$80 cm in women; serum triglycerides $\geq$ 150 mg/dL; HDL-C < 40 mg/dL in men or <50 mg/dL in women; systolic BP $\geq$ 130 and/or diastolic BP $\geq$ 85 mm Hg; and fasting glucose $\geq$100 mg/dL. Participants with medications for diabetes, hypertension or hyperlipidemia were sorted into the group which met the criteria for fasting glucose $\geq$100 mg/dL, BP$\geq$130/85 mmHg or serum TG $\geq$150 mg/dL respectively.

2.2. Selection of single nucleotide polymorphisms

We searched candidate genes and NAFLD-related SNPs on the website of the most up-to-date SNPs reported in the publications of the National Human Genome Research Institute (NHGRI) and the European Bioinformatics Institute (EMBL-EBI) (Supplement A and B). The website of NHGRI-EBI Catalog of Published Genome-Wide Association Studies [21] collects a total of 67 documented SNPs in PNPLA3 and 12 SNPs in SAMM50 which were found to be related to NAFLD. From the HAVO database, a total of 1652 lean controls and 602 lean NAFLD patients were extracted by criteria. Global Screening Array-24 v1.0 BeadChip (Infinium, California, USA) was then used for genotyping. The basic microarray technical data of the Asian Screening Array were downloaded from the Illumina official website [22]. The main sources of ASA chips were from East Asian and Southeast Asian populations, such as China, Japan, South Korea, Mongolia, and Singapore. A total of more than 9000 subjects were enrolled, and whole-gene sequencing data were obtained. A total of approximately 642,824 SNPs were screened. The SNP genotyping panel for replication analysis was created using the Illumina VeraCode Genotyping assay. Data analyses of the custom panel results were conducted with Illumina GenomeStudio. The accuracy of the genotyping panel was verified using five duplicate samples with the Illumina HumanOmni-Quad BeadChip. Samples with a call rate below 0.99, SNPs with more than 2 % missing data, a Hardy-Weinberg P-value less than 0.01, or a minor allele frequency below 0.05 were excluded. For detailed methodology, please refer to previously published paper [23].

After matching 67 SNPs in PNPLA3 and 12 SNPs in SAMM50 to the ASA chip, we excluded 60 SNPs in the PNPLA3 gene and 8 SNPs in the SAMM50 gene. Then, we excluded one SNP for duplication and statistical non-significance and five SNPs in PNPLA3 and 2 SNPs in SAMM50 that had high collinearity (Pearson correlation coefficient > 0.95). As a result, rs738409 in PNPLA3 and rs3761472 in SAMM50 entered further statistical analyses. The flow chart of SNP selection for lean NAFLD is shown in Fig. 1.

2.3. Statistical analysis

Data are presented as the mean±SD for continuous variables and number (percentage) for categorical variables. Differences between the groups were examined using the chi-squared test for categorical variables and Student's t-test or one-way analysis of variance (ANOVA) for continuous variables. Multivariate logistic regression analyses were performed to estimate the relationship between the odds of having lean NAFLD in relation to PNPLA3 rs738409 and SAMM50 rs3761472 after adjustment for age, sex, BMI and metabolic factors (waist circumference, fasting glucose, systolic BP, diastolic BP, triglycerides and HDL-C). We performed receiver operating characteristic (ROC) analysis to determine the diagnostic performance of PNPLA3 rs738409 and SAMM50 rs3761472 for lean NAFLD. All analyses were performed using SPSS statistical software (V.17, SPSS,
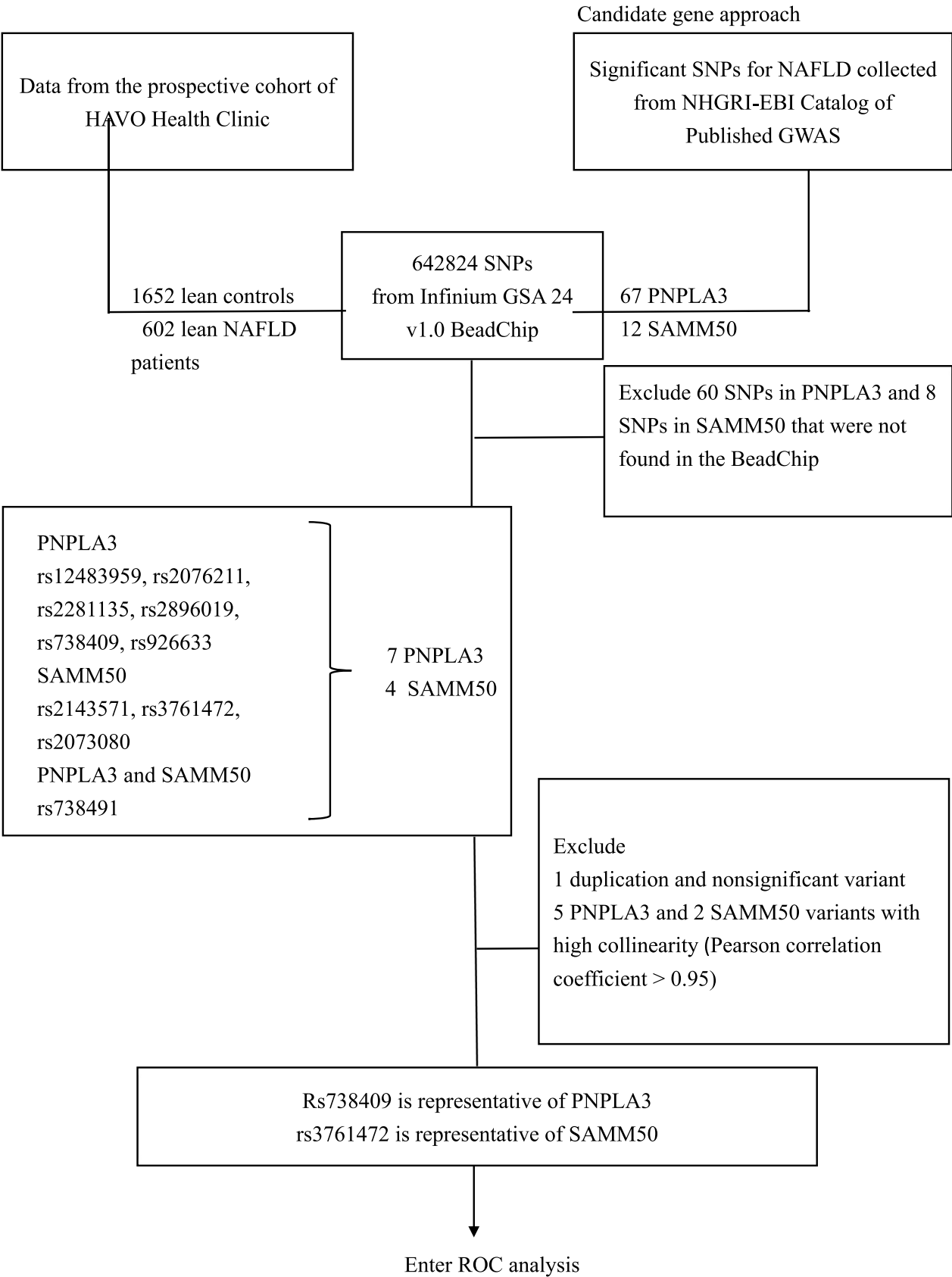
Candidate gene approach

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┌─────────────────────────────┐          ┌──────────────────────────────┐
│ Data from the prospective   │          │ Significant SNPs for NAFLD   │
│ cohort of                   │          │ collected from NHGRI-EBI     │
│ HAVO Health Clinic          │          │ Catalog of Published GWAS    │
└─────────────────────────────┘          └──────────────────────────────┘
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┌──────────────────────────┐
│    642824 SNPs           │
1652 lean controls   │ from Infinium GSA 24     │   67 PNPLA3
602 lean NAFLD       │ v1.0 BeadChip            │   12 SAMM50
patients             └──────────────────────────┘
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┌───┐
│ Exclude 60 SNPs in PNPLA3 and 8   │
│ SNPs in SAMM50 that were not   │
│ found in the BeadChip   │
└───┘

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┌────────────────────────────────────────┐
│ PNPLA3                                  │
│ rs12483959, rs2076211,                  │
│ rs2281135, rs2896019,                   │
│ rs738409, rs926633            7 PNPLA3  │
│ SAMM50                        4  SAMM50 │
│ rs2143571, rs3761472,                   │
│ rs2073080                               │
│ PNPLA3 and SAMM50                       │
│ rs738491                                │
└────────────────────────────────────────┘
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┌───┐
│ Exclude   │
│ 1 duplication and nonsignificant variant   │
│ 5 PNPLA3 and 2 SAMM50 variants with   │
│ high collinearity (Pearson correlation   │
│ coefficient > 0.95)   │
└───┘

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┌───────────────────────────────────────────┐
│ Rs738409 is representative of PNPLA3       │
│ rs3761472 is representative of SAMM50      │
└───────────────────────────────────────────┘
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Enter ROC analysis

Fig. 1. Flow chart of SNP selection for lean NAFLD. Data was gathered from the HAVO Health Clinic cohort, which included 1652 lean controls and 602 lean NAFLD patients. Initially, 642,824 SNPs were identified using the Infinium GSA 24 v1.0 BeadChip. Significant SNPs were collected from the NHGRI-EBI Catalog of Published GWAS, resulting in the examination of 67 PNPLA3 and 12 SAMM50 SNPs. SNPs with duplications, non-significance, or high collinearity (Pearson correlation coefficient $\geq$ 0.95) were excluded. This process resulted in the final representative SNPs: rs738409 for PNPLA3 and rs3761472 for SAMM50. These representative SNPs were then used in the ROC analysis to evaluate their predictive value for lean NAFLD.

Table 1
Basic characteristics and biochemical profiles of the study population.

	Lean Control (n = 1652)	Lean NAFLD (n = 602)	P value
Male (%)	733 (44.4)	397 (65.9)	<0.001
Age (years)	42.0 ± 11.4	48.6 ± 10.3	<0.001
Smoking (%)	332 (20.4)	202 (33.9)	<0.001
BMI (kg/m^2)	21.0 ± 1.83	22.6 ± 1.1	<0.001
WC (cm)	74.8 ± 6.3	81.4 ± 5.0	<0.001
SBP (mmHg)	114.3 ± 12.1	119.5 ± 12.9	<0.001
DBP (mmHg)	70.0 ± 9.1	74.3 ± 9.4	<0.001
Glu AC (mg/dL)	87.5 ± 13.8	95.6 ± 24.1	<0.001
TCHO (mg/dL)	190.6 ± 35.9	201.4 ± 34.7	<0.001
LDL (mg/dL)	120.8 ± 34.0	134.7 ± 33.6	<0.001
TG (mg/dL)	83.7 ± 40.9	136.5 ± 88.2	<0.001
HDL (mg/dL)	59.0 ± 13.8	49.6 ± 11.7	<0.001
MetS (%)	25 (1.5)	63 (10.5)	<0.001

Abbreviations: BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP: diastolic blood pressure; TCHO, total cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol.

Chicago, Illinois, USA) and R software (R-4.2.2). A p value of <0.05 was considered to statistical significance.

2.4. Ethical statement

Informed consent forms were signed, and the study was approved by the Institutional Review Board of National Taiwan University Hospital (IRB NO. 202110005RIND).

3. Results

3.1. Baseline characteristics

A total of 1652 lean controls and 602 lean NAFLD patients were enrolled. The average age was 43.8 ± 11.5 years, and 1130 (50.1 %) patients were male (Table 1). In the lean NAFLD group, the subjects were older, and the percentage of metabolic syndrome was higher than that in the lean control group (case vs. control: 10.5 % vs. 1.5 %). Waist circumference, systolic BP, diastolic BP, fasting glucose and triglycerides were significantly higher and HDL-C was significantly lower in lean NAFLD patients than in lean controls.

3.2. Distribution of genotypes in lean subjects

The distribution of PNPLA3 rs738409 (CC/CG/GG) and SAMM50 rs3761472 (AA/AG/GG) in lean NAFLD patients differed significantly from that in lean controls. (Table 2A) In subjects with the PNPLA3 rs738409 CC/CG/GG genotypes, 193 (22.6 %), 216 (26.1 %)

Table 2A
Correlation between genotype at locus rs738409 (representative of PNPLA3 gene).

	CC (N = 853)	CG (N = 827)	GG (N = 341)	P value
Male (%)	421 (49.4)	426 (51.5)	160 (46.9)	0.407
Age (years)	43.9 ± 11.6	43.3 ± 11.2	44.2 ± 11.5	0.362
Smoking (%)	201 (24.0)	204 (24.8)	75 (22.5)	0.868
BMI (kg/m^2)	21.4 ± 1.8	21.5 ± 1.8	21.4 ± 1.8	0.746
WC (cm)	76.5 ± 6.8	76.8 ± 6.7	76.4 ± 6.5	0.612
SBP (mmHg)	115.6 ± 12.6	115.8 ± 12.3	115.0 ± 12.4	0.771
DBP (mmHg)	71.2 ± 9.3	71.4 ± 9.5	70.8 ± 8.9	0.671
Glu AC (mg/dL)	89.3 ± 16.1	90.0 ± 17.7	89.8 ± 20.2	0.809
TCHO (mg/dL)	192.4 ± 34.1	194.1 ± 36.1	191.3 ± 39.0	0.544
LDL (mg/dL)	123.4 ± 32.4	126.1 ± 34.9	122.0 ± 36.5	0.116
TG (mg/dL)	99.4 ± 71.7	95.0 ± 55.5	96.5 ± 55.8	0.231
HDL (mg/dL)	56.5 ± 14.2	56.4 ± 13.5	56.5 ± 13.4	0.821
MetS (%)	37 (4.4)	34 (4.1)	11 (3.3)	0.563
Fatty liver (%)	193 (22.6)	216 (26.1)	123 (36.1)	<0.001

Table 2B
Correlation between genotype at locus rs3761472 (representative of SAMM50 gene).

	AA (861)	AG (N = 1056)	GG (N = 337)	P value
Male (%)	425 (49.4)	545 (51.6)	160 (47.5)	0.354
Age (years)	43.8 ± 11.6	43.6 ± 11.3	44.2 ± 11.7	0.670
Smoking (%)	195 (23.0)	266 (25.5)	73 (22.2)	0.330
BMI (kg/m^2)	21.4 ± 1.8	21.5 ± 1.8	21.4 ± 1.7	0.896
WC (cm)	76.4 ± 6.7	76.7 ± 6.7	76.5 ± 6.5	0.554
SBP (mmHg)	115.6 ± 12.5	116.0 ± 12.6	115.0 ± 12.4	0.421
DBP (mmHg)	71.2 ± 9.3	71.3 ± 9.5	70.6 ± 8.9	0.509
Glu AC (mg/dL)	89.1 ± 16.1	90.2 ± 17.9	89.6 ± 20.0	0.443
TCHO (mg/dL)	192.4 ± 33.8	194.8 ± 36.4	191.9 ± 39.2	0.243
LDL (mg/dL)	123.3 ± 32.3	126.1 ± 35.3	122.8 ± 36.8	0.124
TG (mg/dL)	99.8 ± 72.2	97.3 ± 54.8	94.2 ± 54.8	0.353
HDL (mg/dL)	56.5 ± 14.2	56.4 ± 13.7	56.7 ± 13.5	0.935
MetS (%)	39 (4.6)	41 (3.9)	8 (2.4)	0.225
Fatty liver (%)	197 (22.9)	287 (27.2)	118 (35.0)	<0.001

and 123 (36.1 %) had lean NAFLD compared with 660 (77.4 %), 611 (73.9 %) and 218 (63.9 %) lean controls, respectively [OR=1.11 (95% CI: 0.90−1.36) for the CG genotype, OR=1.77 (95% CI: 1.36 −2.29) for the GG genotype (reference: CC genotype)]. In subjects with the SAMM50 rs3761472 AA/AG/GG genotypes, 197 (22.9 %), 287 (27.2 %) and 118 (35.0 %) had lean NAFLD compared with 664 (77.1 %), 769 (72.8 %) and 219 (65.0 %) lean controls, respectively [OR=1.26 (95% CI: 1.02−1.55) for the AG genotype, OR=1.82 (95% CI: 1.38−2.39) for the GG genotype (reference: AA genotype)] (data not shown).

3.3. Clinical characteristics according to genotype

We sorted the subjects into categories by genotype at locus rs738409 (representative of the PNPLA3 gene) or locus rs3761472 (representative of the SAMM50 gene). The rs738409 SNP posed a low, moderate and high risk of lean NAFLD in the CC, CG, and GG genotypes, respectively, and the rs3761472 SNP posed a low, moderate and high risk of lean NAFLD in the AA, AG, and GG genotypes, respectively. Among the different gene risks of fatty liver in the lean population, there were no significant differences in body mass index, age and sex. Additionally, none of the metabolic factors, including waist circumference, systolic BP, diastolic BP, fasting glucose, triglycerides and HDL-C, were significantly different among the different genotypes. The only difference among the CC, CG, and GG genotypes of PNPLA3 rs738409 or the AA, AG, and GG genotypes of SAMM50 rs3761472 was the presence or absence of lean NAFLD (Table 2B).

Table 3
The odds of having fatty liver in lean subjects in relation to PNPLA3 (rs738409) and SAMM50 (rs3761472) gene variants after adjustment by logistic regression models.

	Low risk	Moderate risk	High risk	P for trend
	CC	CG	GG	
Model 1	1.00	1.29 (1.03−1.61)	2.17 (1.62−2.92)	<0.001
Model 2	1.00	1.32 (1.04−1.69)	2.49 (1.80−3.44)	<0.001
Model 3	1.00	1.42 (1.09−1.85)	3.06 (2.15−4.37)	<0.001
	AA	AG	GG	
Model 1	1.00	1.30 (1.04−1.62)	2.03 (1.51−2.73)	<0.001
Model 2	1.00	1.36 (1.07−1.73)	2.37 (1.71−3.29)	<0.001
Model 3	1.00	1.40 (1.08−1.83)	2.90 (2.04−4.14)	<0.001

Model 1: adjusted for age and sex.
Model 2: adjusted for variables in Model 1 plus BMI.
Model 3: adjusted for variables in Model 2 plus metabolic factors: waist circumference, fasting glucose, systolic blood pressure, diastolic blood pressure, triglycerides and HDL-C.
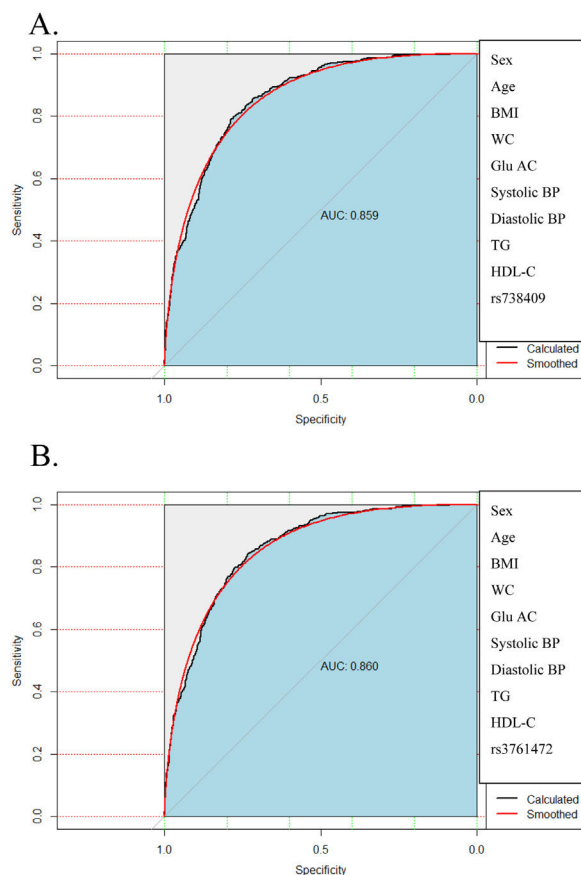
Fig. 2. The area under the ROC curve for gene variants in the detection of lean NAFLD. Other than SNPs, sex, age, body mass index, waist circumference, systolic BP, diastolic BP, HDL and triglycerides were included in the analysis. The areas under the ROC curve for PNPLA3 rs738409 and SAMM50 rs3761472 in the detection of lean NAFLD were 0.859 (95%CI: 0.841, 0.877) and 0.860 (95%CI: 0.843, 0.877), respectively. A. PNPLA3 rs738409 and B. SAMM50 rs3761472.

3.4. Independent risk factors for lean NAFLD

The odds ratios (ORs) of having fatty liver in lean subjects in relation to gene variants of PNPLA3 rs738409 and SAMM50 rs3761472 after adjustment by logistic regression models are shown in Table 3. For the PNPLA3 rs738409 SNP, the OR of having lean NAFLD for the GG group versus the CC group after adjustment for age, and sex was 2.17 (95% CI: 1.62−2.92; P for trend<0.001). After further adjustment for BMI and metabolic factors, i.e., waist circumference, fasting glucose, systolic BP, diastolic BP, triglycerides and HDL-C, the OR of having lean NAFLD for the GG group versus the CC group was 3.06 (95% CI: 2.15−4.37; P for trend <0.001). For the SAMM50 rs3761472 SNP, the OR of having lean NAFLD for the GG group versus the AA group after adjustment for age and sex was 2.03 (95% CI: 1.51−2.73; P for trend<0.001). After further adjustment for BMI and metabolic factors, the OR of having lean NAFLD for the GG group versus the AA group was 2.90 (95% CI: 2.04−4.14; P for trend <0.001).

Therefore, we used age, sex, waist circumference, fasting glucose, systolic BP, diastolic BP, triglycerides and HDL-C to calculate the area under the ROC for lean NAFLD in lean subjects. The AUCs were 0.859 (95%CI: 0.841, 0.877) and 0.860 (95%CI: 0.843, 0.877) for rs738409 and rs3761472, respectively (Fig. 2).

4. Discussion

In our study, we have identified gene variants of G alleles in PNPLA3 rs738409 and SAMM50 rs3761472 that are associated with a higher risk of lean NAFLD than in lean controls. In previous studies,

there were inconsistent results for the association between PNPLA3 rs738409 and lean NAFLD, mostly due to ethnicity differences. Although SAMM50 has been linked to NAFLD, no study to date has explored this relationship. In this comprehensive cohort study, conducted on a large sample size, we observed a higher prevalence of the at-risk genotypes PNPLA3 rs738409 GG and SAMM50 rs3761472 GG in lean individuals with NAFLD compared to lean controls. These two variants, after adjustment for sex, age, BMI, fasting glucose, systolic BP, diastolic BP, triglycerides and HDL-C, had significantly higher odds ratios in lean NAFLD patients than in lean controls. The independent characteristics of PNPLA3 and SAMM50 for lean NAFLD, beyond BMI and metabolic syndrome, reconfirm the unique pathophysiology of lean NAFLD in Asian populations.

The variant PNPLA3 rs738409 was noted to be associated with NAFLD in a meta-analysis [10], among Asian populations [17] and in pediatric patients in Taiwan [14]. However, its association with lean subjects remains under debate in Western countries [2]. In Japanese studies, PNPLA3 rs738409 was strongly associated with the development and progression of nonobese NAFLD rather than obese NAFLD [24], which did not differ in metabolic morbidities and sex [13]. In Hong Kong, among those with NAFLD, lean subjects were more likely to carry the PNPLA3 rs738409 GG genotype than overweight and obese subjects [25]. Similarly, a biopsy-proven NAFLD population in China found that PNPLA3G and SAMM50 may synergistically interact to increase susceptibility to NASH [26]. Furthermore, patients with NAFLD who carry the PNPLA3G allele but are not obese have a higher risk of steatohepatitis or advanced fibrosis [11]. In line with a 7-year prospective community cohort in Sri Lanka, PNPLA3 variants showed higher associations with lean NAFLD [12]. Our study not only supported the higher association between the PNPLA3 rs738409 GG genotype and lean NAFLD in Taiwan but also demonstrated that the association was independent of BMI and metabolic dysfunction.

The role of SAMM50 in NAFLD is thought to be related to fatty acid oxidation and intracellular lipid accumulation [15]. Few studies have demonstrated the association between SAMM50 and NAFLD. In a Chinese Han population, the SAMM50 rs3761472G allele created susceptibility to NAFLD [27]. SAMM50 may interact with PNPLA3 to increase susceptibility to NAFLD in Chinese [28] and Mexican populations [29]. SAMM50 and PNPLA3 may affect the severity of NAFLD in Korean children [30] and adults [31] and the progression of NAFLD [16]. This was the first study to demonstrate that there was an independently higher risk of lean NAFLD among subjects with the SAMM50 rs3761472G allele variant after removing the effects of BMI and metabolic factors.

The 2024 EASL-EASD-EASO Clinical Practice Guidelines delineate the diagnostic criteria for Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) [32]. Upon identifying hepatic steatosis through imaging or biopsy, the guidelines recommend evaluating the presence of cardiometabolic criteria. If the patient meets these cardiometabolic criteria and does not have a history of excessive alcohol consumption, the condition is classified as MASLD [33]. However, it is challenging to recognize whether a patient with normal body weight has fatty liver disease clinically. There is no reliable biomarker, and liver biopsy is not widely used for detecting lean NAFLD [34]. Our study demonstrated that PNPLA3 rs738409 or SAMM50 rs3761472 GG alleles have an approximately 3-fold higher risk of lean NAFLD after adjusting for BMI and metabolic factors. The area under the ROC curve (0.86) showed great performance for PNPLA3 rs738409 and SAMM50 rs3761472 in the detection of lean NAFLD.

There are some limitations of our study. First, it was a case–control study where the exposure was PNPLA3 rs738409 or SAMM50 rs3761472 GG alleles and the outcome was lean NAFLD. Although we tried to collect and adjust for confounding factors such as age, sex, BMI, and every metabolic factor of MetS, potential confounders could not be ruled out. Second, we linked the PNPLA3 rs738409 and

SAMM50 rs3761472 GG alleles to lean NAFLD and demonstrated an independent association of these variants with lean NAFLD. However, we could not distinguish the severity and progression of fatty liver with or without these genetic variants among lean subjects. Future studies should aim to explore the influences of polygenic genes in lean NAFLD. Additionally, our study design does not account for gene-environment interactions, which could also play a significant role in disease development.

5. Conclusions

In conclusion, the PNPLA3 rs738409 and SAMM50 rs3761472 gene polymorphisms are independently associated with a higher risk of fatty liver in lean individuals in Taiwan. Further validation of the association between lean NAFLD and genetic variants in Taiwan as well as Asian populations is warranted.

Author's contribution

CWL, TJC and KCH designed the research; CWL, TJC, TYW, YHL, HJY and KCH conducted the research; CWL and TYW analyzed the data; CWL wrote the manuscript; CWL, TJC, TYW, YHL, HJY and KCH conducted the research; CWL and TYW revised the manuscript.

Availability of data and material

Data will be made available on reasonable request

Funding

Declaration of interests

All authors declare that they have no conflicts of interest.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.aohep.2024.101761.

References

[1] Bellentani S, Marino M. Epidemiology and natural history of non-alcoholic fatty liver disease (NAFLD). Ann Hepatol 2009;8 Suppl 1:S4-8.

[2] Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology (Baltimore, Md) 2016;64(1):73–84.

[3] Eslam M, El-Serag HB, Francque S, Sarin SK, Wei L, Bugianesi E, et al. Metabolic (dysfunction)-associated fatty liver disease in individuals of normal weight. Nat Rev Gastroenterol Hepatol 2022;19(10):638–51.

[4] Valenti L, Pelusi S. Redefining fatty liver disease classification in 2020. Liver Int 2020;40(5):1016–7.

[5] Younes R, Govaere O, Petta S, Miele L, Tiniakos D, Burt A, et al. Caucasian lean subjects with non-alcoholic fatty liver disease share long-term prognosis of non-lean: time for reappraisal of BMI-driven approach? Gut 2022;71(2):382–90.

[6] Lin H, Wong VW. Is lean non-alcoholic fatty liver disease a distinct entity? Hepatol Int 2022;16(2):254–6.

[7] Albhaisi S, Chowdhury A, Sanyal AJ. Non-alcoholic fatty liver disease in lean individuals. JHEP Rep: Innovat Hepatol 2019;1(4):329–41.

[8] Romeo S, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 2008;40(12):1461–5.

[9] Sookoian S, Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of nonalcoholic fatty liver disease. Hepatology (Baltimore, Md) 2011;53(6):1883–94.

[10] Salari N, Darvishi N, Mansouri K, Ghasemi H, Hosseinian-Far M, Darvishi F, et al. Association between PNPLA3 rs738409 polymorphism and nonalcoholic fatty liver disease: a systematic review and meta-analysis. BMC Endocr Disord 2021;21(1):125.

[11] Wei JL, Leung JC, Loong TC, Wong GL, Yeung DK, Chan RS, et al. Prevalence and severity of nonalcoholic fatty liver disease in non-obese patients: a population study using proton-magnetic resonance spectroscopy. Am J Gastroenterol 2015;110(9):1306. -14quiz 15.

[12] Niriella MA, Kasturiratne A, Pathmeswaran A, De Silva ST, Perera KR, Subasinghe S, et al. Lean non-alcoholic fatty liver disease (lean NAFLD): characteristics, metabolic outcomes and risk factors from a 7-year prospective, community cohort study from Sri Lanka. Hepatol Int 2019;13(3):314–22.

[13] Tobari M, Hashimoto E, Taniai M, Ikarashi Y, Kodama K, Kogiso T, et al. Characteristics of non-alcoholic steatohepatitis among lean patients in Japan: not uncommon and not always benign. J Gastroenterol Hepatol 2019;34(8):1404–10.

[14] Lin YC, Chang PF, Hu FC, Yang WS, Chang MH, Ni YH. A common variant in the PNPLA3 gene is a risk factor for non-alcoholic fatty liver disease in obese Taiwanese children. J Pediatr 2011;158(5):740–4.

[15] Li Z, Shen W, Wu G, Qin C, Zhang Y, Wang Y, et al. The role of SAMM50 in non-alcoholic fatty liver disease: from genetics to mechanisms. FEBS 2021;11(7):1893–906.

[16] Kitamoto T, Kitamoto A, Yoneda M, Hyogo H, Ochi H, Nakamura T, et al. Genome-wide scan revealed that polymorphisms in the PNPLA3, SAMM50, and PARVB genes are associated with development and progression of nonalcoholic fatty liver disease in Japan. Hum Genet 2013;132(7):783–92.

[17] Kumar A, Shalimar WGK, Gupta V, Sachdeva MP. Genetics of nonalcoholic fatty liver disease in Asian populations. J Genet 2019;98.

[18] Health Promotion Administration, Ministry of health and welfare, Taiwan. Retrieved August 2, 2024 from https://support.illumina.com/?tab=software.

[19] Pan WH, Flegal KM, Chang HY, Yeh WT, Yeh CJ, Lee WC. Body mass index and obesity-related metabolic disorders in Taiwanese and US whites and blacks: implications for definitions of overweight and obesity for Asians. Am J Clin Nutr 2004;79(1):31–9.

[20] Lu CW, Yang KC, Chi YC, Wu TY, Chiang CH, Chang HH, Huang KC, Yang WS. Adiponectin-leptin ratio for the early detection of lean non-alcoholic fatty liver disease independent of insulin resistance. Ann Med 2023;55(1):634–42.

[21] National Institutes of Health (n.d.) The NHGRI-EBI Catalog of human genome-wide association studies. Retrieved December 30, from https://www.ebi.ac.uk/gwas/home.

[22] Illumina. (n.d.) Illumina Support Center. Retrieved December 30, 2023 from https://support.illumina.com/?tab=software.

[23] Wang HM, Chang TH, Lin FM, Chao TH, Huang WC, Liang C, et al. A new method for post Genome-Wide Association Study (GWAS) analysis of colorectal cancer in Taiwan. Gene 2013;518(1):107–13.

[24] Honda Y, Yoneda M, Kessoku T, Ogawa Y, Tomeno W, Imajo K, et al. Characteristics of non-obese non-alcoholic fatty liver disease: effect of genetic and environmental factors, 46. Hepatology research: the official journal of the Japan Society of Hepatology; 2016. p. 1011–8.

[25] Lin H, Wong GL, Whatling C, Chan AW, Leung HH, Tse CH, et al. Association of genetic variations with NAFLD in lean individuals. Liver Int: Off J Int Assoc Study Liver 2022;42(1):149–60.

[26] Xu K, Zheng KI, Zhu PW, Liu WY, Ma HL, Li G. Interaction of SAMM50-rs738491, PARVB-rs5764455 and PNPLA3-rs738409 increases susceptibility to nonalcoholic steatohepatitis. J Clin Transl Hepatol 2022;10(2):219–29.

[27] Chen L, Lin Z, Jiang M, Lu L, Zhang H, Xin Y, et al. Genetic variants in the SAMM50 gene create susceptibility to nonalcoholic fatty liver disease in a Chinese Han population. Hepat Mon 2015;15(10):e31076.

[28] Xu K, Zheng KI, Zhu PW, Liu WY, Ma HL, Li G, et al. Interaction of SAMM50-rs738491, PARVB-rs5764455 and PNPLA3-rs738409 increases susceptibility to nonalcoholic steatohepatitis. J Clin Transl Hepatol 2022;10(2):219–29.

[29] Larrieta-Carrasco E, Flores YN, Macías-Kauffer LR, Ramírez-Palacios P, Quiterio M, Ramírez-Salazar EG, et al. Genetic variants in COL13A1, ADIPOQ and SAMM50, in addition to the PNPLA3 gene, confer susceptibility to elevated transaminase levels in an admixed Mexican population. Exp Mol Pathol 2018;104(1):50–8.

[30] Lee KJ, Moon JS, Kim NY, Ko JS. Effects of PNPLA3, TM6SF2 and SAMM50 on the development and severity of non-alcoholic fatty liver disease in children. Pediatr Obes 2022;17(2):e12852.

[31] Chung GE, Lee Y, Yim JY, Choe EK, Kwak MS, Yang JI, et al. Genetic polymorphisms of PNPLA3 and SAMM50 are associated with nonalcoholic fatty liver disease in a Korean population. Gut Liver 2018;12(3):316–23.

[32] European Association for the Study of the Liver (EASL). European Association for the Study of Diabetes (EASD), European Association for the Study of Obesity (EASO). EASL–EASD–EASO clinical practice guidelines on the management of metabolic dysfunction-associated steatotic liver disease (MASLD). J Hepatol 2024; j 1–51.

[33] Rinella ME, Lazarus JV, Ratziu V, Francque SM, Sanyal AJ, Kanwal F, et al. A multi-society Delphi consensus statement on new fatty liver disease nomenclature. J Hepatol 2023;79(6):1542–56.

[34] Eslam M, Sanyal AJ, George J. MAFLD: a consensus-driven proposed nomenclature for metabolic associated fatty liver disease. Gastroenterology 2020;158(7):1999–2014.e1.