Title

PNPLA3 rs738409 is associated with renal glomerular and tubular injury in NAFLD patients with persistently normal ALT levels

Short title: *PNPLA3* gene variant and kidney dysfunction

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List of Abbreviations

ALT, alanine aminotransferase; abnALT, abnormal alanine aminotransferase; ANOVA, analysis of variance; AST, aspartate aminotransferase; BMI, body mass index; BUN, blood urea nitrogen; BP, blood pressure; CI, confidence interval; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; HDL-c, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-c, low-density lipoprotein cholesterol; nALT, normal alanine aminotransferase; NASH, non-alcoholic hepatitis; NGAL,

neutrophil gelatinase-associated lipocalin; OR, odds ratio; PLT, platelet count; PNPLA3, patatin-like phospholipase domain-containing protein 3; SD, standard derivation; RTI, renal tubular injury; TC, total cholesterol; TG, triglycerides; u-ACR, urinary albumin-to-creatinine ratio; UA, uric acid; NAFLD, non-alcoholic fatty liver disease; Scr, serum creatinine

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Conflict of Interest Statement

All authors: nothing to declare.

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Abstract

Background & Aim:

Patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) rs738409 polymorphism is associated with NAFLD severity and the *PNPLA3* gene is expressed in the kidneys,but whether *PNPLA3* rs738409 polymorphism is also associated with renal tubular injury is uncertain. We assessed the effect of *PNPLA3* genotypes on biomarkers of renal tubular injury (RTI) and glomerular function in subjects with NAFLD who had either normal (nALT) or abnormal (abnALT) alanine aminotransaminase levels.

Methods:

217 patients with histologically-proven NAFLD, of which 75 had persistently nALT (below upper limit of normal for 3 months) were included. Multivariable regression analyses were undertaken to test associations between *PNPLA3* genotype and biomarkers of kidney dysfunction.

Results:

The nALT patient group had higher urinary neutrophil gelatinase-associated lipocalin levels (u-NGAL, a biomarker of RTI) (P <0.001), higher albuminuria (P =0.039) and greater prevalence of chronic kidney disease (CKD) (P =0.046) than the abnALT group. The association between *PNPLA3* GG genotype and risk of CKD and abnormal albuminuria remained significant after adjustment for kidney risk factors and severity of NAFLD histology, mostly in the nALT group. Similarly, *PNPLA3* GG

genotype was associated with higher u-NGAL levels in the nALT group, even after adjustment for the aforementioned risk factors and glomerular filtration-based markers (β-coefficient: 22.29, 95% CI: 0.99-43.60, *P* =0.041).

Conclusion:

Patients with NAFLD and persistently nALT, who carry the *PNPLA3* rs738409 G allele, are at higher risk of early glomerular and tubular damage. We suggest *PNPLA3* genotyping may help identify patients with NAFLD at higher risk of RTI.

Keywords: Patatin-like phospholipase domain-containing protein 3, non-alcoholic fatty liver disease, renal tubular injury, chronic kidney disease

Lay summary

PNPLA3 rs738409 polymorphism, which is associated with increased risk of NAFLD progression, may also play a role in kidney dysfunction, but the impact of this genetic variant on renal tubular injury in patients with NAFLD is uncertain, regardless of whether they have persistently normal or abnormal serum ALT levels. Our findings show that the rs738409 C>G variant is associated with a higher risk of early renal tubular injury in patients with histologically-confirmed NAFLD and persistently normal ALT levels, independently of established kidney risk factors and severity of NAFLD histology.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is currently recognized not only as a major cause of liver-related morbidity and mortality, but it may be also actively involved in the development and progression of chronic kidney disease (CKD) (1, 2). NAFLD exacerbates systemic/hepatic insulin resistance and promotes the release of several pro-inflammatory, pro-fibrogenic and pro-oxidant mediators, which are also factors potentially involved in the pathophysiology of CKD (3, 4). Recent studies have reported that the rs738409 polymorphism in the patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) gene, (*i.e.*, the major common genetic variant associated with a greater predisposition to progressive forms of NAFLD (5-7)), was associated with decreased estimated glomerular filtration rate (eGFR) and increased albuminuria, irrespective of the presence of NAFLD (6, 7). However, to date, very few of these studies have used liver biopsy for diagnosing and staging NAFLD, and it is not known whether there is an association between *PNPLA3* genotype and renal tubular injury (RTI), independent of glomerular filtration-based markers (such as serum creatinine or albuminuria).

Recent studies have supported a prognostic role of increased urinary neutrophil gelatinase-associated lipocalin (u-NGAL), *i.e.*, an early and reliable biomarker of RTI, for the prediction of future ischemic atherosclerotic cardiovascular events (8, 9). NGAL is a small protein of 178 amino acids produced by activated neutrophils and epithelial cells that is rapidly up-regulated in response to any cellular stress, such as

ischemia or inflammation (10). The primary site of NGAL production in injured kidneys is located in the thick ascending Henle's loop and the intercalated cells of the collecting duct (11). Therefore, u-NGAL is thought to be a more accurate biomarker of RTI than plasma NGAL levels (12).

While most clinicians usually focus their practice on patients with NAFLD and abnormal ALT (abnALT), patients with normal ALT (nALT) and NAFLD represent an important proportion of this patient group. The health consequences of nALT and NAFLD are poorly understood and have been evaluated in only a few studies (13-15). Although it is well known that serum ALT levels have poor sensitivity and specificity for predicting the presence and severity of NAFLD (16), serum ALT levels remain the most frequently used parameter for referral of patients with suspected NAFLD to secondary care clinics both in China and in many other countries. Thus, individuals with nALT (who have NAFLD) are often not referred to specialists for further evaluation of the severity of their liver disease. Thus, our understanding of the severity of NAFLD in individuals with nALT and its relationship with extra-hepatic complications, such as CKD, in this patient group is currently uncertain.

Therefore, the main aim of this cross-sectional study was to ascertain the possible effect of *PNPLA3* rs738409 polymorphisms on markers of glomerular and tubular damage in adult patients with histologically-proven NAFLD and persistently normal ALT levels.

MATERIALS AND METHODS

Patients

A total of 906 adult individuals who had undergone liver biopsy were consecutively recruited at the First Affiliated Hospital of Wenzhou Medical University during a period of nearly two years (December 2016 to February 2019). Patients with suspected NAFLD were classified according to their liver histology data as definitive non-alcoholic hepatitis (NASH), borderline NASH, or simple fatty liver (NAFL) according to the NASH Clinical Research Network classification (17). Among them, 689 subjects were excluded for the following reasons: (1) excessive alcohol intake (>140 g/week for men and >70 g/week for women); (2) presence of viral hepatitis (B or C), autoimmune hepatitis, primary biliary cholangitis or Wilson's disease; (3) chronic use of hepatotoxic drugs, such as non-steroid anti-inflammatory agents, calcium channel blockers, steroids, tamoxifen, amiodarone, isoniazid or methotrexate; (4) prior history of cirrhosis of any etiology or presence of liver failure; (5) other known causes of chronic or acute kidney diseases as well as presence of urinary tract infection; and (6) fatty liver infiltration <5% on liver histology or missing data.

Persistent nALT status was defined as a serum ALT level below the upper limits of normal (local clinical laboratory reference of less than 40U/L) for at least 3 months prior to study entry (14). After applying the aforementioned exclusion criteria, 217 adults with biopsy-proven NAFLD were included in the final analysis. Of these 75 individuals had persistently nALT, whereas the remaining 142 individuals had

abnormal ALT levels.

Written informed consent was obtained from each subject before their participation in the study. Personal information was omitted and personal identifiers were replaced by the health examination number. The research protocol was approved by the ethics committee of the First Affiliated Hospital of Wenzhou Medical University.

Liver histology

An ultrasound-guided liver biopsy was performed under sedation using a 16-gauge Hepafix needle (Gallini, Modena, Italy). All liver biopsy specimens were placed in formalin solution for fixation, embedded in paraffin blocks and stained with hematoxylin-eosin and Masson's trichrome. All biopsies were analyzed by an experienced liver pathologist (XD Wang), who was blinded to clinical and laboratory data of participants. The histologic features of NAFLD were scored according to the NASH Clinical Research Network classification (17). The histologic stage of hepatic fibrosis was quantified according to the Brunt's criteria (18).

Assessment of kidney disease

Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was used to calculate eGFR (19). The CKD-EPI equation is as follows: $eGFR = 141 \times \min(Scr/\kappa, 1)^{\alpha} \times \max(Scr/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018$ [if female], where Scr is serum creatinine, κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411

for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ or 1. CKD stages were defined according to the KDIGO guidelines (20): stage 1, urinary albumin-to-creatinine ratio (u-ACR) ≥30 mg/g with eGFR ≥90 ml/min/1.73m^2; stage 2, u-ACR ≥30 mg/g with eGFR of 60-89 ml/min/1.73m^2; stage 3, eGFR of 30-59 ml/min/1.73m^2 (with or without u-ACR ≥30 mg/g); stage 4, eGFR of 15-29 ml/min/1.73m^2, stage 5, eGFR of <15 ml/min/1.73m^2. In our study, patients were then stratified into two groups as follows: no-CKD (stage 0) and CKD (stage 1 to 5).

Urinary albumin excretion was measured on a morning spot urine sample and expressed as u-ACR; abnormal albuminuria was defined as u-ACR ≥30 mg/g. Urinary and plasma NGAL levels were measured using an up-converting phosphor technology (Hotgen, Beijing) according to the manufacturer's instructions. Reference NGAL values were obtained from a consecutive sample of healthy Chinese volunteers (*n*=25) without known kidney disease; their mean u-NGAL and plasma NGAL levels were 31.18 ±12.77 ng/ml and 31.47 ±11.29 ng/ml, respectively.

Genetic analysis

Blood samples were collected from all patients and approximately 20 ng of genomic DNA from each blood sample was extracted for the genetic analysis. Thirteen representative single nucleoid polymorphisms (SNP) associated with NAFLD were genotyped to investigate the susceptibility of CKD in our NAFLD population.

Genotyping assay for the selected SNPs was designed using the MassARRAY platform (Agena Bioscience, San Diego, CA, USA). Locus-specific PCR and detection primers were designed using Assay Design Suite v3.1. After the DNA samples were amplified via multiplex PCR, allele detection was performed through MALDI-TOF mass spectrometry.

Clinical and laboratory data

Demographic data, anthropometry, clinical parameters and comorbidities were recorded from all participants. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Blood pressure was measured using an automated sphygmomanometer with the subject in a quiet environment and in a sitting position for at least 10 min. Hypertension was defined as blood pressure ≥130/85 mmHg or the use of any anti-hypertensive drugs. Diabetes was defined by a fasting blood glucose ≥7.0 mmol/L or HbA1c ≥6.5% or prescribed hypoglycemic medications. Hyperuricemia was defined as serum uric acid levels (UA) >420 μmol/L for men and >360 μmol/L for women, respectively, or use of allopurinol. Dyslipidemia was defined as any of the following criteria: total cholesterol >5.17 mmol/L; triglycerides >1.70 mmol/L; high-density lipoprotein cholesterol (HDL-c) <1.0 mmol/L for female and <1.3 mmol/L for male and low-density lipoprotein cholesterol (LDL-c) ≥3.4 mmol/L, or use of any lipid-lowering drugs. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using fasting glucose and insulin measurements as follows: [fasting insulin (mU/ml) × fasting

glucose (mmol/L)/2.5]. Biochemical parameters included measurement of albumin, ALT, aspartate aminotransferase (AST), fasting glucose, blood urea nitrogen, creatinine, uric acid, lipid profile, white blood cells, red blood cells and platelet counts. All these parameters were measured by an automated analyzer (Abbott AxSYM), using standard methods.

Statistical analysis

All statistical analyses were conducted using the SPSS version 22.0 (SPSS, Chicago, IL). Data are presented as means ± standard deviation (SD) or frequencies. Baseline characteristics of the study population, stratified by serum ALT levels, were compared using the one-way analysis of variance (ANOVA) for continuous variables and the χ^2-test for categorical variables. The associations of *PNPLA3* rs738409 genotype with CKD (*i.e.,* CKD stage 1-5), abnormal albuminuria (u-ACR ≥30 mg/g) and elevated u-NGAL levels (u-NGAL ≥31.18 ng/ml) in patients with NAFLD, stratified by serum ALT levels, were tested using both an unadjusted logistic regression model and two progressive multivariable regression models with pre-specified adjustments for established kidney risk factors, presence of NASH and stage of liver fibrosis. Finally, we also tested the independent association between *PNPLA3* rs738409 genotype and u-NGAL levels (included as a continuous measure) by using a multivariable linear regression analysis that included the same covariates of the aforementioned logistic regression models *plus* glomerular filtration-based markers (i.e., eGFR and albuminuria). In all these regression models, we have presented results using CG and

GG *PNPLA3* rs738409 genotypes that were included either separately or combined into a single category. All statistical tests were 2-sided and *P*-value of <0.05 (two-tailed) was considered statistically significant.

RESULTS

Baseline characteristics of participants

Overall , the 217 patients with histologically-proven NAFLD included in the study had an average age of 42 years, and 78.8% were men. 34.6% (*n*=75) of these patients had persistently normal ALT levels, but the histologic stages of liver inflammation and fibrosis were not significantly different between the two groups, after stratifying by serum ALT levels **(Table 1)**. Compared to those in the abnALT group, patients in the nALT group also had lower BMI and waist circumstance, besides a fewer histologic grade of hepatic steatosis (all *P* <0.05 or less). In addition, these individuals were more likely to be of female sex, older and to have lower eGFR, higher u-NGAL levels and greater prevalence of abnormal albuminuria, CKD, hypertension and diabetes compared to those belonging to the abnALT group. For further investigation, we also tested thirteen SNPs, which have been reported to be associated with an increased risk of NAFLD. **Figure 1** shows the prevalence of CKD and abnormal albuminuria according to different SNP genotypes among NAFLD patients with persistently nALT. Interestingly, we found that the carriers of *PNPLA3* rs738409 GG genotype were more likely to have both CKD and abnormal albuminuria compared to those carrying GC and CC genotypes (*P* <0.05 or less).

Baseline characteristics in NAFLD patients with persistently nALT according to *PNPLA3* genotype

Table 2 shows the clinical and biochemical characteristics of NAFLD patients with persistently nALT, stratified by *PNPLA3* rs738409 polymorphism. Compared to those with either CC or CG genotypes, carriers of *PNPLA3* GG genotype had significantly higher u-NGAL levels, and a greater prevalence of CKD, hypertension, abnormal albuminuria and dyslipidemia. Sex, obesity, diabetes, plasma lipids, HOMA-IR, fasting glucose and uric acid levels did not significantly differ among the three patient groups. Moreover, in comparison with different *PNPLA3* genotypes of patients with abnALT, patients with GG genotype belonging to the nALT group had the lowest mean value of eGFR, the highest mean value of u-NGAL and the highest prevalence of abnormal albuminuria **(Figure 2)**. Conversely, plasma NGAL levels did not differ significantly between the groups (95.22 ±35.47 *vs*. 103.49 ±78.86 ng/ml, *P*=0.397).

***PNPLA3* GG genotype related to decreased kidney function**

As shown in **Table 3**, *PNPLA3* GG genotype was significantly associated with an increased risk of stage 1-5 CKD in the whole population, even after adjustment for established kidney risk factors (age, sex, adiposity measures, hypertension, diabetes, HOMA-IR), hyperuricemia, presence of NASH and liver fibrosis stage (adjusted-OR =3.42, 95%CI: 1.07-10.85). Moreover, the *PNPLA3* GG genotype was also independently associated with an increased risk of abnormal albuminuria after

adjustment for the aforementioned kidney risk factors (model 3: adjusted-OR =2.87, 95%CI: 0.80-10.28). Notably, after stratifying the patients by serum ALT levels, the significant and independent association we observed between *PNPLA3* GG genotype and risk of CKD and abnormal albuminuria was evident only in the subgroup of those with persistently nALT **(Table 3, model 3)**. Conversely, no significant association between the risk allele G of *PNPLA3* rs738409 and risk of having CKD or abnormal albuminuria was found in the subgroup of those with abnALT **(Table 3)**.

PNPLA3 GG genotype related to increased renal tubular injury in NAFLD patients with persistently nALT

As shown in **Table 2**, we found that in the nALT group, carriers of GG genotype had significantly higher u-NGAL levels than those carrying CC genotype (63.09 ±53.92 *vs*. 38.73 ±18.55 ng/ml, *P*=0.028). To further examine the relationship between *PNPLA3* GG genotype and u-NGAL levels, we performed both linear and logistic regression analyses, where u-NGAL was included as the dependent variable and was modelled either as categorical or as continuous measure **(Tables 3 and 4,** respectively**)**. In the nALT group, the *PNPLA3* GG genotype was significantly associated with higher u-NGAL levels, even after adjusting for age, sex, BMI, waist circumference, hyperuricemia, HOMA-IR, diabetes and hypertension (model 2). After further adjustment for glomerular filtration-based markers (albuminuria and eGFR), presence of NASH and histologic stage of liver fibrosis, the results remained essentially unchanged, showing an independent association between *PNPLA3* GG

genotype and increasing u-NGAL levels, modelled either as categorical variable **(Table 3)** or as continuous variable **(Table 4)**, especially in the nALT subgroup.

DISCUSSION

To our knowledge, this is the first observational study to investigate the relationship between *PNPLA3* rs738409 polymorphism and markers of kidney (glomerular and tubular) function in adults with histologically-confirmed NAFLD who have persistently nALT levels. Patients with NAFLD who had persistently nALT were more likely to have higher u-NGAL levels, abnormal albuminuria and CKD compared to those with abnALT. Furthermore, we also found that *PNPLA3* rs738409 GG genotype was significantly associated with a higher prevalence of CKD and abnormal albuminuria both in the overall population and in the nALT group alone. This association remained statistically significant even after adjustment for established kidney risk factors, HOMA-estimated insulin resistance and histologic severity of NAFLD (*i.e.*, presence of NASH and fibrosis stage). More importantly, we also found that the presence of the risk allele G of rs738409 conferred an additional risk for RTI, mostly in the nALT patient group, thus further contributing to the increased risk of renal disease progression of these patients.

Our findings, obtained in an ethnically homogeneous Chinese cohort, corroborate and expand the results of recent studies published by other investigators in other ethnic groups (5-7). In a preliminary cross-sectional study of nearly 200 Italian non-obese,

nondiabetic elderly individuals (about a third of whom had biopsy-proven non-cirrhotic NAFLD), Musso *et al*. showed that the presence of *PNPLA3* G allele was associated with a higher prevalence of abnormal albuminuria (OR =3.52, 95%CI: 1.19-7.81, *P* =0.028) and CKD (OR =3.87, 95%CI: 1.18-7.30, *P* =0.002), regardless of traditional kidney risk factors (5). Similarly, two other studies, performed in Italy, reported a significant association between *PNPLA3* G allele and presence of kidney dysfunction both in children/adolescents with biopsy-proven NAFLD and in type 2 diabetic women with ultrasound-defined NAFLD (6, 7). Finally, in a health-screening program including 740 Japanese elderly individuals, Oniki *et al*. showed that carriers of *PNPLA3* GG genotype had lower eGFR than those carrying the C/C genotype, independently of traditional kidney risk factors and presence of NAFLD on ultrasonography (21). However, it is important to note that only few of the aforementioned studies used liver biopsy for diagnosing and staging NAFLD (5, 6), and that in none of these studies have the authors included Chinese individuals or, importantly, measured any specific markers of kidney tubular function.

Serum ALT levels are often used in many parts of the world as surrogate biomarkers of liver injury by clinicians (1, 22, 23). However, it is well established that serum ALT levels alone do not correlate well with the histologic severity of NAFLD (24). Thus, patients with persistently nALT, who have NAFLD, are often not referred to hepatologists/gastroenterologists, or are not regularly monitored by most clinicians. Similarly, this group of patients with NAFLD may not be carefully monitored for any

increased risk of potential NAFLD-related complications, including cardiovascular disease and CKD. In fact, in our study, we showed for the first time that there was a significant and independent association between the presence of *PNPLA3* rs738409 genetic variant and the risk of kidney dysfunction not only in terms of glomerular injury (eGFR and albuminuria) but also kidney tubular impairment, especially in patients with NAFLD and persistently nALT.

It is well known that the PNPLA3 protein promotes lipid droplet remodeling in hepatocytes by its hydrolase activity. However, *PNPLA3* G allele encoding the I148M variant reduces this enzymatic activity, and increases the intra-hepatic amount of triglycerides and retinyl esters (25), thus leading to hepatic steatosis (26), NASH (27) and hepatocellular carcinoma (28). Indeed, the *PNPLA3* genotype may not only increase lipid deposition in hepatocytes, but also increase the production of pro-inflammatory and pro-fibrogenic factors in hepatic stellate cells (29). However, the exact mechanisms underlining the association between the G allele of rs738409 and decreased kidney function are poorly understood. Since NAFLD is closely related with risk of CKD, it is possible to hypothesize that *PNPLA3* rs738409 polymorphism is linked to abnormal albuminuria and decreased eGFR, mainly due to the presence and severity of NAFLD. However, some studies recently suggested that rs738409 G allele was associated with lower eGFR and higher albuminuria, regardless of the presence and severity of NAFLD (7, 30). Thus, the evidence from this and the two above-mentioned studies suggests that the G allele of rs738409 itself might exert

some direct adverse effect on kidney function. Pirazzi *et al*. performing an extensive analysis of the *PNPLA3* mRNA expression in human tissues, showed that *PNPLA3* mRNA was expressed not only in the liver but also in the adipose tissue and kidneys (31). Other investigators reported that *PNPLA3* mRNA expression in the liver was ~50-fold to 100-fold lower compared to that in the adipose tissue in regular chow diet-fed mice, however, the hepatic relative *PNPLA3* mRNA expression was increased when feeding a Western-type diet in both C57BL/6 mice and $LDLR^{-/-}$ mice (32). These findings suggest that the PNPLA3 protein might play a role in hepatic lipid metabolism under conditions of lipid excess (31). Therefore, it is reasonable to speculate that PNPLA3 expression in the kidneys might also be stimulated under conditions of lipid excess and then increase ectopic lipid accumulation in renal mesangial and tubular cells. In addition, renal lipotoxicity can also promote kidney tubular impairment, abnormal albuminuria and glomerulosclerosis, thus causing a progressive decline in kidney function (33-36).

Our observation of a strong association between the *PNPLA3* GG genotype and higher u-NGAL levels also implies the possibility that *PNPLA3* rs738409 gene variant might impair not only renal glomeruli but also renal tubules. To date, accumulating evidence suggests that *PNPLA3* rs738409 gene variant is associated with increased pro-inflammatory and pro-fibrogenic factors (37, 38). Moreover, renal pericytes, which have been also supposed to express *PNPLA3* mRNA, may play an important role in regulating renal medullary and promote kidney tubulo-interstitial fibrogenesis

(7, 39). However, we acknowledge that further research is required to better elucidate the role of *PNPLA3* rs738409 polymorphism in the development and progression of kidney disease.

Our study has some important limitations that merit mention. Firstly, the cross-sectional design of the study does not allow for establishing the temporality and causality of the observed associations, although this limitation is mitigated by the fact that the genetic variant under study is inherited and, therefore, reverse causation does not apply. Secondly, in this single-center study we enrolled patients with biopsy-proven NAFLD belonging to a single Asian ethnicity. Hence, additional studies are needed to confirm these findings in other ethnic populations. Thirdly, we have measured common biomarkers of early kidney (glomerular and tubular) dysfunction, but not some specific biomarkers of tubular injury (e.g., urinary kidney injury molecule-1 and calprotectin), and, finally, information on the renal pathology associated with NAFLD was not available in our study.

In conclusion, the results of this cross-sectional study show that *PNPLA3* rs738409 polymorphism is significantly associated with a higher risk of kidney dysfunction, including glomerular and tubular damage, especially in patients with NAFLD who have persistently normal ALT levels. The association between *PNPLA3* GG genotype and kidney dysfunction appears to be independent of established kidney risk factors, insulin resistance, presence of NASH and histologic stage of liver fibrosis. If verified

in other cohorts, our findings suggest that patients with NAFLD who have persistently normal ALT levels , and who carry the *PNPLA3* GG genotype, should be monitored for RTI and albuminuria in order to prevent CKD progression. That said, further studies are now needed to investigate the inter-relationships between *PNPLA3* rs738409 polymorphism, NAFLD and risk of chronic kidney dysfunction.

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LEGEND TO THE TABLES

Table 1. Baseline characteristics of patients with biopsy-proven NAFLD, stratified by serum ALT levels.

Table 2. Baseline characteristics of patients with biopsy-proven NAFLD and persistently normal ALT levels (nALT), stratified by *PNPLA3* rs738409 genotype.

Table 3. Association of *PNPLA3* rs738409 genotype with chronic kidney disease (CKD), abnormal albuminuria (u-ACR) and elevated u-NGAL levels in patients with biopsy-proven NAFLD, stratified by serum ALT levels.

Table 4. Association between *PNPLA3* rs738409 genotype and u-NGAL levels (included as a continuous measure) in patients with biopsy-proven NAFLD, stratified by serum ALT levels.

LEGEND TO THE FIGURES

Figure 1. Heatmap for the prevalence of CKD and abnormal albuminuria in different SNP genotypes. The prevalence of CKD and abnormal albuminuria was significantly higher in patients with rs738409 GG than in those with rs738409 CC or CG genotypes. *$P < 0.05$

Figure 2. eGFR values (A), prevalence of abnormal albuminuria (B), urinary and plasma NGAL levels (C and D) in NAFLD patients with either nALT or abnALT, stratified by *PNPLA3* rs738409 genotype. Data are presented as means ± SEM; *$P <$ 0.05