Gut and Liver
https://doi.org/10.5009/gnl230044
pISSN 1976-2283 eISSN 2005-1212

Original Article

Genetic and Metabolic Characteristics of Lean Nonalcoholic Fatty Liver Disease in a Korean Health Examinee Cohort

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Article Info
Received February 8, 2023
Revised April 5, 2023
Accepted April 10, 2023
Published online August 10, 2023

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Background/Aims: The pathophysiology of lean nonalcoholic fatty liver disease (NAFLD) is unclear but has been shown to be associated with more diverse pathogenic mechanisms than that of obese NAFLD. We investigated the characteristics of genetic or metabolic lean NAFLD in a health checkup cohort.

Methods: This retrospective cross-sectional study analyzed single nucleotide polymorphism data for 6,939 health examinees. Lean individuals were categorized according to a body mass index cutoff of 23 kg/m^2. Single nucleotide polymorphisms were analyzed using genotyping arrays.

Results: The prevalence of lean NAFLD was 21.6% among all participants with NAFLD, and the proportion of lean NAFLD was 18.5% among lean participants. The prevalence of metabolic syndrome and diabetes among lean patients with NAFLD was 12.4% and 10.4%, respectively. Lean NAFLD appeared to be metabolic-associated in approximately 20.1% of patients. The homozygous minor allele (GG) of *PNPLA3* (rs738409) and heterozygous minor alleles (CT, TT) of *TM6SF2* (rs58542926) were associated with lean NAFLD. However, the prevalence of fatty liver was not associated with the genetic variants *MBOAT7* (rs641738), *HSD17B13* (rs72613567), *MARC1* (rs2642438), or *AGXT2* (rs2291702) in lean individuals. Lean NAFLD appeared to be associated with *PNPLA3* or *TM6SF2* genetic variation in approximately 32.1% of cases. Multivariate risk factor analysis showed that metabolic risk factors, genetic risk variants, and waist circumference were independent risk factors for lean NAFLD.

Conclusions: In a considerable number of patients, lean NAFLD did not appear to be associated with known genetic or metabolic risk factors. Further studies are required to investigate additional risk factors and gain a more comprehensive understanding of lean NAFLD. **(Gut Liver 2024;18:316-327)**

Key Words: Non-alcoholic fatty liver disease; Single-nucleotide polymorphism; Metabolic syndrome; Central obesity

INTRODUCTION

The prevalence of nonalcoholic fatty liver disease (NAFLD) in lean individuals ranges from 10% to 20%.[1] Previous studies have shown that lean individuals with NAFLD are male, older, and have a larger waist circumference than lean individuals without NAFLD.[2] Genetic and nongenetic factors, such as single nucleotide polymorphisms (SNPs), metabolic dysfunction, diet, sarcopenia, and the microbiome are presumed to affect the development of lean NAFLD.[2,3] However, the pathogenesis and characteristics of lean NAFLD remain unclear.

Lean patients with NAFLD had a larger waist circumference and unfavorable metabolic parameters than lean

individuals without NAFLD. Moreover, genetic factors, in addition to central obesity and metabolic dysfunction, play a very important role in the pathogenesis of lean NAFLD. Recently, Vilarinho *et al.*[4] proposed classifying lean NAFLD into type I (central obesity or metabolic association; metabolic) and type II (SNP association; genetic). They stressed that most patients with lean NAFLD belong to type I. However, no previous study has revealed the distribution of types I and II in patients with lean NAFLD based on an actual community cohort. In general, patatin-like phospholipase domain containing 3 (*PNPLA3*),[3,5] transmembrane 6 superfamily 2 (*TM6SF2*),[3,6] membrane-bound O-acyltransferase domain containing 7 (*MBOAT7*),[7] hydroxysteroid 17-beta dehydrogenase 13 (*HSD17B13*),[8] mitochondrial amidoxime reducing component 1 (*MARC1*),[9] and alanine-glyoxylate aminotransferase 2 (*AGXT2*)[10] are known to be closely related to fatty liver development in obese individuals. However, it is unclear whether SNPs associated with NAFLD have a similar effect on lean NAFLD. Interestingly, Honda *et al.*[11] suggested that the odds ratio (OR) for SNPs in *PNPLA3* was higher in non-obese patients with NAFLD than in obese patients with NAFLD. Another retrospective study that analyzed 669 patients with biopsy-proven NAFLD reported that a significantly greater proportion of patients with lean NAFLD carried rs58542926 C>T in *TM6SF2* than obese or overweight individuals with NAFLD.[12]

Currently, there is a lack of data on the classification of lean NAFLD according to genetic and nongenetic factors and their characteristics in health checkup cohorts. Therefore, in this study, we aimed to investigate the characteristics of genetic or metabolic lean NAFLD in a health

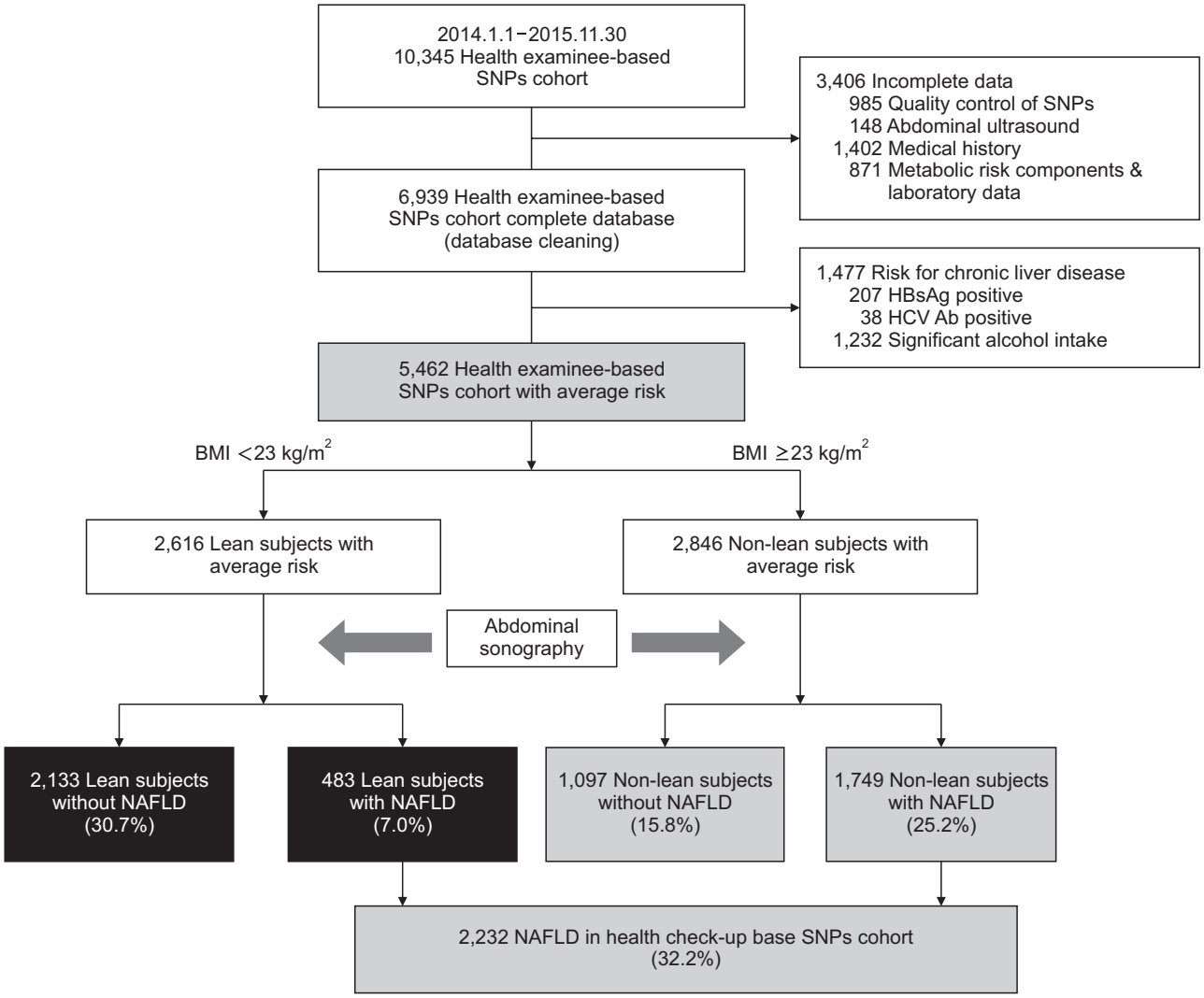


Fig. 1. Study flowchart. SNPs, single nucleotide polymorphisms; BMI, body mass index; NAFLD, nonalcoholic fatty liver disease; HBsAg, hepatitis B surface antigen; HCV Ab, hepatitis C virus antibody.

checkup cohort.

MATERIALS AND METHODS

1. Study design and population

This was a retrospective, cross-sectional study. As a health examinee-based SNPs cohort, we collected data from 10,345 participants from the Gene-Environmental Interaction and Phenotype cohort who visited the Seoul National University Hospital Gangnam Center from 2014 to 2015 for routine health checkup and donated blood samples to the biorepository after providing informed consent (Fig. 1). Detailed baseline characteristics and cohort protocols have been previously described.[13] Information on age, anthropometric data, underlying medical conditions, alcohol consumption, physical activity, serologic data of viral hepatitis, metabolic components, and abdominal sonography was collected using the electronic medical records of the patients, which were acquired during the routine medical checkup. After excluding participants with incomplete data (n=3,406), 6,939 participants were analyzed. After further excluding participants with a risk of chronic liver disease who were positive for hepatitis B virus (n=207), hepatitis C virus (n=38), or had significant alcohol consumption (n=1,232), 5,462 participants with average risk were selected. Lean individuals were categorized according to a body mass index (BMI) cutoff of 23 kg/m^2. This study was approved by the Seoul National University Hospital Institutional Review Board (IRB number: 2105-049-1218), which waived the requirement for informed consent.

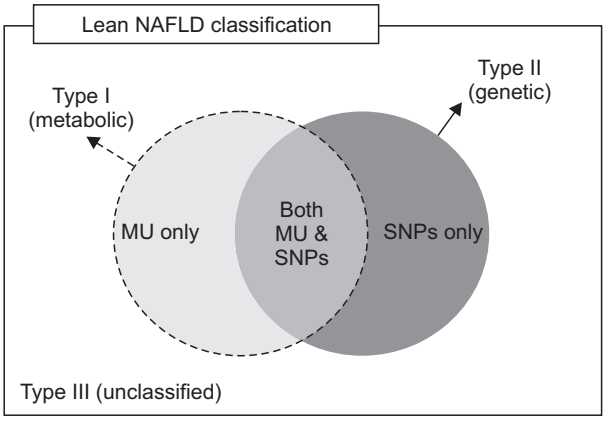
In the risk group, 827 participants who were referred to liver specialists from other departments or primary care clinics at the Hanyang University Medical Center because of liver problems were enrolled (Supplementary Fig. 1). Participants with missing information (n=227) were excluded. We further excluded participants with other chronic liver disease, such as alcoholic liver disease (n=4), viral hepatitis (n=12), and others (n=22). Finally, risk group diagnosed as NAFLD (n=562) was selected. Among them, patients with BMI <23 kg/m^2 were finally analyzed as having lean NAFLD at risk (n=39). This study was approved by the Institutional Review Board (IRB number: 2020-01-012-014) of Seoul Hanyang University Hospital and informed consent was obtained from all patients.

2. Clinical parameters of the participants

Routine questionnaires were administered to every patient during the health checkup. The questionnaires included questions on self-reported personal medical histories of metabolic risk factors (i.e., diagnosis and medication for hypertension, diabetes, and dyslipidemia), subjective signs and symptoms, and lifestyle information. Information regarding alcohol consumption (frequency of alcohol intake and amount of alcohol consumed per week or month) was obtained. Anthropometric measurements including waist circumference, blood pressure, height, weight, total fat mass, and lean mass were also recorded. Additionally, fasting serum glucose, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, aspartate aminotransferase, alanine aminotransferase, and γ-glutamyl transferase levels were measured. The presence of fatty liver was evaluated using ultrasonography. The fibrosis-4 index and NAFLD fibrosis score (NFS) were calculated, and their cut-off values were selected based on previous studies.[14] Body composition was analyzed using bioelectrical impedance analysis (InBody 720 body composition analysis). Exercise status was categorized as minimal, moderate, or health-enhancing physical activity according to the International Physical Activity Questionnaire.[15]

3. Three subtypes of lean NAFLD and their definitions

Lean patients with NAFLD were classified based on the study by Vilarinho *et al.*[4] The presence of a metabolically unhealthy (MU) status (either central obesity or insulin resistance; metabolic syndrome [MS] or diabetes mellitus [DM]) as compatible with type I (metabolic-associated lean NAFLD) in patients with lean NAFLD (Fig. 2). Having common genetic risk variants in any of the six validated genes but without MU status was defined as being compat-



MU: central obesity or metabolic syndrome or diabetes
SNPs: *PNPLA3* (GG) or *TM6SF2* (CT+TT)

Fig. 2. Schematic of the definitions of subtypes in lean NAFLD. NAFLD, nonalcoholic fatty liver disease; MU, metabolically unhealthy; SNPs, single nucleotide polymorphisms; *PNPLA3*, patatin-like phospholipase domain containing 3; *TM6SF2*, transmembrane 6 superfamily 2.

ible with type II (genetic lean NAFLD) in patients with lean NAFLD. Those who were not compatible with either type I or II were classified as having type III NAFLD (unclassified lean NAFLD). Metabolic risk abnormalities were defined as follows:[16,17] (1) central obesity, waist circumference ≥85 cm for females and ≥90 cm for males; (2) high blood pressure, blood pressure ≥130/85 mm Hg and/or intake of antihypertensive medication; (3) high triglyceride level, serum triglyceride level ≥150 mg/dL; (4) low HDL cholesterol level, HDL cholesterol level <50 mg/dL for females and <40 mg/dL for males; and (5) impaired fasting glucose, fasting glucose level outside the range of 100 to 125 mg/dL and/or intake of medication for diabetes. The cutoff values for central obesity were defined according to the Korean Society for the Study of Obesity criteria.[17] MS was defined as having ≥3 metabolic abnormalities.

4. Statistical analyses

A total of 584,061 SNPs that passed the quality control were used in the genome-wide association study. We used a multivariate logistic regression model in the PLINK software package (version 1.07) and ordinal logistic regression in the R statistical software package (version 3.1.1; R Development Core Team; R Foundation for Statistical Computing, Vienna, Austria) to test the association between NAFLD and SNPs in the genome. Age and BMI were used as covariates. The R statistical software package was used for the statistical analysis and to draw a Manhattan plot of the log10 values. Continuous and categorical variables are presented as mean±standard deviation and numbers (%), respectively. Categorical variables were analyzed using either the chi-square test or Fisher exact test, whereas continuous variables were analyzed using the Student t-test. The association between the development of fatty liver and the presence of SNPs or metabolic risk abnormalities such as central obesity, high blood pressure, high serum glucose or triglyceride levels, and low HDL levels were assessed using logistic regression analysis. Multiple models were constructed as follows: Model 1 was adjusted for age, male sex, presence of SNPs, and MU status. Model 2 was further adjusted for five metabolic risk abnormalities, including systolic blood pressure, waist circumference, serum fasting glucose, triglyceride, and HDL levels, as continuous variables, instead of the presence of MU status. Model 3 was adjusted for five metabolic risk abnormalities as categorical variables. Statistical significance was set at $p<0.05$. Statistical analyses were performed using SPSS software (version 26.0; IBM Corp., Armonk, NY, USA).

RESULTS

1. Baseline characteristics

A total of 2,616 lean individuals (BMI <23 kg/m^2) were enrolled out of 6,939 individuals in the health examinee-based SNP cohort. Lean individuals were predominantly women (57.6%). Their mean age and BMI were 46.9 years and 20.6 kg/m^2, respectively (Table 1). The mean number of metabolic risk abnormalities was 0.65; however, 4.1% was consistent with the definition of MS among lean individuals. The prevalence of NAFLD in the health examinee-based SNP cohort was 32.2% (2,232/6,939) (Fig. 1). Fatty liver was present in 20.6% (483/2,616) of the lean individuals. One-fifth (21.6%, 483/2,232) of the patients with NAFLD were lean, with a BMI <23 kg/m^2 according to the definition. Table 1 shows that lean patients with NAFLD were predominantly male and older, and had a higher waist circumference and unfavorable metabolic parameters than lean individuals without NAFLD. MS (2.3% vs 12.4%, $p<0.001$) and DM (2.0% vs 10.4%, $p<0.001$) were more prevalent in patients with fatty liver than among lean individuals.

2. Genetic predisposing factors for fatty liver in lean individuals

Among lean individuals, the prevalence of fatty liver was significantly higher in lean individuals with the homozygous minor allele (GG) than in those with the heterozygous major allele (GC) (25.2% vs 17.8%, $p<0.001$) and the homozygous major allele (CC) in *PNPLA3* (rs738409) (25.2% vs 15.8%, $p<0.001$) (Fig. 3). Similarly, fatty liver was significantly more prevalent in individuals with an increasing number of minor T alleles in *TM6SF2* (rs58542926) (TT, 47.4% and CT, 23.3%) than in those with homozygous major alleles (CC, 17.5%) (TT vs CC, $p=0.001$ and CT vs CC, $p=0.008$). However, the prevalence of fatty liver was not associated with the genetic variants *MBOAT7* (rs641738), *HSD17B13* (rs72613567), *MARC1* (rs2642438), or *AGXT2* (rs2291702) in lean individuals (Fig. 3 and Supplementary Fig. 2). Prevalence of lean NAFLD was higher in individuals with genetic risk variants (homozygous minor alleles in *PNPLA3* [GG] and heterozygous minor alleles in *TM6SF2* [CT, TT]) than in those without these variants (24.9% vs 15.7%, $p<0.001$) (Fig. 4A). However, the coexistence of *PNPLA3* (GG) and *TM6SF2* (CT, TT) did not have an additive effect on the development of NAFLD in lean individuals (Supplementary Fig. 3).

3. MU status and central obesity in lean NAFLD individuals

The prevalence of NAFLD was higher in lean individu-

Table 1. Baseline Characteristics of Lean Individuals According to the Presence of NAFLD

Characteristic	Lean subjects (n=2,616)	Lean subjects without NAFLD (n=2,133)	Lean subjects with NAFLD (n=483)	p-value
Age, yr	46.9±10.3	46.1±10.3	50.5±9.4	<0.001
Male sex	1,108 (42.4)	795 (37.3)	313 (64.8)	<0.001
Body mass index, kg/m^2	20.6±1.6	20.4±1.6	21.6±1.1	<0.001
Waist circumference, cm	76.0±6.0	75.1±5.8	80.3±4.6	<0.001
SBP, mm Hg	110±12	109±12	113±12	<0.001
AST, IU/L	21±9	21±9	23±9	<0.001
ALT, IU/L	18±12	17±11	24±14	<0.001
GGT, U/L	23±22	21±19	33±32	<0.001
Triglyceride, mg/dL	82±47	75±39	114±65	<0.001
HDL, mg/dL	57±12	58±12	51±11	<0.001
Fasting glucose, mg/dL	93±14	92±11	101±20	<0.001
HbA1c, %	5.5±0.4	5.4±0.3	5.7±0.6	<0.001
High blood pressure (BP≥130/85 mm Hg)	483 (18.5)	353 (16.5)	130 (26.9)	<0.001
Central obesity (F≥85 cm, M≥90 cm)	24 (0.9)	16 (0.8)	8 (1.7)	0.059
Impaired fasting glucose (≥100 mg/dL)	557 (22.1)	373 (17.5)	204 (42.2)	<0.001
High triglyceride (≥150 mg/dL)	192 (7.3)	92 (4.3)	100 (20.7)	<0.001
Low HDL (F<50 mg/dL, M<40 mg/dL)	424 (16.2)	298 (14.0)	126 (26.1)	<0.001
No. of metabolic risk abnormality	0.65±0.85	0.53±0.76	1.18±1.04	<0.001
Metabolic syndrome	108 (4.1)	48 (2.3)	60 (12.4)	<0.001
Hypertension	283 (10.8)	201 (9.4)	82 (17.0)	<0.001
Diabetes	92 (3.5)	42 (2.0)	50 (10.4)	<0.001
PNPLA3 (rs738409)				<0.001
CC	860 (32.9)	724 (33.9)	136 (28.2)	
CG	1,292 (49.4)	1,062 (49.8)	230 (47.6)	
GG	464 (17.7)	347 (16.3)	117 (24.2)	
MBOAT7 (rs641738)				0.295
CC	1,649 (63.0)	1,358 (63.7)	291 (60.2)	
CT	864 (33.0)	695 (32.6)	169 (35.0)	
TT	103 (3.9)	80 (3.8)	23 (3.8)	
TM6SF2 (rs58542926)				<0.001
CC	2,245 (85.8)	1,853 (86.9)	392 (81.2)	
CT	352 (13.5)	270 (12.7)	82 (17.0)	
TT	19 (0.7)	10 (0.5)	9 (1.9)	
HSD17B13 (rs72613567)				0.564
TT	1,278 (48.9)	1,044 (48.9)	234 (48.4)	
TTA	1,100 (42.0)	901 (42.2)	199 (41.2)	
TATA	238 (9.1)	188 (8.8)	50 (10.4)	
MARC1 (rs2642438)				0.510
GG	1,878 (71.8)	1,525 (71.5)	353 (73.1)	
GA	671 (25.6)	550 (25.8)	121 (25.1)	
AA	67 (2.6)	58 (2.7)	9 (1.9)	
AGXT2 (rs2291702)				0.296
CC	1,168 (44.6)	937 (43.9)	231 (47.8)	
CT	1,165 (44.5)	963 (45.1)	202 (41.8)	
TT	283 (10.8)	233 (10.9)	50 (10.4)	

Data are presented as mean±SD or number (%). When calculating the p-value between lean individuals with and without NAFLD, a t-test or chi-square test was used for continuous variables or categorical variables, respectively.

NAFLD, nonalcoholic fatty liver disease; SBP, systolic blood pressure; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; HDL, high-density lipoprotein; HbA1c, hemoglobin A1c; BP, blood pressure; F, female; M, male; *PNPLA3*, patatin-like phospholipase domain containing 3; *MBOAT7*, membrane-bound O-acyltransferase domain containing 7; *TM6SF2*, transmembrane 6 superfamily 2; *HSD17B13*, hydroxysteroid 17-beta dehydrogenase 13; *MARC1*, mitochondrial amidoxime reducing component 1; *AGXT2*, alanine-glyoxylate aminotransferase 2.

als with MU status (central obesity, MS, or DM) than in those without (50.0% vs 15.9%, p<0.001) (Fig. 4B). Additionally, the coexistence of MU status and SNPs in generic risk variants resulted in the highest prevalence of NAFLD (57.4%) (Fig. 4C). In line with this, multivariate risk factor analysis showed that MU status (OR, 3.382;

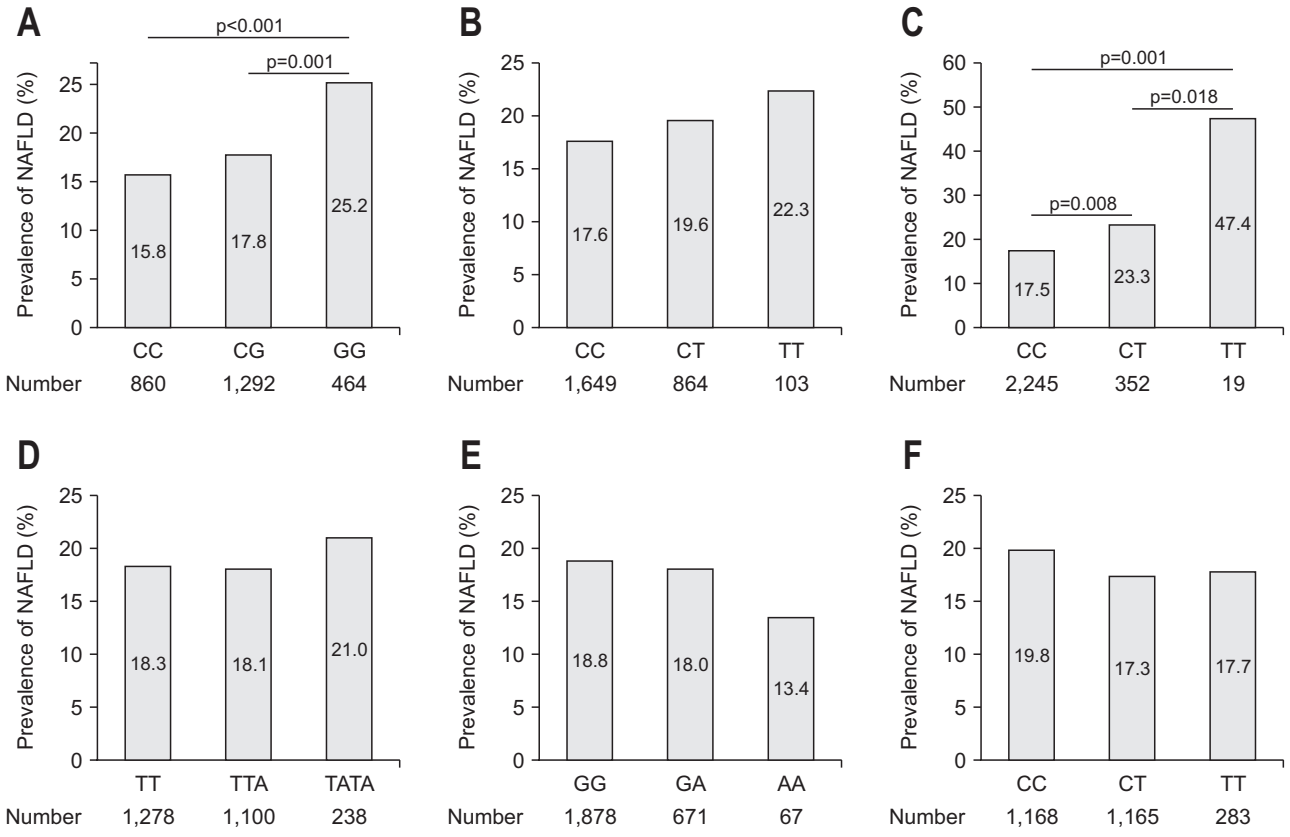


Fig. 3. Prevalence of NAFLD in lean individuals according to SNP genotypes. The SNP genotypes included (A) *PNPLA3* (rs738409), (B) *MBOAT7* (rs641738), (C) *TM6SF2* (rs58542926), (D) *HSD17B13* (rs72613567), (E) *MARC1* (rs2642438), and (F) *AGXT2* (rs2291702). NAFLD, nonalcoholic fatty liver disease; SNP, single nucleotide polymorphism; *PNPLA3*, patatin-like phospholipase domain containing 3; *MBOAT7*, membrane-bound O-acyltransferase domain containing 7; *TM6SF2*, transmembrane 6 superfamily 2; *HSD17B13*, hydroxysteroid 17-beta dehydrogenase 13; *MARC1*, mitochondrial amidoxime reducing component 1; *AGXT2*, alanine-glyoxylate aminotransferase 2.

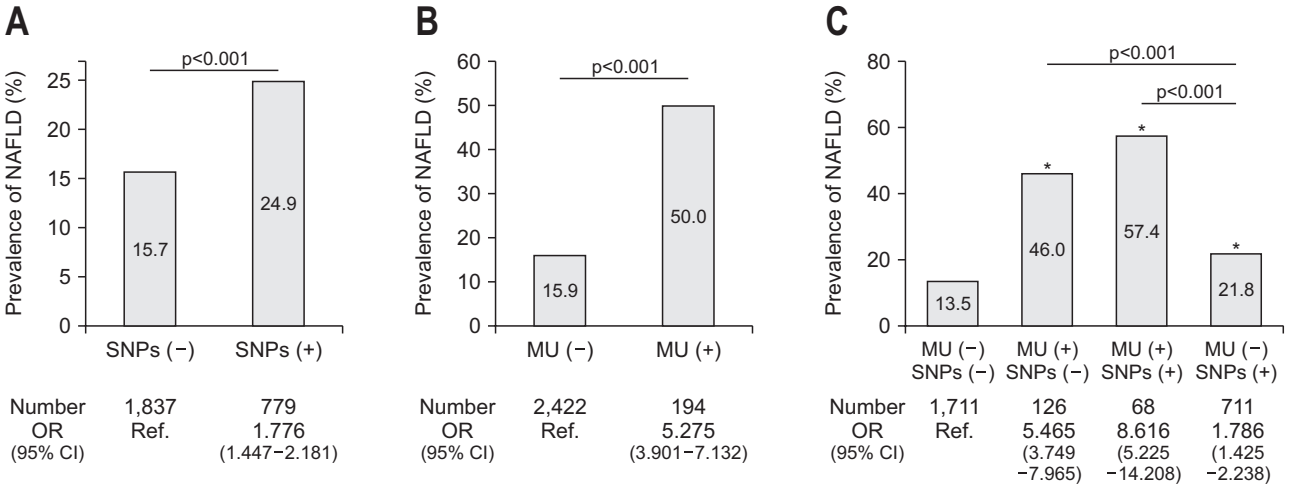


Fig. 4. Prevalence of NAFLD in lean patients with or without metabolic or genetic predisposition. (A) Presence of genetic risk variants, (B) presence of the MU status, and (C) presence of either the MU status or genetic risk variants. Individuals with genetic risk variants were defined as those harboring *PNPLA3* (GG) or *TM6SF2* (CT+TT). The MU status was defined as the presence of central obesity, metabolic syndrome, or diabetes mellitus. NAFLD, nonalcoholic fatty liver disease; MU, metabolically unhealthy; SNPs, single nucleotide polymorphisms; OR, odds ratio; CI, confidence interval; *PNPLA3*, patatin-like phospholipase domain containing 3; *TM6SF2*, transmembrane 6 superfamily 2. *Indicates statistical significance (p<0.001) compared to the prevalence in those without MU or SNPs (MU [–] SNPs [–]).

95% confidence interval [CI], 2.450 to 4.670; p<0.001) and the presence of SNPs in genetic risk variants (OR, 1.870; 95% CI, 1.505 to 2.325; p<0.001) were independent of each other in predisposing lean NAFLD (Table 2). Increased waist circumference as a continuous variable was an independent risk factor for lean NAFLD. However, when waist circumference was classified as a categorical variable according to the definition of MS (conventional cutoff in Koreans; females 85 cm, males 90 cm),[17] it was not an independent risk factor for lean NAFLD (Table 2). The prevalence of central obesity in patients with NAFLD was 45% (1,005/2,232), while that in patients with lean NAFLD was only 1.7% (8/483).

4. Proportion of lean NAFLD according to metabolic and genetic factors

Among the patients with lean NAFLD, the prevalence of metabolic-associated NAFLD (type I, MU status) was

Table 2. Multivariate Risk Factor Analysis for NAFLD in Lean Subjects

Variable	OR (95% CI)	p-value
Model 1		
Age (yr)	1.023 (1.012–1.034)	<0.001
Male sex	2.482 (1.992–3.098)	<0.001
SNPs (*PNPLA3* (GG) or *TM6SF2* (CT+TT))	1.870 (1.505–2.325)	<0.001
Metabolic unhealthy status (CO or DM or MS)	3.382 (2.450–4.670)	<0.001
Model 2		
Age (yr)	1.006 (0.994–1.018)	0.346
Male sex	0.833 (0.634–1.095)	0.189
SNPs (*PNPLA3* (GG) or *TM6SF2* (CT+TT))	1.936 (1.535–2.443)	<0.001
Systolic blood pressure (mm Hg)	0.998 (0.989–1.008)	0.702
Waist circumference (cm)	1.154 (1.123–1.186)	<0.001
Fasting glucose (mg/dL)	1.024 (1.015–1.032)	<0.001
Triglyceride (mg/dL)	1.008 (1.006–1.010)	<0.001
HDL (mg/dL)	0.981 (0.970–0.992)	0.001
Model 3		
Age (yr)	1.018 (1.007–1.030)	0.001
Male sex	2.202 (1.744–2.781)	<0.001
SNPs (*PNPLA3* (GG) or *TM6SF2* (CT+TT))	1.867 (1.494–2.332)	<0.001
High blood pressure (BP≥130/85 mm Hg)	0.978 (0.749–1.278)	0.873
Central obesity (F≥85 cm, M≥90 cm)	2.218 (0.871–5.650)	0.095
Impaired fasting glucose (≥100 mg/dL)	2.116 (1.662–2.696)	<0.001
High triglyceride (≥150 mg/dL)	3.367 (2.420–4.683)	<0.001
Low HDL (F<50 mg/dL, M<40 mg/dL)	1.837 (1.409–2.394)	<0.001

When calculating the OR for NAFLD, logistic regression analysis was executed.
NAFLD, nonalcoholic fatty liver disease; OR, odds ratio; CI, confidence interval; SNPs, single nucleotide polymorphisms; *PNPLA3*, patatin-like phospholipase domain containing 3; *TM6SF2*, transmembrane 6 superfamily 2; CO, central obesity; DM, diabetes mellitus, MS, metabolic syndrome; HDL, high-density lipoprotein; BP, blood pressure; F, female; M, male.

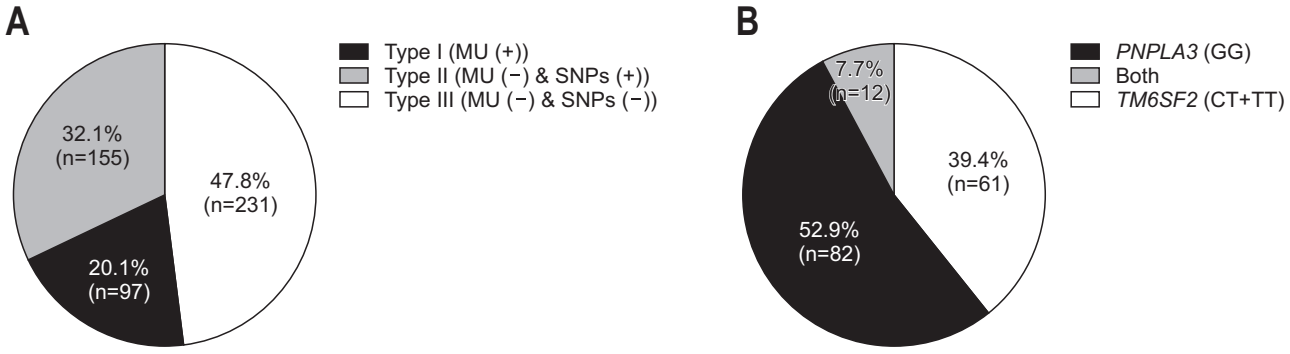


Fig. 5. Pie graph representing the proportion of subgroups. (A) The proportion of subtypes of lean NAFLD and (B) the proportion of SNPs in type II lean NAFLD. Individuals with SNPs were defined as those with *PNPLA3* (GG) or *TM6SF2* (CT+TT). The MU status was defined as the presence of metabolic syndrome or diabetes mellitus. NAFLD, nonalcoholic fatty liver disease; SNPs, single nucleotide polymorphisms; MU, metabolically unhealthy; *PNPLA3*, patatin-like phospholipase domain containing 3; *TM6SF2*, transmembrane 6 superfamily 2.

Table 3. Clinical Characteristics of Lean Subjects with NAFLD According to Subtypes

Characteristic	Type I (A) (n=97)	Type II (B) (n=155)	Type III (C) (n=231)	p-value		
				A vs B	A vs C	B vs C
Age, yr	55.2±8.5	49.1±9.3	49.5±9.2	<0.001	<0.001	0.674
Male sex	77 (79.4)	91 (58.7)	145 (62.8)	0.001	0.003	0.422
Body mass index, kg/m^2	21.8±0.8	21.5±1.0	21.4±1.3	0.015	0.007	0.464
Waist circumference, cm	82.3±4.7	79.8±4.3	79.9±4.6	<0.001	<0.001	0.829
SBP, mm Hg	118±12	112±13	112±12	<0.001	<0.001	0.978
AST, IU/L	25±15	22±8	22±7	<0.001	<0.001	0.947
ALT, IU/L	27±15	24±14	23±13	0.075	0.012	0.504
GGT, U/L	39±31	29±27	33±34	0.049	0.009	0.597
Triglyceride, mg/dL	151±78	105±62	103±54	0.007	0.155	0.184
HDL, mg/dL	48±9	53±11	52±10	<0.001	<0.001	0.748
Fasting glucose, mg/dL	125±32	95±9	95±9	0.001	0.001	0.729
HbA1c, %	6.4±1.0	5.5±0.2	5.5±0.2	<0.001	<0.001	0.810
High blood pressure (BP≥130/85 mm Hg)	58 (59.8)	29 (18.7)	43 (18.6)	<0.001	<0.001	0.981
Central obesity (F≥85 cm, M≥90 cm)	8 (8.2)	0	0	<0.001	<0.001	-
Impaired fasting glucose (≥100 mg/dL)	90 (92.8)	44 (28.4)	70 (30.3)	<0.001	<0.001	0.686
High triglyceride (≥150 mg/dL)	43 (44.3)	21 (13.5)	36 (15.6)	<0.001	<0.001	0.580
Low HDL (F<50 mg/dL, M<40 mg/dL)	49 (50.5)	35 (22.6)	42 (18.2)	<0.001	<0.001	0.289
No. of metabolic risk abnormality	2.5±0.8	0.8±0.7	0.8±0.7	<0.001	<0.001	0.945
Metabolic syndrome	60 (61.9)	0	0	<0.001	<0.001	-
Hypertension	38 (39.2)	18 (11.6)	26 (11.3)	<0.001	<0.001	0.914
Diabetes	50 (51.5)	0	0	<0.001	<0.001	-
FIB-4	1.23±0.57	1.06±0.54	1.03±0.36	0.018	<0.001	0.475
NFS	−1.69±1.18	−2.59±1.04	−2.61±1.11	<0.001	<0.001	0.837
PNPLA3 (rs738409)				<0.001	<0.001	<0.001
CC	37 (38.1)	21 (13.5)	78 (33.8)			
CG	37 (38.1)	40 (25.8)	153 (66.2)			
GG	23 (23.7)	94 (60.6)	0			
MBOAT7 (rs641738)				0.671	0.482	0.609
CC	61 (62.9)	97 (62.6)	133 (57.6)			
CT	30 (30.9)	52 (33.5)	87 (37.7)			
TT	6 (6.2)	6 (3.9)	11 (4.8)			
TM6SF2 (rs58542926)				<0.001	<0.001	<0.001
CC	79 (81.4)	82 (52.9)	231 (100)			
CT	17 (17.5)	65 (41.9)	0			
TT	1 (1.0)	8 (5.2)	0			
HSD17B13 (rs72613567)				0.709	0.573	0.277
TT	49 (50.5)	70 (45.2)	115 (49.8)			
TTA	40 (41.2)	71 (45.8)	88 (38.1)			
TATA	8 (8.2)	14 (9.0)	28 (12.1)			
MARC1 (rs2642438)				0.832	0.557	0.592
GG	74 (76.3)	113 (72.9)	166 (71.9)			
GA	21 (21.6)	38 (24.5)	62 (26.8)			
TT	2 (2.1)	4 (2.6)	3 (1.3)			
AGXT2 (rs2291702)				0.237	0.569	0.102
CC	49 (50.5)	79 (51.0)	103 (44.6)			
CT	41 (42.3)	55 (35.5)	106 (45.9)			
TT	7 (7.2)	21 (13.5)	22 (9.5)			

Data are presented as mean±SD or number (%). When calculating the p-value, a t-test, or chi-square test was used for continuous variables or categorical variables, respectively.

NAFLD, nonalcoholic fatty liver disease; SBP, systolic blood pressure; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; HDL, high-density lipoprotein; HbA1c, hemoglobin A1c; BP, blood pressure; F, female; M, male; FIB-4, fibrosis-4 index; NFS, NAFLD fibrosis score; *PNPLA3*, patatin-like phospholipase domain containing 3; *MBOAT7*, membrane-bound O-acyltransferase domain containing 7; *TM6SF2*, transmembrane 6 superfamily 2; *HSD17B13*, hydroxysteroid 17-beta dehydrogenase 13; *MARC1*, mitochondrial amidoxime reducing component 1; *AGXT2*, alanine-glyoxylate aminotransferase 2.

20.1% and that of genetic NAFLD (type II, genetic risk variants without MU status) was 32.1%. Notably, nearly half (47.8%) of patients with lean NAFLD were unclassified (type III, with neither MU status nor genetic risk variants) (Fig. 5A). A risk variant (GG) in *PNPLA3* (52.9%) and risk variants (CT+TT) in *TM6SF2* (39.4%) were caused by two dominant SNPs in patients with genetic lean NAFLD (Fig. 5B), among whom only 7.7% had both SNPs simultaneously.

5. Clinical characteristics of patients according to three subtypes of lean NAFLD

Patients with type I (metabolic) lean NAFLD were significantly older and more likely to be male than those with type II (genetic) or type III (unclassified) lean NAFLD (Table 3). Patients with type I lean NAFLD showed unfavorable metabolic profiles, unlike those with type II/III lean NAFLD. The fibrosis-4 and NFS scores were highest in patients with type I lean NAFLD. The proportions of hypertension, central obesity, impaired fasting glucose, DM, hypertriglyceridemia, lower HDL, MS, and NFS scores were significantly lower in patients with lean NAFLD who only had SNPs in generic risk variants than in the other two groups with MU status (Supplementary Table 1). The overall clinical characteristics of patients with both MU status and SNPs in the generic risk variants were not significantly different from those in the MU status-only group. Therefore, we classified metabolic (type I) lean NAFLD when the MU status and genetic risk variants coexisted. Moreover, patients with type III lean NAFLD exhibited a phenotype similar to that observed in patients with type II lean NAFLD. However, patients with type III lean NAFLD were older, more likely to be male, and had more unfavorable metabolic risk abnormalities than lean individuals without NAFLD (Supplementary Table 2). There were no significant differences in the exercise quantity or total skeletal muscle mass divided by body weight (%) between patients with type III lean NAFLD and lean individuals without NAFLD.

In patients from the liver clinics at risk of liver disease, different aspects of the proportion of lean NAFLD were observed (Supplementary Table 3). In the at-risk population, type I (76.9%) was more prevalent than the other types (type II, 15.4%; type III, 7.7%). Their characteristics were not distinguished according to type, unlike the results of the health examinee cohort.

DISCUSSION

This is the first large-scale study to show the constitution of metabolic, genetic, and unclassified NAFLD in lean individuals and to compare their characteristics according to subtypes. In this study, 21.6% of patients with NAFLD in Asia were lean. Among individuals with lean NAFLD, 20.1% and 32.1% were classified into metabolic and genetic NAFLD, respectively. Interestingly, only *PNPLA3* (rs738409) and *TM6SF2* (rs58542926) were identified as genetic risk factors associated with lean NAFLD. The result was similar to that of another study using the U.K. biobank wherein only two SNPs were associated with lean NAFLD among the 13 SNPs.[18] Meanwhile, it was difficult to identify the risk factors for half of the lean NAFLD patients.

Recently, Vilarinho *et al.*[4] proposed two broad subtypes of lean NAFLD: type I (individuals with visceral adiposity and insulin resistance but normal BMI) and type II (individuals with hepatic steatosis resulting from a monogenic disorder with rare genetic variants driving the disease). Lean patients with NAFLD are more heterogeneous and complex in pathogenesis than those with obese NAFLD, in whom metabolic factors act more strongly than other factors. Therefore, it is reasonable to manage patients with lean NAFLD according to their etiology, such as metabolic lean NAFLD (type I) or genetic lean NAFLD (type II), as suggested by Vilarinho *et al.*[4] A consensus on the diagnostic criteria for the classification of lean NAFLD is a prerequisite. However, there is an unmet need for classification of lean NAFLD.

First, the definition of type I diabetes (individuals with visceral adiposity and insulin resistance but normal BMI) is uncertain. Vilarinho *et al.*[4] proposed that central obesity is an indicator of visceral adiposity, insulin resistance, and dyslipidemia. However, there is no suggested cutoff value for each risk factor. In this study, the prerequisites for type I disease were central obesity or insulin resistance (MS or DM), referred to as the MU status. MS or DM was used as a synonym of insulin resistance. As a result, 20.1% of all patients with lean NAFLD were classified as having type I. Meanwhile, it is difficult to define the MU status and central obesity, especially in lean individuals, owing to their lean phenotype.[19,20] As there is currently no established cutoff value for abnormal waist circumference in lean individuals, we utilized the same cutoff value for central obesity in both lean and obese patients for the purposes of this study. Nonetheless, it is crucial to conduct further research to develop an appropriate definition of central obesity in lean individuals. Most lean individuals with NAFLD in Asia have a normal abdominal circumference. In this study, the prevalence of central obesity in lean NAFLD was very low (1.7%, 8/483) if a general cutoff was applied (90 cm for males and 85 cm for females in Korea).[21] The prevalence of MS (12.4%) and DM (10.4%), which are

recognized as severe forms of metabolic dysfunction, was much higher than that of central obesity (1.7%). Moreover, the proportion of patients with central obesity and type I lean NAFLD was very low at 8.2% (8/97). Interestingly, in the multivariate risk factor analysis for the development of NAFLD in lean individuals (Table 2), waist circumference (cm) was evaluated as an independent risk factor, unlike the presence of central obesity (categorical variable). These findings raise the question of whether it is appropriate to apply the same cutoff value for diagnosing central obesity in patients with lean NAFLD. Therefore, further research on the appropriate definitions of MU status and central obesity is required.

Second, the definition of genetic lean NAFLD remains unclear. The heterozygous minor allele of *PNPLA3* is the most widely known risk factor for NAFLD. Previous studies have suggested that the heterozygous minor allele is associated with the development of NAFLD[22] and hepatic fibrosis.[23-25] However, our data showed that the heterozygous minor allele of *PNPLA3* had no statistically significant effect, and only the homozygous minor allele of *PNPLA3* increased the risk of NAFLD in lean individuals. When individuals with the heterozygous minor allele of *PNPLA3* were included as risk factors, the proportion of type II increased from 32.1% to 63.8% (Supplementary Fig. 4A). Additionally, the proportion of patients with risk variants of *PNPLA3* increased from 52.9% to 76.3% among the patients with lean NAFLD (Supplementary Fig. 4B). In this study, we classified lean NAFLD using only six well-known SNPs. Type II accounted for the highest proportion of lean NAFLD cases, except for type III with unknown risk factors. Further investigation of more SNPs is needed to define type II for precise classification.

Finally, it is unclear whether patients with both metabolic and genetic risk factors should be classified as type I or type II. In our study, the overall clinical characteristics of patients with both MU status and SNPs in generic risk variants were similar to those of the MU status-only group. Moreover, previous studies have shown that SNP-associated NAFLD is associated with favorable metabolic parameters.[26-28] This implies that the associated mechanism is more likely to be metabolic factors rather than genetic factors in individuals with SNPs and metabolic abnormalities. Vilarinho *et al.*[4] also proposed the selection of type I lean NAFLD first if patients were compatible with the definition of type I (individuals with visceral adiposity and insulin resistance but with normal BMI). Therefore, we assumed that it would be reasonable to classify patients with both metabolic and genetic risk factors as having type I.

The fundamental purpose of classifying lean patients with NAFLD is to understand the pathophysiology of lean NAFLD. However, this study could not clearly answer the above question. Two important clues were presented to answer this question. First, metabolic factors are important risk factors for the development of NAFLD, even in lean individuals. The OR (5.465; 95% CI, 3.749 to 7.965; p<0.001) for the development of lean NAFLD in the MU status was higher than that (3.526; 95% CI, 3.010 to 4.130; p<0.001) for non-lean NALFD in the MU status (Fig. 4C and Supplementary Fig. 5). The MU status and metabolic risk abnormalities were also evaluated as independent risk factors for the development of lean NAFLD (Table 2). In line with this, previous studies have shown that lean patients with NAFLD had poor histological findings when metabolic abnormalities were present.[29,30] Second, generic risk variants such as *PNPLA3* and *TM6SF2* are important risk factors not only in lean individuals but also in non-lean individuals. The OR (1.786; 95% CI, 1.425 to 2.238; p<0.001) for lean NAFLD in only SNP status was not different from that (1.781; 95% CI, 1.391 to 2.280; p<0.001) for non-lean NALFD in only SNP status (Fig. 4 and Supplementary Fig. 5). Finally, a considerable proportion of lean NAFLD cases (type III) was not associated with metabolic or genetic risk factors. This implies that there are still a lot of risk factors that must be identified. Sarcopenia, diet, circulating metabolites, gut microbiome, and epigenetic factors are possible risk factors.[31,32] Further studies on additional risk factors are necessary to better understand the pathophysiology of lean NAFLD.

This study had several limitations. First, insulin resistance was defined as the presence of MS or DM, because data on serum insulin levels are not routinely obtained in real-life practice. In this setting, having either MS or diabetes could be a reasonable substitute for the presence of insulin resistance.[33] Second, as the cohort analyzed in this study consisted of health checkup examinees from a single center in Korea, the results would not represent the general population in Korea or other parts of the globe. Notwithstanding, the health checkup cohort reflects the characteristics of the general Korean population, as all employees or adults over 40 years of age are required to receive a health checkup every 1 or 2 years by law in Korea. Meanwhile, the indistinct characteristics according to type in the at-risk group may be related to the small number of patients in each group. Third, fibrosis-4 and NFS are less accurate methods than transient elastography or magnetic resonance elastography used to evaluate the hepatic fibrosis burden. Additional studies, including the determination of hepatic fibrosis burden and liver disease outcomes according to the subtypes of lean NAFLD, are needed. Fourth, we were unable to objectively evaluate the amount of exercise and alcohol intake because the data were based on a self-

reported questionnaire.

In conclusion, the proportions of patients with type I and type II lean NAFLD were 20.1% and 32.1% among lean patients with NAFLD, respectively. A considerable proportion of lean NAFLD cases is not associated with metabolic or genetic risk factors (type III). Definitions of visceral obesity and insulin resistance in lean individuals are required to clearly classify the subtypes of lean NAFLD according to etiology. Further research on additional genetic risk variants or other mechanisms associated with lean NAFLD is required to better understand the disease.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGEMENTS

This work was supported by a Research Program funded by the Korea National Institute of Health (grant number: 2022-ER0906-00 to D.W.J.).

AUTHOR CONTRIBUTIONS

Study concept and design: D.W.J. Data acquisition: M.K., W.H., H.L.K., G.E.C., E.K.C., J.H.B., S.H.C. Data analysis and interpretation: D.W.J., E.L.Y. Drafting of the manuscript: H.P. Critical revision of the manuscript for important intellectual content: D.W.J., S.Y.Y. Statistical analysis: W.H., H.P. Obtained funding: D.W.J. Administrative, technical, or material support; study supervision: D.W.J., S.Y.Y. Approval of final manuscript: all authors.

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SUPPLEMENTARY MATERIALS

Supplementary materials can be accessed at https://doi.org/10.5009/gnl230044.

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