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ARTICLE

The *PNPLA3* rs738409 C>G variant interacts with changes in body weight over time to aggravate liver steatosis, but reduces the risk of incident type 2 diabetes

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Abstract

Aims/hypothesis The rs738409 C>G variant of the patatin-like phospholipase domain containing 3 gene (*PNPLA3*) increases the risk of non-alcoholic fatty liver disease (NAFLD) with no predisposition for insulin resistance. In this study, we aimed to investigate the influence of *PNPLA3* polymorphisms on liver fat content (LFC) and glucose metabolic variables, and the associations between these, during the natural course of body weight changes in a Chinese adult cohort.

Methods The LFC, measured using a quantitative ultrasound method, was prospectively monitored in 2189 middle-aged and elderly adults from the Shanghai Changfeng Study, together with changes in body weight and metabolic variables. General linear models were used to detect interactive effects between the *PNPLA3* rs738409 genotype and 4 year changes in body weight on liver steatosis and glucose metabolism.

Results The *PNPLA3* homozygous GG genotype dissociated the changes in the LFC and OGTT 2 h post-load blood glucose (PBG) in relation to 4 year changes in body weight. *PNPLA3* GG genotype carriers showed greater increases in the LFC and serum alanine aminotransferase (ALT) but lower PBG elevation and incident diabetes than *PNPLA3* wild-type (CC) genotype carriers exhibiting the same degree of body weight increase. The interactions between the *PNPLA3* genotype and changes in body weight on the LFC (false discovery rate [FDR]-adjusted $p_{interaction} = 0.044$) and ALT (FDR-adjusted $p_{interaction} = 0.044$) were significant. Subgroup analyses showed that the effect of the *PNPLA3* GG genotype on changes in the LFC and PBG was only observed in metabolically unhealthy participants with insulin resistance or abdominal obesity.

Conclusions/interpretation The *PNPLA3* GG genotype interacted with changes in body weight to aggravate liver steatosis but reduced the risk of incident type 2 diabetes in metabolically unhealthy participants.

Keywords Body weight · Diabetes · Gene–environment interaction · NAFLD · *PNPLA3* gene variant

Ming-Feng Xia and Huan-Dong Lin contributed equally to this work.

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Research in context

What is already known about this subject?

- The *PNPLA3* rs738409 G variant is the strongest genetic risk factor for non-alcoholic fatty liver disease (NAFLD)
- Synergy between adiposity and the *PNPLA3* rs738409 G variant promotes NAFLD progression
- Individuals with NAFLD due to the *PNPLA3* rs738409 G variant are not predisposed to type 2 diabetes and cardiovascular disease

What is the key question?

- Will the *PNPLA3* rs738409 G variant influence the changes in the liver fat content and glucose metabolic variables, and the associations between these, in response to long-term body weight changes?

What are the new findings?

- The *PNPLA3* homozygous GG genotype dissociated the changes in the liver fat content from the OGTT 2 h post-load blood glucose in a Chinese adult cohort
- The *PNPLA3* homozygous GG genotype interacted with body weight increases to aggravate liver steatosis but protected against an increase in OGTT 2 h blood glucose and incident diabetes
- The effect of the *PNPLA3* GG genotype on changes in liver fat content and OGTT 2 h post-load blood glucose was significant in metabolically unhealthy but not in metabolically healthy participants

How might this impact on clinical practice in the foreseeable future?

- Our study indicates that participants with the *PNPLA3* rs738409 GG genotype respond differently to changes in body weight; thus, the *PNPLA3* genotype may be a potent biomarker for guidance of personalised treatment of NAFLD and associated diabetes

Abbreviations

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
CVD	Cardiovascular disease
FBG	Fasting blood glucose
FDR	False discovery rate
^{1}H-MRS	Proton magnetic resonance spectroscopy
LFC	Liver fat content
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
PBG	Post-load blood glucose
PNPLA3	Patatin-like phospholipase domain containing 3

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease worldwide and affects approximately 25% of the population [1]. In persons with NAFLD, the liver disease- and cardiovascular disease (CVD)-specific mortality rates are 0.77 and 4.79, respectively, per 1000 person-years [1]. NAFLD is closely associated with obesity, the metabolic syndrome, dyslipidaemia and type 2 diabetes [2]. However, a proportion of individuals develop NAFLD in the absence of obesity [3] and susceptibility to NAFLD clearly varies in part due to genetic polymorphisms [4, 5].

The rs738409 C>G variant of the gene encoding patatin-like phospholipase domain containing 3 (PNPLA3) protein is the first and strongest common variant that modifies genetic susceptibility to NAFLD [6]. The rs738409 C>G variant has been fully demonstrated to be a strong determinant of liver fat deposition independent of age [7], ethnicity [8], features of the metabolic syndrome and other risk factors for steatosis [6]. In several meta-analyses, this gene variant was shown to increase the risk of non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis and hepatocellular carcinoma [9]. However, the effect of *PNPLA3* gene variants on diabetes is still uncertain. Most studies have indicated that *PNPLA3* C>G variant carriers with NAFLD are not predisposed to insulin resistance [10] and type 2 diabetes [11]. An inverse correlation between the *PNPLA3* G variant and the risk of diabetes was found in the NASH Clinical Research Network cohort [11]. In one large-scale cohort study in Germany, the *PNPLA3* rs738409 G variant was found to be associated with increased liver disease-associated mortality but reduced coronary heart disease-associated mortality [12]. Adiposity can amplify the effect of the *PNPLA3* rs738409 C>G variant on liver fat accumulation according to recent studies [13–15]. Weight gain is the most

important risk factor for both NAFLD [16] and type 2 diabetes [17]. Weight loss is the mainstay for NAFLD treatment [18] and is an approved effective method to prevent diabetes [19]. Thus, we can obtain a better understanding of the role of the *PNPLA3* C>G variant in the natural progression and remission of NAFLD and diabetes by investigating changes in the liver fat content (LFC) and metabolic variables in relation to changes in body weight in different *PNPLA3* genotype carriers.

In this study, we prospectively monitored the natural course of changes in body weight and investigated their impact on the LFC and glucose and lipid metabolic variables in 2189 middle-aged and elderly individuals with different *PNPLA3* genotypes from the Shanghai Changfeng Study [20].

Methods

Participants The Shanghai Changfeng Community Study recruited 4300 Chinese community residents (1652 men, 2648 women) aged >45 years from May 2010 to December 2012. Participants with medical records or self-reports of previous viral hepatitis, excessive alcohol consumption and chronic liver diseases other than NAFLD were excluded [14]. In the current study, we report the first-round follow-up results of the Changfeng large-scale community study. Among the 4300 participants at baseline, a total of 156 participants died before November 2014 according to registration data from the Shanghai Center for Disease Control. The remaining 4144 participants were invited to attend the first-round follow-up appointment by phone call, letter or email from November 2014 to March 2017. Finally, 3262 (78.7%) participants responded to the invitation. After excluding 685 participants being treated with lipid-lowering medication, 232 with hypoglycaemic medication, 75 with a combination of hypoglycaemic and lipid-lowering medication and 81 with hepatoprotectants (e.g. polyene phosphatidylcholine, silymarin, adenosylmethionine, reduced glutathione), 2189 participants (889 men and 1300 women) with an average follow-up period of 4.2 years were included in the analysis (see electronic supplementary material [ESM] Fig. 1). The baseline characteristics of the enrolled 2189 participants were similar to those of the 4300 overall participants and could well represent the whole community population (ESM Table 1). The study was approved by the Research Ethics Committees of the Shanghai Health Bureau, China, and each participant provided written informed consent.

Anthropometric and serum biochemical measurements A uniform questionnaire about the past history of diabetes, medications, smoking status and alcohol consumption for each participant was completed in a face-to-face interview. Body height and weight were measured with the participants wearing no shoes or outer clothing. The BMI was calculated by dividing the weight (kg) by the square of the height (m^2). Waist circumference was measured using a soft tape at the midpoint between the lowest rib and the iliac crest in a standing position. For blood pressure, the mean of three resting measurements was used for the analysis. After at least a 12 h overnight fast, a venous blood sample was collected for the biochemical examinations. The serum total cholesterol, HDL-cholesterol and triacylglycerol levels were measured by an oxidase method and alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured by the ultraviolet lactate and malate dehydrogenase methods, respectively, on a model 7600 automated bio-analyzer (Hitachi, Tokyo, Japan). LDL-cholesterol was calculated using the Friedewald equation [21]. All participants underwent a 75 g OGTT. The fasting and OGTT 2 h post-load blood glucose (PBG) concentrations were measured using the glucose oxidase method. An electrochemiluminescence immunoassay was used to measure the serum insulin concentrations. HOMA-IR was calculated by multiplying the fasting blood glucose (FBG) (mmol/l) by fasting insulin (pmol/l) and dividing by 156.3.

***PNPLA3* genotype** The *PNPLA3* rs738409 C/G variants were genotyped at the baseline examination using primer extension of multiplex products with detection by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry using the MassARRAY platform (MassARRAY Compact Analyzer; Sequenom, San Diego, CA, USA).

Determination of LFC LFC was determined using a quantitative ultrasound method. Trained ultrasonographers who were unaware of the clinical data performed the ultrasound examinations. Ultrasound images were captured using the GE Logiq P5 scanner (GE Healthcare, Milwaukee, WI, USA), analysed using imaging software (ImageJ 1.41o; National Institutes of Health, Bethesda, MD, USA) and standardised using a tissue-mimicking phantom (Model 057; Computerized Imaging Reference Systems, Norfolk, VA, USA). The LFC was calculated using the following equation: LFC (%) = (62.592 × standardised US hepatic:renal ratio) + (168.076 × standardised US hepatic attenuation rate) − 27.863 [22].

Diagnosis of diabetes For both the baseline and follow-up evaluations, diabetes was defined according to the 1999 WHO criteria [23] as follows: (1) FBG ≥7.0 mmol/l or (2) OGTT 2 h PBG ≥11.1 mmol/l.

Definition of metabolically healthy and unhealthy status The waist circumference measurement and HOMA-IR have been used to define metabolic health status in previous epidemiological studies [24]. For the subgroup analyses in this study, we divided the participants into metabolically healthy and unhealthy groups according to a combination of the final

HOMA-IR and waist circumference as follows: metabolically healthy group, HOMA-IR <2.5 and waist circumference <90 cm in men and <80 cm in women; metabolically unhealthy group, HOMA-IR ≥2.5 or waist circumference ≥90 cm in men and ≥80 cm in women.

Statistical analysis All statistical analyses were performed using SPSS software (SPSS 18.0 software, SPSS Inc., Armonk, NY, USA; URL https://www.ibm.com/products/spss-statistics). The data are presented as the mean ± SD, except for skewed variables, which are presented as the median with the interquartile range (25–75%) reported in parentheses. Four-year changes in body weight were calculated by dividing the changes in body weight (in kg) by the follow-up times in 4 year periods. Participants were divided into quintiles based on the distribution of the 4 year changes in body weight during the follow-up: quintile 1, 4 year weight loss >2.6 kg; quintile 2, 4 year weight loss 0.9–2.6 kg; quintile 3, 4 year weight loss <0.9 kg or weight gain <0.5 kg; quintile 4, 4 year weight gain 0.5–2.1 kg; quintile 5, 4 year weight gain >2.1 kg. General linear models were used for comparisons of baseline continuous data among groups, whereas the linear-by-linear association χ^2 test was used for comparisons of categorical variables. The distributions of the baseline LFC, triacylglycerol, insulin and HOMA-IR were highly skewed to the right and were log-transformed to approximate normality before entering into the general linear models. Our primary outcomes of interest were the hepatic outcomes (including changes in LFC and serum ALT) and glucose outcomes (including FBG and OGTT 2 h PBG); secondary outcomes included changes in other NAFLD-related metabolic variables (waist circumference, triacylglycerol, HDL-cholesterol and HOMA-IR). We examined the associations of changes in the LFC, ALT and metabolic variables with quintiles of the 4 year changes in body weight and different *PNPLA3* genotypes using general linear models. Potential confounders considered in the multivariable models were age, sex, alcohol consumption, cigarette smoking, baseline body weight and baseline value of the investigated metabolic variable. Changes in LFC were further adjusted in the multivariable models on the associations between *PNPLA3* genotypes and changes in glucose and lipid metabolic variables. Furthermore, we evaluated the interactions between changes in body weight (as an ordered categorical variable) and the *PNPLA3* genotype and their effects on changes in the LFC, plasma glucose and lipid variables by inclusion of interaction terms into the models. Given the fact that the primary outcomes in the study included both hepatic and glucose outcomes, four tests (changes in LFC, ALT, FBG and OGTT 2 h PBG) were controlled in the primary interaction analyses. The interaction of *PNPLA3* genotype and body weight change on other metabolic outcomes was evaluated as exploratory tests. False discovery rate (FDR) was used to adjust for multiple testing of the primary interaction analyses and the associations of *PNPLA3* genotypes and quintiles of body weight change with changes in LFC and all metabolic variables [25]. A correlation coefficient heatmap was used to depict the correlations between changes in the LFC and changes in various metabolic variables in participants with different *PNPLA3* genotypes. In the secondary analyses, logistic regression models were used to estimate ORs for incident diabetes in 1741 non-diabetic participants with different *PNPLA3* genotypes. Age, sex, alcohol consumption, cigarette smoking, baseline body weight and LFC and changes in body weight and LFC were adjusted in the multivariable logistic regression models. Since our previous cross-sectional study indicated that *PNPLA3* gene variants increased the risk of NAFLD in a metabolic factor-dependent manner [14], subgroup analyses were performed in participants with different metabolic health statuses. Values of $p < 0.05$ were considered statistically significant for all analyses.

Results

Baseline characteristics and changes in body weight and metabolic variables A total of 889 men and 1300 women were included in the study, with average age of 62.2 years, BMI 24.1 kg/m^2 and LFC 7.8%. There were 841 (38.4%) *PNPLA3* CC homozygotes, 1036 (47.3%) CG heterozygotes and 312 (14.3%) GG homozygotes. The baseline characteristics of the study participants across categories of *PNPLA3* genotypes and changes in body weight are shown in ESM Table 2 and Table 1, respectively. The *PNPLA3* G variant carriers showed significantly higher LFCs and serum AST levels but lower serum triacylglycerol levels at baseline. Compared with participants in the lower quintiles of body weight change, those in the higher quintiles had lower baseline body weights, LFCs, FBG, OGTT 2-h PBG, HOMA-IR, and serum ALT, AST and triacylglycerol levels and higher serum HDL-cholesterol levels (all $p < 0.05$). The frequencies of the *PNPLA3* CC, CG, and GG genotypes were similar among the groups with different grades of body weight changes ($p = 0.254$, Table 1). Despite their initially better metabolic status when compared with participants in the lower quintiles of body weight change, those in the higher quintiles showed greater increases in the LFC, OGTT 2 h PBG, HOMA-IR and serum levels of ALT, triacylglycerol and LDL-cholesterol and a reduction in the HDL-cholesterol levels (ESM Table 3). Except for a lack of association between changes in body weight and changes in PBG and LDL-cholesterol in the *PNPLA3* GG genotype carriers, an association between change in body weight and changes in the LFC and metabolic variables was found in all participants regardless of their *PNPLA3* genotype, even after adjustment for age, sex, alcohol consumption, cigarette

Table 1 Baseline characteristics of the study participants according to 4 year changes in body weight

Characteristic	Quintiles of 4 year change in body weight					*p* for trend
	Quintile 1 (loss >2.6 kg)	Quintile 2 (loss 0.9–2.6 kg)	Quintile 3 (loss <0.9 kg or gain <0.5 kg)	Quintile 4 (gain 0.5–2.1 kg)	Quintile 5 (gain >2.1 kg)	
No. of participants (*n* men)	441 (210)	437 (157)	439 (154)	430 (171)	442 (197)	0.760
Age, years	63.1 ± 8.5	62.0 ± 8.6	62.4 ± 8.8	62.2 ± 7.9	61.2 ± 8.0	0.026
Follow-up time, years	4.2 ± 0.4	4.3 ± 0.5	4.2 ± 0.5	4.2 ± 0.5	4.2 ± 0.5	0.092
Smoking, *n* (%)	110 (24.9)	82 (18.8)	79 (18.0)	92 (21.4)	110 (24.9)	0.685
Alcohol consumer, *n* (%)	76 (17.2)	68 (15.6)	57 (13.0)	73 (17.0)	84 (19.0)	0.374
Body weight, kg	65.9 ± 10.6	62.8 ± 9.7	61.5 ± 9.7	62.2 ± 9.9	62.1 ± 10.2	<0.001
BMI, kg/m^2	24.8 ± 3.1	24.2 ± 3.0	23.8 ± 3.0	23.9 ± 3.2	23.5 ± 3.3	<0.001
Waist circumference, cm	85.9 ± 9.2	83.5 ± 9.2	82.3 ± 8.8	83.2 ± 9.8	82.2 ± 9.5	<0.001
LFC, %	6.6 (3.0–13.6)	5.5 (2.1–12.5)	5.8 (2.6–10.8)	5.6 (2.5–11.6)	5.0 (2.7–9.3)	<0.001
FBG, mmol/l	6.0 ± 1.8	5.5 ± 1.2	5.3 ± 1.0	5.5 ± 1.4	5.4 ± 1.4	<0.001
OGTT 2 h PBG, mmol/l	8.3 ± 3.8	7.5 ± 3.0	7.2 ± 2.7	7.5 ± 4.1	7.1 ± 2.4	<0.001
Triacylglycerol, mmol/l	1.6 (1.1–2.3)	1.5 (1.1–2.0)	1.4 (1.1–1.9)	1.4 (1.0–1.9)	1.3 (1.0–1.8)	<0.001
Total cholesterol, mmol/l	5.1 ± 0.9	5.2 ± 1.0	5.1 ± 0.9	5.0 ± 0.9	5.0 ± 1.0	0.089
HDL-cholesterol, mmol/l	1.3 ± 0.4	1.4 ± 0.4	1.5 ± 0.4	1.4 ± 0.4	1.5 ± 0.4	<0.001
LDL-cholesterol, mmol/l	2.9 ± 0.8	2.9 ± 0.8	2.9 ± 0.7	2.8 ± 0.8	2.8 ± 0.8	0.261
SBP, mmHg	136 ± 18	133 ± 19	133 ± 17	134 ± 19	132 ± 18	0.011
DBP, mmHg	77 ± 10	76 ± 10	75 ± 10	75 ± 10	75 ± 10	0.001
Insulin, pmol/l	59.9 (36.9–89.2)	55.0 (38.3–76.6)	50.8 (36.2–75.9)	54.3 (37.6–80.1)	50.1 (32.7–71.7)	<0.001
HOMA-IR	2.1 (1.3–3.4)	1.9 (1.3–2.6)	1.7 (1.2–2.7)	1.8 (1.2–2.9)	1.7 (1.1–2.5)	<0.001
ALT, U/l	20.2 ± 12.4	19.6 ± 11.5	19.1 ± 12.5	18.4 ± 10.0	17.5 ± 10.9	<0.001
AST, U/l	22.3 ± 8.2	22.2 ± 7.0	22.3 ± 9.6	21.0 ± 7.1	21.0 ± 7.4	<0.001
PNPLA3 polymorphism						
CC, *n* (%)	184 (41.7)	156 (35.7)	160 (36.4)	167 (38.8)	174 (39.4)	0.254
CG, *n* (%)	207 (46.9)	219 (50.1)	215 (49.0)	197 (45.8)	198 (44.8)	
GG, *n* (%)	50 (11.4)	62 (14.2)	64 (14.6)	66 (15.4)	70 (15.8)	

Data are presented as mean ± SD, except for skewed variables, which are presented as the median with the interquartile range given in parentheses
DBP, diastolic blood pressure; SBP, systolic blood pressure

smoking, baseline body weight and baseline LFC or the investigated metabolic variable (ESM Table 3).

Effect of the interaction between the *PNPLA3* GG genotype and changes in body weight on NAFLD and liver enzymes As shown in Fig. 1a, participants with the *PNPLA3* GG genotype had significantly greater increases in the LFC than *PNPLA3* CC homozygotes in quintiles 1, 2, 4 and 5 (all FDR-adjusted $p < 0.05$). For participants with decreases in body weight during the follow-up period, the LFC was significantly decreased in the *PNPLA3* CC homozygotes but was still increased in the *PNPLA3* GG homozygotes. The average change in the LFC of the CG heterozygotes was between that of the homozygote groups. An interaction between the changes in body weight and *PNPLA3* polymorphisms was also observed (FDR-adjusted $p_{interaction} = 0.044$) (Fig. 1a).

Liver enzyme markers, especially ALT, are the most commonly used variables for evaluating liver inflammation. Participants with the *PNPLA3* GG genotype had a greater increase or lesser reduction in their ALT levels than the *PNPLA3* CC carriers for all participants with body weight changes (all FDR-adjusted $p < 0.05$) (Fig. 1b). The effect of the interaction between changes in body weight and the *PNPLA3* genotype on serum ALT was statistically significant (FDR-adjusted $p_{interaction} = 0.044$) (Fig. 1b).

Effect of the interaction between the *PNPLA3* GG genotype and changes in body weight on glucose metabolic variables We also assessed the possible interactions between *PNPLA3* genotypes and changes in body weight on other metabolic characteristics related to NAFLD. In participants with a body weight gain of 0.5–2.1 kg and >2.1 kg, the *PNPLA3* GG genotype carriers showed a significantly lower increase in the OGTT 2 h PBG (Fig. 2b) than the *PNPLA3* CC genotype carriers. The *PNPLA3* CG genotype carriers with a body-weight gain >2.1 kg also showed a lower increase in the

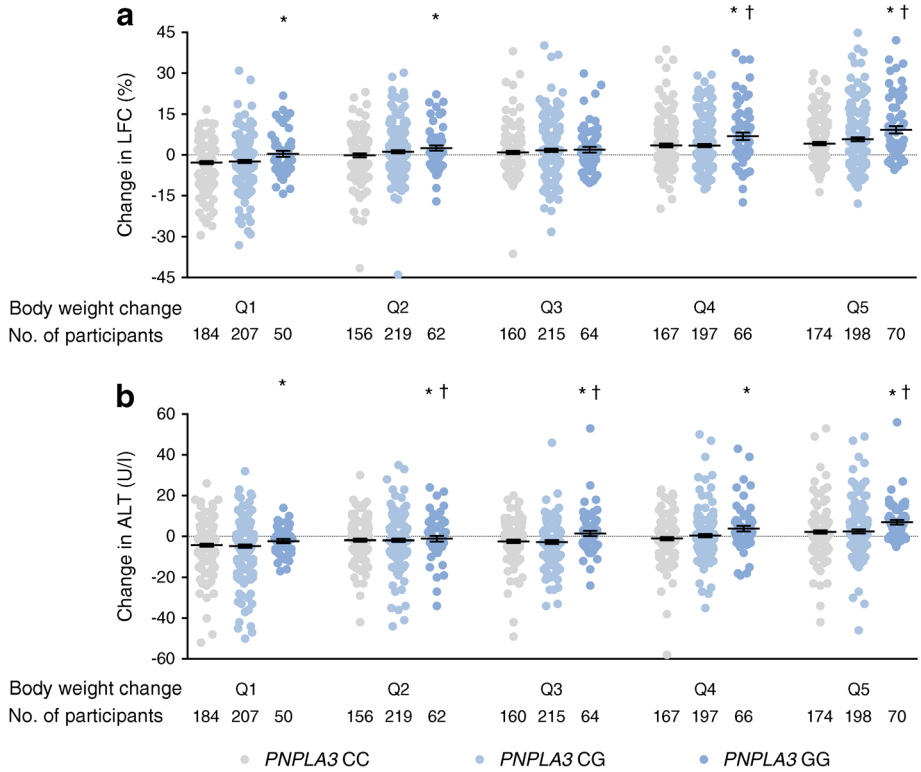

Fig. 1 Changes in LFC (**a**) and serum ALT (**b**) according to changes in body weight and *PNPLA3* genotype. Data are presented as the mean ± SEM; *$p < 0.05$ (FDR adjusted) compared with *PNPLA3* CC genotype carriers; †$p < 0.05$ (FDR adjusted) compared with the *PNPLA3* CG genotype carriers. *PNPLA3* GG genotype carriers had significantly greater increases in the LFC in the first, second, fourth and fifth quintiles of body weight changes (**a**) and greater increases or smaller reductions in ALT than the *PNPLA3* wild-type (CC) carriers in all quintiles (**b**). An interaction was found between changes in body weight and *PNPLA3* polymorphisms that affected changes in the LFC (FDR-adjusted $p_{interaction} = 0.044$) (**a**) and serum ALT (FDR-adjusted $p_{interaction} = 0.044$) (**b**). Quintiles of change in body weight over 4 years: quintile 1 (Q1), weight loss >2.6 kg; quintile 2 (Q2), weight loss 0.9–2.6 kg; quintile 3 (Q3), weight loss <0.9 kg or weight gain <0.5 kg; quintile 4 (Q4), weight gain 0.5–2.1 kg; quintile 5 (Q5), weight gain >2.1 kg

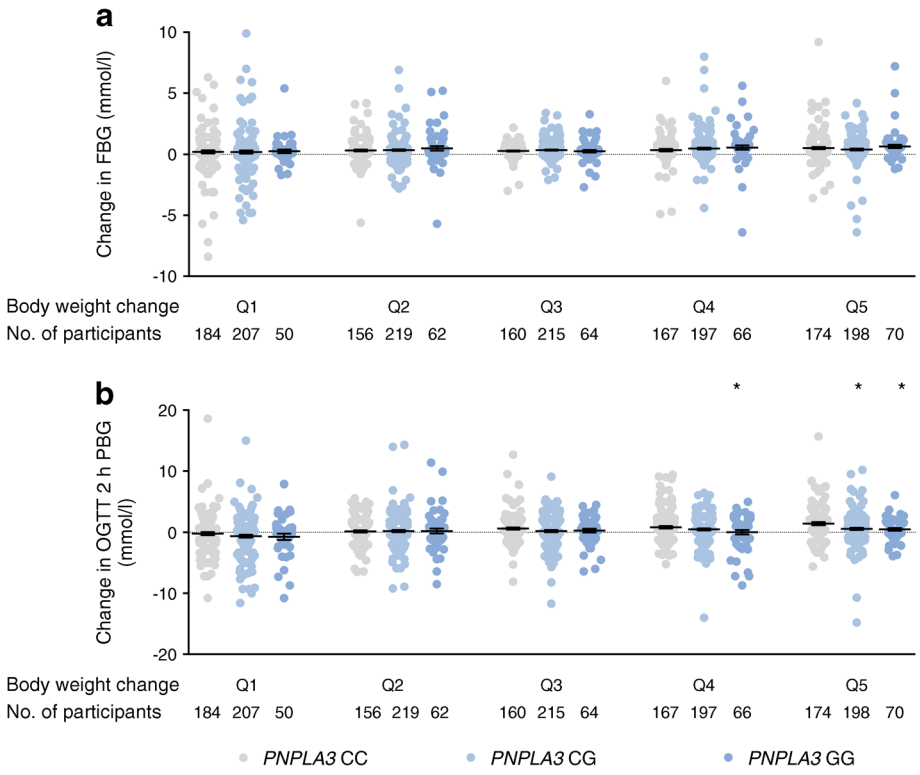

Fig. 2 Changes in FBG (**a**) and OGTT 2 h PBG (**b**) according to changes in body weight and the *PNPLA3* genotype. Data are presented as the mean ± SEM; *$p < 0.05$ (FDR adjusted) compared with *PNPLA3* CC genotype carriers. *PNPLA3* GG genotype carriers in the fourth and fifth quintiles and CG genoytpe carriers in the fifth quintile had significantly smaller increases in OGTT 2 h PBG compared with *PNPLA3* wild-type (CC) carriers (**b**). There were no interactions between changes in body weight and *PNPLA3* genotypes that affected blood glucose. Quintiles of change in body weight over 4 years: quintile 1 (Q1), weight loss >2.6 kg; quintile 2 (Q2), weight loss 0.9–2.6 kg; quintile 3 (Q3), weight loss <0.9 kg or weight gain <0.5 kg; quintile 4 (Q4), weight gain 0.5–2.1 kg; quintile 5 (Q5), weight gain >2.1 kg

Table 2 ORs (95% CIs) for incident diabetes in participants with different *PNPLA3* genotypes

Variable	*PNPLA3* genotype			*p* value
	CC (*n* = 672)	CG (*n* = 829)	GG (*n* = 240)	
FBG ≥7.0 mmol/l				
New-onset diabetes, *n* (%)	28 (4.2)	22 (2.7)	13 (5.4)	
Crude OR (95%CI)	1	0.627 (0.355, 1.106)	1.317 (0.671, 2.587)	0.905
Adjusted OR (95%CI)[a]	1	0.575 (0.303, 1.094)	1.014 (0.449, 2.290)	0.605
OGTT 2 h PBG ≥11.1 mmol/l				
New-onset diabetes, *n* (%)	50 (7.4)	51 (6.2)	9 (3.8)	
Crude OR (95% CI)	1	0.815 (0.544, 1.221)	0.485 (0.235, 1.000)	0.048
Adjusted OR (95% CI)[a]	1	0.758 (0.485, 1.185)	0.335 (0.149, 0.751)	0.008
FBG ≥7.0 mmol/l or OGTT 2 h PBG ≥11.1 mmol/l				
New-onset diabetes, *n* (%)	60 (8.9)	59 (7.1)	16 (6.7)	
Crude OR (95% CI)	1	0.782 (0.537, 1.137)	0.729 (0.411, 1.291)	0.167
Adjusted OR (95% CI)[a]	1	0.736 (0.483, 1.122)	0.509 (0.260, 0.998)	0.033

[a] ORs were calculated by multivariable logistic regression analysis, with adjustment for age, sex, alcohol consumption, cigarette smoking, baseline body weight and LFC and changes in body weight and LFC

OGTT 2 h PBG than *PNPLA3* CC genotype carriers. However, no significant differences were found in the changes in waist circumference, HOMA-IR, FBG, serum triacylglycerol or HDL-cholesterol among the *PNPLA3* CC, CG, and GG genotype carriers with the same degree of body weight changes (Fig. 2a and ESM Fig. 2a–d). No interactions between changes in body weight and the *PNPLA3* genotype were identified for changes in the FBG, OGTT 2 h PBG or other lipid metabolic traits.

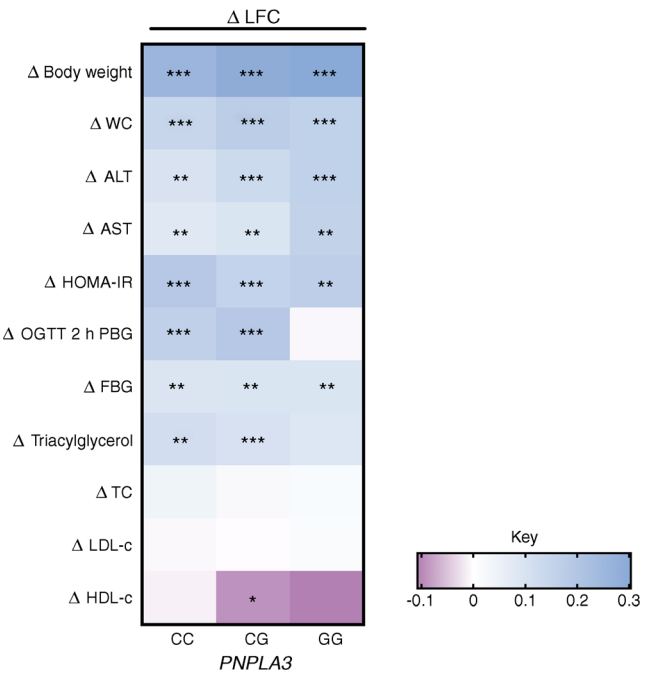

Fig. 3 Correlation coefficient heatmap for the correlations between changes in the LFC and changes in metabolic variables among participants with different *PNPLA3* genotypes. Changes in the LFC were significantly correlated with changes in the OGTT 2 h PBG and triacylglycerol in participants with the *PNPLA3* CC and CG genotypes but not those with the *PNPLA3* GG genotype. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$. HDL-c, HDL-cholesterol; LDL-c, LDL-cholesterol; TC, total cholesterol; WC, waist circumference

Among the 1741 participants without diabetes at baseline, the rate of incident diabetes diagnosed by both FBG and the OGTT 2 h PBG was 8.9%, 7.1% and 6.7% for the *PNPLA3* CC, CG and GG genotype carriers, respectively (Table 2). The incident diabetes rate diagnosed by the OGTT 2 h PBG alone was 3.8% in the *PNPLA3* GG genotype carriers, only half that in the *PNPLA3* CC genotype carriers. The multivariable logistic regression analyses showed that the multivariate-adjusted ORs were 0.509 (0.260, 0.998) for incident diabetes diagnosed by FBG and OGTT 2 h PBG and 0.335 (0.149, 0.751) for incident diabetes diagnosed by OGTT 2 h PBG alone in the *PNPLA3* GG genotype carriers (Table 2).

The *PNPLA3* GG genotype dissociates changes in the LFC and the OGTT 2 h PBG Changes in the LFC were positively associated with changes in body weight, waist circumference, HOMA-IR and serum ALT, AST and FBG in all participants regardless of their *PNPLA3* genotype. However, the changes in LFC were significantly correlated with changes in the OGTT 2 h PBG and triacylglycerol only in participants with the *PNPLA3* CC and CG genotype, and not in those with the *PNPLA3* GG genotypes (Fig. 3).

Subgroup analyses of participants with differing metabolic health status We divided all participants into categories according to metabolic health (healthy vs unhealthy) based on their final HOMA-IR and final waist circumference. In the metabolically healthy participants with a final HOMA-IR <2.5 and no abdominal obesity, no significant differences

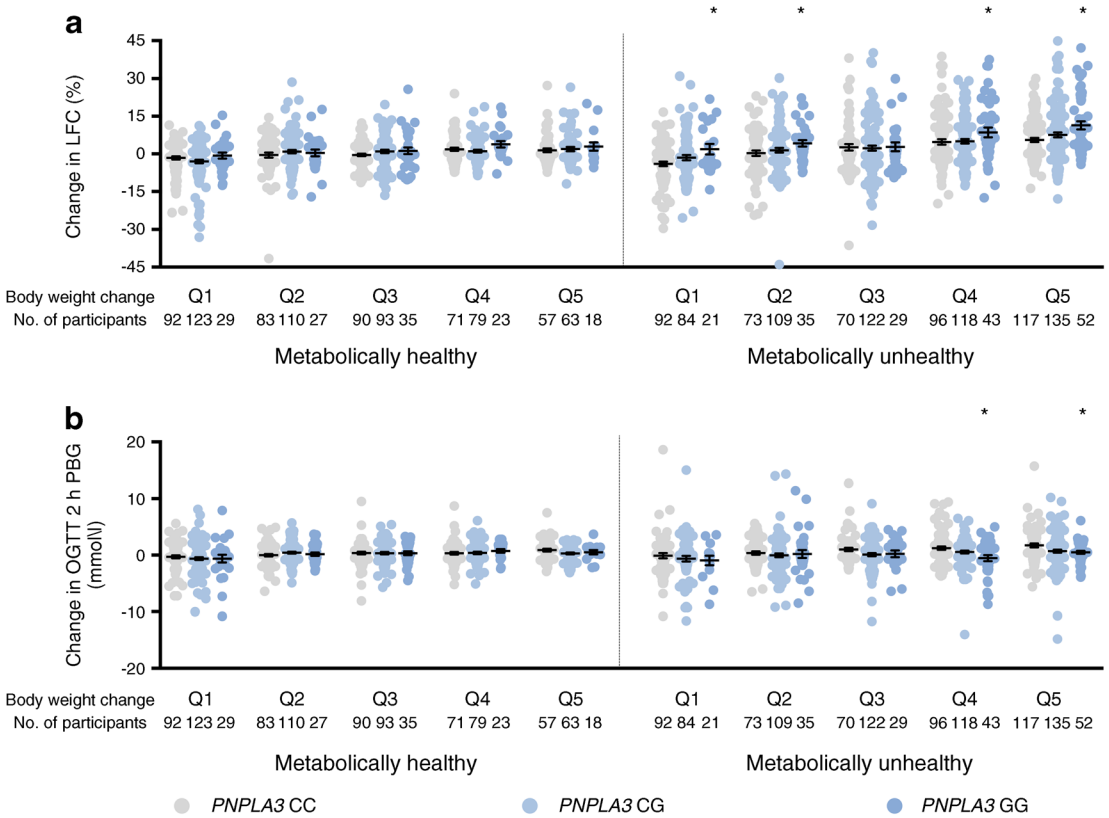

Fig. 4 Changes in the LFC (**a**) and OGTT 2 h PBG (**b**) according to changes in body weight and the *PNPLA3* genotype in subgroups with a metabolically healthy (final HOMA-IR <2.5 and waist circumference <90 cm in men and <80 cm in women) and unhealthy (final HOMA-IR ≥2.5 or waist circumference ≥90 cm in men and ≥80 cm in women) status. Data are presented as the mean ± SEM; *$p < 0.05$ (FDR adjusted) compared with the *PNPLA3* CC genotype carriers. For metabolically healthy participants, the *PNPLA3* GG genotype had no effect on changes in the LFC or PBG, whereas for metabolically unhealthy participants, the *PNPLA3* GG genotype was associated with a greater increase in LFC in the first, second, fourth and fifth quintiles of weight change (**a**), but a smaller increase in the OGTT 2 h PBG in the fourth and fifth quintiles of weight change (**b**). Quintiles of change in body weight over 4 years: quintile 1 (Q1), weight loss >2.6 kg; quintile 2 (Q2), weight loss 0.9–2.6 kg; quintile 3 (Q3), weight loss <0.9 kg or weight gain <0.5 kg; quintile 4 (Q4), weight gain 0.5–2.1 kg; quintile 5 (Q5), weight gain >2.1 kg

were found in the changes in the LFC and OGTT 2 h PBG among the different *PNPLA3* genotype groups displaying the same degree of changes in body weight (Fig. 4a, b). However, in the metabolically unhealthy participants with a final HOMA-IR ≥2.5 or abdominal obesity, *PNPLA3* GG genotype carriers showed a significantly greater increase in the LFC in the first, second, fourth and fifth quintiles of body weight changes (all FDR-adjusted $p < 0.05$) but smaller increase in the OGTT 2 h PBG with 4 year weight gain over 0.5 kg when compared with the *PNPLA3* CC genotype carriers (Fig. 4a, b).

Discussion

An interaction between the *PNPLA3* genotype and obesity has been previously reported to affect liver steatosis and increase the serum ALT level. The major finding of this study is that the LFC and OGTT 2 h PBG in participants with different *PNPLA3* genotypes respond differently to long-term spontaneous changes in body weight. In a cohort of middle-aged and elderly Chinese individuals from a community population from the Shanghai Changfeng Study, the *PNPLA3* homozygous GG genotype dissociated the changes in the LFC and OGTT 2 h PBG. This variant was also associated with seemingly paradoxically greater increases in the LFC but less elevation of the OGTT 2 h PBG and diabetes incidence when compared with individuals having the *PNPLA3* CC genotype and the same degree of weight gain. Furthermore, an interaction between change in body weight and *PNPLA3* genotype on the change in LFC and serum ALT was discovered. Subgroup analyses indicated that the effect of the *PNPLA3* GG genotype on changes in the LFC and PBG was significant in metabolically unhealthy participants but not in those who were metabolically healthy. Taken together, these results indicate that the *PNPLA3* genotype plays an important role in influencing individual hepatic and glucose metabolic outcomes related to the natural course of body weight change.

Several recent studies support a close association between body weight changes and the risk of NAFLD [26], type 2 diabetes and CVD [27]. Our previous study in the same cohort also showed that changes in body weight could predict risk of

impaired glucose regulation and diabetes [28]. In the current study, we further found that the *PNPLA3* rs738409 C>G variant interacted with changes in body weight and influenced the clinical outcomes of NAFLD and diabetes. In fact, several previous studies also reported that individuals with NAFLD and the *PNPLA3* GG genotype responded differently to weight-loss interventions when compared with *PNPLA3* CC genotype carriers. Several previous studies have indicated that *PNPLA3* GG genotype carriers benefit most from body weight reduction resulting from an intensive short-term low-energy low-carbohydrate diet [29], 12 month lifestyle intervention programme with >10% body weight reduction [30], or bariatric surgery with an average weight loss of 40 kg [31]. Other intervention studies showed that *PNPLA3* GG genotype variant carriers displayed changes in the LFC similar to those seen in *PNPLA3* CC homozygotes, with an average of 3 kg of weight loss over 6 months in a Finnish population [32] and a 5% reduction in body weight over 6 months in children with severe obesity [33]. The results from the Wessex Evaluation of Fatty Liver and Cardiovascular Markers in NAFLD with Omacor Therapy (WELCOME) trial even showed that the LFC was increased after docosahexaenoic acid and eicosapentaenoic acid treatment and weight loss in *PNPLA3* GG homozygotes, in contrast to the changes seen in *PNPLA3* CC and CG genotype carriers [34]. In our subgroup analysis, we found that the effect of the *PNPLA3* G variant on the natural course of changes in the LFC was dependent on the individual's final metabolic health status. In participants with central obesity or insulin resistance, the *PNPLA3* GG genotype interacted with changes in body weight and aggregated liver steatosis, whereas in metabolically healthy participants, presence of the *PNPLA3* GG genotype had no effect on the changes in the LFC.

The reasons for the discrepancies in the effect of *PNPLA3* polymorphisms on the progression and remission of NAFLD under different metabolic conditions are not known. Possibly, the expression of harmful proteins resulting from *PNPLA3* mutation is regulated by insulin and the nutritional status. *PNPLA3* has been demonstrated to be directly regulated by the insulin-regulated transcription factor sterol regulatory element binding protein-1c (SREBP-1c), and pathogenic *PNPLA3* C>G mutant products accumulate under conditions of insulin resistance or central obesity, thereby exacerbating liver steatosis and inflammation [35]. In accordance with our findings in the current study, previous laboratory studies found that the *PNPLA3* G variant alone was insufficient to cause liver steatosis in chow-fed mice but elicited a two- to threefold increase in the risk of NAFLD in sucrose-fed mice [36]. The regulation of *PNPLA3* expression by metabolic status also provides a theoretical foundation for the interaction between *PNPLA3* gene variant and changes in body weight.

We found that participants with the *PNPLA3* GG genotype who gained body weight displayed smaller increase in the OGTT 2 h PBG than the *PNPLA3* CC genotype carriers despite baseline OGTT 2 h PBG and changes in FBG and HOMA-IR being similar among the different *PNPLA3* genotype carriers. Several cross-sectional studies have shown that the *PNPLA3* G variant is correlated with better insulin sensitivity [37, 38]. However, the beneficial effect of the *PNPLA3* G variant on glucose metabolism was not able to be detected by the baseline FBG, OGTT 2 h PBG, HOMA-IR or even euglycaemic–hyperinsulinaemic clamp in all participants. Only in participants with a high percentage of body fat could a better insulin sensitivity status precisely measured by OGTT be found in *PNPLA3* G variant carriers [37]. An interaction of *PNPLA3* G variant with obesity on insulin sensitivity was reported previously [39]. This might explain our finding of a lower blood glucose level and diabetes incidence in *PNPLA3* GG genotype carriers with a metabolically unhealthy status, when compared with the *PNPLA3* CC homozygotes with the same degree of body weight change. Since *PNPLA3* expression levels are extremely low in a fasting status [35] and an increased PBG is better correlated with insulin resistance than the FBG [40], it is understandable that the *PNPLA3* G variant has more influence on the OGTT 2 h PBG than the FBG. The mechanism underlying the beneficial effect of *PNPLA3* gene variants on glucose metabolism may be related to changes in the liver lipid composition from saturated triacylglycerol to polyunsaturated triacylglycerol and a marked reduction in insulin-resistance-inducing ceramides [41]. One recent study indicated that PNPLA3 functions as a very-long-chain polyunsaturated fatty acid-specific triacylglycerol hydrolase, which promotes transfer of polyunsaturated fatty acids from triacylglycerol to phosphatidylcholine and that the *PNPLA3* C>G variant causes an 80% reduction in PNPLA3 activity and a reduction in the ratio of saturated to polyunsaturated triacylglycerol in the liver [42].

To the best of our knowledge, our current study might be the first large-scale community population-based cohort study to evaluate the influence of *PNPLA3* genotype on the association between natural changes in body weight and the progression or remission of NAFLD and metabolic complications. Several limitations are associated with our current study. First, information regarding diet and exercise during the follow-up period was not recorded. Therefore, our results did not permit evaluation of the different hepatic responses to changes in weight for specific reasons among different *PNPLA3* genotype carriers. Second, this study was performed in a Chinese cohort aged >45 years and the results need to be confirmed in participants with different ethnicities and in different age groups. Third, the LFC was quantified using a quantitative ultrasound method, which is not as accurate as a liver biopsy or proton magnetic resonance spectroscopy (^{1}H-MRS). However, invasive liver biopsy and ^{1}H-MRS cannot be carried out routinely in large-scale prospective population studies, and previous studies have indicated that the LFC

measured by our quantitative ultrasound method is suitable for large-scale human studies and agrees well with LFC measurement determined by ^{1}H-MRS ($r = 0.85$, $p < 0.001$) and the histological liver steatosis grades ($r = 0.79$, $p < 0.001$) [43].

In conclusion, the *PNPLA3* GG genotype and its interaction with body weight change aggravates liver steatosis but protects against increased risk of incident diabetes. The interaction between the *PNPLA3* GG genotype and changes in body weight on NAFLD is highly dependent on an individual's metabolic status. Since no formal recommendations exist for the treatment of NAFLD associated with *PNPLA3* gene variant at present, our data indicate the need for personalised treatment of NAFLD in those with the *PNPLA3* rs738409 C>G variant.

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Data availability Data are available on request from the corresponding author.

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Contribution statement MX and HL designed and performed the study and drafted the manuscript. LC, LW, HM, QL and QA performed the study, collected data and revised the manuscript. WH conducted the ultrasound examination, interpreted data and revised the manuscript. JG analysed and interpreted the data and wrote the statistical methods section of the manuscript. YH and HB interpreted the data and revised the discussion section of the manuscript. XL designed the study and revised the manuscript. XG designed the study, provided funding and revised the manuscript. All authors approved the final version. XG is the guarantor of this work and, as such, has full access to all of the data, takes responsibility for the integrity of the data, and controlled the decision to publish.

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