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Adverse effect of *PNPLA3* p.I148M genetic variant on kidney function in middle-aged individuals with metabolic dysfunction

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Summary

Background: The *PNPLA3* p.I148M variant is the main genetic determinant of nonalcoholic fatty liver disease, and PNPLA3 silencing is being evaluated to treat this liver condition. Data suggest that the p.I148M variant predisposes to kidney damage, but the relative contribution to kidney function, compared to overall genetic susceptibility, is not defined.

Aims: We aimed to assess the effect of *PNPLA3* p.I148M on the estimated glomerular filtration rate (eGFR) in individuals with metabolic dysfunction.

Methods: We included 1144 middle-aged individuals from the Liver-Bible-2022 cohort. Glomerular filtration rate (eGFR) was estimated using the Chronic Kidney Disease Epidemiology Collaboration equation. The effect of *PNPLA3* p.I148M on $eGFR_{CKD\text{-}EPI}$ levels was tested under additive genetic models adjusted for clinical predictors, ethnicity and a polygenic risk score of chronic kidney disease (PRS-CKD). In a subset of 144 individuals, we examined the effect of *PNPLA3* p.I148M on $eGFR_{CKD\text{-}EPI}$ over a median follow-up of 17 months.

Results: The p.I148M variant was associated with lower $eGFR_{CKD\text{-}EPI}$ levels (−1.24 mL/min/1.73 m^2 per allele, 95% CI: −2.32 to −0.17; $p = 0.023$), independent of age, sex, height, waist circumference, systolic blood pressure, LDL-cholesterol, transaminases, fasting insulin, albuminuria, lipid-lowering drugs, ethnicity and PRS-CKD score. In the prospective evaluation, the p.I148M variant was independently associated with faster $eGFR_{CKD\text{-}EPI}$ decline ($\Delta eGFR_{CKD\text{-}EPI}$ −3.57 mL/min/1.73 m^2 per allele, 95% CI: −6.94 to −0.21; $p = 0.037$).

Conclusions: We found a detrimental impact of the *PNPLA3* p.I148M variant on $eGFR_{CKD\text{-}EPI}$ levels in middle-aged individuals with metabolic dysfunction. This association was independent of established risk factors, ethnicity and genetic predisposition to CKD. *PNPLA3* p.I148M silencing may protect against kidney damage progression in carriers.

Alessandro Mantovani, Serena Pelusi are equal contributors. Daniele Prati, Giovanni Targher and Luca Valenti are senior authors.

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1 | INTRODUCTION

The rs738409 C>G single nucleotide polymorphism in the patatin-like phospholipase domain-containing protein-3 (*PNPLA3*) gene, which encodes for the p.Ile148Met aminoacidic change (p.I148M), accounts for the largest fraction of heritability of hepatic fat content and liver disease at population level, and is associated with a greater susceptibility to nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH) and hepatocellular carcinoma.[1,2] The *PNPLA3* gene encodes for a lipase that has a hydrolase and possibly trans-acylase activity on triglycerides, phospholipids and retinyl esters. PNPLA3 is highly expressed on lipid droplets of hepatocytes and hepatic stellate cells.[3–5] The p.I148M substitution causes a loss-of-function in PNPLA3 enzymatic activity, but also induces PNPLA3 accumulation on hepatocyte lipid droplets, where the mutated protein can interfere with the enzymatic activity of other lipases.[3–5] In the long-term, these mechanisms may promote liver injury and fibrosis.[3–5] Carriage of the p.I148M variant is associated with adverse liver-related outcomes and may also affect overall mortality, especially in individuals with obesity.[6,7] Therefore, *PNPLA3* p.I148M silencing could be a promising approach for a precision medicine treatment of NAFLD, which has now reached phase 2 clinical trials.[8,9]

Interestingly, recent cross-sectional studies have reported that the *PNPLA3* p.I148M variant is significantly associated with lower levels of estimated glomerular filtration rate (eGFR) in both adults and adolescents, regardless of the presence of type 2 diabetes (T2D) or NAFLD.[10–17] For example, in a cross-sectional study of 157 Caucasian individuals with T2D, we recently showed that homozygosity for the p.I148M variant was associated with lower levels of eGFR and higher likelihood of chronic kidney disease (CKD). Notably, these associations did not attenuate after adjustment for established renal risk factors, diabetes-related variables and use of medications.[10] Thus, it has been suggested that *PNPLA3* p.I148M variant might also have a direct detrimental effect on kidney function, irrespective of T2D. The proposed underlying mechanism(s), by which the *PNPLA3* p.I148M variant may adversely affect kidney function, might be either directly mediated via the expression of the PNPLA3 p.I148M protein in glomerular pericytes and podocytes, or indirectly through the promotion of more advanced NAFLD that, in turn, may induce kidney dysfunction.[10,18] These findings are also in line with a Delphi-based consensus statement reached from a multidisciplinary panel of international experts, supporting the existence of a significant association between *PNPLA3* p.I148M variant and risk of kidney dysfunction.[19]

To our knowledge, there is currently little information about the association between the *PNPLA3* p.I148M variant and decreasing levels of eGFR, even within the normal range, in individuals with metabolic dysfunction and without advanced kidney disease. Hence, the main aim of our cross-sectional and exploratory longitudinal study was to investigate the association between the *PNPLA3* p.I148M variant and eGFR levels in middle-aged individuals belonging to Liver-Bible-2022 cohort, which includes a biobank of apparently healthy individuals with metabolic dysfunction, most of whom had normal or near-normal kidney function and did not have T2D.

2 | MATERIALS AND METHODS

2.1 | Participants

The present study was conducted on 1144 middle-aged individuals participating in the Liver-Bible-2022 cohort, who were consecutively enrolled from July 2019 to July 2022, and for whom information on genomic data was available.[20,21] In particular, the Liver-Bible-2022 cohort included apparently healthy blood donors, aged 40–65 years, who were selected for a comprehensive liver disease, metabolic and cardiovascular screening, due to the presence of at least three metabolic risk abnormalities, among overweight/obesity (defined as body mass index [BMI] ≥25 kg/m^2), hypertension (blood pressure ≥130/85 mm Hg or anti-hypertensive treatment), dysglycaemia (fasting glucose level ≥100 mg/dL or use of glucose-lowering agents), low plasma HDL-cholesterol (<45 mg/dL in men and <55 mg/dL women) or high plasma triglycerides (≥150 mg/dL or lipid-lowering treatment).[22] Individuals with chronic degenerative diseases (such as, e.g. advanced kidney disease (eGFR < 30 mL/min/1.73 m^2), cirrhosis or active cancer), except for well-controlled arterial hypertension, treated hypothyroidism and well-compensated T2D (glycated haemoglobin <64 mmol/molHb) not requiring pharmacotherapy (except for metformin), were excluded from the cohort at first evaluation. The overall goal of this ongoing biobank study was primarily to examine the role of genetic factors and other non-invasive biomarkers of NAFLD in the risk prediction of cardiometabolic diseases, to provide the framework to design precision medicine approaches to prevent these cardiometabolic conditions.

2.2 | Clinical and laboratory data

Participants completed a self-report survey in which recorded lifestyle information and clinical characteristics, including smoking status, and use of medications or supplements. All participants were negative for serum markers of hepatitis B and C infection, and none of them had excessive alcohol consumption (defined as ≥60 g/day in men or ≥40 g/day in women). All participants underwent evaluation of anthropometric parameters (BMI and waist circumference), as well as levels of alcohol intake (drinks/weeks), sweetened beverages (drinks/week) and physical activity (h/week). Blood samples were collected after an overnight fast. For plasma collection, blood was centrifugated at 2000 ***g*** for 15 min and immediately stored at −80°C at the Fondazione Biological Resource Center (POLI-MI Biobank, which is part of the Italian node of Biobanking and Biomolecular Resources Research Infrastructure, BBMRI). In all participants, we measured fasting

plasma glucose, insulin, glycated haemoglobin (HbA1c), lipid profile (total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), C-reactive protein, creatinine, complete blood count and spot albuminuria (abnormal albuminuria was defined as >30 mg/L). An HbA1c of ≥48 mmol/mol (HbA1c ≥ 6.5%) was used as the cut point for diagnosing T2D. The estimated glomerular filtration rate (eGFR) was calculated according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation based on serum creatinine, revised in 2021.[23] All participants also underwent vibration-controlled transient elastography (Fibroscan®) for measurement of controlled attenuation parameter (CAP) and liver stiffness (LSM) to non-invasively estimate liver fat and fibrosis respectively[24]

As a part of the study protocol, participants could be re-assessed yearly (at least 10 months) since the last study evaluation. At the time of data analysis, a subset of 144 individuals underwent a first follow-up evaluation (median of 17 months; interquartile range [IQR]: 13–28 months).

The study was approved by the Ethical Committee of the Fondazione IRCCS Ca′ Granda of Milan, and each participant signed a written informed consent (ID: 1650, revision June 23rd, 2020).

2.3 | Genotyping and imputation

DNA was extracted from peripheral blood collected at the time of enrolment by the QIASymphony (Qiagen). Genotyping was performed by Illumina Global Screening Array (GSA)-24 v3.0 plus Multidisease Array (Illumina), which contains 712,189 variants before quality control, at the FIMM Institute for Molecular Medicine. To maximise genetic coverage, we performed single-nucleotide polymorphism (SNP) imputation on genome build GRCh38 using the Michigan Imputation Server and 194,512 haplotypes generated by the Trans-Omics for Precision Medicine (TOPMed) programme (freeze 5).[21,25] At the time of analysis, genomic data passing quality control were available for 1144 individuals. The patatin-like phospholipase domain-containing-3 (*PNPLA3*) rs738409 C>G variant (p.I148M) was directly typed by the GSA Array.

To adjust for a genetic predisposition towards CKD development, we exploited the most comprehensive available cross-ancestry genome-wide polygenic risk score for CKD (namely the PRS-CKD score).[26] This PRS-CKD score encompasses the evaluation of 41,124 common inherited risk variants to predict levels of eGFR and the risk of CKD in different populations, which was tested and validated in 15 independent cohorts of different ethnicity (including 3 cohorts of European ancestry, 6 cohorts of African ancestry, 4 cohorts of Asian ancestry and 2 admixed Latin cohorts), with the top 2% of the PRS-CKD score being associated with an almost three-fold increased risk of CKD across all ancestries.[26] The PRS-CKD score was standardised to zero mean and unit variance based on ancestry-matched population controls.

2.4 | Statistical analysis

For descriptive statistics, categorical variables are shown as number and proportion. Continuous variables are shown as means and standard deviation (SD), or medians and interquartile ranges (IQR), as appropriate. Variables that were not normally distributed were log-transformed before entering statistical analyses.

To evaluate the effect of the *PNPLA3* rs738409 C>G variant (p.I148M) on continuous levels of eGFR (or eGFR change at the first study follow-up in a small subset of individuals), associations were performed by fitting data to generalised linear models (GLM). GLM analyses were adjusted for age, sex, ethnicity, PRS-CKD score, albuminuria and clinical factors significantly associated at univariate analysis (selecting the most robust determinant in each category to avoid multicollinearity). For the main analysis (including eGFR levels as a continuous measure), the effect of *PNPLA3* p.I148M variant was also adjusted for covariates associated with this genetic variant at univariate analyses (including the use of lipid-lowering medications and serum aminotransferase levels). Interaction terms of the selected variables with *PNPLA3* p.I148M were also tested and retained when $p < 0.10$.

Statistical analyses were carried out using the JMP Pro 16.0 Statistical Analysis Software (SAS Institute), and R statistical analysis software version 4.1.3 (http://www.R-project.org/). $p < 0.05$ (two-tailed) were considered to be statistically significant.

3 | RESULTS

3.1 | Characteristics of participants

Among the 1144 middle-aged participants of the Liver-Bible-2022 cohort (>90% Caucasian; 36.5% men; mean age 54 ± 6 years), 608 (53.1%) individuals had *PNPLA3* p.I148M I/I genotype, 444 (38.8%) had I/M genotype and 92 (8.1%) had M/M genotype (p = not significant for violation of Hardy–Weinberg equilibrium). Of these individuals, 404 (35.1%) had CKD stage 1 (defined as $eGFR_{CKD\text{-}EPI}$ ≥ 90 mL/min/1.73 m^2), 708 (61.5%) had CKD stage 2 ($eGFR_{CKD\text{-}EPI}$ 89–60 mL/min/1.73 m^2) and 40 (3.5%) had CKD stage 3A ($eGFR_{CKD\text{-}EPI}$ 59–45 mL/min/1.73 m^2). No patients had CKD stage ≥3B (eGFR < 45 mL/min/1.73 m^2). Given the inclusion criteria of the study, about 85% of these participants had arterial hypertension, 61% were overweight, 33% were obese. In addition, on the basis of the strict inclusion criteria of the study requiring the presence of good glycaemic control and no pharmacotherapy except for metformin, only 1.4% of these participants had established T2D. The frequency distribution of $eGFR_{CKD\text{-}EPI}$ levels is shown in Figure 1A. As expected, PRS-CKD score was normally distributed (median:−0.159, IQR: −0.343 to 0.0195, range: −1.003 to 0.614). The frequency distribution of PRS-CKD score is presented in Figure 1B.

Table 1 shows the clinical and biochemical characteristics of the study participants, stratified by rs738409 C>G (*PNPLA3* p.I148M)

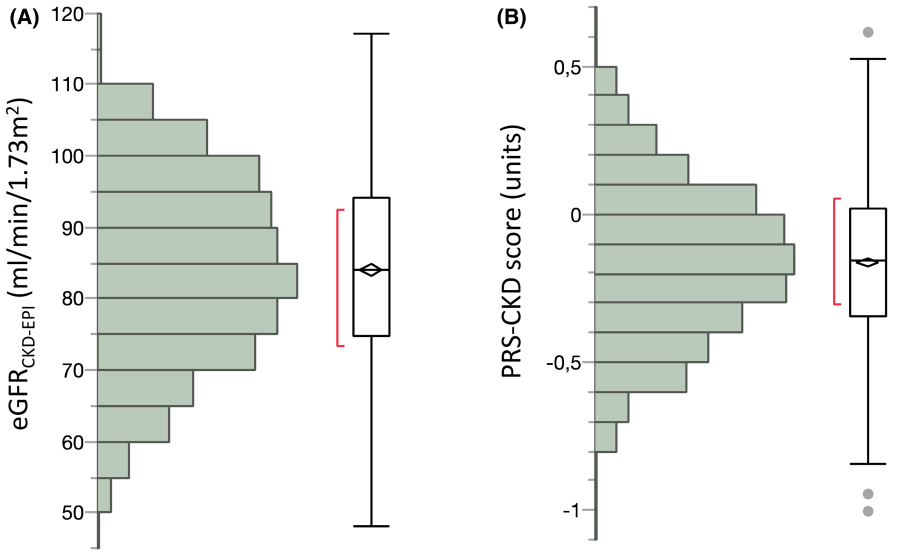

FIGURE 1 Frequency distribution of (A) $eGFR_{CKD\text{-}EPI}$ levels, and (B) polygenic risk score of chronic kidney disease (PRS-CKD score) among 1144 participants with metabolic dysfunction belonging to the Liver-Bible-2022 cohort. Positive values denote a higher genetic predisposition to CKD compared to the population average, whereas negative values denote protection against CKD.

variant genotype. Under additive genetic models, the *PNPLA3* p.I148M allele was significantly associated with higher levels of serum aminotransferases, but lower LDL-cholesterol levels and with a lower likelihood of being treated with lipid-lowering medications. Conversely, age, sex, anthropometric parameters, blood pressure, GGT, platelet count, HDL-cholesterol, triglycerides, C-reactive protein, HbA1c, fasting glucose, insulin levels and Fibroscan®-assessed CAP and LSM, as well as the prevalence of T2D, abnormal albuminuria and PRS-CKD score did not significantly differ across *PNPLA3* p.I148M genotypes. Figure 2 shows the distribution of $eGFR_{CKD\text{-}EPI}$ levels according to *PNPLA3* rs738409 C>G genotype, encoding for the p.I148M variant, in the whole cohort of participants, stratified by age and PRS-CKD score. Levels of $eGFR_{CKD\text{-}EPI}$ decreased across *PNPLA3* p.I148M genotypes (see next paragraph).

Results remained unchanged when we included Fibroscan®-assessed CAP or LSM, instead of serum ALT levels in this regression model (estimate: −1.25, 95% CI: −2.33 to −0.17; $p = 0.022$; and estimate: −1.29, 95% CI: −2.36 to −0.2; $p = 0.018$ respectively). Notably, CAP and LSM values were not independently associated with lower $eGFR_{CKD\text{-}EPI}$ levels ($p > 0.1$). Results remained unchanged even when we excluded from the analysis the subjects with established T2D ($n = 16$) (data not shown).

In another sensitivity analysis in which the presence of T2D and hypertension were included as covariates in the multivariable model (instead of fasting insulin levels and systolic blood pressure), the *PNPLA3* p.I148M variant remained independently associated with lower $eGFR_{CKD\text{-}EPI}$ (estimate −1.18, 95% CI: −2.26 to −0.10; $p = 0.031$).

3.2 | *PNPLA3* p.I148M was associated with lower $eGFR_{CKD\text{-}EPI}$ levels in cross-sectional analysis

Table 2 shows the independent predictors of $eGFR_{CKD\text{-}EPI}$ levels (included as a continuous measure) in the whole cohort of participants ($n = 1144$). The *PNPLA3* p.I148M variant was significantly associated with lower eGFR levels, independent of age, sex, height, waist circumference, systolic blood pressure, fasting insulin level (as a proxy of insulin resistance), LDL-cholesterol, lipid-lowering treatment, aminotransferase levels, albuminuria and importantly also ethnicity and PRS-CKD score. Other variables that were independently associated with lower $eGFR_{CKD\text{-}EPI}$ (along with the *PNPLA3* p.I148M variant) were age, height, fasting insulin, LDL-cholesterol levels, albuminuria, ethnicity and PRS-CKD score. At multivariable ordinal regression analysis adjusted for the same variables as in the GLM, under an additive genetic model the presence of *PNPLA3* p.I148M variant was significantly associated with a higher likelihood of being classified as having more severe stages of CKD (estimate 0.22, 95% CI: 0.02–0.43; $p = 0.037$).

3.3 | *PNPLA3* p.I148M was associated with faster $eGFR_{CKD\text{-}EPI}$ decline in prospective analysis

Table 3 shows the effect of *PNPLA3* p.I148M variant on $eGFR_{CKD\text{-}EPI}$ changes over time in a subset of participants ($n = 144$). During a median follow-up of 17 months, the *PNPLA3* p.I148M variant was associated with a faster $eGFR_{CKD\text{-}EPI}$ decline, independently of sex, height, systolic blood pressure, LDL-cholesterol, ALT aminotransferase, albuminuria and PRS-CKD score.

4 | DISCUSSION

The main findings of our cross-sectional and exploratory prospective study that included a cohort of well-characterised middle-aged individuals with metabolic dysfunction belonging to Liver-Bible-2022 cohort, are as follows: (a) the *PNPLA3* p.I148M variant was significantly associated with lower $eGFR_{CKD\text{-}EPI}$ levels; (b) this significant association persisted even after adjustment for established renal

TABLE 1 Clinical and biochemical characteristics of 1144 participants with metabolic dysfunction belonging to the Liver-Bible-2022 cohort, stratified by the rs738409 C>G (*PNPLA3* p.I148M) variant genotype.

	Subjects with I/I genotype ($n = 608$)	Subjects with I/M genotype ($n = 444$)	Subjects with M/M genotype ($n = 92$)	*p* value
Age (years)	54 ± 6	54 ± 6	54 ± 7	0.92
Sex (men) (%)	45.1	31.6	6.6	0.15
Caucasian ethnicity (%)	95.2	94.6	89.1	**0.007**
BMI (kg/m^2)	28.6 ± 3.1	28.6 ± 3.3	28.8 ± 3.2	0.62
Waist circumference (cm)	102.8 ± 8.9	102.3 ± 9.0	102 ± 9.0	0.39
Obesity (BMI > 30 kg/m^2) (%)	32.1	33.6	35.9	0.43
Hypertension (%)	86.8	84.2	82.6	0.16
Systolic blood pressure (mm Hg)	138 ± 13	137 ± 12	137 ± 12	0.55
Diastolic blood pressure (mm Hg)	86 ± 8	86 ± 8	86 ± 9	0.94
AST (U/L)	23 ± 6	25 ± 9	26 ± 8	**0.002**
ALT (U/L)	29 ± 12	30 ± 15	34 ± 18	**0.005**
GGT (U/L)	29 ± 21	27 ± 19	30 ± 27	0.28
Platelet count (×100,000/mm^3)	236 ± 51	231 ± 50	234 ± 52	0.20
Glucose (mg/dL)	97 ± 15	96 ± 17	98 ± 12	0.97
HbA1c (mmol/molHb)	36 ± 5	36 ± 5	36 ± 4	0.77
Insulin (mIU/L)	15 ± 10	15 ± 9	15 ± 10	0.84
HOMA-IR score	3.6 ± 2.4	3.5 ± 2.4	3.7 ± 2.4	0.96
Type 2 diabetes (%)	1.8	0.9	1.1	0.28
LDL-cholesterol (mg/dL)	127 ± 31	124 ± 30	119 ± 33	**0.014**
HDL-cholesterol (mg/dL)	46 ± 10	45 ± 10	46 ± 11	0.92
Triglycerides (mg/dL)	164 ± 84	161 ± 70	163 ± 125	0.72
Albuminuria (mg/L)	12 ± 30	11 ± 34	9 ± 10	0.30
Abnormal albuminuria (%)	3.3	1.6	0.3	0.11
C-reactive protein (mg/L)	0.2 ± 0.3	0.2 ± 0.3	0.2 ± 0.5	0.74
Alcohol (drinks/week)	4.5 ± 5.8	4.5 ± 6.1	4.5 ± 6.6	0.98
Physical activity (h/week)	1.7 ± 3.0	1.6 ± 2.7	1.4 ± 2.3	0.49
Sweetened beverages (*n*/week)	0.9 ± 2.1	1.0 ± 2.4	0.9 ± 2.2	0.78
Current smokers (%)	7.1	10.8	5.4	0.06
Anti-hypertensive drug users (%)	32.4	27.2	32.6	0.31
Lipid-lowering drug users (%)	13.2	8.1	7.6	**0.007**
Fibroscan®-measured CAP (dB/m)	274 ± 42	276 ± 42	276 ± 42	0.50
Fibroscan®-measured LSM (kPa)	4.9 ± 1.3	5.0 ± 1.2	5.1 ± 1.4	0.67
PRS-CKD (units)	−0.16 ± 0.26	−0.16 ± 0.27	−0.20 ± 0.26	0.32

Note: Sample size, $n = 1144$. Data are expressed as means ± SD, or percentages. The impact of the *PNPLA3* p.I148M genetic variants on the reported phenotypes was tested by GLM (logistic regression was applied for binary outcomes) under an additive genetic model. Not normally distributed variables were log-transformed before statistical analyses. For the sake of clarity, significant *p*-values were highlighted in bold.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; GGT, gamma-glutamyltransferase; HbA1c, glycated haemoglobin; HOMA-IR, homeostatic model assessment for insulin resistance; LSM, liver stiffness measurement; PRS-CKD, polygenic risk score of kidney disease.

risk factors and potential confounders, including age, sex, ethnicity, height, systolic blood pressure (or hypertension), serum transaminases, fasting insulin, LDL-cholesterol, albuminuria, use of lipid-lowering medications or pre-existing type 2 diabetes; and (c) in a subset of these individuals with available follow-up data, the *PNPLA3* p.I148M variant was also associated with a faster $eGFR_{CKD\text{-}EPI}$ decline during a median follow-up of 17 months, independently of established renal risk factors and potential confounders. Last, but not least, we also showed, for the first time, that the association between *PNPLA3* p.I148M variant and lower $eGFR_{CKD\text{-}EPI}$ levels was independent of other genetic determinants, including ethnicity (which is a known determinant of the frequency of the p.I148M variant) and

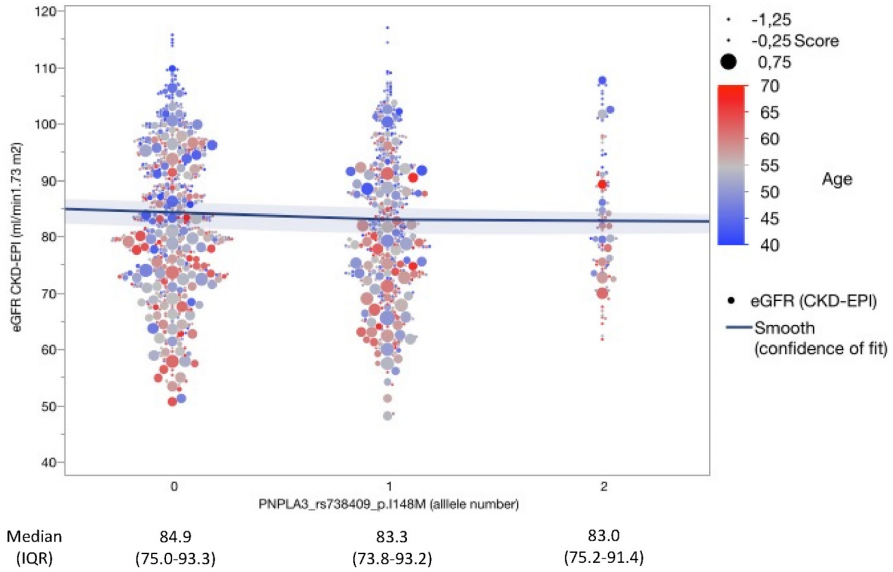

FIGURE 2 Distribution of $eGFR_{CKD\text{-}EPI}$ levels according to *PNPLA3* rs738409 C>G genotype, encoding for the p.I148M variant, in 1144 participants with metabolic dysfunction, with stratification by age (colour code) and PRS-CKD score (dot size). The linear trend and 95% confidence intervals are also reported.

TABLE 2 Independent predictors of $eGFR_{CKD\text{-}EPI}$ levels in individuals with metabolic dysfunction from the Liver-Bible-2022 cohort (cross-sectional analysis).

Multivariable GLM analysis	Beta coefficient	Standard error	95% CIs	*p* value
Sex (women vs. men)	−0.72	0.61	−1.85 to 0.55	0.24
Age (years)	−0.69	0.06	−0.80 to −0.58	**2E-29**
Height (cm)	−0.29	0.06	−0.41 to −0.16	**0.009**
Waist circumference (cm)	0.08	0.04	−0.01 to 0.16	0.09
Systolic blood pressure (mm Hg)	−0.03	0.03	−0.08 to 0.03	0.33
Alanine aminotransferase (log U/L)	0.62	0.93	−1.22 to 2.45	0.51
Fasting insulin (log IU/L)	−2.04	0.73	−3.49 to −0.60	**0.005**
Microalbuminuria (log mg/L)	1.07	0.33	0.41 to 1.72	**0.001**
LDL-cholesterol (mg/dL)	−0.04	0.01	−0.06 to −0.01	**0.001**
Lipid-lowering therapy (yes vs. no)	0.54	0.58	−0.59 to 1.67	0.33
Ethnicity				
Sub-Saharan African	Ref.	Ref.	Ref.	
Asian	1.60	1.97	−2.27 to 5.47	0.35
Caucasian	−5.71	1.51	−8.67 to −2.74	**0.0002**
North African	0.87	2.74	−4.52 to 6.26	0.75
PNPLA3 rs738409 p.I148M (alleles)	−1.24	0.54	−2.32 to −0.17	**0.023**
PRS-CKD (units)	−10.0	1.32	−12.6 to −7.40	**1E-14**

Note: Sample size, $n = 1144$. Data are expressed as beta coefficients, standard errors and 95% confidence intervals as tested by multivariable GLM. Continuous values of $eGFR_{CKD\text{-}EPI}$ (for every 1 mL/min/1.73 m^2) were included as the dependent variable in multivariable GLM model. Ref. = reference category. For the sake of clarity, significant *p*-values were highlighted in bold.

the most comprehensive PRS-CKD available to date, summarising the genetic predisposition to CKD due to carriage of common genetic risk factors.

Collectively, therefore, the findings of our study corroborate and expand the results of recent cross-sectional studies showing a significant association of the *PNPLA3* p.I148M variant with lower eGFR levels and greater prevalence of CKD, both in adults and adolescents as well as in patients with and in those without T2D.[10–17] For instance, in a small cross-sectional study enrolling approximately 200 Caucasian nonobese non-diabetic adults, Musso et al. showed that homozygosity for the *PNPLA3* p.I148M variant was independently associated with lower eGFR levels and abnormal albuminuria.[17] In another small cross-sectional study of 227 Chinese individuals with biopsy-proven NAFLD, Sun et al. reported that homozygosity for the *PNPLA3* p.I148M variant was associated with a higher prevalence of CKD, abnormal albuminuria, as well as with higher levels of urinary neutrophil gelatinase-associated lipocalin (a marker of renal tubular injury), even after adjustment for age, sex, hypertension, T2D and

TABLE 3 Independent predictors of $eGFR_{CKD\text{-}EPI}$ change at follow-up in a subset of 144 participants of the Liver-Bible-2022 cohort followed for a median of 17 months (prospective analysis).

Multivariate GLM analysis	Beta coefficient	Standard error	95% CIs	*p* value
Sex (women vs. men)	−3.01	1.48	−5.94 to −0.08	**0.042**
Height (cm)	−0.13	0.13	−0.40 to 0.12	0.30
Microalbuminuria (log mg/L)	0.02	0.05	−0.06 to 0.28	0.84
LDL-cholesterol (mg/dL)	0.09	0.03	0.03 to 0.15	**0.002**
Alanine aminotransferase (log IU/L)	4.67	3.00	−1.30 to 10.64	0.12
PNPLA3 rs738409 p.I148M (alleles)	−3.57	1.60	−6.94 to −0.21	**0.037**
PNPLA3 rs738409 p.I148M * Sex	−3.22	1.72	−6.63 to 0.19	0.062
PRS-CKD (units)	−8.10	4.23	−16.5 to 0.28	0.056

Note: Sample size, $n = 144$. Data are expressed as beta coefficients, standard errors and 95% confidence intervals as tested by multivariable GLM. For the sake of clarity, significant *p*-values were highlighted in bold.

severity of NAFLD.[12] In another study including 740 Japanese adults undergoing a health screening programme, Oniki et al. reported that homozygosity for the *PNPLA3* p.I148M variant was associated with lower levels of eGFR, independent of common renal risk factors.[11] Similar to the results of our study, these authors also showed that homozygosity for the *PNPLA3* p.I148M variant was associated with faster eGFR decline in a small subgroup of subjects followed for a mean period of 5 years.[11] Almost identical results were found in another pilot prospective study of 46 postmenopausal T2D women with preserved kidney function showing that the *PNPLA3* p.I148M variant was associated with a faster eGFR decline during a 5-year follow-up period, independent of annual changes in common renal risk factors and use of certain glucose-lowering medications.[27] Finally, in a recent experimental and clinical study, our research group, beside confirming that the p.I148M variant homozygosity was strongly associated with lower eGFR levels, also showed that PNPLA3 mRNA levels were expressed at high levels both in the liver and in the kidney. In particular, among the cellular types of the kidney, podocytes showed higher PNPLA3 mRNA and protein expression levels than tubular cells.[10]

Here we also confirmed that genetic predisposition, as detected by the PRS-CKD score, had a large and robust impact on eGFR levels also in our Liver-Bible-2022 cohort of individuals with metabolic dysfunction at risk for liver disease, accounting for an $eGFR_{CKD\text{-}EPI}$ variation of 16.5 mL/min/1.73 m^2 between the extremes of its distribution. In our cohort, each *PNPLA3* p.I148M allele carried by participants had an impact of about 7.6% of the whole PRS-CKD spectrum. It could therefore be speculated that in the presence of overweight/obesity and insulin resistance, the phenotypic expression of the *PNPLA3* p.I148M variant on kidney function is mainly triggered by hyperinsulinaemia.[28,29] It is possible to hypothesise that the absence of triggers, the protection against hypercholesterolaemia, and the predisposition conferred towards competing liver-related or other diabetes-related events, which is observed at population level,[30,31] may account for the fact that the *PNPLA3* gene has not yet emerged among the genome-wide significant *loci* for CKD.[26]

To date, whilst the adverse effect of the *PNPLA3* p.I148M variant on the risk of NAFLD development and progression is well established, the biological mechanisms that are potentially implicated in the link between the *PNPLA3* p.I148M variant and kidney dysfunction are not fully understood. The most obvious explanation for our findings is that the association between *PNPLA3* p.I148M variant and lower eGFR levels could be mediated by the adverse effect of *PNPLA3* p.I148M variant on the liver (favouring hepatic steatosis, inflammation and fibrosis). However, in our multivariable regression analyses, the adjustment for liver damage evaluated either by circulating aminotransferase levels or by Fibroscan®-measured CAP and LSM values did not attenuate the significant association between *PNPLA3* p.I148M variant and lower $eGFR_{CKD\text{-}EPI}$ levels or faster eGFR decline, despite this genetic variant had a robust impact on serum aminotransferase levels in our cohort. Noteworthy, recent cross-sectional studies[10–17] also suggested that the association between *PNPLA3* p.I148M variant and kidney dysfunction might be mediated by a direct adverse effect of the mutated PNPLA3 protein on glomerular podocytes. In this regard, we have recently demonstrated that *PNPLA3* protein expression in glomerular podocytes is high and comparable to that of hepatic stellate cells.[10] In the kidney, chronic activation of glomerular podocytes and pericytes, that are stromal cells playing a role both in angiogenesis and in regulating renal medullary and cortical blood flows, may promote kidney fibrogenesis and glomerulosclerosis.[32] In addition, some experimental evidence also suggests that glomerular podocytes may accumulate lipid droplets and retinol esters and that this mechanism might be implicated in CKD pathogenesis.[33] However, further prospective and mechanistic studies are required to better understand the long-term effect of *PNPLA3* p.I148M variant on retinol and lipid metabolism in the kidneys.

Our findings may have some important clinical implications, as they support the possibility that *PNPLA3* genotyping might be useful among individuals with metabolic dysfunction not only for identifying those at higher susceptibility to NAFLD development and progression, but also those at higher risk of developing kidney dysfunction over time. As antisense oligonucleotides targeting the *PNPLA3* p.I148M variant for degradation have recently entered phase 2 clinical trials conducted in patients with fibrotic NASH,[8,9,34] our results also suggest that *PNPLA3* silencing might have beneficial

effects on the development and progression of CKD in this clinical setting.

Our study has some important limitations that should be mentioned. First, our longitudinal analysis was performed only in a small subset of individuals. Second, although we did not directly measure GFR (by plasma iohexol disappearance), we used the most widely accepted serum creatinine-based equation for estimating GFR in clinical practice and large epidemiological studies. Third, our Liver-Bible-2022 cohort comprised individuals with metabolic dysfunction (most of whom were of Caucasian ethnicity, had normal or near-normal kidney function, that is, CKD stage 1 or stage 2, and did not have established T2D) and, consequently, our results cannot be necessarily extrapolated to the general population or other specific cohorts of individuals. Finally, we cannot exclude residual confounding as a result of unmeasured risk factors.

It is important to note that the present results do not exclude that NAFLD can have a detrimental impact on kidney function, independently of carriage of the *PNPLA3* p.I148M genetic variant. Several factors may account for the fact that we could not observe any significant impact of NAFLD on kidney function in our cohort of individuals with metabolic dysfunction. These include the lack of possibility to directly quantify hepatic fat accumulation by gold-standard approaches based on magnetic resonance imaging. In addition, the inclusion of a population not selected for liver disease and at a low baseline risk of advanced liver fibrosis precludes the possibility to systematically examine the liver damage by histology.

Notwithstanding the aforementioned limitations, our study has important strengths, such as the large number of individuals included in the cross-sectional analysis, the consecutive enrolment of the study population, the completeness of the database, the adjustment for multiple renal risk factors and potential confounding factors, including the most updated instrument to measure the genetic predisposition to CKD, as well as the exclusion of subjects with important comorbidities (e.g. advanced kidney disease, cirrhosis or active cancer). We believe that the inclusion of subjects with such comorbidities might have confounded the interpretation of data.

In conclusion, the results of our cross-sectional study showed that the *PNPLA3* p.I148M variant was significantly associated with lower $eGFR_{CKD\text{-}EPI}$ levels (an average loss of 1.24 mL/min/1.73 m^2 of $eGFR_{CKD\text{-}EPI}$ for each *PNPLA3* risk allele) in a cohort of well-characterised middle-aged individuals with metabolic dysfunction. Notably, this association remained statistically significant even after adjustment for established renal risk factors and potential confounders, including ethnicity and the most comprehensive PRS-CKD available to date, summarising the genetic predisposition to CKD due to carriage of common genetic risk factors. In addition, the results of our exploratory prospective study confirmed that the *PNPLA3* p.I148M variant was associated with a faster $eGFR_{CKD\text{-}EPI}$ decline over a median follow-up of 17 months. Given the potential translational relevance of the association between the *PNPLA3* p.I148M variant and lower eGFR levels for the design of precision medicine approaches, we believe that further research is needed to better elucidate the possible detrimental effect of this common genetic variant on the risk of kidney dysfunction.

AUTHOR CONTRIBUTIONS

Alessandro Mantovani: Conceptualization (supporting); data curation (equal); investigation (supporting); methodology (equal); writing – original draft (equal); writing – review and editing (equal). **Serena Pelusi:** Data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); resources (equal); supervision (equal); writing – original draft (equal); writing – review and editing (equal). **Sara Margarita:** Data curation (equal); formal analysis (supporting); methodology (equal); project administration (supporting); resources (equal); supervision (supporting); writing – review and editing (supporting). **Francesco Malvestiti:** Data curation (supporting); formal analysis (lead); investigation (supporting); methodology (equal); software (equal); writing – review and editing (equal). **Michela Dellalma:** Formal analysis (supporting); methodology (supporting); software (supporting); writing – review and editing (equal). **Cristiana Bianco:** Data curation (supporting); methodology (supporting); resources (supporting); writing – review and editing (supporting). **Luisa Ronzoni:** Data curation (supporting); methodology (supporting); resources (supporting); supervision (supporting); writing – review and editing (supporting). **Daniele Prati:** Funding acquisition (supporting); project administration (supporting); resources (supporting); supervision (supporting); writing – review and editing (supporting). **Giovanni Targher:** Conceptualization (supporting); supervision (supporting); writing – original draft (supporting); writing – review and editing (equal). **Luca Valenti:** Conceptualization (lead); data curation (equal); formal analysis (equal); funding acquisition (lead); investigation (equal); methodology (equal); resources (equal); supervision (lead); visualization (equal); writing – original draft (equal); writing – review and editing (equal).

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest relevant to the present study. LV has received speaking fees from MSD, Gilead, AlfaSigma and AbbVie, served as a consultant for Gilead, Pfizer, AstraZeneca, Novo Nordisk, Intercept, Diatech Pharmacogenetics, Ionis Pharmaceuticals, Boeringher Ingelheim, and received research grants from Gilead. DP served as a consultant for, and has received speaking fees, travel grants and research grants from Macopharma, Ortho Clinical Diagnostics, Grifols, Terumo, Immucor, Diamed, Diatech Pharmacogenetics.

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