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NAFLD as a driver of chronic kidney disease

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1. Summary (unstructured abstract)

Nonalcoholic fatty liver disease (NAFLD) and chronic kidney disease (CKD) are two worldwide public health problems, affecting up to 25-30% (NAFLD), and up to 10-15% (CKD) of the general population. Recently, it has also become established that there is a strong association between NAFLD and CKD, regardless of the presence of potential confounding diseases such as obesity, hypertension and type 2 diabetes (T2DM). Since NAFLD and CKD are two common diseases and both conditions often occur with other metabolic conditions, such as T2DM or metabolic syndrome (MetS), elucidating the relative impact of NAFLD on risk of incident CKD presents a substantial challenge to investigators working in this research field. A growing body of epidemiological evidence to date suggests that NAFLD is an independent risk factor for CKD and recent evidence also suggests that associated factors such as MetS, dysbiosis, unhealthy diets, platelet activation and processes associated with ageing could also contribute mechanisms linking NAFLD and CKD. This narrative review provides an overview of the literature on: a) the evidence for an association and causal link between NAFLD and CKD and b) the underlying mechanisms by which NAFLD (and factors strongly linked with NAFLD) may increase risk of developing CKD.

2. Introduction

Readers of the *Journal* will be very familiar with nonalcoholic fatty liver disease (NAFLD) and how to define it, but will perhaps be less familiar with chronic kidney disease (CKD) and how to define this condition.

Both diseases are progressive chronic conditions that represent a spectrum of diseases extending from relatively mild disease, with only modest changes in function, to severe debilitating disease with end-stage organ damage, necessitating either chronic dialysis or organ transplantation in order to sustain life. NAFLD encompasses a histopathological spectrum of metabolic liver conditions encapsulating simple steatosis alone (nonalcoholic fatty liver, i.e. NAFL); steatosis, inflammation and ballooning of hepatocytes, with or without liver fibrosis (nonalcoholic steatohepatitis, i.e. NASH), and cirrhosis[1, 2]. When advanced fibrosis or cirrhosis occurs the risk of hepatocellular carcinoma may also increase markedly. Chronic kidney disease (CKD) on the other hand, is a complex, progressive chronic condition that is defined by either abnormalities of kidney structure or function present for ≥3 months, with serious implications for health[3, 4]. Either markers of kidney damage or decreased glomerular filtration rate may be present.

The National Kidney Foundation has identified five stages of CKD from 1 to 5 (as also shown in **Figure 1**) [5]. In the presence of an urinary albumin-to-creatinine ratio (ACR) that is normal or very mildly increased (i.e., urinary ACR <30 mg/g) and an estimated glomerular filtration rate (eGFR) above 60 ml/min/1.73 m^2, risk of progression to end-stage renal disease is very low and such patients usually do not undergo regular surveillance. Clinicians therefore should identify CKD stage 3 or above, because these stages of CKD are associated with a high or very high risk of disease progression. For defining CKD stage ≥3, markers of kidney damage can include the presence of one of: abnormal albuminuria (ACR ≥30 mg/g) or overt proteinuria, urine sediment abnormalities and other abnormalities due to tubular disorders, abnormalities detected by kidney histology, structural abnormalities detected by imaging, or a history of renal transplantation. For decreased eGFR, CKD is defined by an eGFR value <60 ml/min/1.73 m^2 [3, 4].

NAFLD and CKD are two public health problems with an increasing prevalence and incidence, poor outcomes, and high costs. Indeed, NAFLD affects up to ~25-30% [1, 6] and

CKD affects up to ~10-15% of the general adult population in many parts of the world [7, 8]. It is well established that CKD is also a major risk factor for cardiovascular disease (CVD) and all stages of CKD are associated with an increased risk of cardiovascular morbidity, premature mortality and decreased quality of life[9]. Recently, it has also been shown that NAFLD is an independent risk factor for CVD, regardless of the coexistence of cardiometabolic risk factors, such as obesity, hypertension, type 2 diabetes mellitus (T2DM) or metabolic syndrome (MetS) [10, 11]. Therefore, since NAFLD and CKD often occur with features of the MetS that adversely affect the kidney, elucidating the relative impact of NAFLD on risk of incident CKD presents a substantial challenge to investigators working in this field of research.

The main aims of this narrative review are to discuss: a) the evidence for an association and causal link between NAFLD and CKD and b) the putative mechanisms by which NAFLD (and factors strongly linked with NAFLD) may increase risk of developing CKD.

3. Evidence of an association between NAFLD and CKD

Since patients with NAFLD exhibit multiple traditional and non-traditional risk factors for CKD (as summarized in **Figure 1)** [12-18], it is not surprising that these patients also have a higher prevalence and incidence of CKD compared with subjects who do not have steatosis.

3.1 Cross-sectional studies

Several hospital-based and community-based studies have documented that NAFLD, as assessed by imaging techniques or liver biopsy, is significantly associated with an increased prevalence of CKD (defined as eGFR <60 ml/min/1.73 m^2, abnormal albuminuria or overt proteinuria) [19-30]. As reviewed extensively elsewhere [31], in these studies the prevalence of CKD ranged from approximately 20% to 55% among patients with NAFLD compared to 5%-30% among their counterparts without NAFLD. Notably, in most of these studies the significant association between NAFLD and increased prevalence of CKD persisted, both in patients with T2DM and in those without diabetes, even after adjustment for common risk factors for CKD. Some smaller case-control studies using liver biopsy to diagnose NAFLD have also shown a significant, graded

association between the histologic severity of NAFLD (mainly the hepatic fibrosis stage) and the presence of either decreased eGFR or abnormal albuminuria [27-30]. For example, in a previous case-control study [27], we found that patients with biopsy-confirmed NASH had a higher prevalence of both CKD and abnormal albuminuria than age-, sex- and body mass index-matched control subjects, and that the histologic stage of liver fibrosis was associated with decreasing eGFR values, independently of age, sex, adiposity measures, hypertension, plasma triglyceride concentrations and HOMA-estimated insulin resistance (**Figure 2**).

3.2 Cohort studies

Although the current evidence from the cross-sectional studies for the existence of an association between NAFLD and increased prevalence of CKD is robust and consistent across different ethnicities and patient populations, whether NAFLD is also a "*driving force*" for the development and progression of CKD remains uncertain [32, 33]. However, to date, an ever-increasing number of retrospective and prospective cohort studies, with a reasonably long duration of follow-up, have consistently documented that NAFLD (diagnosed either by abnormal levels of serum liver enzymes or by imaging techniques) is significantly associated with an increased incidence of CKD [34-46] (**Table 1**). Notably, in most of these studies the significant association between NAFLD and increased incidence of CKD persisted even after adjustment for age, sex, obesity, hypertension, T2DM and other potential confounding factors. For example, in the Valpolicella Heart Diabetes Study, including 1,760 outpatients with T2DM who had preserved kidney function at baseline, the presence of NAFLD on ultrasonography was associated with a nearly 50%-increased risk of incident CKD (adjusted-hazard ratio 1.49; 95% CI 1.1-2.2) over a follow-up period of 6.5 years, independent of age, sex, adiposity measures, blood pressure, smoking, duration of diabetes, haemoglobin A1c, plasma lipids, baseline eGFR, microalbuminuria, and the use of hypoglycaemic, lipid-lowering, antihypertensive or antiplatelet drugs [36]. Similar results have been also found in adults with type 1 diabetes and in other large community-based cohort studies of different ethnicities (**Table 1**).

3.3 Systematic reviews and meta-analyses

In a meta-analysis of 33 observational (20 cross-sectional and 13 longitudinal) studies published in 2014, Musso *et al.* examined the association between NAFLD and risk of prevalent and incident CKD (defined as eGFR <60 ml/min/1.73 m^2, abnormal albuminuria

or both)[47]. Meta-analysis of the data from 20 cross-sectional studies (involving nearly 30,000 individuals) showed that NAFLD was associated with a two-fold increased prevalence of CKD (random-effects odds ratio [OR] 2.12, 95%CI 1.69-2.66). More interestingly, meta-analysis of data from the 13 longitudinal studies (involving a total of nearly 28,500 individuals) showed that NAFLD was associated with a nearly 80% increased risk of incident CKD (random-effects hazard ratio [HR] 1.79, 95%CI 1.65-1.95). Similarly, in a subgroup analysis of individual patient data from five small studies (involving a total of ~430 adults with biopsy-confirmed NAFLD with only 86 incident CKD cases), the authors also suggested that the presence of advanced hepatic fibrosis was associated with a higher prevalence (random-effects OR 5.20, 95%CI 3.14-8.61) and incidence (random-effects HR 3.29, 95%CI 2.30-4.71) of CKD than either non-advanced fibrosis or simple steatosis, respectively[47]. In all of the aforementioned analyses, the presence and severity of NAFLD were associated with a higher prevalence and incidence of CKD even after adjustment for pre-existing T2DM and other common risk factors for CKD, such as age, ethnicity, body mass index and smoking history[47].

Recently, we have also performed a comprehensive systematic review and meta-analysis that involved a total of nine observational cohort studies (published up to August 2017) with aggregate data on ~96,500 middle-aged individuals (34.1% with NAFLD) of predominantly Asian descent and ~5,000 new cases of incident CKD (stage ≥3, defined as occurrence of eGFR <60ml/min/1.73 m^2, with or without accompanying proteinuria) over a median follow-up period of 5.2 years[48]. No studies with biopsy-proven NAFLD were available for the analysis. As shown in **Figure 3**, this updated meta-analysis confirmed that NAFLD (detected by serum liver enzymes, fatty liver index or ultrasonography) was associated with a nearly 40% increase in the long-term risk of incident CKD (random-effects HR 1.37, 95%CI 1.20-1.53; I^2=33.5%), a risk that appeared to increase further with greater severity of NAFLD as assessed by the NAFLD fibrosis score or other non-invasive markers of advanced fibrosis (*n*=2 studies; random-effects HR 1.50, 95%CI 1.25-1.74; I^2=0%), and remained significant in those studies where analysis was adjusted for common risk factors and potential confounders (i.e., age, sex, body mass index, hypertension, smoking, diabetes, baseline eGFR and use of certain medications). In addition, as also shown in **Figure 3**, when the analysis was stratified by the type of study population, the association between NAFLD and risk of incident CKD was essentially consistent for both patients with diabetes, and those without diabetes at

baseline[48]. In the few studies involving patients with T2DM the association between NAFLD and risk of CKD remained significant even after adjusting for duration of diabetes, glycemic control, hypertension and other established risk factors for CKD[47]. In addition, when the analysis was stratified either by study country (Asian vs. European countries), the association between NAFLD and the risk of incident CKD appeared to be stronger in studies performed in Asian populations (*n*=5 studies; random-effects HR 1.40, 95%CI 1.22-1.58; I^2=36.5%) than in European populations (*n*=3 studies; random-effects HR 1.29, 95%CI 0.82-1.76; I^2=33.5%).

Taken together, the findings of these two meta-analyses[47, 48] clearly support the assertion that NAFLD identifies a group of individuals, who are at increased risk of CKD, and who need more careful surveillance and treatment to reduce their risk of developing CKD. The results of these two meta-analyses also suggest that it is advanced NAFLD that carries a greater risk of incident CKD. This finding is in line with the results of a comprehensive meta-analysis supporting a strong link between the severity of NAFLD and increased risk of fatal and nonfatal cardiovascular outcomes [10]. However, this question remains still largely unsolved, and further prospective studies in larger cohorts of both Asian and non-Asian patients with biopsy-confirmed NAFLD are needed, in order to definitely prove whether the severity of NAFLD adversely affects risk of developing CKD. That said, we believe that the evidence from these two meta-analyses and other more recent follow-up studies published in 2018 and 2019 (as listed in **Table 1**) calls for a more active and systematic search for CKD in patients with NAFLD with a view to implementing an earlier and more aggressive treatment whenever indicated, and also suggests that there is a need now to include renal outcomes (such as temporal changes in eGFR and albuminuria or CKD development) in future randomised controlled trials focussed on testing the efficacy and safety of novel treatments for NAFLD or NASH.

In line with these observations, Vilar-Gomez *et al*. found that the histologic resolution of NASH and improvement in liver fibrosis stage were independently associated with an increase in eGFR values in a post-hoc analysis of a published clinical trial that included 261 patients with biopsy-confirmed NASH, who were treated with lifestyle modifications during a period of 52 weeks [49]. Recently, Onnerhag *et al*. examined the risk of overall mortality in patients with biopsy-proven NAFLD with the aim of investigating whether any increase in all-cause mortality was due to the presence of CKD [50]. The authors

measured eGFR values both at baseline and at the end of follow up in a cohort of 120 middle-aged Sweden patients with biopsy-proven NAFLD, who were followed for a mean period of 19.5 years. The authors found that although NAFLD patients with CKD had significantly higher crude overall mortality rate than NAFLD patients without CKD, the increased mortality risk was more strongly explained by an increased prevalence of metabolic comorbidities (including T2DM) rather than CKD.

3.4 Study limitations

It should be noted that the observational design of the available studies (**Table 1**) does not allow establishing a causal association between NAFLD and risk of CKD stage ≥3, and that it remains currently uncertain whether NASH or NAFLD with advanced fibrosis carry a higher risk of incident CKD than simple steatosis. Most of the available studies used ultrasonography, which is the recommended first-line imaging method for detecting NAFLD in clinical practice. No studies used liver biopsy that is considered the 'gold standard' for diagnosing and staging NAFLD. Moreover, as shown in **Table 1**, the published cohort studies employed varying degrees of baseline adjustments for risk factors of CKD. In particular, almost all studies adjusted their results for BMI, but only a few of these studies additionally adjusted their results for body fat distribution, which plays a key role in the pathogenesis of NAFLD and CKD. An accurate assessment of abdominal visceral fat accumulation would be particularly important to better understand whether the association between CKD and NAFLD is affected by this metabolic risk factor. Other limitations include the use of the Modification of Diet in Renal Disease or the CKD-Epidemiology Collaboration study equations to calculate eGFR, neither of which are reliable in the presence of severe obesity or cirrhosis[51]. Furthermore, most of the available cohort studies have been conducted in Asian countries, where large populations undergo regular health check-up programs, including liver ultrasonography. Since Asian and non-Asian populations have different genetic/cultural backgrounds, dietary factors and adipose tissue distributions, we believe that additional studies should be conducted in non-Asian populations. Another potential limitation is that no large prospective studies are available that have examined the rates of CKD progression to kidney failure (stage 5 CKD) neither in cohorts of NAFLD patients nor in cohorts of advanced CKD patients. Finally, none of these studies have used renal biopsy to examine the specific renal pathology associated with NAFLD. So, it is currently uncertain if NAFLD is associated

with a specific type of kidney disease, although we suggest that it is reasonable to assume that NAFLD may promote kidney injury, mostly through accelerated atherothrombosis.

Nevertheless, it is important to consider that in a recent analysis of the Third National Health and Nutrition Survey database that included a total of ~11,700 United States individuals, Paik *et al*. showed that amongst patients with ultrasound-detected NAFLD, the presence of moderate to advanced stages of CKD were independently associated with increased all-cause mortality over a mean follow-up period of 19 years [52]. These findings point out that identification of CKD in patients with NAFLD has important prognostic implications. As also suggested by the authors, these data should inform clinicians and policy makers to identify who are at the highest risk for adverse outcomes for appropriate management strategies.

4. Putative mechanisms linking NAFLD with CKD

a) T2DM and metabolic syndrome

Although not conclusive, as discussed above the current epidemiological evidence suggests that NAFLD is an independent risk factor for CKD and the presence of NAFLD and associated features of the MetS[53] may be causally involved, at least in part, in the development and progression of CKD.

More than a third of patients with NAFLD have impaired renal function and impaired renal function in patients with NAFLD is also associated with the severity of liver disease and presence of T2DM [54, 55]. When two common diseases co-exist and share common risk factors, it can be difficult to disentangle causal relationships and understand the role of potential confounders. Such confounding conditions linking NAFLD and CKD could be T2DM or MetS. In centrally obese subjects with T2DM, insulin resistance frequently occurs with other cardiometabolic risk factors that increase risk of both NAFLD and CKD. The clustering of cardiometabolic risk factors occurring with visceral obesity and insulin resistance, are encapsulated within the features of MetS, such as atherogenic dyslipidaemia, increased blood pressure or dysglycaemia[53].

As mentioned in the Introduction section, CKD stage ≥3 is defined by either abnormal albuminuria or decreased eGFR values. Whereas abnormal albuminuria (or overt

proteinuria) is strongly associated with microvascular damage in renal glomeruli in diabetes and is a classical microvascular complication of diabetes, decreased eGFR values are more strongly associated with macrovascular damage. Macrovascular disease is strongly associated with CVD risk factors occurring with MetS such as hypertension, atherogenic dyslipidaemia (and other risk factors not related to MetS, such as increased LDL-cholesterol concentrations). Thus, the clustering of cardiometabolic risk factors occurring with NAFLD and commonly referred to as the MetS, has the potential to cause both microvascular and macrovascular damage, giving rise to CKD.

CKD also commonly occurs with the MetS[56, 57]. Consequently, it can be very difficult to disentangle the differential effects of insulin resistance, visceral obesity and the linked macrovascular and microvascular risk factors on the kidneys, from the consequences of liver disease *per se*. That said, this may be a moot point, given the close inter-relationships of insulin resistance, visceral adiposity and other MetS features with liver disease in NAFLD. Given that all these MetS risk factors are closely inter-related in NAFLD, even in the presence of known genetic modifiers of NAFLD severity, such as the two common genetic variants in *patatin-like phospholipase domain-containing 3 (PNPLA3*) and *transmembrane 6 superfamily member 2 (TM6SF2*) [58, 59], it may be an arcane point to try and dissect out the relative contributions of one NAFLD-associated hepatic or cardiometabolic risk factor from another, on the kidneys. In fact, since these risk factors cluster tightly together and NAFLD may also promote the development of these cardiometabolic risk factors[60], we reason that it is perhaps more appropriate to refer to NAFLD as a metabolic liver disease with cardiovascular and metabolic risk factors; many of which have the potential for causing kidney dysfunction [61].

Whether liver fibrosis in NAFLD is associated with an even greater risk of extra-hepatic complications than simple steatosis or NASH is uncertain. Since NASH can only be satisfactorily diagnosed by examination of liver histology, and there are far too few cohort studies that have utilized vibration-controlled transient elastography, or validated non-invasive liver fibrosis biomarkers to diagnose liver fibrosis[62, 63], the answer to this question remains unresolved. We have previously attempted to address the question of whether liver fibrosis with NAFLD further increases risk of either incident CVD or incident T2DM and although these data suggest that liver fibrosis is associated with a further increase in the risk of incident CVD events [10] compared to steatosis alone; liver fibrosis

did not seem to further increase the risk of incident T2DM, when compared to the risk increase associated with liver steatosis alone [64].

As mentioned above, visceral obesity is a classical feature of the MetS. Many potential mechanisms by which accumulation of visceral fat causes chronic inflammation are well described[65-68] and a detailed discussion of these is beyond the scope of this review. However, briefly, with increases in visceral obesity and ectopic fat accumulation, there is an increase in plasma concentrations of non-esterified fatty acids (NEFA) and a failure to adequately suppress NEFA concentrations during hyperinsulinaemia [69-72]. With the increase in NEFA supply to the liver, a link has been proposed between NEFA overflow from the expanded and dysfunctional visceral adipose tissue and activation of hepatic macrophages that is independent of body mass index [73]. With activation of hepatic macrophages and hepatic inflammation, there is an increase in proinflammatory cytokines[74] and hepatic/systemic insulin resistance[75, 76], increased activity of the renin-angiotensin-aldosterone system [77] and oxidative stress mediated by proinflammatory and profibrotic mediators[78]. The liver-kidney crosstalk in NAFLD also includes the role of the energy sensor 5'-AMP activated protein kinase (AMPK) and its regulation of fetuin-A and adiponectin. In liver and kidney, AMPK is pivotal to directing hepatocytes and renal podocytes to compensatory and potentially deleterious pathways, leading to inflammatory and profibrotic cascades culminating in end-organ damage[79]. Collectively, NAFLD and CKD share common proinflammatory and profibrotic mechanisms of disease progression [80, 81] Experimental evidence also supports a role of inflammosome and innate immune system in CKD [32, 82]. Therefore, all of these factors and pathways could mediate a link between NAFLD and CKD and potentially explain a causal link by which NAFLD increases risk of incident CKD.

Not only are many traditional risk factors shared between NAFLD, CKD and T2DM/MetS, but it has now become clear that newer and emerging risk factors are also frequently present with each of these conditions. These newer risk factors include perturbation of the intestinal microbiota (dysbiosis) with associated inflammation and intestinal dysfunction and platelet activation and the role of these newer risk factors in NAFLD and CKD will be discussed below.

b) Dysbiosis and perturbed intestinal function affecting NAFLD and CKD.

Emerging experimental evidence suggests a role of the intestinal microbiota in the pathogenesis of both CKD[32, 83] and NAFLD [84-87]. **Figure 4** shows possible mechanisms and factors potentially linking intestinal dysbiosis, visceral adipose tissue dysfunction, NAFLD and CKD. With perturbation of the gut microbiota (dysbiosis), there is an increase in Gram –ve organisms, lipopolysaccaride, gut permeability, secondary bile acids and renal toxins that may increase risk of development and progression of both NAFLD[88, 89] and CKD[32, 84]. Intestinal microbiota-generated production of uraemic toxins (e.g., trimethylamine, cresol and indole) [83, 90-93] has the potential to further damage renal, hepatic and cardiovascular function through inflammatory, oxidative and fibrotic pathways. The metabolism of the amino acids tyrosine and phenylalanine by a variety of obligate or facultative anaerobes, including the genera *Bacteroides*, *Lactobacillus*, *Enterobacter*, *Bifidobacterium*, and especially *Clostridium difficile,* results in the increased production of *para*-cresyl and the conjugate *para*-cresyl sulfate [94, 95]. *Escherichia coli* has been shown to metabolize tryptophan resulting in the production of indole that is metabolized in the liver to the uraemic toxin indoxyl sulfate (IS) [94, 95]. A variety of other potentially nephrotoxic metabolites are also produced, such as ammonia, thiols and phenols[83].

A complex interaction also exists between the gut microbiota and bile acid metabolism in NAFLD[89] to produce a diverse range of bile acids (BAs) that can be detected in the plasma and that have the potential to influence development and progression of NAFLD. Secondary BAs are generated from the 1-5% of primary BAs that are not re-absorbed in the jejunum. These BAs enter the ileum and colon and are modified by the gut microbiota hydrolases and dehydroxylases to create secondary BAs, such as deoxycholic acid and lithocholic acid. Further bacterial enzymes that include epimerases, oxidases and esterases, are capable of further modifying BAs before they are excreted in the stool. There is now evidence that the dynamic interaction existing between the microbiota and the BA pool can be modified by certain microbiota species to change the BA profile[96]. Specifically, NASH is associated with changes in the intestinal microbiota composition and metabolome, an intestinal and systemic inflammatory response, and BA profiles[97], and it has been also suggested that the composition of the gut microbiome associated with dysregulation of BA biosynthetic pathways may contribute to persistence of NAFLD[89]. Modification of the BA profile may be important in the treatment of NAFLD [98] but mechanistic studies are required to elucidate causal links between intestinal dysbiosis, NAFLD and CKD.

c) Dietary changes mediating a link between NAFLD, dysbiosis and CKD

Increased consumption of sugar-sweetened beverages is linked with the development of NAFLD, hypertension, MetS and T2DM in both laboratory animals and humans[99, 100] although the association may be confounded by excess calorie intake or by unhealthy lifestyles. Today the most commonly consumed sugar is high fructose corn syrup. However, a causal role of excessive fructose consumption in the development of these metabolic diseases remains still debated and the molecular mechanisms by which fructose elicits effects on dysregulated liver metabolism remain not completely understood [99, 100]. Increased dietary fructose intake is associated with NASH and increased dietary fructose intake is also associated with increased serum uric acid concentrations in children and adolescents [101]. Emerging experimental data suggest that increased dietary fructose might induce NAFLD, at least in part, due to the generation of uric acid during fructose metabolism that results in mitochondrial oxidative stress and impairment in ATP production[102-104]. Although it has been thought that most fructose in the body is derived from dietary fructose intake (principally sugar/corn syrup sweetened drinks), it has recently been shown that endogenous fructose can also be generated in the liver with activation of the polyol pathway. In this pathway, glucose is converted to sorbitol by aldose reductase and sorbitol is converted to fructose by sorbitol dehydrogenase [105]. Aldose reductase is a nicotinamide adenine dinucleotide phosphate (NADPH)-dependent aldo-keto reductase, best known as the rate-limiting enzyme of the polyol pathway. Recently, the effect of uric acid on aldose reductase expression and oxidative stress has been investigated[104]. In this experimental study, it was shown that uric acid dose-dependently stimulated aldose reductase expression, and this phenomenon was associated with increased endogenous fructose production and hepatic triglyceride accumulation. This stimulatory mechanism was mediated by uric acid–induced oxidative stress and stimulation of the transcription factor nuclear factor of activated T-cells 5 (NFAT5)[104]. Although aldose reductase transcripts and protein are detected at low levels in healthy livers, in contrast, levels of aldose reductase mRNA and protein are increased in diseased human livers[106]. Aldose reductase is expressed in both sinusoidal lining cells and Kupffer cells and also in the fibrous septa of cirrhotic livers[106]. Overall, these data suggest that in NASH a positive feedback loop may exist, whereby increased dietary fructose consumption may increase hepatic *de novo* lipogenesis and uric acid production, generating oxidative stress. The increase in uric acid concentrations leads to a further increase in endogenous fructose production by stimulating

aldose reductase in the polyol pathway [104], with the potential for not only uric acid-mediated kidney damage, but also fructose-mediated liver disease.

d) Platelet activation as a mediator of the link between NAFLD and CKD

The hepatic microenvironment plays a crucial role in liver disease development, as hepatic stellate cells, resident liver macrophages (Kupffer cells), endothelial cells, extracellular matrix and a variety of immune cells or platelets may interact in complex and intertwined signaling pathways[107]. As mentioned above dyslipidaemia is a key feature of MetS, NAFLD and CKD and the specific dyslipidaemia involves an increase in hepatic-derived triglyceride-rich lipoproteins. Oxidative stress is a key feature of NAFLD [108-110] and CKD [111-113]; and both oxidative stress and an increase in triglyceride-rich lipoproteins (such as very low density lipoprotein and remnant lipoproteins), are key regulators of platelet activation [114-117]. With oxidative stress and kidney dysfunction, there is a reduction in anti-oxidant protective factors produced by the kidneys, such as the Klotho protein[118], and generation of metabolites such as plasma F2-isoprostanes, 8-oxo-7,8-dihydro-2'-deoxyguanosine, malonyldialdehyde (MAD), advanced oxidation protein products (AOPPs), carbamylated proteins, asymmetric dimethylarginine (ADMA) and oxidized lipoprotein particles [119-122]. When platelets are activated, alpha granules and dense granules are released containing multiple proinflammatory cytokines, chemokines and growth factors. These include chemokine (CXC motif) ligand 4 (CXCL4), endothelial growth factor (EGF), interleukin-6 (IL-6), platelet-derived growth factor (PDGF), serotonin, insulin-like growth factor 1 (IGF-1), transforming growth factor (TGF)-beta, tumour necrosis factor (TNF)-alpha, vascular endothelial growth factor A (VEGF-A), hepatocyte growth factor (HGF) and fibroblast growth factor (FGF). The release of TGF-beta, PDGF, serotonin and CXCL4 is capable of causing progression of liver disease by activating stellate cells with a consequent increase in extracellular matrix production. With CKD, there is also increased platelet activation, and an attenuated response to dual antiplatelet therapy, compared to patients without CKD[123]. Dysbiosis may also act to promote increased platelet activation since indoxyl sulfate (IS) activates platelets [124]. Decreased urinary Klotho protein level has been identified as one of the earliest biomarkers of CKD progression [125], and the Klotho protein is also able to modulate the effect of IS on platelet hyperactivity and thrombus formation, protecting against IS-induced atherosclerosis in mice with CKD[124]. Thus, increased oxidative stress, intestinal dysbiosis, an increase in hepatic-derived triglyceride-rich lipoproteins, platelet activation, NAFLD and CKD are all closely inter-related with the

potential for a “vicious” spiral of worsening liver and kidney disease in NAFLD and CKD. Recently, Malehmir *et al*. showed that platelet number, platelet activation and platelet aggregation are increased in NASH but not in simple steatosis or insulin resistance. Antiplatelet therapy (aspirin/clopidogrel, ticagrelor) but not nonsteroidal anti-inflammatory drug treatment with sulindac also prevented NASH and subsequent development of hepatocellular carcinoma in a murine hIL4r-alpha-/GP1b-alpha transgenic mouse model of NASH [126]. In addition, intra-vital microscopy also showed that antiplatelet therapy reduced intra-hepatic platelet accumulation and the frequency of platelet-immune cell interaction, thereby limiting hepatic immune cell trafficking. Taken together, these experimental results suggest that blocking platelet activation might ameliorate NASH and subsequently decrease the risk of developing hepatocellular carcinoma[127].

e) Premature ageing and age-related changes

Older age is a risk factor for NAFLD[1], CKD[128] and T2DM[129]. Decreased urinary Klotho protein occurs with ageing, and with decreased Klotho protein excretion, there is an associated vascular phenotype of medial calcification, intima hyperplasia, endothelial dysfunction, arterial stiffening, hypertension and impaired angiogenesis [130]. Decreased urinary Klotho protein has been also identified as one of the earliest biomarkers of CKD progression and the Klotho gene was identified first as a putative aging-suppressor gene that extended life span when overexpressed, and accelerated aging-like phenotypes when disrupted in mice[125]. As mentioned above, the Klotho protein modulates the effect of IS on platelet hyperactivity [124], and thus there is the potential for low levels of Klotho protein to mediate the increase in platelet reactivity that occurs with ageing[131]. Older patients with NAFLD are at a higher risk of CKD as a function of their increased age, but with advancing age, obesity and increased serum uric acid concentrations there is also increased risk of developing CKD[132]. Age-related changes in the liver may also occur with alterations in hepatic sinusoidal endothelial cells [133], increases in the hepatokine fetuin-A and decreases in adiponectin, potentially linking MetS, NAFLD and CKD[79].

In summary, these new mechanistic data suggest plausible mechanisms and new pathways linked to MetS, intestinal dysbiosis, excessive fructose consumption, platelet activation and ageing that might, at least in part, mediate links between NAFLD and risk of CKD. However, more research is needed to better understand if experimental models of NAFLD/NASH, initiated by primary changes in lipid storage in the liver, ultimately lead to CKD.

5. EFFECT OF *PNPLA3* POLYMORPHISM ON RENAL FUNCTION

Several susceptibility gene variants predisposing to NAFLD have been consistently identified in different populations[58, 59]. Among the genetic factors that may influence the onset and progression of NAFLD, the minor allele G of rs738409, i.e., a non-synonymous single nucleotide polymorphism in the *PNPLA3* gene encoding an Ile148Met change, has been recognized to be the major common genetic variant associated with a greater predisposition to NASH and progressive liver fibrosis in both paediatric and adult populations[58, 59].

Emerging evidence is now suggesting that the G allele of rs738409 is significantly associated with decreased eGFR values, irrespective of established renal risk factors and presence of NAFLD, across different ethnicities and patient populations. Indeed, as summarized in **Table 2**, there are now half a dozen studies that have examined whether, and to what extent, *PNPLA3* rs738409 polymorphism is associated with decreasing kidney function in both adults and children or adolescents [134-140]. For instance, our group recently showed for the first time that the presence of the risk allele (G) of rs738409 was strongly associated with both decreasing eGFR and increasing 24-hour urinary protein excretion in a sample of 142 Italian overweight children/adolescents with biopsy-proven NAFLD [138]. Notably, these associations were independent of sex, age, measures of adiposity, blood pressure, HOMA-estimated insulin resistance and also the histologic severity of NAFLD (i.e., NASH and liver fibrosis stage) [138]. Similarly, Sun *et al*. reported that *PNPLA3* GG genotype was significantly associated with a higher risk of prevalent CKD, abnormal albuminuria or increased levels of urinary neutrophil gelatinase-associated lipocalin (i.e., a reliable marker of renal tubular injury) in 217 Chinese adults with biopsy-confirmed NAFLD [137]. Also in this study, *PNPLA3* GG genotype remained significantly associated with renal glomerular and tubular injury after adjusting for age, sex, body mass index, waist circumference, hypertension, diabetes, HOMA-estimated insulin resistance, hyperuricemia, and histologic severity of NAFLD [137]. In another study involving 740 Japanese elderly individuals, Oniki *et al*. found that *PNPLA3* GG genotype was associated with lower levels of eGFR, independently of common renal risk factors and presence of ultrasound-detected NAFLD, especially in subjects with normal body weight [134]. Notably, in a subgroup of these subjects the authors also showed that the *PNPLA3* G/G genotype was associated with a significant decline in eGFR over a mean follow-up of 5.5 years [134].

To date, the putative mechanisms underlying the association between the G allele of rs738409 and decreasing kidney function are not entirely understood. The published studies [134-140] show that the association between *PNPLA3* G/G genotype and kidney dysfunction was largely independent of the shared renal/metabolic risk factors. It is reasonable to hypothesize that the G allele of rs738409, which is highly expressed in liver sinusoidal pericytes [141], might also exert direct adverse effects on the kidneys. These nephrotoxic effects may occur via activation of renal pericytes, as renal pericytes are stromal cells that play a key role in angiogenesis and in regulating renal medullary and cortical blood flow promoting renal fibrogenesis and glomerulosclerosis [142, 143]. However, further research is needed to better understand the role of the *PNPLA3* rs738409 polymorphism on the development of glomerular and interstitial fibrosis.

If confirmed in future larger studies, we believe that the results of these studies may have important clinical implications, because they support the notion that *PNPLA3* genotyping might be useful not only for identifying subjects with greater susceptibility to NAFLD development and progression, but also for those patients with NAFLD who may be at higher risk of CKD, thus promoting the implementation of specific prevention programs and treatment strategies for CKD among carriers of the *PNPLA3* rs738409 G/G genotype.

6. Conclusions

This review article outlines the existence of a strong association between the presence and severity of NAFLD and increased prevalence and incidence of CKD, independent of obesity, hypertension, T2DM and other common cardio-renal risk factors. The data also suggest that *PNPLA3* genotyping might be useful not only for identifying subjects with greater susceptibility to NAFLD development and progression, but also for identifying a subgroup of patients with NAFLD who are at higher risk of developing CKD. Despite the convincing evidence linking NAFLD with a higher risk of CKD, it remains to be definitely established whether a causal association also exists. Moreover, it should also be noted that none of the available studies have used renal biopsy to examine the specific renal morphology/pathology associated with NAFLD, and therefore it is uncertain if NAFLD is associated with a specific type of kidney disease, although it is reasonable to assume that NAFLD may promote kidney damage, mostly through accelerated atherothrombosis. We suggest that future prospective and interventional studies of well-characterized cohorts of

patients with biopsy-proven NAFLD are required to try and better elucidate whether it is the presence and severity of NAFLD, or whether it is the presence of co-existing risk factors that increases risk of incident CKD. In the meantime, however, given the close link between NAFLD and CKD, more careful surveillance of these patients will be needed.

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FIGURE LEGENDS

Figure 1. **Risk factors in patients with NAFLD and CKD.**
In the figure are also reported the stages of CKD. CKD is defined as either kidney damage or estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m^2 for at least three months or more. Kidney damage is defined as pathologic abnormalities or markers of damage, including abnormalities in blood or urine tests or in imaging methods.

Figure 2. **Renal function parameters in patients with NASH and control subjects**. Panel A shows the prevalence of both CKD (defined as eGFR <60 ml/min/1.73 m^2 and/or abnormal albuminuria) and abnormal albuminuria (i.e., urinary albumin-to-creatinine ratio ≥30 mg/g) in 80 patients with biopsy-proven NASH and 80 non-steatotic control individuals who were matched for age, sex and body mass index. Panel B shows the adjusted means (±standard deviations) of eGFR values in patients with NASH according to the histologic stage of hepatic fibrosis. Data have been adjusted for age, sex, measures of adiposity (body mass index and waist circumference), hypertension, plasma triglyceride levels, and HOMA-estimated insulin resistance. Data are from [27]. Reproduced with permission.

Figure 3. **Risk of CKD in patients with NAFLD.**
Forest plot and pooled estimates of the effect of the presence of NAFLD on the risk of incident CKD (stage ≥3) in observational cohort studies, stratified either by different non-invasive methodologies for the diagnosis of NAFLD (panel a), or by study population (diabetes *vs*. no-diabetes) (panel b). Panel c shows the forest plot and pooled estimates of the effect of the severity of NAFLD (defined by either high-intermediate NAFLD fibrosis score [i.e., NFS ≥-1.455] or increased serum gamma-glutamyltransferase levels [i.e., serum GGT levels ≥109 U/l] in patients with ultrasound-detected NAFLD) on the risk of incident CKD (stage ≥3). Data are from [48]. Reproduced with permission.

Figure 4. Potential mechanisms and factors linking intestinal dysbiosis, visceral adipose tissue dysfunction, NAFLD and CKD.
A calorie dense, fibre-poor diet is associated with changes in the intestinal microbiota (dysbiosis). With dysbiosis, production of incretins, fermentation of carbohydrate and metabolism of amino acids is affected leading to changes in concentrations of short chain fatty acids (SCFAs), lipopolysaccaride (LPS) and the production of potentially hepatotoxic molecules such as ethanol and nephrotoxic molecules [i.e., hippuric acid, phenylacetic acid, trimethylamine (TMA), cresol and indole]. Certain of these molecules (e.g. TMA) are oxidized, or conjugated (e.g., cresol and indole) within the liver, to potentially nephrotoxic compounds. Development and progression of liver disease occurs with visceral adipose tissue dysfunction. Hepatic *de novo* lipogenesis is stimulated by high levels of long-chain fatty acids and low levels of adiponectin from expanded and dysfunctional visceral adipose tissue, producing an excess of intrahepatic molecules, such as di-acyl glycerols (DAGs) and ceramides that promote hepatic insulin resistance, necro-inflammation (by Kupffer cell activation that enhances the production of multiple pro-inflammatory cytokines and hepatokines) with subsequent activation of hepatic stellate cells and increased production of collagen matrix and progression of liver disease. The inflammatory activation of hepatic stellate and Kupffer cells also results in the chemokine-mediated infiltration of neutrophils, monocytes, natural killer and natural killer T cells that are implicated in hepatic fibrogenesis.

Abbreviations: VLDL, very low-density lipoprotein; PAI-1, plasminogen activator inhibitor 1; TMAO, trimethylamine oxide.

Table 1. Principal observational cohort studies examining the association between NAFLD and risk of development or progression of CKD stage ≥3 (ordered by publication year).

Authors, Country, Year (Ref.)	Study design and population	Diagnosis of NAFLD	Diagnosis of CKD and number of incident CKD cases	Covariate adjustments	Main results
Ryu S et al., South Korea, 2007 [34]	Community-based cohort study: 10,337 nondiabetic and non-hypertensive male workers with normal kidney function and no overt proteinuria at baseline. Follow-up: 3 years	Serum liver enzymes (serum GGT concentrations)	eGFR <60 ml/min/1.73 m^2 and/or overt proteinuria (urinary dipstick ≥1); 366 patients developed incident CKD during follow-up	Age, BMI, alcohol intake, smoking, baseline eGFR, triglycerides, HDL-cholesterol, C-reactive protein, HOMA-IR, and incident cases of hypertension and diabetes	NAFLD (i.e., top quartile of serum GGT concentrations) was independently associated with increased risk of incident CKD (aHR 1.87; 95% CI 1.31-2.67)
Chang Y et al., South Korea, 2008 [35]	Community-based cohort study: 8,329 nondiabetic and non-hypertensive men with normal kidney function and no overt proteinuria at baseline. Follow-up: 3.2 years	Ultrasonography; the prevalence of NAFLD was 30.2%	eGFR <60 ml/min/1.73 m^2 and/or overt proteinuria (by urinary dipstick); 324 patients developed incident CKD during follow-up	Age, BMI, alcohol intake, hypertension, smoking, fasting glucose, baseline eGFR, triglycerides, HDL-cholesterol, LDL-cholesterol, HOMA-IR, C-reactive protein, incident cases of hypertension and diabetes	NAFLD was independently associated with increased risk of incident CKD (aHR 1.60; 95% CI 1.3-2.0)
Targher G et al., Italy, 2008 [36]	Prospective cohort study (Valpolicella Heart Diabetes Study): 1,760 type 2 diabetic outpatients with preserved kidney function and no overt proteinuria, free of cardiovascular disease and known chronic liver diseases at baseline. Follow-up: 6.5 years	Ultrasonography; prevalence of NAFLD was 73.2%	eGFR <60 ml/min/1.73 m^2 and/or overt proteinuria; 547 patients developed incident CKD during follow-up (428 developed decreased eGFR alone, 112 developed proteinuria, irrespective of eGFR, and 7 developed kidney failure; no patients developed nephrotic syndrome)	Age, sex, BMI, waist circumference, blood pressure, smoking, diabetes duration, hemoglobin A1c, plasma lipids, baseline eGFR, use of antihypertensive, lipid-lowering, antiplatelet or hypoglycemic agents	NAFLD was independently associated with increased risk of incident CKD (aHR 1.49; 95% CI 1.1-2.2)
Arase Y et al., Japan, 2011 [37]	Retrospective cohort study: 5,561 middle-aged individuals with NAFLD and normal kidney function without overt proteinuria at baseline. Follow-up: 5.5 years	Ultrasonography and serum liver enzymes (serum GGT concentrations). Prevalence of NAFLD was 100%	eGFR <60 ml/min/1.73 m^2 and/or overt proteinuria (urinary dipstick); 263 patients developed incident CKD during follow-up	Age, sex, hypertension, diabetes, total cholesterol, triglycerides, HDL-cholesterol, liver enzymes, hemoglobin, white blood cell count, platelet count, baseline eGFR	Among patients with NAFLD, elevated serum GGT concentrations were independently associated with an increased risk of incident CKD (aHR 1.35; 95% CI 1.02-1.8)
Targher G et al., Italy, 2014 [38]	Prospective cohort study: 261 type 1 diabetic adult outpatients with normal kidney function, free of cardiovascular disease and known chronic liver	Ultrasonography; prevalence of NAFLD was 50.2%	eGFR <60 ml/min/ 1.73 m^2 and/or overt proteinuria; 61 patients developed incident CKD during follow-up (28 developed decreased eGFR with abnormal albuminuria,	Age, sex, diabetes duration, hemoglobin A1c, hypertension, baseline eGFR, presence of microalbuminuria	NAFLD was independently associated with an increased risk of incident CKD (aHR 1.85; 95% CI 1.03-3.3). Measurement of NAFLD provided incremental risk

	diseases at baseline. Follow-up: 5.2 years		21 developed reduced eGFR alone, and 12 developed macroalbuminuria alone; no patients developed kidney failure; no patients developed nephrotic syndrome)		reclassification beyond that of conventional CKD risk factors
Huh JH et al., South Korea, 2017 [39]	Prospective cohort study: 4,761 adults with normal kidney function and no overt proteinuria and free of cardiovascular disease and known chronic liver diseases at baseline. Mean follow-up: 10 years	Fatty liver index (FLI); prevalence of NAFLD (defined as FLI ≥60) was 12.6%	eGFR <60 ml/min/1.73 m^2; 724 individuals developed incident CKD during follow-up	Age, sex, smoking, diabetes status, physical exercise, alcohol intake, protein intake, systolic blood pressure, total cholesterol, C-reactive protein, baseline eGFR	NAFLD (FLI ≥60) was independently associated with increased risk of incident CKD (aHR 1.46; 95% CI 1.19-1.79). FLI provided incremental risk reclassification beyond that of traditional renal risk factors
Shen ZW et al., China, 2017 [40]	Prospective cohort study: 21,818 adults with normal kidney function and no overt proteinuria at baseline, who received routine health examination. Follow-up: 5 years	Serum liver enzymes (serum GGT concentrations)	eGFR <60 ml/min/1.73 m^2 and/or overt proteinuria (urinary dipstick); 1,456 individuals developed incident CKD during follow-up	Age, sex, BMI, alcohol intake, serum creatinine, albumin, alanine aminotransferase, hemoglobin, white blood count, triglycerides, total cholesterol, hypertension, smoking, history of cardiovascular disease, history of diabetes	NAFLD (i.e., top quartile of serum GGT levels) was independently associated with an increased risk of incident CKD (aHR 1.33, 95% CI 1.07-1.64)
Kunutsor SK et al., Finland, 2017 [41]	Prospective cohort study (Kuopio Ischemic Heart Disease Study): 2,338 middle-aged men with normal kidney function at baseline. Median follow-up: 25.6 years	Serum liver enzymes (serum GGT concentrations)	eGFR <60 ml/min/1.73 m^2; 221 individuals developed incident CKD during follow-up	Age, BMI, systolic blood pressure, history of hypertension, smoking, history of coronary heart disease, diabetes, total cholesterol, HDL-cholesterol, alcohol intake, baseline eGFR	NAFLD (i.e., top quartile of serum GGT concentrations) was not independently associated with increased risk of incident CKD (aHR 0.97, 95% CI 0.64-1.47)
Sinn DH et al., South Korea, 2017 [42]	Retrospective cohort study: 41,430 adults with normal kidney function and no overt proteinuria at baseline, free from known chronic liver diseases. Follow-up: 4.2 years	Ultrasonography; advanced NAFLD fibrosis assessed by the NFS (≥-1.455), FIB4 score (≥1.45) or APRI index (≥0.5); prevalence of NAFLD was 34.3%	eGFR <60 ml/min/1.73 m^2; 691 participants developed incident CKD during follow-up	Age, sex, BMI, smoking, alcohol intake, systolic blood pressure, hemoglobin A1c, LDL-cholesterol, use of hypoglycemic and lipid-lowering medications, baseline eGFR, time-varying development of diabetes and hypertension over the follow-up	NAFLD was independently associated with increased risk of incident CKD (aHR 1.21, 95% CI 1.03-1.44). The association between NAFLD and CKD was consistent in all subgroups analyzed. In addition, advanced NAFLD fibrosis (as detected by a NFS ≥-1.455) was associated with even a higher risk of incident CKD (aHR 1.59, 95%CI 1.31-

					1.93). When NAFLD participants were classified according to APRI index and FIB4 score, those with higher APRI index or FIB4 score also had an increasing risk of incident CKD
Jang HR et al., South Korea, 2018 [43]	Retrospective cohort study: 1,525 adults with CKD (baseline mean eGFR 59 ml/min/1.73 m^2, 26% with overt proteinuria) free from known liver diseases, who underwent repeated health check-up examinations. Mean follow-up: 6.5 years	Ultrasonography; advanced NAFLD fibrosis assessed by the NFS (≥-1.455); prevalence of NAFLD was 40.9%	Annual percent decline in eGFR	Age, sex, year of visit, smoking, alcohol intake, BMI, hypertension, diabetes, dyslipidemia, systolic blood pressure, hemoglobin A1c, LDL cholesterol, triglycerides	NAFLD was independently associated with CKD progression. In multivariable adjusted models, the average difference in annual percent change in eGFR decline comparing patients with NAFLD to those without NAFLD was −1.06% (−1.73%, −0.38%; p=0.002). The decline in eGFR associated with NAFLD was greater in patients with higher NFS, in those with proteinuria or with low eGFR (<45 ml/min/1.73 m^2) at baseline
Wilechansky RM et al., United States, 2019 [44]	Prospective cohort study (Framingham Heart Study): 688 adults with normal kidney function and abnormal albuminuria at baseline, free from known liver diseases. Median follow-up: 12.5 years	Multidetector computed tomography	eGFR <60 ml/min/1.73 m^2; microalbuminuria; number of incident CKD cases was not reported	Age, sex, BMI, smoking, drinks per week, systolic/diastolic blood pressure, use of antihypertensive medications, HDL, total cholesterol, regular aspirin use, diabetes and follow‐up interval	Liver fat (measured by the average liver attenuation on CT) was significantly associated with incident microalbuminuria and CKD in age- and sex-adjusted models. These relationships were not significant in multivariable-adjusted models. However, there was a discrepancy between the timing of baseline kidney function measurements (1998‐2001) and CT assessment of liver fat (2002‐2005) of a median of 4.1 years
Önnerhag K et al., Sweden, 2019 [45]	Retrospective cohort study (identified from a computerized register in Malmö): 144 adult patients with biopsy-proven NAFLD. Mean follow-up: 18.8 years	Non-invasive fibrosis scoring systems (i.e., FIB-4-index, NFS, APRI and BARD score)	eGFR <60 ml/min/1.73 m^2; 47 participants developed incident CKD during follow-up	Age, sex, overweight/obesity, prior cardiovascular disease, hypertension, liver fibrosis stage	Both the intermediate and high-risk category of NFS and FIB-4 scores were independently associated with increased risk of incident CKD
Park H et al., United States, 2019 [46]	Retrospective propensity-matched cohort analysis of the Truven Health MarketScan Database	International Classification of Diseases (ICD-9) codes	CKD stages 3‐5 identified by the ICD‐9‐CM codes; There were 5,766 new CKD cases in the NAFLD cohort	Age, sex, diabetes, hypertension, obesity, hyperlipidemia, coronary artery disease, peripheral	NAFLD was independently associated with increased risk of incident CKD (aHR 1.41, 95% CI 1.36-1.46). In the sensitivity

	(2006-2015): 262,619 newly diagnosed patients with NAFLD and 769,878 propensity (1:3)-matched non-NAFLD patients. Follow-up: 9 years		and 8,655 new CKD cases in non‐NAFLD cohort	vascular disease, cerebrovascular disease, heart failure and chronic obstructive pulmonary disease, use of angiotensin‐converting‐enzyme inhibitors and angiotensin II receptor blockers, mean number of outpatient visits and mean number of inpatient visits, cirrhosis, decompensated cirrhosis and hepatocellular carcinoma	analysis adjusting for time‐varying covariates after NAFLD diagnosis, NAFLD persisted as a significant CKD risk factor (aHR 1.58, 95% CI 1.52-1.66)

Abbreviations: aHR, adjusted hazard ratio; AST, aspartate aminotransferase; APRI, AST to platelet ratio index; BMI, body mass index; CI, confidence interval; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; FIB4, fibrosis-4 score; GGT, gamma-glutamyltransferase; HOMA-IR, homeostasis model assessment-insulin resistance; NFS, NAFLD fibrosis score.

Note: eGFR was estimated by using either the four-variable Modification of Diet in Renal Disease (MDRD) study equation or the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) study equation (that was used by the last nine studies reported in this table).

Table 2. Association between *PNPLA3* rs738409 polymorphism and kidney dysfunction both in adults and in children or adolescents (ordered by publication year).

Authors, Country, Year (Ref.)	Study design and population	Kidney outcome measures	Covariate adjustments	Main results
Adult individuals				
Oniki K et al., Japan, 2015 [134]	Cross-sectional of 591 elderly subjects without known liver diseases (472 without NAFLD and 119 with NAFLD on ultrasonography) recruited during a health screening program and selected from an initial cohort of 740 subjects. Among these subjects, a retrospective longitudinal analysis with a median follow-up of 5.0 (range: 1.0–6.0 years) was also performed in 341 subjects (51 with NAFLD and 290 without NAFLD)	$eGFR_{CKD\text{-}EPI}$ (considered as continuous measure)	Age, sex, BMI, diabetes, hypertension, dyslipidemia and presence of NAFLD on ultrasonography	*PNPLA3* GG genotype was independently associated with lower eGFR values compared with carriers of the CC genotype, only in the subgroup of subjects with normal body weight (but not in those with overweight or obesity) in cross-sectional analyses. This association was also replicated in the longitudinal analyses
Musso G et al., Italy, 2015 [135]	Cross-sectional study of 202 nonobese, nondiabetic adults (61 non-cirrhotic biopsy-proven NAFLD and 81 control subjects)	CKD (i.e., $eGFR_{CKD\text{-}EPI}$ <60 ml/min/1.73 m^2 and/or microalbuminuria); microalbuminuria (30 - 300 mg/g)	Not specified	*PNPLA3* GG or CG genotype (combined) was significantly associated with a higher risk of prevalent CKD, lower eGFR or microalbuminuria both in NAFLD patients and in control subjects
Mantovani A et al., Italy, 2019 [136]	Cross-sectional study of 101 Caucasian post-menopausal women with non-insulin treated type 2 diabetes mellitus without known liver diseases	CKD (i.e., $eGFR_{CKD\text{-}EPI}$ <60 ml/min/1.73 m^2 and/or abnormal albuminuria); abnormal albuminuria ≥30 mg/g	Age, duration of diabetes, hemoglobin A1c, HOMA-IR, systolic blood pressure, hypertension treatment and presence of NAFLD on ultrasonography	*PNPLA3* GG genotype was independently associated with lower eGFR values and higher prevalence of CKD compared with the CC or GC genotype
Sun DQ et al., China, 2019 [137]	Cross-sectional study of 217 adults with non-cirrhotic biopsy-proven NAFLD	CKD (i.e., $eGFR_{CKD\text{-}EPI}$ <60 ml/min/1.73 m^2 and/or abnormal albuminuria); abnormal albuminuria ≥30 mg/g, urinary NGAL levels ≥31.2 ng/ml	Age, sex, BMI, waist circumference, hypertension, diabetes, HOMA-IR, hyperuricemia, presence of NASH (i.e., defined as a NAS ≥ 5) and histologic stage of fibrosis	*PNPLA3* GG genotype was independently associated with a higher risk of prevalent CKD, abnormal albuminuria or increased NGAL levels, especially in patients with persistently normal serum alanine-aminotransferase levels
Overweight/obese children or adolescents				
Targher G et al., Italy, 2019 [138]	Cross-sectional study of 142 Caucasian children/adolescents with biopsy-proven NAFLD	eGFR (using the Bedside Schwartz equation); 24-hour proteinuria (both considered as continuous measures)	Age, sex, systolic blood pressure, waist circumference, presence of NASH (i.e., defined as a NAS ≥ 5) and histologic stage of liver fibrosis	*PNPLA3* GG genotype was independently associated with lower eGFR values and increasing 24-hour proteinuria
Marzuillo P et al., Italy, 2019 [139]	Cross-sectional study of 591 Caucasian children/adolescents with obesity	eGFR (using the Schwartz equation) considered as continuous measure	Sex, duration of obesity, HOMA-IR score, BMI, LDL-	*PNPLA3* GG genotype was independently associated with lower eGFR values compared

			cholesterol, triglycerides	with the CC or GC genotype, only in the subgroup of children with NAFLD (defined by ultrasonography and/or serum ALT >40 IU/l))
Di Costanzo et al., Italy, 2019 [140]	Cross-sectional study of 230 overweight/obese children (105 with NAFLD defined as liver fat fraction ≥5% by magnetic resonance imaging)	eGFR (using the Schwartz equation); abnormal albuminuria ≥30 mg/g	Age, sex, pubertal status, diastolic blood pressure, waist circumference and presence of NAFLD on magnetic resonance imaging	*PNPLA3* G risk allele was not independently associated with eGFR <90 ml/min/1.73 m^2 and/or abnormal albuminuria

NB: In all aforementioned studies, the *PNPLA3* genotyping was determined on blood samples.

Abbreviations: BMI, body mass index; CKD, chronic kidney disease; $eGFR_{CKD\text{-}EPI}$, estimated glomerular filtration rate (estimated by the CKD-EPI equation); HOMA-IR, homeostasis model assessment-insulin resistance; NAFLD, non-alcoholic fatty liver disease; NAS, NAFLD activity score; NASH, non-alcoholic steatohepatitis; NGAL, neutrophil gelatinase-associated lipocalin; PNPLA3, patatin-like phospholipase domain-containing protein-3.

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