Nonalcoholic Fatty Liver Disease Increases Risk of Incident Chronic Kidney Disease: a Systematic Review and Meta-Analysis.

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ABSTRACT

Background - Recent studies examined the prognostic impact of nonalcoholic fatty liver disease (NAFLD) on the risk of incident chronic kidney disease (CKD). However, the extent to which NAFLD may confer risk of incident CKD is uncertain. We performed a meta-analysis of relevant studies to quantify the magnitude of the association between NAFLD and risk of incident CKD.

Methods - We searched PubMed, Scopus and Web of Science from January 1, 2000 to August 31, 2017 using pre-defined keywords to identify large observational cohort studies with a follow-up duration of at least 1 year, in which NAFLD was diagnosed by biochemistry, fatty liver index or ultrasonography. No studies with biopsy-proven NAFLD were available for the analysis. Data from selected studies were extracted, and meta-analysis was performed using random-effects modeling.

Results - A total of 9 observational studies with 96,595 adult individuals (34.1% with NAFLD) of predominantly Asian descent, and 4,653 cases of incident CKD stage •3 (*i.e.*, defined as occurrence of estimated glomerular filtration rate <60 ml/min/1.73 m^2, with or without accompanying overt proteinuria) over a median period of 5.2 years were included in the final analysis. Patients with NAFLD had a significantly higher risk of incident CKD than those without NAFLD (random-effects hazard ratio [HR] 1.37, 95%CI 1.20-1.53; I^2=33.5%). Patients with more 'severe' NAFLD (according to ultrasonography and non-invasive fibrosis markers) were also more likely to develop CKD (n=2 studies; random-effects HR 1.50, 95%CI 1.25-1.74; I^2=0%); this risk appeared to be even greater among those with ultrasound-diagnosed NAFLD and high-intermediate NAFLD fibrosis score (n=1 study; random-effects HR 1.59, 95%CI 1.31-1.93). Sensitivity analyses did not alter these findings. Funnel plot and Egger's test did not reveal significant publication bias.

Conclusions - This largest and most updated meta-analysis to date shows that NAFLD (detected by biochemistry, fatty liver index or ultrasonography) is associated with a nearly 40% increase in the long-term risk of incident CKD. However, the observational nature of the eligible studies does not allow for proving causality. Our findings pave the way for future large, prospective, histologically-based studies.

Keywords: NAFLD; CKD risk; meta-analysis

LIST OF ABBREVIATIONS USED

CKD-EPI, chronic kidney disease epidemiology collaboration

eGFR, estimated glomerular filtration rate

FIB4, fibrosis-4

FLI, fatty liver index

GGT, gamma-glutamyltransferase

MDRD, modification of diet in renal disease

NAFLD, nonalcoholic fatty liver disease

NASH, nonalcoholic steatohepatitis

NFS, NAFLD fibrosis score

NOS, Newcastle–Ottawa scale

1. INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is an umbrella term that encompasses a histologic spectrum of liver diseases, ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), cirrhosis and even hepatocellular carcinoma, occurring in a dysmetabolic milieu, though in the absence of excessive alcohol consumption and other competing etiologies of liver disease. NAFLD is the most common liver disease worldwide (affecting up 25%–30% of the general adult population in the United States and Europe) and its prevalence is projected to rise dramatically over the next decade [1-3]. However, the clinical and economic burden of NAFLD is not only restricted to progressive liver disease, but also embraces cardiovascular disease and other major extra-hepatic complications that have a significant impact on healthcare expenditures [4-6].

In this regard, the close relationship between NAFLD and chronic kidney disease (CKD) recently has gained considerable scientific interest [7]. CKD is defined by a sustained reduction in glomerular filtration rate (GFR) or evidence of structural or functional abnormalities of the kidneys on urinalysis, imaging or biopsy [8]. A five-stage classification system for CKD has been established internationally to guide identification of cases and facilitate the management [8]. This five-stage classification system is based predominantly on the use of validated creatinine-based GFR estimating equations, although the risk of related complications at a given rate is modified substantially by the amount of proteinuria [8].

CKD is a major risk factor for end-stage kidney disease, cardiovascular disease and premature death [8,9], and in 2010 the age-standardized global prevalence of CKD stages 1-5 in adults aged 20 years and older was nearly 10% in men and 12% in women, respectively [10]. However, the worldwide rise in the prevalence of CKD and consequent end-stage kidney disease necessitating renal replacement therapy is projected to reach epidemic proportions over the next decade [10]. Therefore, the high mortality, morbidity, and healthcare costs associated with CKD have led investigators to seek novel and potentially modifiable risk factors.

NAFLD and CKD share many cardiometabolic risk factors and proinflammatory and profibrotic molecular pathways [11]. In recent years, several cross-sectional studies have consistently shown that the prevalence of stage 3 CKD (defined as eGFR <60 ml/min/1.73 m^2 and/or over proteinuria) is markedly increased among patients with NAFLD [7]. Notably, in most of these published studies the significant association between NAFLD and increased CKD prevalence persisted even after adjustment for multiple common risk factors for CKD [7].

Although the association between NAFLD and increased prevalence of CKD is strong and has been consistently replicated across different ethnicities and patient populations, whether NAFLD is causally linked to the development and progression of CKD remains currently uncertain [7,11]. The validation of NAFLD as an independent risk factor of CKD would have direct relevance for primary preventive strategies against CKD.

Thus, we herein report the results of a comprehensive systematic review and meta-analysis of large observational cohort studies that has examined the association between the presence and severity of NAFLD (detected by serum liver enzymes, fatty liver index or liver ultrasonography) and the risk of incident CKD (stage 3 or more). The aim of this meta-analysis was to gauge precisely the nature and magnitude of the association between NAFLD and the risk of incident CKD. Clarification of the magnitude of risk of incident CKD associated with the different stages of liver disease within the spectrum of NAFLD may have important clinical implications for the diagnosis, prevention and treatment of CKD.

2. MATERIALS AND METHODS

2.1 Registration of review protocol

The protocol for this systematic review was registered in advance with PROSPERO (International Prospective Register of Systematic Reviews, *no*. CRD42017075092).

2.2 Data sources and searches

Studies were included if they were observational (prospective or retrospective) cohort studies that reported the risk of incident of CKD among adult individuals (>18 years old) with NAFLD as compared with those without NAFLD. Study participants were of either sex with no restrictions in terms of race, ethnicity or comorbidities. Only large (*n* ≥250) observational studies with a follow-up duration of at least 1 year were included. The diagnosis of NAFLD was based on biochemistry [*i.e*., serum liver enzymes or fatty liver index (FLI); FLI is an algorithmically-derived score to diagnose the presence of hepatic steatosis that utilises measurements of serum gamma-glutamyltransferase (GGT) levels along with body mass index, waist circumference and serum triglyceride concentration] or ultrasonography, in the absence of excessive alcohol consumption and other competing causes of chronic liver disease. No studies using serum aminotransferase levels or biopsy for diagnosing NAFLD were available in the literature for the analysis. Based on data from the eligible studies, 'severe' NAFLD was defined by the coexistence of ultrasonographic hepatic steatosis with either high-intermediate NAFLD fibrosis score (NFS) [12] or increased serum GGT levels, which may be also a surrogate marker of advanced NAFLD fibrosis [13].

In all eligible studies, incident CKD stage •3 was defined as the occurrence of eGFR <60 ml/min/1.73 m^2 [as estimated either by the four-variable Modification of Diet in Renal Disease (MDRD) or by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) study equations], with or without accompanying overt proteinuria during the follow-up [14].

Exclusion criteria of the meta-analysis were as follows: 1) reviews, editorials, abstracts, case reports, practice guidelines and cross-sectional studies; 2) studies conducted in paediatric populations (≤18 years old); 3) studies with a sample size of less than 250 individuals, or with a follow-up duration less than 1 year; 4) studies performed in liver donors or patients with NAFLD who received liver transplants; 5) studies which did not exclude individuals with excessive alcohol consumption and other known causes of chronic liver disease; 6) studies in which the outcome measure was defined as occurrence of early stages of CKD (stages 1 and 2; *i.e*., abnormal albuminuria with eGFR ≥60 ml/min/1.73 m^2); and 7) studies which did not specifically report any hazard ratio and 95% confidence interval for the outcome measure.

Included and excluded studies were collected following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram. Additionally, because the included studies were observational in design, we followed the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) guidelines for the meta-analysis of these studies [15].

2.3 Data extraction and quality assessment

Relevant studies were identified by systematically searching PubMed, Scopus and Web of Science from January 1, 2000 to August 31, 2017 (date last searched) using the free text terms "fatty liver" (OR "nonalcoholic fatty liver disease" OR "NAFLD" OR "nonalcoholic steatohepatitis") AND "chronic kidney disease" OR "incident CKD". No language restriction was applied. Reference lists of relevant papers and previous review articles were hand searched for other relevant studies. Two investigators (AM and GT) independently examined all titles and abstracts, and obtained full texts of potentially relevant papers. Working independently and in duplicate, we read the papers and determined whether they met inclusion criteria. Discrepancies were resolved by consensus, referring back to the original article, in consultation with a third author. For all studies, we extracted information on study design, study size, source of data, population characteristics, duration of follow-up, outcome

of interest, matching and confounding factors. Additionally, in the case of multiple publications, we included the most up-to-date or comprehensive information.

Two authors (AM and GT) assessed the risk of bias independently. Any discrepancies were addressed by a revaluation of the original article by a third author. Since all the included observational studies had a cohort design, the Newcastle-Ottawa Scale (NOS) was used to judge study quality, as recommended by the Cochrane Collaboration [16]. This scale uses a star system to assess the quality of a study in three domains: selection, comparability, and outcome/exposure. The NOS assigns a maximum of four stars for selection, two stars for comparability, and three stars for outcome/exposure. We judged studies that received a score of nine stars to be at low risk of bias (*i.e.*, thus reflecting the highest quality), studies that scored seven or eight stars to be at medium risk, and those that scored six or less to be at high risk of bias.

2.4 Data synthesis and analysis

As previously reported, the outcome measure of the meta-analysis was the risk of incident CKD stage ≥3 (as defined above) in individuals with NAFLD in comparison with the risk of incident CKD in individuals without NAFLD. When possible, we pooled adjusted hazard ratios (HR) with their 95% confidence intervals. In the case of studies reporting HRs with varying degrees of covariate adjustment, we always used the fully adjusted HR estimates.

Visual inspection of the forest plots was used to investigate the possibility of statistical heterogeneity. Statistical heterogeneity was assessed by the I^2 statistic, which provides an estimate of the percentage of variability across studies that is due to heterogeneity rather than chance alone. According to Higgins and Thompson [17], a rough guide to interpretation is as follows: I^2 values of approximately 25% represent low heterogeneity; approximately 50% represent medium heterogeneity; and approximately 75% represent high heterogeneity.

The adjusted HRs of all eligible studies were pooled, and an overall estimate of effect size (ES) was calculated using a random-effects model, as this methodology takes into account any differences between studies even if there is no statistically significant heterogeneity.

Publication bias was evaluated using the funnel plot and the Egger's regression test [18].

Given the expected heterogeneity of the eligible studies, stratification-sensitivity or subgroup analyses were also carried out to relate the outcome measure (*i.e*., incident CKD) with the individual study design characteristics. Specifically, based on the data from the eligible studies, the prognostic impact of NAFLD on the risk of incident CKD was assessed by stratifying the eligible studies according to the study country (Asia *vs*. Europe), the follow-up duration (>5 *vs*. ≤5 years), the severity of NAFLD (based mainly on the use of the NFS values or increasing serum GGT levels among patients with ultrasound-diagnosed NAFLD), whether the studies included only patients with established diabetes, whether they had eight or nine stars on the NOS scale (*i.e*., the "high-quality" studies), or whether the studies had full adjustment for covariates [*i.e*., those studies adjusting at least for age, sex, body mass index, hypertension, smoking, baseline eGFR, diabetes (or fasting glucose levels) and dyslipidemia (or plasma lipid levels)]. Additionally, we tested for possibly excessive influence of individual studies using a meta-analysis influence test that eliminated each of the included studies at a time.

All statistical tests were two sided and used a significance level of $p<0.05$. We used STATA® 14.0 (StataCorp, College Station, Texas, USA) for all statistical analyses.

3. RESULTS

3.1 Characteristics of included studies

Based on the titles and abstracts of 293 citations, we initially identified 11 potentially relevant studies. Of these, we excluded 2 studies as specified in the PRISMA flow diagram

(**supplementary Figure 1**). Thus, 9 unique, observational cohort studies were eligible for inclusion in the meta-analysis and were assessed for quality. In the **supplementary Table 1** we showed the characteristics of the excluded studies (at the stage of eligibility according to the PRISMA flow diagram) with the exact reason(s) for exclusion.

As summarized in **Table 1**, all the eligible studies had an observational (prospective or retrospective) design [19-27]. The eligible studies recruited participants either from approximately general populations [19,20,22,24-27] or from outpatient cohorts of adults with type 1 or type 2 diabetes [21,23]. Most of these studies were carried out in Asia (South Korea, China and Japan); a community-based cohort study was carried out in Finland, whereas two outpatient cohorts of adults with type 1 or type 2 diabetes were carried out in Italy. Most of these studies included middle-aged individuals predominantly of male sex.

Overall, in the 9 observational cohort studies included in the meta-analysis there were 96,595 adult individuals (34.1% with NAFLD; *n*=32,898) with 4,653 cases of incident CKD over a median follow-up of 5.2 years (interquartile range: 3.7-8.3 years). Five studies used ultrasonography for the diagnosis of NAFLD (*n*=57,341 individuals included), three studies used serum GGT levels (*n*=34,393 individuals included), and a single study of South Korean individuals used the FLI index (*n*=4,761 individuals included). No studies with biopsy-proven NAFLD were available for the analysis. In all eligible studies, the subjects had preserved kidney function without overt proteinuria at baseline, whereas the occurrence of incident CKD during the follow-up was defined as at least stage 3 CKD (*i.e.*, eGFR <60 ml/min/1.73 m^2 estimated either by the four-variable MDRD or by the CKD-EPI study equations), with or without accompanying overt proteinuria.

Of the 9 eligible studies (**supplementary Table 2**), three studies received at least eight stars on the NOS (indicating that those studies had a low risk of bias), three studies received seven stars (*i.e.*, being at medium risk of bias) and three studies received six stars (*i.e.*, being at high risk of bias), thus indicating an overall medium-high risk of bias.

3.2 NAFLD increases risk of incident CKD

The distribution of studies by estimate of the association between NAFLD and risk of incident CKD is plotted in **Figure 1**. Eight studies (involving a total of 91,034 individuals with 4,392 cases of incident CKD; approximately 30% had NAFLD at baseline) provided data suitable for the pooled primary analysis [19-21,23-27]. The study by Arase *et al*. [22] was excluded from the pooled primary analysis because it only included individuals with ultrasound-diagnosed NAFLD; this study was used in a secondary analysis for examining the association between the severity of NAFLD and risk of CKD.

NAFLD was significantly associated with an increased risk of incident CKD [*n*=8 studies; random-effects HR 1.37, 95%CI 1.20-1.53. Heterogeneity: Tau^2=0.0175; Chi^2 10.53, d.f.= 7 (*p*=0.16), I^2=33.5%. Test for overall effect: Z=16.23 (*p*<0.0001)]. Notably, since we have always used the fully adjusted HR estimates for each eligible study (as specified in **Table 1**), this random-effects HR was independent of a (relatively) large number of common risk factors and potential confounders (*e.g.,* age, sex, body mass index, plasma lipids, hypertension, smoking, baseline eGFR, diabetes status and use of certain medications).

As also shown in **Figure 1**, when the comparison was stratified by various methodologies used for the diagnosis of NAFLD, the significant association between NAFLD and the risk of incident CKD was substantially consistent in studies using ultrasonography [*n*=4 studies; random-effects HR 1.40, 95%CI 1.15-1.65; I^2=33.5%. Test for overall effect: Z=10.79 (*p*<0.0001)], FLI index [*n*=1 study; random-effects HR 1.46, 95%CI 1.16-1.76. Test for overall effect: Z =9.54 (*p*<0.0001)], or serum GGT levels [*n*=3 studies; random-effects HR 1.32, 95%CI 0.92-1.72; I^2=61.2%. Test for overall effect: Z=6.44 (*p*<0.0001)].

As shown in **Figure 2**, when the comparison was stratified by the length of follow-up period, the significant association of NAFLD with the risk of incident CKD was essentially comparable

between studies with more than 5 years of follow-up [*n*=4 studies; random-effects HR 1.34, 95%CI 1.04-1.65; I^2=36.8%. Test for overall effect: Z=8.62 (*p*<0.0001)] and those with ≤5 years of follow-up [*n*=4 studies; random-effects HR 1.40, 95%CI 1.17-1.63; I^2=48.1%. Test for overall effect: Z=11.88 (*p*<0.0001)].

As shown in **Figure 3**, when the comparison was stratified by the type of study population, the association between NAFLD and the risk of incident CKD appeared to be (slightly) stronger in those studies that enrolled exclusively individuals with type 1 or type 2 diabetes [*n*=2 studies; random-effects HR 1.56, 95%CI 1.07-2.05; I^2=0%. Test for overall effect: Z=6.19 (*p*<0.0001)].

As shown in **Figure 4**, when the comparison was stratified by the study country, the significant association between NAFLD and the risk of incident CKD appeared to be slightly stronger in Asian populations [*n*=5 studies; random-effects HR 1.40, 95%CI 1.22-1.58; I^2=36.5%. Test for overall effect: Z=15.33 (*p*<0.0001)] than in European populations [*n*=3 studies; random-effects HR 1.29, 95%CI 0.82-1.76; I^2=43.4%. Test for overall effect: Z=5.38 (*p*<0.0001)].

Limiting the analysis to 'high-quality' studies with full adjustment for covariates provided overall estimates consistent with the pooled primary analysis (*n*=3 studies; random-effects HR 1.38, 95%CI 1.11-1.65; I^2=45% - see **supplementary Table 2**). Finally, eliminating each of the eligible studies from the analysis had no significant effect on the overall risk of incident CKD (data not shown).

As shown in **supplementary Figure 2,** the Egger's regression test did not show statistically significant asymmetry of the funnel plot (*p*=0.39), thus suggesting that publication bias was unlikely.

3.3 'Severe' NAFLD increases risk of incident CKD

Two cohort studies (involving a total of 46,991 Asian individuals with 954 cases of incident CKD) reported data on patients with more 'severe' NAFLD, defined either by a high-intermediate NFS (*i.e.*, ≥-1.455) or by elevated GGT levels (*i.e.*, ≥109 U/l) among individuals with ultrasound-diagnosed NAFLD [22,27]. The distribution of studies by estimate of the association between more 'severe' NAFLD and the risk of incident CKD is plotted in **Figure 5**. The presence of more 'severe' NAFLD was significantly associated with a 50% increased risk of incident CKD [*n*=2 studies; random-effects HR 1.50, 95%CI 1.25-1.74. Heterogeneity: Tau^2=0.00; Chi^2 0.89, d.f.= 1 (*p*=0.35), I^2=0%. Test for overall effect: Z=12.09 (*p*<0.0001)]. This risk appeared to be even higher among patients with advanced NAFLD fibrosis as estimated by a NFS ≥-1.455 (*n*=1 study; random-effects HR 1.59, 95%CI 1.31-1.93); in the same study, when patients with NAFLD were classified according to APRI (AST to platelet ratio index) or FIB4 (fibrosis-4) score, those with higher APRI index or FIB4 score also had an increasing risk of incident CKD [27]. Also in such case, since we always used the fully adjusted HR estimates for each eligible study (as specified in **Table 1**), this random-effects HR was independent of a wide range of common risk factors and potential confounders. However, only a single cohort study from South Korea assessed NAFLD severity by using the NFS (or other non-invasive fibrosis markers), and no studies involving European or American individuals were available for this analysis, thus limiting the generalizability of the finding to other settings or to other ethnicities.

4. DISCUSSION

Our meta-analysis provides evidence for a significant association between NAFLD and the long-term risk of incident CKD. Indeed, the meta-analysis involves a total of 9 unique, observational cohort studies with aggregate data on 96,595 middle-aged individuals (34.1% with NAFLD) and nearly 5,000 new cases of incident CKD (stage ≥3) over a median follow-up of 5.2 years. To our knowledge, ours is the largest and most updated meta-analysis aimed at examining the prognostic role of NAFLD on the long-term risk of incident CKD.

We found that the presence of NAFLD (detected by serum GGT levels, FLI index or ultrasonography) conferred a nearly 40% increased risk of incident CKD, a risk that appeared to increase further with greater severity of NAFLD (as assessed by the NFS or other non-invasive fibrosis markers) and remained significant in those studies where analysis was fully adjusted for potentially confounding factors. In addition, when the analysis was stratified either by study country or by study population, the association between NAFLD and the risk of incident CKD appeared to be stronger in studies performed in Asian countries and in studies that enrolled exclusively patients with established diabetes.

Most of the published studies that used liver biopsy to diagnose NAFLD (*i.e*., the reference method for diagnosing and staging NAFLD) did not have a control group and cannot, therefore, be included in the meta-analysis. Additionally, and most importantly, none of these liver biopsy studies reported any specific data on temporal changes in eGFR or albuminuria/proteinuria over the follow-up.

Collectively, our findings confirm and extend on a threefold larger sample size the results of a previous meta-analysis (published by Musso *et al*. in 2014) that incorporated both cross-sectional and longitudinal studies using biochemistry, imaging or histology to diagnose NAFLD [28]. Meta-analysis of data from the 20 cross-sectional eligible studies (involving a total of nearly 30,000 individuals) indicated that NAFLD was associated with a two-fold increased risk of prevalent (pre-existing) CKD (random-effects HR 2.12, 95%CI 1.69-2.66). More interestingly, meta-analysis of data from the eleven longitudinal eligible studies (involving a total of 28,680 individuals with nearly 2,000 cases of incident CKD) indicated that NAFLD was associated with a nearly 80% increased risk of incident CKD (random-effects HR 1.79, 95%CI 1.65-1.95) [28]. Thus, the magnitude of the NAFLD-related effect on incident CKD risk, observed in the meta-analysis by Musso *et al.,* appears to be greater than that observed in our meta-analysis (random-effects HR 1.37, 95% CI 1.20-1.53). We hypothesize that this may be partly due to the shorter follow-up duration of the included studies and the lower overall sample size of the meta-analysis of Musso *et al.* (*n*=28,680 *vs*. 96,595); moreover, at variance with our meta-

analysis that included only longitudinal studies with incident cases of (at least) CKD stage 3, Musso *et al*. also included at least a couple of longitudinal studies that included exclusively incident cases with stages 1 or 2 CKD (*i.e*., abnormal albuminuria with only mildly decreased eGFR values) [29,30].

Interestingly, in a subgroup analysis of individual patient data of five small studies (involving a total of only 429 individuals with biopsy-proven NAFLD with 86 incident CKD cases [31-35]), Musso *et al*. also suggested the existence of a higher risk of incident CKD in NASH and advanced fibrosis *vs*. simple steatosis and non-advanced fibrosis, respectively [28]. In our meta-analysis, we have not included these five small studies of patients with biopsy-proven NAFLD because they lacked an adequate control group [31-35]. Also, and most importantly, the data on changes in eGFR values among these patients during the follow-up and the corresponding unadjusted/adjusted HRs for incident CKD have never been published in the original articles [31-35], but included for the first time by the same co-authors in the Musso meta-analysis [28]. Nevertheless, the findings of Musso *et al*. are consistent with the results of our meta-analysis that included two large Asian cohort studies (involving a total of nearly 47,000 individuals) using non-invasive markers of NAFLD severity (**Figure 5**).

The issue of whether the increased risk for incident CKD is restricted to patients with more severe NAFLD (*i.e*., NASH and/or advanced fibrosis, estimated to represent up to 10-15% of the overall NAFLD population) [1-3] or applies to all patients with NAFLD, is particularly relevant in view of the disease burden that NAFLD represents and might impact on the healthcare resources needed to survey and manage these patients adequately. The results of our meta-analysis (based exclusively on studies involving Asian individuals and using non-invasive markers of fibrosis) suggest that it is advanced NAFLD that carries a higher risk of developing CKD. This is also consistent with the conclusion of a comprehensive meta-analysis supporting a strong link between NAFLD severity and risk of fatal and nonfatal cardiovascular events [36]. Such findings fuel the expectation that new pharmacologic agents that target inflammation and fibrosis in the early clinical stages of NASH and CKD could also reduce the

progression of both disease conditions [37]. However, this question remains largely unsolved, and further prospective studies in larger cohorts of both Asian and non-Asian individuals with biopsy-confirmed NAFLD are needed in order to prove whether the severity of NAFLD affects risk of incident CKD.

That said, we believe that the findings of our meta-analysis are clinically relevant and provide further support for the view that a diagnosis of NAFLD identifies a subset of individuals, who are at higher risk of incident CKD, and who need more intensive surveillance and early treatment to potentially decrease the risk of developing CKD. For example, in a post-hoc analysis of a randomized controlled trial, Vilar-Gomez *et al*. reported that improvement of NAFLD histology due to 1-year lifestyle modification was independently associated with improved kidney function in a cohort of 261 patients with biopsy-proven NASH [38]. Collectively, these findings emphasise that there is a real need now to include outcomes such as development of CKD, and changes in eGFR and albuminuria/proteinuria in large randomised placebo-controlled trials focussed on testing the efficacy of novel therapies for NASH.

To date, there is convincing evidence of biological plausibility that NAFLD could increase risk of incident CKD. Indeed, the liver and kidneys share a number of pathophysiological pathways that are intrinsically linked to each other [8,34]. Growing experimental and clinical evidence indicates that NAFLD, especially NASH with varying amounts of liver fibrosis, may exacerbate hepatic insulin resistance (mostly through the secretion of multiple hepatokines, such as fetuin-A and fibroblast growth factor-21), promotes hypertension, induces atherogenic dyslipidemia and releases a myriad of proinflammatory molecules (*e.g*., C-reactive protein, interleukin-6, tumour necrosis factor-alpha), procoagulant factors (e.g., fibrinogen, plasminogen activator inhibitor-1), prooxidant (*e.g*., reactive oxygen species) and profibrogenic (*e.g*., transforming growth factor-beta, connective tissue growth factor) mediators that play important roles in the pathophysiology of CKD and other extra-hepatic vascular complications [5-7,11,39-44].

Our meta-analysis has some important limitations (strictly inherent to the nature of the included studies) that should be mentioned. Firstly, the observational design of the eligible studies does not allow establishing a causal relationship between NAFLD and incident CKD, and the quality of these studies is not consistently high (as shown in **supplementary Table 2**). Secondly, none of the eligible studies used liver biopsy for the diagnosis of NAFLD, which is the reference standard for diagnosing and staging NAFLD [1,2]. Moreover, the few eligible studies using surrogate markers of NAFLD (*i.e*., serum GGT levels or FLI index) should be interpreted with caution. Conversely, most of the eligible studies used ultrasonography, which is the recommended first-line imaging method for detecting NAFLD in clinical practice, given that this imaging technique accurately detects mild-to-moderate hepatic steatosis as assessed histologically [45,46]. Thirdly, regarding the association between NAFLD severity and risk of incident CKD, these data are derived from very few studies, and the use of non-invasive markers of advanced NAFLD fibrosis (such as the NFS) has been insufficiently validated in the general population [12]. Fourthly, most of the eligible studies originate from Asian countries [19,20,22,24,25,27], where large populations undergo regular health check-ups, including liver ultrasonography. As Asian and non-Asian populations have different adipose tissue distributions and genetic/cultural backgrounds, further studies should be conducted in non-Asian populations. Fifthly, all the eligible studies used creatinine-based GFR estimating equations (which do not perform well in patients with severe obesity or cirrhosis) [14], instead of direct GFR measurements to diagnose CKD, and no detailed information was available in these studies about specific renal pathology/morphology associated with NAFLD. Finally, the varying degree of confounder adjustment across the individual studies hampered a systematic assessment of the effect of established risk factors on the outcome of interest. As shown in **Table 1**, some studies reported incomplete adjustments for established risk factors and potential confounding variables (*e.g*., waist circumference or use of certain medications); as such, it was not possible to combine models in studies that adjusted for the same set of potential confounders.

Despite these limitations, our study has also several important strengths. As discussed previously, this meta-analysis provides the most comprehensive assessment to date on the prognostic impact of NAFLD per se on the long-term risk of incident CKD. These results, obtained by analyzing 4,653 new cases of incident CKD among nearly 100,000 individuals (incorporating data from observational cohort studies that are likely to be an accurate reflection of NAFLD patients commonly observed in clinical practice), provide clear evidence that risk of incident CKD of individuals with NAFLD is significantly higher than that of individuals without NAFLD (with a medium-low level of heterogeneity for the pooled primary analysis, but with a medium-high risk of bias according to the NOS scale of the available studies). Moreover, we have employed standardized risk estimates from all eligible studies to allow a consistent combination of estimates across studies. The large number of incident cases of CKD has provided high statistical power to quantitatively assess the association between NAFLD and CKD risk. Finally, selective reporting bias of studies was not a major concern in our analyses, as our comprehensive search have made it unlikely that any published report was missed and visual inspection of plots and formal tests demonstrated no statistical evidence of publication bias (**supplementary Figure 2**).

In conclusion, the results of this comprehensive meta-analysis show that NAFLD (as detected by serum GGT levels, FLI or ultrasonography) is significantly associated with a nearly 40% increase in the long-term risk of incident CKD (stage •3). No studies with biopsy-proven NAFLD were available for the analysis. Therefore, it remains uncertain whether NASH or NAFLD with advanced fibrosis carry a higher risk of incident CKD than simple steatosis, and whether NAFLD causally increases CKD risk or is a simple marker of other shared cardio-renal risk factors. Future well-designed, adequately powered prospective and randomized clinical studies of cohorts of patients with biopsy-proven NAFLD from both Asian and non-Asian countries are urgently needed to establish whether the presence and severity of NAFLD increases the risk of incident CKD, and to clarify whether improvement (or resolution) of (fibrosing) NAFLD will ultimately prevent or delay the development and progression of CKD.

Conflict of Interest: All authors declare no conflicts of interest.

Author's Contributions: study concept and design: GT; acquisition of data: AM, GT; statistical analysis of data: AM, GT; analysis and interpretation of data: AM, GT; drafting of the manuscript: AM, GT; critical revision of the manuscript for important intellectual content: GZ, CDB, AL, GZ, EB.

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FIGURE LEGENDS

Figure 1. Forest plot and pooled estimates of the effect of NAFLD on the risk of incident chronic kidney disease (CKD stage 3 or higher) in eight eligible observational cohort studies, stratified by different non-invasive methodologies for the diagnosis of NAFLD (*i.e*., serum liver enzymes [specifically increasing gamma-glutamyltransferase levels], fatty liver index [FLI] or liver ultrasonography).

Figure 2. Forest plot and pooled estimates of the effect of NAFLD on the risk of incident chronic kidney disease in eight eligible observational cohort studies, stratified by follow-up duration (based on the median follow-up of the eligible studies).

Figure 3. Forest plot and pooled estimates of the effect of NAFLD on the risk of incident chronic kidney disease in eight eligible observational cohort studies, stratified by study population (diabetes *vs*. no-diabetes).

Figure 4. Forest plot and pooled estimates of the effect of NAFLD on the risk of incident chronic kidney disease in eight eligible observational cohort studies, stratified by study country (Asian *vs*. European countries).

Figure 5. 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 ≥109 U/l] among patients with ultrasound-diagnosed NAFLD) on the risk of incident chronic kidney disease in two eligible observational cohort studies from South Korea and Japan. The NFS is an algorithmically-derived score to diagnose the presence of advanced hepatic fibrosis that utilises measurements of age, body mass index, impaired fasting glucose/diabetes status, platelet count, serum albumin concentration, and the ratio between the concentrations of the enzymes aspartate transaminase (AST) and alanine transaminase (ALT).

Figure S1. The PRISMA flow diagram of the meta-analysis.

Figure S2. Funnel plot of standard error by log-odds ratio for risk of incident CKD. Egger's regression test: *p*-value=0.39.