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NAFLD-related gene polymorphisms and all-cause and cause-specific mortality in an Asian population: the Shanghai Changfeng Study

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Summary

Background: The PNPLA3 and TM6SF2 gene variants have been found to cause NAFLD with a favourable cardiovascular risk profile.

Aims: To investigate the effects of the NAFLD risk alleles on the all-cause and cause-specific mortality in 5581 Chinese adults.

Methods: The genome-wide genotypes were detected using a genotyping array and serum lipoprotein profiles were examined using 1H NMR platform. Liver fat content (LFC) was measured using a quantitative ultrasound method. The vital status was determined using official registration data.

Results: Genome-wide association analysis showed that a series of variants in PNPLA3 were associated with LFC, including rs738409 C>G variant ($P = 8.6 \times 10^{-7}$). Further analyses validated the associations of TM6SF2 rs58542926 C>T and MBOAT7 rs641738 C>T variants with NAFLD. During 29 425.1 person-years of follow-up, the overall mortality was 816 per 100 000 person-years, where 299 deaths were attributable to cardiovascular disease and 85 to liver disease. The PNPLA3 rs738409 C>G variant was independently associated with increased liver-specific mortality (P for trend = 0.034) but reduced cardiovascular mortality (P for trend = 0.047). A

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composite genetic-predisposition score of PNPLA3, TM6SF2, and MBOAT7 risk alleles presented similar opposite effects on liver-specific and cardiovascular mortality. Moreover, interactions of the NAFLD risk alleles with adiposity for liver-specific mortality were found ($P_{interaction} < 0.05$). The reduced serum VLDL1 concentration was responsible for the increased liver-specific mortality related to NAFLD risk alleles.

Conclusion: The PNPLA3 rs738409 C>G variant and its combination with TM6SF2 rs58542926 C>T and MBOAT7 rs641738 C>T variants increase liver-specific mortality but reduce cardiovascular mortality in overweight/obese Chinese.

1 | INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease that affects a quarter of the world's population.[1] NAFLD, especially its severe subtype of nonalcoholic steatohepatitis (NASH), was associated with increased risk of overall, liver-specific and cardiovascular mortality, with estimates of cardiovascular mortality incidence at 479 per 100 000 person-years and liver-specific mortality incidence at 77 per 100 000 person-years.[2] Given the rapidly growing prevalence of NAFLD in parallel with the dramatic escalation in obesity and insulin resistance,[3] the NAFLD-related mortality will undergo a steep increase,[4] which deserves extensive attention globally.

Usually, NAFLD arises in the context of metabolic disorders, and cardiovascular disease is the primary cause of death in patients with NAFLD.[5] Lifestyle intervention targeting metabolic disorders has been indicated to reduce NAFLD-related mortality.[6] However, a proportion of individuals developed NAFLD in the absence of obesity and metabolic syndrome,[7] and there is a significant genetic contribution to the development of these lean NAFLD.[8] Several gene variants, such as patatin-like phospholipase domain-containing 3 (PNPLA3) rs738409 C>G variant and transmembrane 6 superfamily member 2 protein (TM6SF2) rs58542926 C>T variant, have been reported to cause NAFLD with a favourable cardiovascular risk profile.[9,10]

The effects of these NAFLD-related gene variants on individual overall and cause-specific mortality have been rarely studied, except for a few studies showing increased liver-related mortality and inconsistent changes of overall and cardiovascular mortality in the PNPLA3 gene variant carriers.[11-15]

However, none of the previous studies has investigated the relationship between NAFLD-related gene variants and cause-specific mortality in the Asian population. Moreover, previous studies found that adiposity[16] and metabolic dysfunction, as defined by the new criteria of metabolic dysfunction-associated fatty liver disease (MAFLD),[17,18] could amplify the effect of multiple genetic variants on fatty liver disease. An interaction of NAFLD risk alleles with diabetes and obesity on liver-specific mortality was also indicated in the European individuals recruited by the UK Biobank.[19] Thus, in the current study, we investigated the effects of multiple NAFLD-related gene variants as well as their interaction with adiposity and metabolic disorders on all-cause and cause-specific mortality in a Han Chinese population, and attempted to explore the underlying in vivo mechanism by measuring serum lipoprotein profile using ^{1}H nuclear magnetic resonance (1H-NMR).

2 | PATIENTS AND METHODS

2.1 | Patients selection

Based on the official residential registration data of the Shanghai Changfeng community, a total of 10 070 participants aged over 45 years old were planned to be recruited in the study from 13 sub-communities of the Shanghai Changfeng community, and 6595 participants (responding rate 65.5%) were enrolled consecutively into the Shanghai Changfeng Study, a community-based prospective cohort study of multiple chronic diseases in a middle-aged and elderly Chinese population, from June 2009 to December 2012.[20] The inclusion criteria of participants were (1) aged over 45 years old and (2) lived in Shanghai Changfeng community for at least 5 years. As shown in the participant flow diagram (Figure S1), the vital status data of 6591 participants were available according to registration data from the Shanghai Center for Disease Control (CDC) at the end of 2016.[21] Among them, a total of 5689 participants were genotyped with an Illumina Infinium BeadChip genotyping array (707 180 markers), and after excluding 60 participants with unqualified genotype data and 48 with missing of serum biochemical or NMR-based lipoprotein profile data, a total of 5581 participants were included for analysis, and 4645 participants with both liver ultrasonography and fibrosis 4 score (FIB-4) data available were selected for subgroup analysis. The study was approved by the Research Ethics Committees of Zhongshan Hospital, Fudan University (No. 2008-119 and B2013-132), and each participant provided written informed consent.

2.2 | Anthropometric and biochemical measurements

The past history, smoking status and alcohol consumption of each participant were collected in a face-to-face interview with a trained

investigator using a standardized questionnaire. Height, weight, waist/hip circumference, systolic blood pressure (SBP), diastolic blood pressure (DBP) and serum biochemical parameters were measured at baseline after a 12-hour overnight fasting as detailed in the Supplemental Methods. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of height in metres (kg/m^2).

2.3 | Quantitative ultrasonography for liver fat content

Hepatic ultrasound examination was carried out by an experienced ultrasonographer (who was unaware of the participants' clinical details and laboratory findings) using a GE LOGIQ P5 ultrasound machine (GE Healthcare) with a 4-MHz probe. Liver fat content (LFC) was quantified using an ultrasound quantitative method,[22] which was described in detail in Supplemental Methods.

2.4 | Evaluation of liver fibrosis by FIB-4

The FIB-4 was used to evaluate the liver fibrosis grades, which was also recommended in EASL-EASD-EASO Clinical Practice Guidelines for the management of NAFLD[23]:

$$\text{FIB - 4} = \text{age (years)} \times \text{as partate transaminase (AST)(U/L)} / \left[\text{platelets } (10^9/\text{L}) \times \sqrt{\text{alanine transaminase (ALT)(U/L)}}\right].$$

Advanced liver fibrosis could be excluded if FIB-4 < 1.30, and confirmed if FIB-4 ≥ 2.67.

2.5 | Genome-wide association study

After the quality control procedure (Supplemental Methods), the participants with both genotyping and LFC data were selected and LFC values were normalized using rank-based inverse normal transformation prior to Genome-wide association study (GWAS). A linear mixed model was used to test the association of autosomal genetic variants with LFC assuming an additive allelic effect using GEMMA software. Age, sex and BMI were also included as covariates. FDR correction was used and $P < 1.05 \times 10^{-6}$ was considered significant. Manhattan plot and QQ plot were generated with the R package qqman v0.1.4.

2.6 | Lipoprotein profile examination using ^{1}H-NMR

Serum lipoprotein profile was examined using a 600 MHz AVANCE III NMR spectrometer equipped with a BBI probe (Bruker Biospin GmbH, Germany) with the same method reported previously.[24] Each serum sample was stored at −80°C and thawed at 24°C within 30 minutes, and all subsequent operations were performed upon the ice. About 350 μl of each serum sample was mixed with 350 μl phosphate buffer (0.085 M, pH 7.4) containing 10% D_2O in a 1.5 ml Eppendorf tube, and 600 μl of the mixture was transferred into a 5 mm diameter NMR tube. The NMR tube with the mixture was then put into the NMR Sample Jet (Bruker Biospin) and maintained at 4°C for metabolomic profiling. All NMR spectra were recorded with a standard NOESYGPPR1D pulse sequence into 98k data points at 310K with 32 transients. A total of 112 lipoprotein parameters (including particle number, main fractions, subclasses, and compositional components therein) were quantified using the Bruker IVDr Lipoprotein Subclass Analysis BILISA™ software package (Bruker Biospin). In addition, 19 cholesterol ester components were then obtained by subtracting the free cholesterol components from the total cholesterols in different lipoprotein particles. The missing values of the lipoprotein parameters were filled with half of their minimum values.

2.7 | Causes of mortality

The vital status of all participants was determined according to registration data from the Shanghai CDC. The causes of death were coded according to the 10th Revision of International Classification of Diseases (ICD-10). The main endpoints during the follow-up included death due to cardiovascular disease (ICD-10 codes I00-I99), liver disease (ICD-10 codes C22.0, C22.2-C22.9 and K70-K76), extrahepatic cancers (ICD-10 codes C00-C21, C22.1 and C23-C97), and other causes. Since NAFLD is a disease spectrum ranging from simple steatosis to hepatocellular carcinoma, deaths from primary liver cancer were accounted into liver disease mortality.[13]

2.8 | Definitions

A BMI over 24 and 28 kg/m^2 were diagnosed as overweight and obesity according to the optimal cutoff for Chinese.[25] Hypertension was defined as a SBP/DBP ≥140/90 mm Hg or self-reported history of hypertension or current use of antihypertensive medications. As detailed in the Supplemental Methods, metabolic syndrome was defined according to the updated NCEP ATPIII criteria,[26] diabetes was diagnosed according to the 1999 WHO criteria, and hyperlipidaemia was diagnosed according to 2016 Chinese guideline for the management of dyslipidemia.[27] Fatty liver was diagnosed when LFC by ultrasonography exceeded the cut-off value of 9.15%.[22] Participants with viral hepatitis, autoimmune hepatitis or other chronic liver diseases were identified according to their past history, and participants with LFC by ultrasonography over 9.15% and excessive alcohol intake (≥140 g per week for men or ≥70 g per week for women) were diagnosed as alcoholic fatty liver disease.[28]

2.9 | Statistical analysis

All statistical analyses were performed using the R software version 3.6.2. The continuous parameters with normal distribution are presented as the means ± SD and skewed parameters are presented as the median with the interquartile range (25%-75%) given in parentheses. LFC values were normalized using rank-based inverse normal transformation. General linear models were used to compare LFC among the participants carrying different candidate NAFLD risk gene variants as listed in Table S1, after adjustment for age, sex and BMI.

TABLE 1 Baseline characteristics of the study population (N = 5581)

		PNPLA3 rs738409			
Parameters	**Total**	**CC (N = 2198)**	**CG (N = 2626)**	**GG (N = 757)**	***P* value**
Age, year	63.7 ± 9.6	63.6 ± 9.6	63.7 ± 9.6	63.8 ± 9.5	0.929
Male gender, n (%)	2382 (42.7)	928 (42.2)	1110 (42.3)	344 (45.4)	0.255
Weight, kg	63.3 ± 10.7	63.4 ± 10.8	63.4 ± 10.7	63.0 ± 10.5	0.789
Height, cm	161.5 ± 8.4	161.4 ± 8.2	161.4 ± 8.6	161.7 ± 8.2	0.697
BMI, kg/m^2	24.2 ± 3.3	24.3 ± 3.3	24.3 ± 3.4	24.1 ± 3.1	0.283
Waist circumference, cm	84.2 ± 9.7	84.2 ± 9.8	84.2 ± 9.5	84.1 ± 9.8	0.924
Hip circumference, cm	93.1 ± 6.8	93.3 ± 6.7	93.1 ± 6.8	92.7 ± 6.9	0.125
Cigarette smoking, n (%)	983 (17.6)	378 (17.2)	477 (18.2)	128 (16.9)	0.585
Alcohol drinking, n (%)	706 (12.7)	279 (12.7)	326 (12.4)	101 (13.3)	0.793
FPG, mmol/L	5.6 ± 1.5	5.6 ± 1.5	5.7 ± 1.6	5.6 ± 1.4	0.569
PPG, mmol/L	7.7 ± 3.3	7.6 ± 3.4	7.7 ± 3.3	7.6 ± 3.2	0.531
HbA1c, %	5.9 ± 0.9	5.8 ± 0.9	5.9 ± 1.0	5.8 ± 0.9	0.111
HOMA-IR	1.9 (1.3-3.0)	1.9 (1.2-3.0)	1.9 (1.3-3.0)	1.9 (1.3-2.9)	0.566
Triglyceride, mmol/L	1.4 (1.0-2.0)	1.4 (1.0-2.0)	1.4 (1.1-2.0)	1.3 (1.0-1.9)	0.003
Total cholesterol, mmol/L	5.1 ± 0.9	5.1 ± 0.9	5.1 ± 0.9	5.0 ± 0.9	0.008
HDL cholesterol, mmol/L	1.43 ± 0.37	1.44 ± 0.37	1.43 ± 0.37	1.42 ± 0.38	0.204
LDL cholesterol, mmol/L	2.88 ± 0.80	2.90 ± 0.79	2.88 ± 0.82	2.86 ± 0.78	0.440
SBP, mm Hg	136 ± 19	136 ± 19	135 ± 19	136 ± 19	0.551
DBP, mm Hg	76 ± 10	76 ± 10	76 ± 10	76 ± 10	0.806
ALT, U/L	16 (12-22)	15 (12-21)	16 (12-23)	16 (12-23)	0.001
AST, U/L	20 (17-24)	20 (17-23)	20 (17-24)	21 (18-24)	0.001
Platelet, 10^9/L	213.2 ± 56.7	214.0 ± 53.1	213.9 ± 59.4	208.2 ± 57.0	0.047
Liver fat content, %*	5.4 (2.4-11.5)	5.0 (2.3-10.7)	5.7 (2.5-11.7)	6.2 (2.6-12.8)	<0.001
Metabolic syndrome, n (%)	2222 (39.8)	892 (40.6)	1046 (39.8)	284 (37.5)	0.331
Obesity, n (%)	671 (12.0)	283 (12.9)	309 (11.8)	79 (10.4)	0.176
Hypertension, n (%)	2163 (38.8)	845 (38.4)	1023 (39.0)	295 (39.0)	0.925
Diabetes, n (%)	1172 (21.0)	444 (20.2)	557 (21.2)	172 (22.7)	0.354
Fatty liver, n (%)*	1512 (32.6)	524 (28.6)	747 (34.1)	241 (38.6)	<0.001
Liver fibrosis grades*					
FIB-4 < 1.30 or no fatty liver	3783 (81.5)	1546 (84.4)	1753 (80.0)	484 (77.6)	<0.001
FIB-4: 1.30 ~ 2.67	781 (16.8)	264 (14.4)	397 (18.1)	120 (19.2)	
FIB-4 ≥ 2.67	81 (1.7)	21 (1.2)	40 (1.8)	20 (3.2)	

Notes: The continuous parameters with normal distribution were presented as the means ± SD, skewed parameters were presented as the median with the interquartile range (25%-75%) given in parentheses and the categorical parameters were presented as the frequency with the percentage given in parentheses. FIB-4, fibrosis 4 score. No advanced fibrosis: FIB-4 < 1.30, uncertain fibrosis: FIB-4 1.30 ~ 2.67, advanced fibrosis: FIB-4 ≥ 2.67.

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; FPG, fasting plasma glucose; HDL, high-density lipoprotein-cholesterol; HOMA-IR, homeostasis model assessment for insulin resistance; LDL, low-density lipoprotein-cholesterol; PPG, post-load plasma glucose.

*A total of 4645 participants with liver ultrasonography and NAFLD fibrosis score data available.

A genetic-predisposition score for NAFLD was calculated on the basis of the PNPLA3 rs738409, TM6SF2 rs58542926 and membrane bound O-acyltransferase domain-containing 7 [MBOAT7]) rs641738 single-nucleotide polymorphisms (SNPs).[29] Each SNP was weighted according to its relative effect size (β coefficient) on LFC, as shown in Table S2. The genetic-predisposition score (GPS) was rescaled to represent one effect allele per point of the score. The GPS was calculated using the following equation: GPS = 1.64 × rs738409 C>G allele number +1.08 × rs58542926 C>T allele number +0.56 × rs641738 C>T allele number. We

TM6SF2 rs58542926				MBOAT7 rs641738			
CC (N = 4895)	CT (N = 659)	TT (N = 27)	*P* value	CC (N = 3158)	CT (N = 2070)	TT (N = 353)	*P* value
63.7 ± 9.6	63.4 ± 9.1	66.2 ± 12.5	0.306	63.6 ± 9.5	63.7 ± 9.6	64.0 ± 9.6	0.827
2071 (42.3)	299 (45.4)	12 (44.4)	0.323	1326 (42.0)	901 (43.5)	155 (43.9)	0.486
63.3 ± 10.7	63.5 ± 10.6	59.9 ± 11.0	0.221	63.2 ± 10.6	63.4 ± 10.9	63.7 ± 10.1	0.640
161.4 ± 8.4	161.7 ± 8.5	160.0 ± 9.2	0.518	161.4 ± 8.4	161.5 ± 8.4	161.6 ± 8.5	0.942
24.2 ± 3.3	24.3 ± 3.2	23.3 ± 2.9	0.305	24.2 ± 3.3	24.3 ± 3.4	24.4 ± 3.3	0.599
84.2 ± 9.7	84.3 ± 9.8	82.7 ± 8.1	0.710	84.2 ± 9.6	84.2 ± 9.7	84.4 ± 9.7	0.896
93.1 ± 6.8	93.1 ± 6.6	91.1 ± 7.2	0.300	93.1 ± 6.7	93.1 ± 6.8	93.4 ± 7.2	0.738
868 (17.7)	112 (17.0)	3 (11.1)	0.604	553 (17.5)	366 (17.7)	64 (18.1)	0.954
624 (12.7)	78 (11.8)	4 (14.8)	0.759	385 (12.2)	270 (13.0)	51 (14.4)	0.382
5.6 ± 1.5	5.7 ± 1.5	5.7 ± 1.7	0.540	5.6 ± 1.6	5.6 ± 1.5	5.5 ± 1.3	0.170
7.7 ± 3.3	7.9 ± 3.5	6.5 ± 2.7	0.097	7.7 ± 3.4	7.7 ± 3.3	7.6 ± 2.9	0.757
5.9 ± 0.9	5.9 ± 0.9	5.9 ± 1.2	0.985	5.9 ± 0.9	5.9 ± 0.9	5.8 ± 0.9	0.398
1.9 (1.3-3.0)	1.9 (1.3-2.9)	1.4 (1.1-2.2)	0.253	1.9 (1.3-3.0)	1.9 (1.3-2.9)	1.9 (1.3-2.9)	0.846
1.4 (1.1-2.0)	1.3 (1.0-1.9)	1.0 (0.8-1.6)	<0.001	1.4 (1.1-2.0)	1.4 (1.0-2.0)	1.5(1.1-2.0)	0.472
5.1 ± 0.9	5.0 ± 0.9	4.7 ± 1.2	0.026	5.1 ± 0.9	5.0 ± 0.9	5.1 ± 0.9	0.049
1.43 ± 0.37	1.44 ± 0.38	1.48 ± 0.41	0.660	1.43 ± 0.36	1.44 ± 0.39	1.43 ± 0.38	0.727
2.89 ± 0.80	2.85 ± 0.80	2.70 ± 1.02	0.265	2.90 ± 0.82	2.85 ± 0.78	2.86 ± 0.79	0.014
135 ± 19	136 ± 20	138 ± 21	0.463	135 ± 19	135 ± 19	136 ± 21	0.675
76 ± 10	77 ± 10	75 ± 9	0.402	76 ± 10	76 ± 10	76 ± 10.3	0.906
16 (12-22)	16 (12-23)	15 (13-18)	0.291	16 (12-22)	16 (12-22)	15 (12-20)	0.273
20 (17-24)	20 (17-24)	20 (18-25)	0.634	20 (17-24)	20 (18-24)	20 (17-23)	0.717
213.3 ± 56.9	212.4 ± 54.0	205.2 ± 75.4	0.724	214.4 ± 59.8	211.1 ± 52.3	214.7 ± 52.0	0.124
5.4 (2.4-11.3)	6.7 (2.7-12.7)	6.2 (3.2-11.5)	0.001	5.4 (2.4-11.4)	5.4 (2.4-11.4)	6.5 (2.8-12.8)	0.030
1958 (40.0)	258 (39.2)	6 (22.2)	0.159	1267 (40.1)	817 (39.5)	138 (39.1)	0.859
591 (12.1)	79 (12.0)	1 (3.7)	0.411	346 (11.0)	280 (13.5)	45 (12.7)	0.018
1885 (38.5)	266 (40.4)	12 (44.4)	0.548	1224 (38.8)	796 (38.5)	143 (40.5)	0.764
1028 (21.0)	154 (22.0)	2 (7.4)	0.230	663 (21.0)	439 (21.2)	71 (20.2)	0.915
1297 (31.7)	208 (38.9)	7 (29.2)	0.004	852 (32.3)	545 (31.6)	115 (40.1)	0.017
3353 (82.1)	412 (77.0)	18 (75.0)	0.005	2154 (81.7)	1413 (82.0)	216 (75.3)	0.165
664 (16.3)	112 (20.9)	5 (20.8)		436 (16.5)	278 (16.1)	67 (23.3)	
69 (1.7)	11 (2.1)	1 (4.2)		45 (1.7)	32 (1.9)	4 (1.4)	

divided the genetic-predisposition score into tertiles for further analysis: Tertile 1, GPS ≤ 1.0; Tertile 2, GPS > 1.0 and GPS ≤ 2.2; Tertile 3, GPS > 2.2.

Multivariate Cox proportional hazard models were used to estimate the hazard ratios (HRs) and 95% confidence interval (95% CI) for the overall and cause-specific mortality in participants carrying different risk alleles and genetic predisposition of NAFLD. The interactive effect of the NAFLD risk alleles with multiple metabolic comorbidities was investigated by entering the relative interactive items into the statistical models. To take all competitive events of death into consideration, the competitive risk models were also used to compare the cause-specific mortality among the participants carrying different NAFLD-related genotypes. All the participants were censored at death or the end of 2016. The period between enrolment and censoring was defined as follow-up length. Subgroup analyses were performed in 4645 participants with LFC and FIB-4 score information available and 5182 participants without other known chronic liver diseases. Potential confounders adjusted in the multivariate Cox proportional hazard models and competitive risk models included age, sex, cigarette smoking, alcohol drinking, BMI, SBP, fasting plasma glucose (FPG), serum triglycerides (TG) and high-density lipoprotein (HDL) cholesterol. LFC was further adjusted for in the subgroup with LFC information.

Each component of the serum lipoprotein profile was transformed to normality via rank-based inverse normal transformation before analysis. The effect of each PNPLA3 risk allele and each point of NAFLD genetic-predisposition score on the lipoprotein profile was estimated using linear regression models after multiple adjustment. Serum TG and HDL cholesterol were not adjusted due to their co-linearity with several components of lipoprotein profile. We considered statistical significance at $P < 0.0071$ (0.05/7), where 7 is the number of principal components explaining 95% of the variation in the NMR lipoprotein profile data. The effect of each component of lipoprotein profile on liver-specific and cardiovascular mortality was examined using Cox proportional hazard model, with multiple adjustment and false discovery rate (FDR) correction. Cox proportional hazard models were further used to compare the liver-specific mortality among the participants with genetic risk of NAFLD with low and high serum VLDL1-TG levels after multivariate adjustment.

All statistical analyses were two-sided and $P < 0.05$ was considered statistically significant unless otherwise stated.

3 | RESULTS

3.1 | Baseline characteristics

A total of 5581 participants were enrolled from the Shanghai Changfeng Study. The baseline characteristics of the study participants were shown in Table 1. All the participants were aged over 45 years with an average age of 63.7 years old and BMI of 24.2 kg/m^2. The prevalence of diabetes and metabolic syndrome by ATPIII criteria in this study population was 21.0% and 39.8% respectively. Subgroup analysis was performed in 4645 participants with both LFC and FIB-4 score data available, whose median LFC was 5.4% and prevalence of fatty liver was 32.6%.

3.2 | NAFLD-related gene variants in Shanghai Changfeng Cohort

Genome-wide association analysis showed a series of variants in PNPLA3 was strongly associated with LFC, including rs738409 C>G variant ($P = 8.6 \times 10^{-7}$), as shown in Figure 1A. After adjustment for age, sex and BMI, the homozygous PNPLA3 rs738409 C>G and MBOAT7 rs641738 C>T variants and the heterozygous PNPLA3 rs738409 C>G and TM6SF2 rs58542926 C>T variants were significantly associated with increased LFC in Shanghai Changfeng population (Figure 1B-D). However, other candidate gene variants in Table S1 showed no significant correlation with LFC in our study population and the frequency of SERPINA1 rs28929474 C>T variant was extremely low in Chinese (Figure S2). Thus, the PNPLA3, TM6SF2 and MBOAT7 gene variants were selected for further analysis.

Consistent with the changes in liver steatosis, the PNPLA3 rs738409 C>G and TM6SF2 rs58542926 C>T variants carriers also presented more severe liver fibrosis grades determined by FIB-4, but their serum cholesterol and triglycerides were significantly lower than those without PNPLA3 or TM6SF2 variant (Table 1). The other clinical metabolic parameters, such as body weight, waist circumference, blood pressure and plasma glucose, showed no difference among the participants carrying different PNPLA3, TM6SF2 or MBOAT7 genotypes (Table 1).

3.3 | NAFLD-related gene variants and overall and cause-specific mortality

The prospective analysis covered 29 425.1 person-years in 5581 participants. The mean follow-up time was 5.27 years (range: 0.13-7.58 years). At the end of follow-up, the all-cause mortality was 816 per 100 000 person-years, including 85 per 100 000 person-years from liver diseases and 299 per 100 000 person-years from cardiovascular diseases. After excluding participants with other known chronic liver diseases, the liver-specific mortality was 62 per 100 000 person-years. The HRs and 95% CI of all-cause and cause-specific mortality of different NAFLD-related gene variant carriers were listed in Table 2. For the PNPLA3 rs738409 C>G variant carriers, the liver-specific mortality increased from 43 (14-101) per 100 000 person-years in PNPLA3 CC genotype carriers to 149 (55-325) per 100 000 person-years in PNPLA3 GG genotype carriers (multivariate HR, 3.41; 95% CI, 1.03-11.30). Intriguingly, the cardiovascular mortality decreased from 371 (268-499) per 100 000 person-years in PNPLA3 CC genotype carriers to 174 (70-359) per 100 000 person-years in

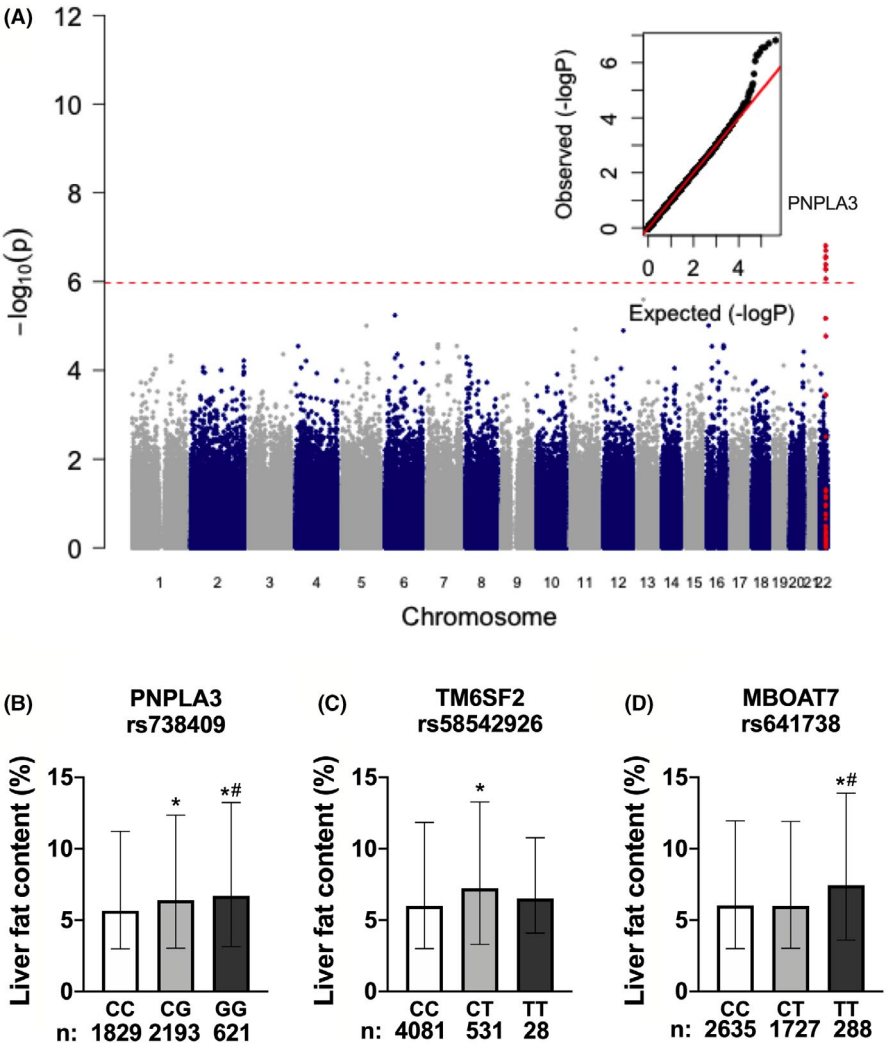


FIGURE 1 NAFLD risk gene variants in Shanghai Changfeng Study population. A, Quantile-quantile plot and Manhattan plot of *P* values for genome-wide association study of liver fat content in Shanghai Changfeng Study. Level of significance: $P < 1.05 \times 10^{-6}$ after FDR correction. (Linear mixed model adjusting for age, sex and BMI). B-D, Liver fat content (median and interquartile range) in participants with different PNPLA3, TM6SF2 and MBOAT7 gene variants. Level of significance: $P < 0.05$ (generalized linear models adjusting for age, sex and BMI, and liver fat contents were normalized using rank-based inverse normal transformation before analysis). $^{*}P < 0.05$ compared with gene variant non-carriers. $^{\#}P < 0.05$ compared with heterozygous gene variant carriers

PNPLA3 GG genotype carriers (multivariate-adjusted HR, 0.48, 95% CI, 0.22-1.07). In an additive genetic model, each PNPLA3 rs738409 C>G allele was significantly associated with increased liver-specific mortality (*P* for trend = 0.034) and decreased cardiovascular mortality (*P* for trend = 0.047) after full adjustment (Figure 2A,B). Competitive risk models were also used to eliminate the influence of competitive risk to the cause-specific mortality, and the effect of the PNPLA3 rs738409 C>G allele on liver-specific and cardiovascular mortality was consistent with the result of the Cox regression analysis (Table 2). For other NAFLD-related gene variants, there were no significant associations of TM6SF2 and MBOAT7 gene variants with the overall or cause-specific mortality, except for a reduced overall mortality (multivariate-adjusted HR, 0.58; 95% CI, 0.35-0.95) and cardiovascular mortality (multivariate-adjusted HR, 0.35; 95% CI, 0.13-0.97) in heterozygous TM6SF2 rs58542926 C>T variant carriers.

The effect of PNPLA3 gene variant on liver-specific and cardiovascular mortality remained significant even after adjustment for LFC in the subgroup with liver fat quantification information and the subgroup without other liver diseases (such as viral hepatitis, autoimmune hepatitis and alcoholic fatty liver diseases) (Tables S3 and S4).

3.4 | Adiposity interacted with the PNPLA3 risk alleles to promote liver-specific mortality

We further divided the study population into subgroups according to their age, sex, BMI and metabolic comorbidities. As shown in Figure 2C, the associations of PNPLA3 gene variant with liver-specific mortality were similar between the male and female participants, and the participants aged ≤65 and >65 years. However, the effect of the PNPLA3 gene variant on liver-specific mortality was significant only in the participants with BMI ≥24 kg/m^2 or the presence of diabetes, hypertension, hyperlipidaemia or metabolic syndrome, and an interaction between adiposity and PNPLA3 risk alleles on liver-specific mortality was found ($P_{interaction}$ = 0.045). The PNPLA3 rs738409 C>G variant was associated with approximately a two-fold increase in the risk of liver-specific mortality in the overweight/obese (BMI ≥ 24 kg/m^2) participants (multivariate-adjusted HR, 2.83; 95% CI, 1.34-5.98), but the association was not detected in the lean participants (BMI < 24 kg/m^2). Similarly, a significant reduction in cardiovascular mortality was observed in the PNPLA3 risk allele carriers with diabetes, hypertension and aged over 65. No interaction was found between the PNPLA3 risk allele and adiposity/metabolic disorders on the cardiovascular mortality (Figure 2C).

TABLE 2 Hazard ratios and 95% confidence interval of all-cause and cause-specific mortality according to NAFLD-related gene variants (N = 5581)

	PNPLA3 rs738409			TM6SF2 rs58542926			MBOAT7 rs641738		
Cause of death	CC (N = 2198)	CG (N = 2626)	GG (N = 757)	CC (N = 4895)	CT (N = 659)	TT (N = 27)	CC (N = 3158)	CT (N = 2070)	TT (N = 353)
All-cause mortality									
Mortality/100 000 person-years	767 (616-944)	870 (721-1040)	771 (524-1094)	856 (747-977)	490 (285-784)	1429 (173-5160)	804 (676-955)	826 (664-1015)	858 (490-1393)
Cox proportional hazards models									
Age-adjusted HR (95% CI)	Reference	1.12 (0.85-1.47)	1.00 (0.66-1.50)	Reference	0.60 (0.37-0.99)	1.11 (0.28-4.49)	Reference	1.01 (0.77-1.32)	1.01 (0.60-1.69)
P value		0.417	0.984		0.043	0.931		0.930	0.970
Multivariate-adjusted HR (95% CI)	Reference	1.14 (0.86-1.50)	0.99 (0.66-1.50)	Reference	0.58 (0.35-0.95)	0.66 (0.16-2.72)	Reference	0.99 (0.75-1.29)	1.11 (0.66-1.86)
P value		0.367	0.977		0.030	0.540		0.940	0.694
Liver-specific mortality (including primary hepatocarcinoma)									
Incidence/100 000 person-years	43 (14-101)	101 (56-170)	149 (55-325)	81 (50-124)	115 (31-295)	0 (0-2630)	72 (37-126)	101 (50-181)	107 (13-387)
Cox proportional hazards models									
Age-adjusted HR (95% CI)	Reference	2.36 (0.85-6.54)	3.35 (1.02-10.97)	Reference	1.47 (0.50-4.28)	—	Reference	1.40 (0.62-3.16)	1.40 (0.31-6.25)
P value		0.100	0.046		0.484	—		0.421	0.659
Multivariate-adjusted HR (95% CI)	Reference	2.47 (0.88-6.89)	3.41 (1.03-11.30)	Reference	1.33 (0.45-3.92)	—	Reference	1.33 (0.58-3.03)	1.53 (0.34-6.85)
P value		0.086	0.045		0.604	—		0.491	0.576
Competitive risk models									
Age-adjusted HR (95% CI)	Reference	2.36 (0.85-6.57)	3.38 (1.03-11.05)	Reference	1.51 (0.51-4.40)	—	Reference	1.41 (0.62-3.20)	1.44 (0.33-6.42)
P value		0.099	0.044		0.453	—		0.410	0.640
Multivariate-adjusted HR (95% CI)	Reference	2.41 (0.88-6.61)	3.34 (1.01-11.17)	Reference	1.41 (0.48-4.17)	—	Reference	1.36 (0.59-3.14)	1.56 (0.36-6.85)
P value		0.088	0.047		0.530	—		0.470	0.550
Cardiovascular disease mortality									
Incidence/100 000 person-years	371 (268-499)	275 (195-378)	174 (70-359)	325 (260-403)	115 (31-295)	0 (0-2630)	294 (218-389)	285 (193-404)	429 (185-845)
Cox proportional hazards models									
Age-adjusted HR (95% CI)	Reference	0.74 (0.48-1.15)	0.45 (0.20-1.00)	Reference	0.37 (0.14-1.02)	—	Reference	0.95 (0.61-1.50)	1.37 (0.65-2.89)
P value		0.184	0.049		0.054	—		0.836	0.413
Multivariate-adjusted HR (95% CI)	Reference	0.77 (0.49-1.18)	0.48 (0.22-1.07)	Reference	0.35 (0.13-0.97)	—	Reference	0.90 (0.57-1.41)	1.43 (0.67-3.02)
P value		0.218	0.072		0.043	—		0.647	0.355
Competitive risk models									
Age-adjusted HR (95% CI)	Reference	0.73 (0.47-1.12)	0.46 (0.21-1.00)	Reference	0.38 (0.14-1.04)	—	Reference	0.96 (0.61-1.50)	1.41 (0.67-2.94)
P value		0.150	0.049		0.059	—		0.860	0.370
Multivariate-adjusted HR (95% CI)	Reference	0.75 (0.48-1.15)	0.47 (0.21-1.06)	Reference	0.37 (0.13-1.02)	—	Reference	0.92 (0.58-1.45)	1.46 (0.70-3.05)
P value		0.180	0.069		0.055	—		0.710	0.320

Note: The multivariate model was adjusted for age, sex, alcohol drinking, cigarette smoking, BMI, SBP, fasting plasma glucose, serum triglycerides and HDL cholesterol.

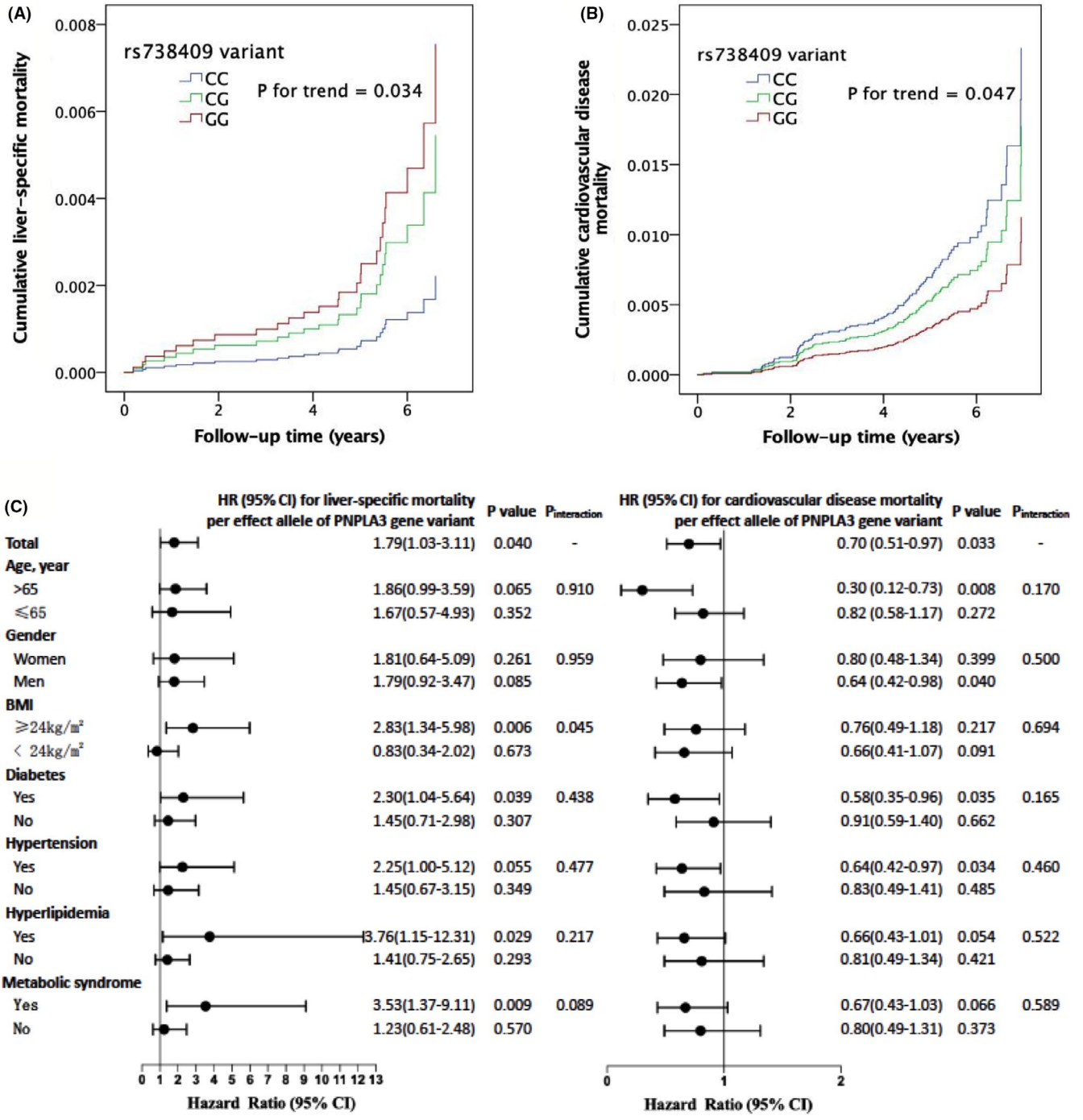


FIGURE 2 PNPLA3 gene variants and liver and cardiovascular disease mortality. A, B, Cumulative mortality from liver and cardiovascular disease in participants with different PNPLA3 genotypes. Level of significance: $P < 0.05$ (Cox proportional hazard models after full adjustment for age, sex, cigarette smoking, alcohol drinking, BMI, SBP, FPG, TG and HDL cholesterol). C, Subgroup analyses of risks of liver-specific and cardiovascular mortality per effect allele of PNPLA3 gene variant in the participants divided by age, sex, BMI, the presence of diabetes, hypertension, hyperlipidaemia and metabolic syndrome by ATPIII criteria. Level of significance: $P < 0.05$ (Cox proportional hazard models after adjustment for age, sex, cigarette smoking, and alcohol drinking)

3.5 | Serum very low-density lipoprotein 1 (VLDL1) level was related to liver-specific mortality in the overweight/obese PNPLA3 gene variant carriers

NMR_based serum lipoprotein profile was further examined. Each effect allele of PNPLA3 rs738409 C>G gene variant was significantly associated with reduced components of the serum very low-density lipoprotein 1 (VLDL1) and low-density lipoprotein 2 (LDL2) in overweight/obese participants (Figure 3, left panel). However, none of the lipoprotein components in the VLDLs or LDLs was associated with the PNPLA3 risk allele in the lean participants (Figure 3, right panel).

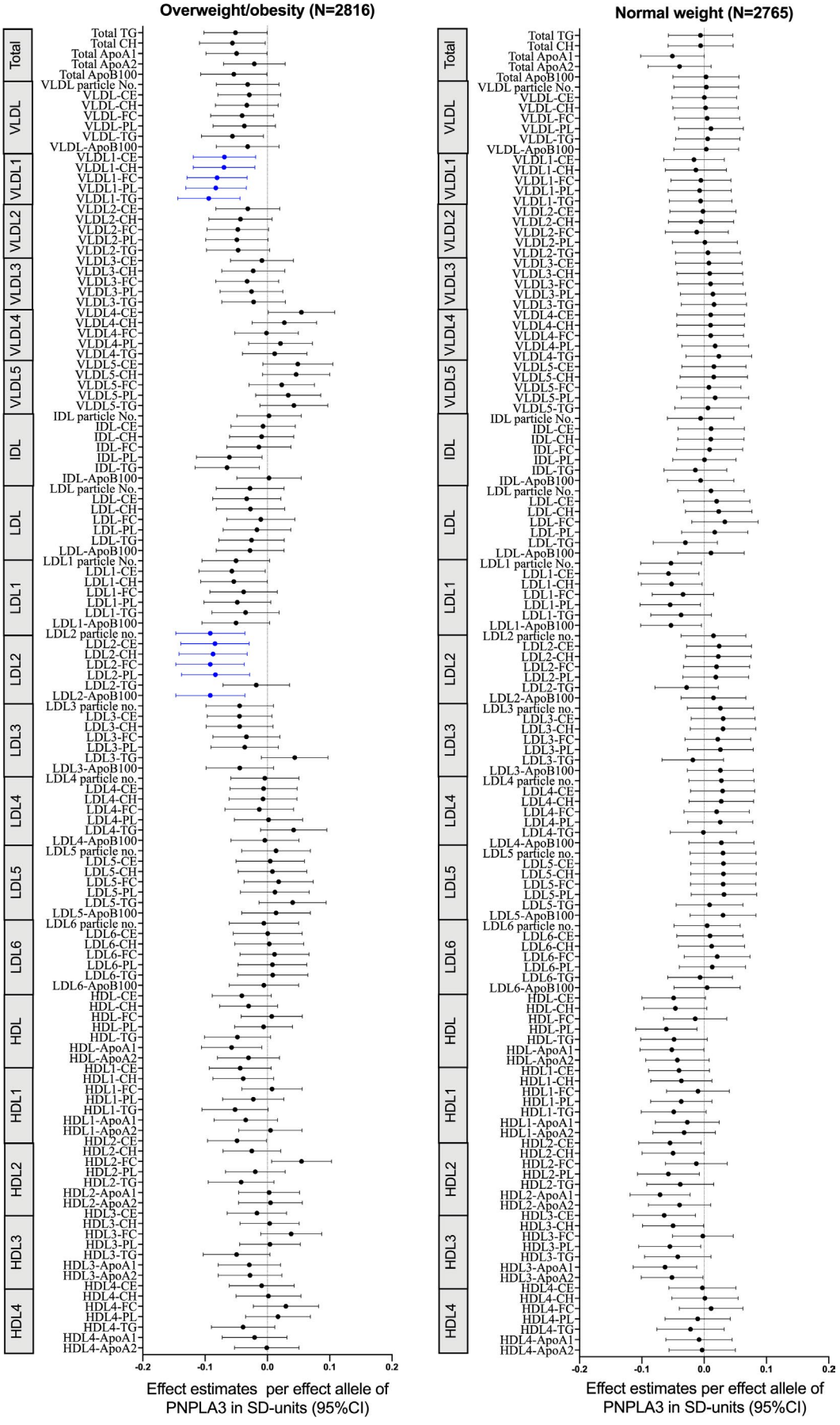


FIGURE 3 Serum lipoprotein profile related to PNPLA3 gene variant in participants divided by BMI levels. Effect estimates of each effect allele of PNPLA3 gene variant on serum lipoprotein profiling in overweight/obese (N = 2816) and lean (N = 2765) participants. The effect estimates with 95% CI were standardized in SD-units. Level of significance: $P < 0.0071$ (generalized linear models adjusting for age, sex, cigarette smoking, alcohol drinking, BMI, SBP and FPG)

We further analysed the associations of all lipoprotein profile components with their liver-specific and cardiovascular mortality as shown in Table S5. As shown in Table 3, we found that the reduced components in VLDL1 were associated with the increased risk of liver-specific mortality after full adjustment (FDR < 0.05), and this kind of reduction was only observed in the overweight/obese but not lean participants (Figure 3), which could well explain the adiposity-dependent risk of liver-specific mortality in PNPLA3 gene variant carriers. The cardiovascular mortality was associated with several components of the serum LDL1 and LDL2 but not the serum VLDLs, and the reduced LDL2 components in the PNPLA3 gene variant carriers might correlate with their reduced cardiovascular mortality (Table S5).

3.6 | Composite effect of the PNPLA3, TM6SF2 and MBOAT7 gene variants on liver-specific and cardiovascular mortality

Among the 3383 PNPLA3 rs738409 C>G variant carriers, only 49.4% carried single PNPLA3 variant, and the other 45.4% and 5.2% carried double and triple NAFLD-related variants (TM6SF2 and/or MBOAT7 gene variants) respectively. A genetic-predisposition score for NAFLD was calculated on the basis of the PNPLA3, TM6SF2 and MBOAT7 risk alleles. A higher genetic-predisposition score indicated higher genetic risk of NAFLD. As shown in Figure S3, the higher tertiles of genetic-predisposition score were associated with higher LFC (P for trend < 0.001) and serum ALT levels (P for trend = 0.001), but lower serum triglycerides (P for trend = 0.002) and cholesterol (P for trend = 0.024). The participants with high genetic predisposition score were associated with significantly increased liver-specific mortality (P for trend = 0.022) but reduced mortality from cardiovascular diseases (P for trend = 0.017), after multivariate adjustment (Figure 4A,B). Subgroup analyses indicated that the participants with genetic risk of NAFLD had increased liver-specific mortality in the presence of obesity, hypertension, hyperlipidaemia and metabolic syndrome (Figure 4C). An interaction of NAFLD genetic-predisposition score with adiposity (P for interaction = 0.046) and metabolic syndrome (P for interaction = 0.041) for liver-specific mortality was also found. In addition, the participants with genetic risk of NAFLD showed reduced risk of cardiovascular mortality only in the presence of adiposity, diabetes, hypertension, hyperlipidaemia or metabolic syndrome (all $P < 0.05$), as shown in the right panel of Figure 4C.

Lipoprotein profile analysis indicated that each NAFLD genetic risk allele was associated with significant reductions in the total cholesterol, components of serum VLDL1 and IDL in overweight/obese participants (Figure 5, left panel), which was partially responsible for the increased liver-specific mortality (Table 3) and reduced mortality from cardiovascular disease in participants with high genetic risk of NAFLD (Table S5).

TABLE 3 Association of serum VLDL1 components with liver disease mortality

Lipoprotein component (per SD)	Liver-specific mortality		
	Hazard ratios (95% CI)	*P* value	FDR *P* value
VLDL1-CE	0.62 (0.36-1.09)	0.097	0.149
VLDL1-CH	0.62 (0.36-1.08)	0.093	0.147
VLDL1-FC	0.54 (0.30-0.97)	0.039	0.075
VLDL1-PL	0.52 (0.30-0.90)	0.020	0.049
VLDL1-TG	0.53 (0.32-0.90)	0.018	0.049

Notes: Hazard ratios and their 95% CI were standardized in SD-units. Age, sex, cigarette smoking, alcohol drinking, BMI, fasting plasma glucose and systolic blood pressure were adjusted in the Cox regression model.

Abbreviation: FDR, false discovery rate.

3.7 | Serum VLDL1-TG concentration determined the liver-specific mortality in patients with genetic risk of NAFLD

Serum VLDL1-PL and VLDL1-TG concentrations were independently and inversely associated with liver-specific mortality after multiple adjustment (FDR < 0.05) (Table 3). Compared with the PNPLA3 CC genotype carriers or the participants with low NAFLD genetic-predisposition score, the PNPLA3 CG and GG genotype carriers or the participants with high NAFLD genetic-predisposition score had a more than 3-fold higher risk of liver-specific mortality if their serum VLDL1-TG levels were below the median value of the whole population (Figure 6A,B). However, for the participants with serum VLDL1-TG levels above the median, there was no significant difference in the liver-specific mortality among the PNPLA3 CC, CG and GG genotype carriers, and the participants with high and low genetic-predisposition score of NAFLD (Figure 6A,B).

4 | DISCUSSION

To the best of our knowledge, our current study is the first study on the associations of NAFLD-related gene variants with all-cause and cause-specific mortality in a large-scale Asian population.

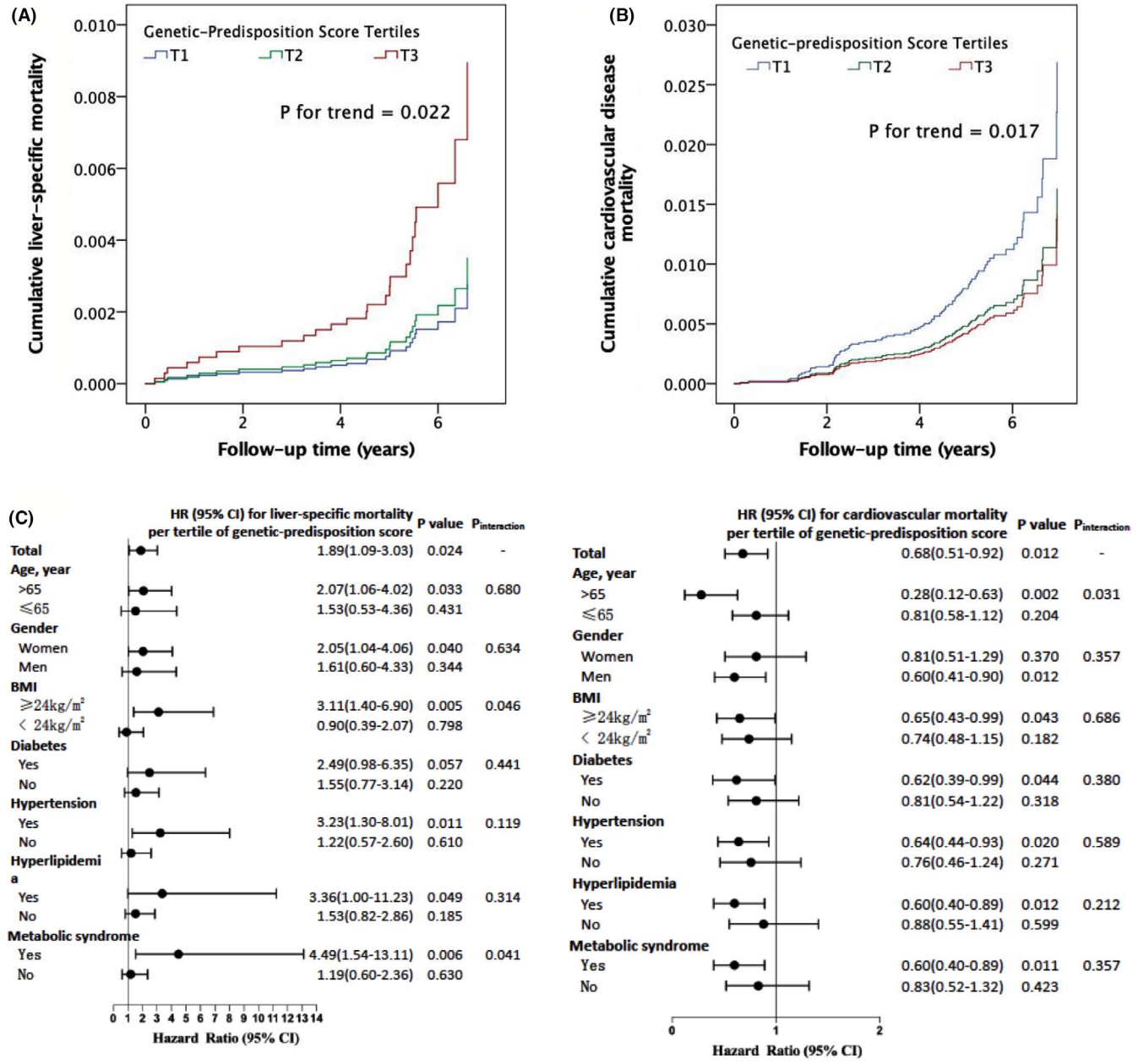
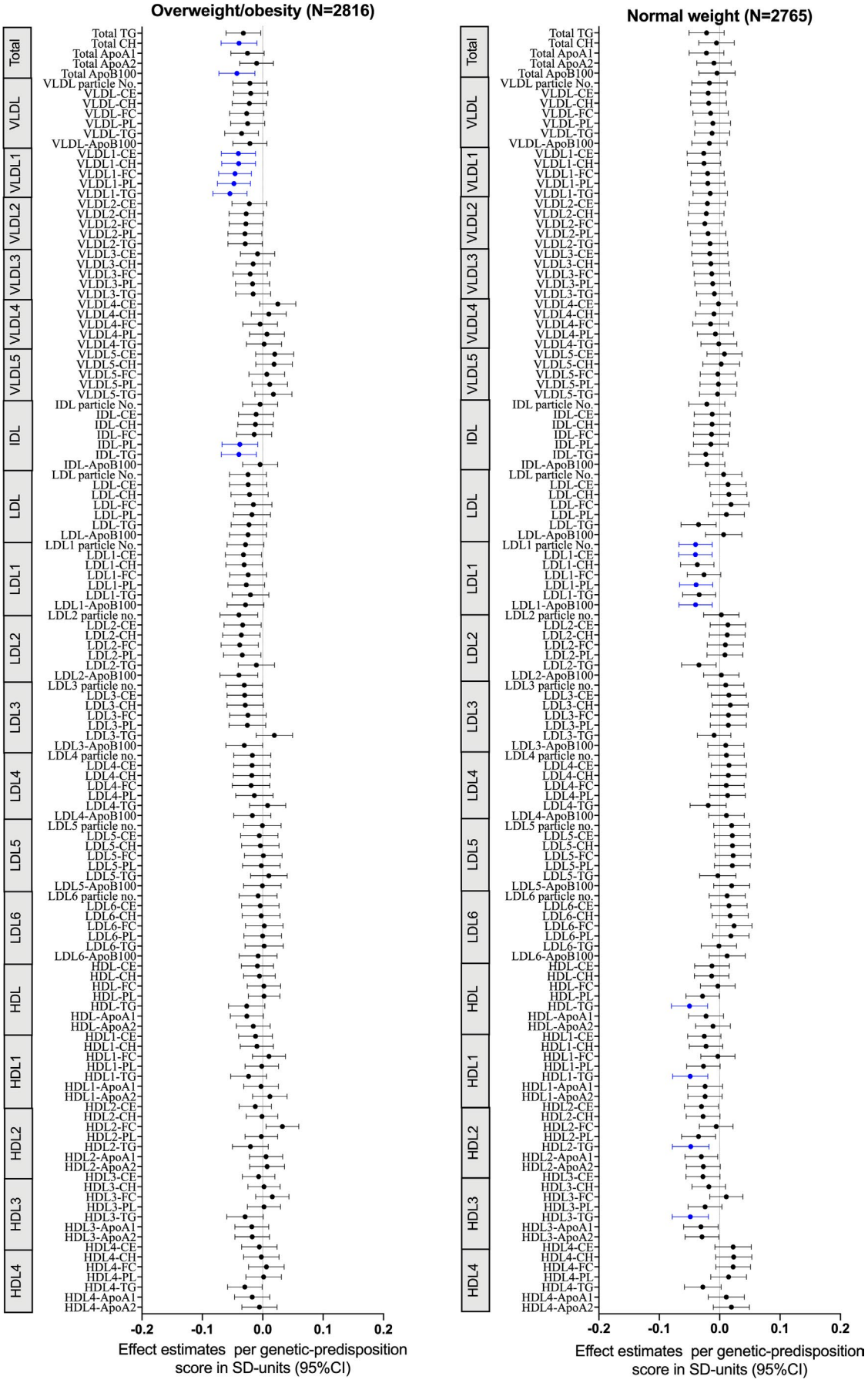


FIGURE 4 Composite effect of PNPLA3, TM6SF2 and MBOAT7 gene variants on liver-related and cardiovascular mortality. A, B, Cumulative mortality from liver and cardiovascular disease in participants with tertiles of genetic-predisposition score. Level of significance: $P < 0.05$ (Cox proportional hazard models after full adjustment for age, sex, cigarette smoking, alcohol drinking, BMI, SBP, FPG, TG and HDL cholesterol). C, Subgroup analyses of risks of liver-specific and cardiovascular mortality per tertile of NAFLD genetic-predisposition score in participants divided by age, sex, BMI, the presence of diabetes, hypertension, hyperlipidaemia and metabolic syndrome by ATPIII criteria. Level of significance: $P < 0.05$ (Cox proportional hazard models after adjustment for age, sex, cigarette smoking, and alcohol drinking)

With the advantage of our complete genome-wide genotyping, serum biochemical, NMR-based lipoprotein profiling and mortality registration database, we found that PNPLA3 rs738409 C>G variant and a composite genetic-predisposition score of PNPLA3, TM6SF2 and MBOAT7 risk alleles were associated with increased liver-specific mortality but reduced cardiovascular mortality in the Chinese population with adiposity or metabolic disorders. Adiposity interacted with the NAFLD risk alleles to promote

FIGURE 5 Serum lipoprotein profile related to NAFLD genetic-predisposition score in participants divided by BMI levels. Effect estimates of each point of genetic-predisposition score on serum lipoprotein profiling in overweight/obese (N = 2816) and lean (N = 2765) participants. The effect estimates with 95% CI were standardized in SD-units. Level of significance: $P < 0.0071$ (generalized linear models adjusting for age, sex, cigarette smoking, alcohol drinking, BMI, SBP and FPG)



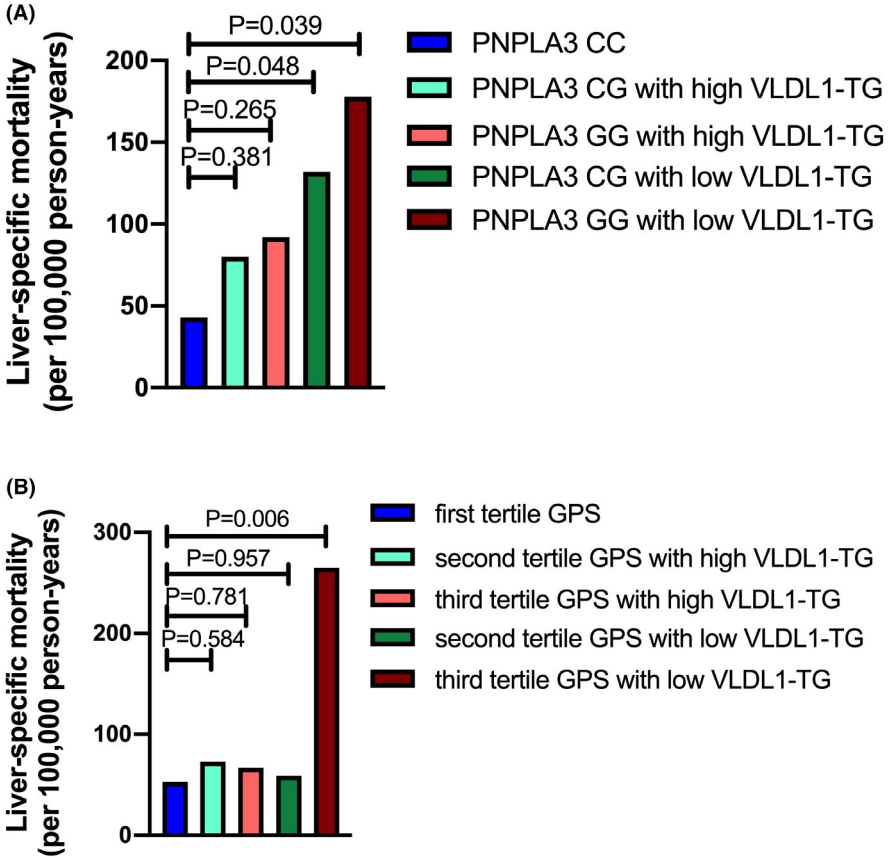


FIGURE 6 Liver-specific mortality in patients with genetic risk of NAFLD divided by serum VLDL1-TG concentration. A, Comparison of liver-specific mortality among the PNPLA3 CC genotype carriers and PNPLA3 CG and GG genotype carriers with low and high VLDL1-TG levels. B, Comparison of liver-specific mortality among the participant with low genetic-predisposition score (first tertile of GPS) and the participants with median and high genetic-predisposition score (second and third tertiles of GPS) with low and high VLDL1-TG levels. Level of significance: $P < 0.05$ (Cox proportional hazard models after adjustment for age, sex, cigarette smoking and alcohol drinking). GPS, genetic-predisposition score

liver-specific mortality through reducing the export of the VLDL1 particles from the liver. Thus, a measurement of the serum concentration of VLDL1 components in patients with genetic risk of NAFLD could help to identify individuals with increased risk of liver-specific mortality.

The heritability of hepatic steatosis is approximately 50%,[30] and the main inherited determinant of NAFLD is the polymorphism of PNPLA3.[31] Previous studies have demonstrated that the PNPLA3 rs738409 C>G variant increases the risk of the entire spectrum of NAFLD (including steatosis, fibrosis and hepatocellular carcinoma),[32] but reduces the serum lipids levels and risk of cardiovascular disease.[33] More recently, two European studies and one multiethnic study from NHANES III found that PNPLA3 variant was associated with increased liver-related mortality.[11-14] Consistently, our current study found that PNPLA3 rs738409 C>G variant increased liver-related mortality in Chinese adults.

PNPLA3, also named adiponutrin, is a 481 amino-acids protein that functions as an enzyme with lipase activity towards triglycerides and retinyl esters, and acyltransferase activity on phospholipids.[34] The rs738409 C>G variant of PNPLA3 causes the loss of enzymatic function of triglycerides hydrolysis in hepatocytes.[35,36] Consistent with the animal studies and in vivo VLDL kinetic study in 55 overweight/obese participants,[37] our lipoprotein profiling data also found that PNPLA3 rs738409 C>G variant reduced the export of triglycerides-enriched VLDL1 from the liver to the peripheral circulation in the presence of adiposity, and contributed to the increased liver disease mortality. In addition, several components in LDL1/2 were also decreased in PNPLA3 variant carriers, which might be secondary to the VLDL change and responsible for the reduced cardiovascular mortality in the PNPLA3 gene variant carriers.

It is noteworthy that PNPLA3 gene variant increased liver-related mortality only in participants with BMI ≥ 24 kg/m^2 or metabolic disorders. Previous human study has demonstrated that the effect of PNPLA3 on liver steatosis could be regulated and amplified by the degree of adiposity.[16] A very recent study also indicated that diabetes and obesity amplified the effect of NAFLD-related gene variants on liver-related mortality in Europeans.[19] However, another study from the NHANES III multi-ethnic population found no interaction of obesity with PNPLA3 gene variant on liver-specific mortality.[13] Our study in Han Chinese further confirmed the interaction of adiposity with PNPLA3 gene variant on liver-specific mortality in Asians. Laboratory research found that the expression of PNPLA3 I148M protein was directly regulated by the insulin-regulated transcription factor sterol regulatory element-binding protein-1c (SREBP-1c) and lead to liver steatosis only under dietary conditions that expose the liver to high levels of insulin.[38] In line with the animal studies, we found that the effect of PNPLA3 gene variant on hepatic VLDL export was only observed in the presence of adiposity in Han Chinese from the Shanghai Changfeng community. Consistently, another recent study in Finns also showed by NMR lipoprotein analysis that the PNPLA3 and TM6SF2 variants confer an antiatherogenic lipid profile only in insulin-resistant individuals.[39]

Similar to PNPLA3 gene variant, animal studies on TM6SF2 KO mice indicated that TM6SF2 was required to mobilize neutral lipids for VLDL assembly.[40] Our current study indicated that PNPLA3, TM6SF2 and MBOAT7 gene variants could jointly amplify the risk of liver-specific mortality. Although different NAFLD-related variants showed divergent metabolic profiling signature,[41] a composite genetic-predisposition score of PNPLA3, TM6SF2 and MBOAT7 gene variants was associated with reduced export of VLDL1 from the liver, and contributed to the liver-related death. An interaction between NAFLD genetic-predisposition score and adiposity was also discovered. This interesting finding indicated the necessity to examine multiple NAFLD-related gene variants for better prediction of individual risk of liver-specific mortality.

NAFLD has been identified as an independent risk factor for cardiovascular disease.[42] Although the mechanisms underlying the relationship between NAFLD and cardiovascular disease have not been completely clarified, at least several pathophysiological procedures in NAFLD patients, such as hepatic insulin resistance, proinflammatory state and excessive lipid efflux, could promote and facilitate atherogenesis and cardiovascular diseases. However, in NAFLD patients with PNPLA3 rs738409 C>G variant, insulin resistance is dissociated with liver steatosis,[43] and the secretion of very low-density lipoprotein into the circulation is reduced.[18] Therefore, PNPLA3 rs738409 C>G variant, with its potential protective effect against hyperlipidaemia, could partially reverse the NAFLD-related cardiovascular disease. In the current study, the cardiovascular disease mortality was reduced in Han Chinese adults with PNPLA3 rs738409 C>G variant and metabolic disorders. This result was consistent with the Study of Health in Pomerania in North-Eastern Germany.[11] Another two studies from the NHANES III multi-ethnic population (N = 4874) and an Italian hospitalized cohort (N = 471) found no association between PNPLA3 gene variant and cardiovascular mortality/events.[12,13] The effect of PNPLA3 gene variant on cardiovascular mortality seemed to be influenced by the ethnicity. Several previous studies found the PNPLA3 rs738409 C>G variant increased vascular damage in high risk Hispanic patients with NAFLD,[44] but showed no or protective effect on cardiovascular disease in Asians[45] and a larger population from CARDIoGRAMplusC4D database.[46] PNPLA3 rs738409 C>G variant probably has pleiotropic effects on glucose, lipid metabolism and inflammation,[47] thus its effect on cardiovascular mortality might be influenced by a series of competing factors, which deserves further investigation. The TM6SF2 rs58542926 C>T variant has also been found to correlate with reduced LDLs components previously.[18] In the current study, we found the TM6SF2 rs58542926 C>T variant conferred significant protection against all-cause and cardiovascular mortality, while having no independent effect on liver-specific mortality in Asian adults. This result indicated that the protective effect of TM6SF2 rs58542926 C>T variant on cardiovascular mortality was more important than its deleterious effect on the liver, which was in contrast with the PNPLA3 rs738409 C>G variant with its dominant effect to increase liver-specific mortality. A dissociation of liver triglycerides/cholesterol and serum cholesterol was also found in our study participants with MBOAT7 rs641738 C>T variant and a chow-diet MBOAT7 liver-specific knockout mouse model.[48] Thus, the composite effect of PNPLA3, TM6SF2 and MBOAT7 variants might contribute to reduced cardiovascular mortality in Chinese adults.

The Shanghai Changfeng Study is a community-based cohort study for the outcomes of multiple diseases in China, and the result can be rationally generalized to the large Han Chinese population in the urban area. Several limitations are associated with our current study. First, the histological information for liver steatosis, inflammation and fibrosis was not available, and the LFC was quantified using a quantitative ultrasound method that was not as accurate as liver biopsy or proton magnetic resonance spectroscopy (^{1}H-MRS), which may preclude detection of the SNPs which confer only a minor LFC increase compared to PNPLA3 rs738409 C>G variant. Second, the number of TM6SF2 rs58542926 TT genotype carriers was very low, which was consistent with the extreme low frequency in the Dallas Heart Study[49] and precluded analysis of the effect of TM6SF2 rs58542926 homozygous variant on liver-specific and cardiovascular mortality. Third, this study investigated the influence of NAFLD-related gene variants on mortality in middle and later life in an Asian population, and further studies were required to expand the conclusion to youth death in participants from different ethnicities. Finally, the mean follow-up period was 5.27 years in our current study, and further study was still required to test whether our findings could be extended to a longer time of follow-up.

5 | CONCLUSION

The PNPLA3 rs738409 C>G variant and a combination of NAFLD genetic risk alleles predicted higher liver-specific mortality but lower cardiovascular mortality in Han Chinese with adiposity or metabolic disorders. Therefore, special attention should be paid to the liver-related rather than cardiovascular outcomes in the NAFLD patients with high genetic predisposition. The interactive effect of NAFLD-related gene variants with adiposity on liver-specific mortality raised the possibility that consideration of metabolic and genetic risk of NAFLD jointly may improve the prediction of individual risk of liver-specific mortality.

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AUTHORSHIP

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DATA AVAILABILITY STATEMENT

Our study was supported from several national and local government research funds. According to the relevant policy of data management, the datasets generated and/or analysed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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SUPPORTING INFORMATION

Additional supporting information will be found online in the Supporting Information section.

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