Commentary

**PNPLA3 rs738409 polymorphism and kidney dysfunction: an association beyond nonalcoholic fatty liver disease?**

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Abstract

This commentary is primarily devoted to a recent observational study by Mantovani and colleagues (Aliment Pharmacol Ther. 2023; 57: 1093-102) examining the adverse effect of the patatin-like phospholipase domain-containing protein-3 (PNPLA3) rs738409 G allele on the kidney function in a cohort of 1,144 middle-aged Italian individuals with metabolic dysfunction. In this study, the authors found that the PNPLA3 rs738409 G allele was significantly associated with lower levels of estimated glomerular filtrate rate (eGFR), even after adjusting for not only common anthropometric and cardiometabolic risk factors but also ethnicity, serum liver enzymes, use of drugs against dyslipidemia and chronic kidney disease polygenic risk score. Additionally, in a subgroup of 144 patients followed for a median of 17 months, the authors also found that the PNPLA3 rs738409 G allele was independently associated with a faster eGFR decline. Commenting on the cohort study by Mantovani et al., we also summarized the rapidly expanding evidence linking the PNPLA3 rs738409 variant with the risk of kidney disease. Furthermore, we discussed the potential research implications of these findings.

Keywords: Nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, glomerular filtration rate, kidney function, chronic kidney disease, PNPLA3 rs738409

The PNPLA3 gene, also known as adiponutrin, encodes for a protein of 481 amino acids mainly located in
lipid droplets of hepatocytes and hepatic stellate cells\cite{1,2}. The PNPLA3 protein exerts a hydrolase activity on triglycerides and a transacylase activity on polyunsaturated fatty acids in phospholipids\cite{3,4}. The rs738409 C > G single nucleotide polymorphism in the PNPLA3 gene leads to an Ile148Met substitution (i.e., 148 isoleucine to methionine protein variant)\cite{5,6,7}, causing a loss of function in the enzymatic activity of the PNPLA3 protein. Such loss of function in the PNPLA3 protein leads to an accumulation of lipid droplets in hepatocytes and hepatic stellate cells mainly due to a reduction in very-low-density lipoprotein (VLDL) secretion and a lack of proteasomal degradation, thus inducing liver damage and fibrosis over time\cite{8}. It is universally acknowledged that the most extensive proportion of heritability in hepatic fat content among adults from the general population, increased proneness to developing nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis, cirrhosis, and primary liver cancer is accounted for by the PNPLA3 rs738409 polymorphism\cite{9,10}.

As summarized in Table 1 many observational studies performed both in adults and adolescents\cite{11,12,13}, although not all\cite{15-17}, have reported that carriers of the PNPLA3 rs738409 G allele have an increased risk of developing NAFLD and its more advanced forms, but also an increased risk of decreased eGFR and chronic kidney disease (CKD stage ≥ 3), regardless of the presence or absence of type 2 diabetes mellitus (T2DM) and hepatic steatosis. For instance, a cross-sectional analysis conducted in 227 Chinese adults with biopsy-proven NAFLD has shown that the PNPLA3 rs738409 G allele was significantly associated with an increased prevalence of CKD, abnormal albuminuria, and higher levels of urinary neutrophil gelatinase-associated lipocalin (i.e., a biomarker of kidney tubule damage), irrespective of sex, adiposity measures, hepatic and cardiometabolic risk factors\cite{18}.

In a clinical and experimental study of 157 Italian postmenopausal women with T2DM, Mantovani et al. showed that homozygous carriers of the PNPLA3 rs738409 G allele had lower eGFR levels and a higher prevalence of CKD than those carrying the PNPLA3 rs738409 C allele\cite{19}. In a large cross-sectional study of 1,022 Chinese patients with chronic hepatitis virus C infection (22% of whom had coexisting CKD), Liu et al. reported that the PNPLA3 rs738409 G allele was significantly associated with an increased risk of prevalent CKD, after controlling for BMI, HOMA-estimated insulin resistance, hypertension, plasma lipids, C-reactive protein levels, and hepatic steatosis\cite{20}. Conversely, there is very little information about a possible association between the PNPLA3 rs738409 G allele and the presence of abnormal albuminuria\cite{21}.

To date, there are no clinical studies examining the risk of developing renal dysfunction in carriers of the PNPLA3 rs738409 G allele among individuals with metabolic dysfunction and normal or near-normal kidney function. Mantovani et al. examined the nexus linking the rs738409 G allele of the PNPLA3 gene and renal function in a cohort of 1,144 Italian adults with dysmetabolic features belonging to the Liver-Bible-2022 cohort (that enrolled volunteers with either fully preserved or nearly normal renal function at baseline who were submitted to extensive screening for hepatic and cardio-metabolic conditions)\cite{22}. In this cohort, the authors reported that the PNPLA3 rs738409 G allele was significantly associated with lower eGFR levels after controlling for sex, ethnicity, adiposity measures, cardiometabolic risk factors, serum liver enzymes, lipid-lowering medication use, albuminuria and a CKD polygenic risk score (PRS)\cite{23}. It should be noted that the statistical significance of this association remained unaltered even after controlling for Fibroscan®-assessed controlled attenuation parameter (CAP) or liver stiffness measurement (LSM)\cite{24}. Notably, in a subset of 144 individuals followed for a median period of 17 months, the authors also found that the PNPLA3 rs738409 G allele was independently associated with a faster decline in eGFR (delta eGFR: -3.57 mL/min/1.73 m² per allele, 95% confidence interval: -6.94 to -0.21; \( P = 0.037 \))\cite{25}.
Table 1. Principal observational studies examining the association between the PNPLA3 rs738409 G allele (or other NAFLD-associated genetic risk variants) and the risk of kidney dysfunction

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Study characteristics</th>
<th>NAFLD diagnosis</th>
<th>Genetic polymorphism</th>
<th>eGFR equation</th>
<th>Covariate adjustments</th>
<th>Main findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oniki et al. [6] 2015</td>
<td>Cross-sectional analysis: 740 Japanese and retrospective longitudinal study of 393 Japanese (follow-up 5.5 years)</td>
<td>Ultrasound</td>
<td>PNPLA3 rs738409 G allele</td>
<td>MDRD equation</td>
<td>Age, sex, BMI, T2DM, hypertension, dyslipidemia, and hepatic steatosis</td>
<td>PNPLA3 rs738409 G allele was linked with lower eGFR levels</td>
</tr>
<tr>
<td>Musso et al. [6] 2015</td>
<td>Cross-sectional analysis: 202 individuals who were free of obesity and T2DM</td>
<td>LB</td>
<td>PNPLA3 rs738409 G allele</td>
<td>CKD-epidemiology collaboration equation</td>
<td>Age, sex, BMI, and MetS</td>
<td>PNPLA3 rs738409 G allele was linked with higher risks of microalbuminuria and CKD</td>
</tr>
<tr>
<td>Mantovani et al. [7] 2019</td>
<td>Cross-sectional study: 101 Italian postmenopausal women with T2DM</td>
<td>FLI ≥ 60 (ultrasound in a subset of patients)</td>
<td>PNPLA3 rs738409 G allele</td>
<td>CKD-epidemiology collaboration equation</td>
<td>Age, T2DM duration, HbA1c, IR, systolic BP, hypertension treatment, and FLI ≥ 60</td>
<td>PNPLA3 rs738409 G allele was associated with lower eGFR and a higher prevalence of CKD</td>
</tr>
<tr>
<td>Targher et al. [8] 2019</td>
<td>Cross-sectional analysis: 142 NAFLD cases in Italian adolescents/children</td>
<td>LB</td>
<td>PNPLA3 rs738409 G allele</td>
<td>Bedside schwartz equation</td>
<td>Age, sex, systolic BP, adiposity, IR, NASH, and stage of hepatic fibrosis</td>
<td>PNPLA3 rs738409 G allele was linked with reduced renal function and proteinuria</td>
</tr>
<tr>
<td>Martuzillo et al. [9] 2019</td>
<td>Cross-sectional study: 591 Italian obese children</td>
<td>Ultrasound</td>
<td>PNPLA3 rs738409 G allele</td>
<td>Bedside schwartz equation</td>
<td>Sex, duration of obesity, ALT, IR, and lipids</td>
<td>PNPLA3 rs738409 G allele was linked with lower eGFR levels</td>
</tr>
<tr>
<td>Di Costanzo et al. [10] 2019</td>
<td>Cross-sectional study: 230 Italian overweight or obese children</td>
<td>MRI</td>
<td>PNPLA3 rs738409 G allele</td>
<td>Bedside schwartz equation</td>
<td>Age, sex, pubertal status, WC, diastolic BP, and liver steatosis</td>
<td>No significant difference was observed among PNPLA3 rs738409 alleles</td>
</tr>
<tr>
<td>Sun et al. [11] 2020</td>
<td>Cross-sectional study: 227 Chinese patients with NAFLD</td>
<td>LB</td>
<td>PNPLA3 rs738409 G allele</td>
<td>CKD-epidemiology collaboration equation</td>
<td>Age, sex, BMI, WC, hyperuricemia, IR, hypertension, T2DM, and hepatic fibrosis assessed histologically</td>
<td>PNPLA3 rs738409 G allele was linked with an increased risk of glomerular and tubular injuries</td>
</tr>
<tr>
<td>Mantovani et al. [12] 2020</td>
<td>Cross-sectional analysis: 157 Italian postmenopausal women with T2D</td>
<td>Ultrasound and VCTE</td>
<td>PNPLA3 rs738409 G allele</td>
<td>CKD-epidemiology collaboration equation</td>
<td>Diabetes duration, HbA1c, hypertension, presence of significant fibrosis (on VCTE), and abnormal albuminuria</td>
<td>PNPLA3 rs738409 G allele was linked with lower eGFR levels and a higher prevalence of CKD</td>
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<tr>
<td>Koo et al. [13] 2020</td>
<td>Cross-sectional study: 396 South Korean individuals with biopsy-proven NAFLD from the Boramae NAFLD study</td>
<td>LB</td>
<td>PNPLA3 rs738409 G allele; TM6SF2 rs58542926 T allele; MBOAT7 rs641738 T allele</td>
<td>CKD-epidemiology collaboration equation</td>
<td>Age, sex, BMI, and MetS</td>
<td>MBOAT7 rs641738 T allele was linked with a higher prevalence of CKD. Conversely, CKD was not linked with PNPLA3 rs738409 G or TM6SF2 rs58542926 T alleles</td>
</tr>
<tr>
<td>Baratta et al. [14] 2022</td>
<td>Cross-sectional analysis: 538 individuals with NAFLD (in whom data regarding kidney function were available) were recruited on an outpatient basis</td>
<td>Ultrasound</td>
<td>PNPLA3 rs738409 G allele; MBOAT7 rs641738 T allele; TM6SF2 rs58542926 T allele; GCKR rs780094 T allele</td>
<td>CKD-epidemiology collaboration equation</td>
<td>BMI, MetS, and liver fibrosis (as assessed by FIB-4 index)</td>
<td>Deterioration of renal function was not associated with any of NAFLD-related polymorphisms</td>
</tr>
</tbody>
</table>
| Mantovani et al. [15] 2023 | Panel data analysis: 46 postmenopausal T2DM women with preserved kidney function at baseline | Ultrasound and VCTE | PNPLA3 rs738409 G allele | CKD-epidemiology | Annual changes in age, HbA1c, hypertension, albuminuria, and proteinuria | PNPLA3 rs738409 G allele was linked with a faster eGFR decline during a 5-
The study by Mantovani et al. is the first large cohort study reporting a nexus linking the PNPLA3 rs738409 G allele with reduced renal function, irrespective of ethnicity, and the PRS-CKD\(^{[13]}\), which includes an evaluation of nearly 41,000 genetic predictors of CKD risk\(^{[18]}\). Collectively, therefore, the results of this study show a detrimental impact of the PNPLA3 p.I148M variant on eGFR levels in middle-aged individuals with metabolic dysfunction and preserved kidney function, suggesting that the identification of the PNPLA3 genotype might help to triage subjects who are at greater odds of progressive NAFLD forms and, at the same time, individuals who are at higher risk of CKD. This issue has also been recently recognized in a Delphi-based consensus statement\(^{[19]}\). In addition, as insulin resistance (more than other metabolic traits) appears to exacerbate the PNPLA3-rs738409-G genetic risk for NAFLD\(^{[20]}\), it is reasonable to hypothesize that improving insulin resistance might offer additional clinical benefits in this patient population.

What is the net effect of PNPLA3 rs738409 polymorphism on kidney function? The study by Mantovani et al. tried to answer this research question, showing that compared to the wild-type genotype, heterozygous and homozygous carriages of the PNPLA3 rs738409 G allele had a reduction of -1.24 and -2.48 mL/min/1.73 m\(^2\) in eGFR levels, respectively\(^{[13]}\). In addition, in this specific cohort of Italian adults with metabolic dysfunction, the PNPLA3 rs738409 G allele explained approximately 8% of the spectrum of eGFR variability dysfunction\(^{[19]}\). However, the presumed mechanisms underlying the nexus linking the PNPLA3 rs738409 G allele and kidney dysfunction are not fully understood. In a previous experimental study, the same group of investigators reported that the concentrations of mRNA of the PNPLA3 were expressed at the maximal levels both intrahepatically and intrarenally and that renal podocytes had the highest expression of mRNA and protein of the PNPLA3 gene compared to other renal cells\(^{[11]}\). Other experimental findings suggest that podocytes of renal glomeruli can store fatty substrates, such as retinol esters and lipid droplets, thereby promoting fatty kidney disease\(^{[21-24]}\). However, although these experimental findings are fascinating, they are preliminary data. As recently discussed by Pirola et al., some important research questions remain open: (i) Could different

\[\text{LT: alanine transferase; BMI: body mass index; BP: blood pressure; CAP: controlled attenuation parameter; CKD: chronic kidney disease; eGFR: estimated glomerular filtration rate; FIB-4: Fibrosis-4; FLI: fatty liver index; GCKR: glucokinase regulatory protein; HOMA-IR: homeostasis model assessment-estimated insulin resistance; IR: insulin resistance; LB: liver biopsy; MBOAT7: membrane-bound O-acyltransferase domain containing 7; MetS: metabolic syndrome; MRI: magnetic resonance imaging; NAFLD: nonalcoholic fatty liver disease; NASH: nonalcoholic steatohepatitis; PNPLA3: patatin-like phospholipase domain-containing 3; PRS-CKD: polygenic risk score of chronic kidney disease; T2DM: type 2 diabetes; VCTE: vibration-controlled transient elastography; TM6SF2: trans-membrane 6 superfamily member 2; WC: waist circumference.}\]
PNPLA3 gene expression patterns in the kidney explain the association between the PNPLA3 rs738409 G allele and kidney dysfunction\(^{[24]}\). (ii) Does current experimental data support a direct adverse effect of the PNPLA3 rs738409 G allele on kidney function? (iii) Does the adverse effect of the PNPLA3 rs738409 G allele on kidney function occur, at least partially owing to this genetic polymorphism affecting the liver? Growing evidence indicates a significant link between NAFLD and the risk of both prevalent and incident CKD, irrespective of common metabolic risk factors, such as diabesity\(^{[22,25-28]}\). Hence, additional pathogenic investigation is required to better understand the long-term effect(s) of the PNPLA3 rs738409 G allele on the risk of kidney dysfunction.

The results of the cohort study by Mantovani et al. should be interpreted with caution, considering the possible inherent limitations of the study\(^{[13]}\). First, while the cross-sectional analysis was performed on the entire cohort of individuals (\(n = 1,144\)), prospective assessment was restricted only to a subset of individuals. Moreover, renal function was not assessed with direct measurements but with a validated creatinine-based equation estimating eGFR, as usually done in routine clinical practice. Finally, the Liver-Bible-2022 cohort enrolled Italian individuals without pre-existing T2DM and with normal or near-normal kidney function. As a result, the findings of this study could not apply to the general adult population, other ethnic groups, or other selected patient populations.

In conclusion, the findings of the recent observational study by Mantovani et al. support a detrimental effect of the PNPLA3 p.I148M variant on eGFR levels in a large cohort of Caucasian middle-aged individuals with metabolic dysfunction\(^{[13]}\). This association was independent of established renal risk factors, presence/severity of NAFLD (as assessed by hepatic transient elastography), ethnicity, and genetic predisposition to CKD. Given the possible translational importance for personalized medicine approaches of the relationship linking the polymorphism p.I148M of the PNPLA3 gene to a faster decline of renal function, additional studies are required to exhaustively clarify the effect of this genetic polymorphism on the risk of CKD. Future research is also needed to examine whether PNPLA3 p.I148M silencing might protect against kidney damage progression in carriers.

**DECLARATIONS**

**Author’s contribution**

Made substantial contributions to the conception and design of the study and performed data analysis and interpretation: Mantovani A, Targher G.

Performed data acquisition, as well as providing administrative, technical, and material support: Mantovani A, Targher G.

**Availability of data and materials**

Not applicable.

**Financial support and sponsorship**

None.

**Conflicts of interest**

All authors declared that there are no conflicts of interest.

**Ethical approval and consent to participate**

Not applicable.
Consent for publication
Not applicable.

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