Association of common gene variants in glucokinase regulatory protein with cardiorenal disease: A systematic review and meta-analysis

Pomme I. H. G. Simons, Nynke Simons, Coen D. A. Stehouwer, Casper G. Schalkwijk, Nicolaas C. Schaper, Martijn C. G. J. Brouwers

1 Department of Internal Medicine, Division of Endocrinology, Maastricht University Medical Center, Maastricht, The Netherlands, 2 Department of Internal Medicine, Division of General Internal Medicine, Laboratory for Metabolism and Vascular Medicine, Maastricht University Medical Center, Maastricht, The Netherlands, 3 CARIM, School for Cardiovascular Diseases, Maastricht, The Netherlands, 4 Department of Internal Medicine, Division of General Internal Medicine, Maastricht University Medical Center, Maastricht, The Netherlands, 5 School for Public Health and Primary Care (CAPHRI), Maastricht, The Netherlands

These authors contributed equally to this work.

* mcgj.brouwers@mumc.nl

Abstract

Background

Small-molecules that disrupt the binding between glucokinase and glucokinase regulatory protein (GKRP) in the liver represent a potential new class of glucose-lowering drugs. It will, however, take years before their effects on clinically relevant cardiovascular endpoints are known. The purpose of this study was to estimate the effects of these drugs on cardiorenal outcomes by studying variants in the GKRP gene (GCKR) that mimic glucokinase-GKRP disruptors.

Methods

The MEDLINE and EMBASE databases were searched for studies reporting on the association between GCKR variants (rs1260326, rs780094, and rs780093) and coronary artery disease (CAD), estimated glomerular filtration rate (eGFR), and chronic kidney disease (CKD).

Results

In total 5 CAD studies (n = 274,625 individuals), 7 eGFR studies (n = 195,195 individuals), and 4 CKD studies (n = 31,642 cases and n = 408,432 controls) were included. Meta-analysis revealed a significant association between GCKR variants and CAD (OR:1.02 per risk allele, 95%CI:1.00–1.04, p = 0.01). Sensitivity analyses showed that replacement of one large, influential CAD study by two other, partly overlapping studies resulted in similar point estimates, albeit less precise (OR:1.02; 95%CI:0.98–1.06 and OR: 1.02; 95%CI: 0.99–1.04). GCKR was associated with an improved eGFR (+0.49 ml/min, 95%CI:0.10–0.89, p = 0.01) and a trend towards protection from CKD (OR:0.98, 95%CI:0.95–1.01, p = 0.13).
**Background**

In the current area of precise medicine, there is an ongoing search for new anti-diabetic medication with different modes of action. Drugs that modulate the function of glucokinase have been the scope of diabetes research for more than a decade now [1–4]. Glucokinase plays a pivotal role in regulating pancreatic insulin secretion and hepatic glucose uptake, owing to its unique enzymatic actions [5]. It catalyzes the conversion of glucose to glucose-6-phosphate, the first step in glycolysis. To date, however, clinical trials with glucokinase activators in patients with type 2 diabetes have been disappointing, since the glucose-lowering effects were non-sustained and accompanied by an increased risk of hypoglycemia and hypertriglyceridemia [1]. Hepatoselective glucokinase activators could theoretically bypass some of these side-effects, in particular the risk of hypoglycemia [6].

An alternative way to increase hepatic glucokinase activity is to disrupt the binding between glucokinase and glucokinase regulatory protein (GKRP). GKRP is a liver-specific protein located in the nucleus that binds—and hence inactivates—glucokinase in the fasting state. In the postprandial state, glucokinase dissociates from GKRP and subsequently migrates towards the cytosolic space where it facilitates phosphorylation of glucose [7, 8]. Lloyd and colleagues previously demonstrated that small molecules that disrupt the glucokinase-GKRP complex reduce plasma glucose levels without causing hypoglycemia in mice [9]. Although promising, it will probably take years before this new drug can be tested in a clinical setting.

Genetic epidemiology can be helpful to gain more insight into the clinical effects of glucokinase-GKRP disruption in humans. Since individuals are ‘randomized’ at birth to receive a wildtype allele or a variant that encodes GKRP that binds glucokinase less effectively, the effects of this variant on clinical endpoints can be studied as a surrogate for glucokinase-GKRP disruptors. Such a Mendelian randomization approach has been proven to be effective in predicting the (un)intended effects of new drugs [10].

We previously reviewed current literature on the cardiometabolic effects of variants in the glucokinase regulatory protein gene (GCKR) [11]. Individuals carrying the variant that binds glucokinase less effectively are indeed characterized by reduced fasting plasma glucose levels, but this is accompanied by an increased risk of nonalcoholic fatty liver disease (NAFLD), hypertriglyceridemia, and gout [12–14]. Of interest, there are studies suggesting that the same variant protects from chronic kidney disease (CKD) [15]. Given these opposing effects it is difficult to predict what the net effect will be on coronary artery disease (CAD), one of the most clinically relevant outcomes in type 2 diabetes.

The aim of the present study was therefore to elucidate the association between GCKR and CAD and CKD by conducting a systematic review and meta-analysis.

**Methods**

**Data sources, searches, and study selection**

The MEDLINE and EMBASE databases were searched for: 1) original, genetic association studies addressing the relationship between common variants in GCKR (rs1260326, rs780094,
or rs780093) and CAD; and 2) genome-wide association studies (GWAS) on CAD, as they are likely to include the variants of interest (see S1 Table for search strategy and S1 Fig for flow-chart). CAD was defined as myocardial infarction (MI), significant stenosis (i.e. \( \geq 50\% \)) in one or more main coronary arteries, or coronary intervention, including coronary artery bypass grafting (CABG) and percutaneous coronary intervention (PCI).

A second search was performed for the association between the common variants in GCKR and renal function. Studies reporting serum creatinine levels, eGFR (based on serum creatinine or cystatin C), or presence of CKD were considered eligible (see S2 Table for search strategy and S2 Fig for flowchart).

Cross-sectional articles, written in English, German, or Dutch, were included. No publication date or publication status restrictions were imposed. The electronic searches were conducted by one researcher (P.I.H.G.S.) and completed on March 6, 2018.

**Meta-analyses**

Two separate systematic reviews and three meta-analyses were conducted to determine the association between 1) common variants in GCKR and CAD; and 2) common variants in GCKR and renal function, i.e. estimated glomerular filtration (eGFR) and chronic kidney disease (CKD; based on dichotomized eGFR). Selection of variants was primarily based on functionality, i.e. the variant has been shown to be functional and mimics the effects of glucokinase-GKRP disruptors (i.e. rs1260326 [16, 17]). In addition, variants that are in strong linkage disequilibrium with this functional variant, i.e. rs780094 or rs780093, were included as well (\( r^2 \approx 0.92 \) for both SNPs in both Europeans and East Asians; source: 1000 Genomes project phase 3). The systematic reviews and meta-analyses were performed according to the PRISMA statement with the only exception of a (registered) review protocol (see S1 Checklist) [18].

**Data extraction and quality assessment**

Data extraction was done in a two-step, standardized fashion where one researcher (P.I.H.G. S.) extracted the data, which was subsequently checked by two other researchers (N.S. and M. C.G.J.B.). The following variables were extracted from the included studies: odds ratios or unstandardized beta coefficients, with 95% confidence intervals or standard errors. Authors were contacted in case of missing data (in particular for the GWAS). In case of non-response, a reminder was sent three weeks later. When more than one GCKR variant was reported, the functional variant (rs1260326) was chosen. The additive model was the preferred model of inheritance, based on previous GCKR association studies [19]. Finally, given our interest in the systematic effects of GCKR per se, we aimed to obtain the crude outcome variables, i.e. without adjustment for potential mediators (e.g. plasma lipids levels).

To avoid inclusion of study cohorts that were reported more than once, in particular in GWAS consortia, special attention was paid to the origin of the individual study populations. In case of overlap, the study that contained the highest number of participants was included. The quality of the study and the risk of bias were assessed by two independent researchers (P.I.H.G.S. and N.S.) according to the Newcastle-Ottawa Scale (NOS) [20].

**Data synthesis and analysis**

Back-transformation of the log-transformed difference in eGFR between the two GCKR alleles was done as described elsewhere [21]. Odds ratios and beta coefficients were meta-analyzed based on a random-effects model, using the DerSimonian-Laird method to incorporate between-study heterogeneity. Funnel plots were visually examined for asymmetry and analyzed by means of regression (Egger’s test).
Since most studies (in particular GWAS) only reported the principal summary measures (i.e. odds ratios) instead of individualized data, it was not feasible to adjust for potential environmental effects, nor was it possible to assess Hardy-Weinberg equilibrium or linkage disequilibrium for each study.

Sensitivity analyses were performed to assess the impact of studies that included subjects with different ancestries, studies with low quality (defined as a NOS score <5 stars), and studies that did not report crude (or age- and/or sex-adjusted) estimates. All analyses were conducted with the R’s statistical software (R Developmental Core Team) using the metaphor package [22].

Results
Systematic review and meta-analysis of the association between common variants in GCKR and CAD

The electronic search identified 3,051 unique records, which eventually resulted in five studies that were used for the meta-analysis [23–27] (see S1 Fig for flowchart and reasons for exclusion). All included studies were written in English. Twenty-six studies showed overlap with one of the included studies, i.e. the combined UK Biobank, CARDIoGRAMplusC4D 1000 genomes-based GWAS, and Myocardial Infarction Genetics and CARDIoGRAM Exome dataset [24], and were therefore not included in the meta-analysis (S3 Table). The genetic variants of interest were often not reported in the main article, but could be found in the (online) supplementary materials of the article. For one GWAS, the authors were contacted and the requested data were kindly provided [25].

The characteristics of the included studies are shown in Table 1. In total, 274,625 subjects were included. In some, mainly Asian studies, the GCKR effect allele—defined as the allele that predisposes to reduced fasting plasma glucose levels (similar to the effect of a glucokinase-GKRP disruptor)—was the predominant allele. The overall quality of the studies was good (S4 Table).

Meta-analysis demonstrated that the GCKR effect allele was significantly associated with CAD (OR: 1.02, 95%CI: 1.00–1.04, p = 0.01) (Fig 1). Heterogeneity was low (Q = 3.30, I² = 0%) [28]. Due to the low number of included studies, a funnel plot (or testing for funnel plot asymmetry) was not included, according to previous recommendations [29, 30]. Since the meta-analysis was dominated by one large study—which is composed of 76 sub-studies [31]–we conducted several sensitivity analyses to test the robustness of our findings. First, this large study

| Author      | Year | Ancestry                  | Population type | Number of cases | Number of controls | Covariates adjusted for | SNP           | EAF     | Outcome |
|-------------|------|----------------------------|-----------------|-----------------|---------------------|------------------------|---------------|---------|---------|
| Lian [23]   | 2013 | Asian                      | Hospital        | 568             | 494                 | -                      | rs780093      | 0.52    | CHD     |
| Nelson [24] | 2017 | European + non-European    | General + hospital | 268,744*        | -                   | Array and population structure/ancestry | rs1260326  | 0.40    | CAD     |
| Raffield [25] | 2015 | European                   | Type 2 diabetes  | 212             | 771                 | Age, sex               | rs1260326    | 0.39    | MI      |
| Takeuchi [26] | 2012 | Asian                      | Hospital        | 1,347           | 1,337               | Not specified          | rs780094     | 0.56    | CAD     |
| Zhou [27]   | 2015 | Asian                      | General + hospital | 555             | 597                 | -                      | rs1260326    | 0.42    | CAD     |

*Number of cases refers to the overall population.

Abbreviations: SNP: single nucleotide polymorphism; EAF: effect allele frequency; CHD: coronary heart disease; MI: myocardial infarction.
was replaced by another large study that combined the CARDIoGRAMplusC4D 1000 genomes-based GWAS dataset with an additional 56,354 samples (n = 260,365 subjects in total, S3 Table) [32]. The subsequent meta-analysis revealed a similar, but less precise point estimate (OR: 1.02, 95%CI: 0.98–1.06, p = 0.37, S3 Fig). The initial large study was also replaced by the CARDIoGRAMplusC4D Metabochip data [33, 34], which overlaps for ~55% with the CARDIoGRAMplusC4D 1000 genomes-based GWAS data (S3 Table) [35]. This also allowed a better sensitivity analysis stratified by ancestry, since data for Europeans only have been presented separately [34]. Again, the overall meta-analysis showed a similar, but non-significant point estimate (OR: 1.02, 95%CI: 0.99–1.05, p = 0.27, S4 Fig).

The GCKR effect allele was significantly associated with CAD in studies that included subjects of European ancestry only (n = 3) (OR: 1.02, 95%CI: 1.00–1.05, p = 0.02), but not in studies that included subjects of Asian ancestry only (OR: 1.06, 95%CI: 0.98–1.15, p = 0.13; S4 Fig). Of note, these effect sizes were not statistically different (p = 0.36). Repeat analysis without the study with low quality [25] (i.e. NOS score <5 stars) did not affect the primary outcome.

### Systematic review and meta-analysis of the association between common variants in GCKR and eGFR and CKD

Of the 661 eligible records that were selected by our initial search, eight studies fulfilled all in- and exclusion criteria and were used for the meta-analyses (see S2 Fig for flowchart and

| Author, Year | CAD | Control | Odds Ratio [95% CI] |
|--------------|-----|---------|-------------------|
| Lian, 2013 | 568 | 494 | 0.97 [0.82, 1.15] |
| Nelson, 2017 | 268744* | | 1.02 [1.00, 1.04] |
| Raffield, 2015 | 212 | 771 | 1.12 [0.90, 1.39] |
| Takeuchi, 2012 | 1347 | 1337 | 1.10 [0.99, 1.23] |
| Zhou, 2015 | 555 | 597 | 1.08 [0.91, 1.27] |
| Model (Q = 3.30, df = 4, p = 0.51; I² = 0.0%) | | | 1.02 [1.00, 1.04] |

Fig 1. Meta-analysis of the relationship between the GCKR effect allele and coronary artery disease (CAD). * Number of individuals refers to the overall population.

https://doi.org/10.1371/journal.pone.0206174.g001
reasons for exclusion, and S5 Table for duplicate studies). All included studies were written in English. The genetic variants of interest were often not reported in the main article, but could be found in the (online) supplementary materials of the article. For two GWAS, the authors were contacted and the requested data were kindly provided [36, 37]. Six studies reported data on creatinine-based eGFR [36, 38–42], one on cystatin C-based eGFR [15], and four on CKD [36, 37, 40, 42]. Study characteristics of the eGFR and CKD studies are provided in Table 2. All studies used only the (creatinine-based) eGFR criterion to define CKD. Quality assessment of the included studies yielded an average score of five out of nine stars (S6 Table). Many studies reporting on eGFR scored low on ‘comparability’, i.e. the analyses were adjusted for covariates more than age and/or sex, whereas we aimed to obtain the crude relationship between GCKR and eGFR.

Meta-analysis, including 195,195 individuals, showed that the GCKR effect allele was significantly associated with an increased eGFR (0.49 ml/min, 95%CI: 0.10–0.89, p = 0.01) (Fig 2). Heterogeneity was high (Q = 43.12, I^2 = 88.4%). The only study that reported on cystatin C-based eGFR observed similar effect sizes, which was statistically significant in the discovery cohort (p = 0.006), but not in the replication cohort (p = 0.16) [15].

The meta-analysis for CKD, including 31,642 cases and 408,432 controls, showed a protective effect of the GCKR effect allele on CKD, albeit not statistically significant (OR: 0.98, 95% CI: 0.95–1.01, p = 0.13; Q = 5.54, I^2 = 45.9%) (Fig 3). The forest plot identified one outlying study that explained the moderate heterogeneity (Fig 3). Repeat analysis without this study [40] resulted in a significant, negative relationship (OR: 0.97, 95%CI: 0.95–0.99, p = 0.003). The same study also accounted for the non-significant relationship with CKD when sensitivity analyses were conducted for Asian studies only (S5 Fig). All CKD studies were of sufficient quality (NOS score ≥ 5 stars) and did not adjust for co-variates other than age and/or sex.

**Discussion**

Glucokinase regulatory protein (GKRP) is a liver-specific protein that plays an important role in the regulation of hepatic glucose uptake and, consequently, de novo lipogenesis, one of the principal pathways in the development of NAFLD [11]. By studying the systemic effects of common variants in GCKR it is possible to gain more insight into the interaction between hepatic glucose metabolism and cardiorenal disease. Moreover, it allows an evaluation of small-molecule disruptors of the glucokinase-GKRP complex as a potential new glucose-lowering treatment. In three meta-analyses using data from at least ~200,000 individuals, we showed that the GCKR effect allele—which encodes a GKRP protein that binds glucokinase less effectively—appeared to be associated with CAD, whereas a protective effect was observed for eGFR.

Previous studies have shown that the GCKR effect allele is associated with an atherogenic lipid profile, i.e. higher plasma triglycerides and apolipoprotein B levels, reduced HDL cholesterol levels and the presence of small-dense LDL particles [12, 43, 44]. In that respect it is of no surprise that we did observe a positive association of GCKR on CAD in our primary analysis. If, however, one would take into account the effect of GCKR on only plasma triglycerides, it would be anticipated to already result in an odds ratio of 1.05 to develop CAD [45]. The smaller effect estimate that was found in this study (OR: 1.02, 95%CI: 1.00–1.04) should therefore be accounted for by another, protective factor that blunts the plasma lipid-mediated effects of GCKR on CAD risk. GCKR has previously been associated with reduced fasting plasma glucose levels [12]. The hitherto reported protective effect of GCKR on eGFR could be another explanatory factor. Previous epidemiological studies have shown that CKD is an independent cardiovascular risk factor [46].
The current meta-analyses were confined to creatinine-based renal outcome measures (eGFR and CKD), since these were most frequently reported. Köttgen and colleagues showed that the positive relationship between GCKR and (creatinine-based) eGFR was also observed for cystatin C-based eGFR [15]. The same authors suggested that another gene, which is in linkage disequilibrium with GCKR, is actually responsible for the association with renal function [15]. However, previous experiments in liver-specific glucokinase knockout mice—which are metabolically opposite to increased glucokinase-GKRP disruption—are characterized by increased kidney damage [47], which is in line with the current study.

The mechanism by which enhanced glucokinase-GKRP disruption exerts its renoprotective effects remains to be elucidated. The GCKR effect allele has been associated with increased NAFLD risk, low HDL cholesterol levels, and higher urate levels [12, 13, 43, 44, 48], which in turn have been associated with deterioration of renal function [49–51]. These factors should therefore be outbalanced by factors that protect the kidney, such as lower plasma glucose levels. We cannot exclude that there are also other, yet unknown factors that contribute to the renoprotective effect of the GCKR effect allele. Further research is needed to identify these factors as it may have important clinical implications.

The present study may provide a glimpse into the future of what the cardiorenal effects of small-molecule disruptors of the glucokinase-GKRP complex will be as a potential new glucose-lowering drug. Although the protective effect on eGFR and CKD appears to be promising at first sight, it may be outbalanced by an increased risk to develop CAD. Furthermore, a synergistic effect between GCKR and type 2 diabetes on CAD risk cannot be ruled out. We previously demonstrated that the effects of the GCKR effect allele on plasma lipid levels were more pronounced in patients with type 2 diabetes when compared to healthy individuals [52].

### Table 2. Characteristics of included studies on eGFR and CKD.

| Author Year | Ancestry | Population type | Number of cases | Number of controls | Adjusted covariates | SNP | EAF | Definition of outcome |
|-------------|----------|-----------------|-----------------|--------------------|---------------------|-----|-----|----------------------|
| **eGFR (creatinine-based)** | | | | | | | | |
| Bonetti [38] | 2011 | European | T2D | 474 | - | Age, sex, BMI | rs780094 | 0.47 | MDRD |
| Deshmukh [39] | 2013 | European | T2D | 2,970 | - | Age, sex, BMI, SBP, HbA1c, T2DM duration | rs1260326 | MDRD |
| Hishida [40] | 2014 | Asian | General | 3,324 | - | Age, sex | rs1260326 | 0.61 | Modified MDRD |
| Okada [41] | 2012 | Asian | General + hospital | 42,451 | - | Age, sex, alcohol, smoking, BMI | rs1260326 | 0.52 | Modified CKD-EPI |
| Pattaro [42] | 2016 | European | General + T2D | 133,413 | - | Age, sex | rs1260326 | 0.42 | MDRD |
| Yamada [36] | 2013 | Asian | Hospital | 12,563 | - | Age, sex | rs1260326 | 0.57 | Modified MDRD |
| **eGFR (cystatin C-based)** | | | | | | | | |
| Köttgen [15] | 2010 | European | General + T2D | 20,907 | - | Age, sex | rs1260326 | 0.41 | 76.7 × (serum cystatin C)−1.19 |
| Hishida [40] | 2014 | Asian | General | 578 | 2,746 | - | rs1260326 | 0.61 | eGFR < 60 ml/min/1.73m2 |
| Pattaro [42] | 2016 | European | General + T2D | 12,385 | 104,780 | Age, sex | rs1260326 | 0.42 | eGFR < 60 ml/min/1.73m2 |
| Svein-Bjornsson [37] | 2014 | European | Hospital | 15,594 | 291,428 | Age, sex | rs1260326 | 0.35 | eGFR < 60 ml/min/1.73m2 |
| Yamada [36] | 2013 | Asian | Hospital | 3,085 | 9,478 | Age, sex | rs1260326 | 0.57 | eGFR < 50 ml/min/1.73m2 |

*Number of cases for the eGFR trait refers to the overall population.

Abbreviations: SNP: single nucleotide polymorphism; EAF: effect allele frequency; BMI: body mass index; SBP: systolic blood pressure; MDRD: modification of diet in renal disease; CKD-EPI: chronic kidney disease epidemiology collaboration.

https://doi.org/10.1371/journal.pone.0206174.t002
similar interaction between GCKR and type 2 diabetes on CAD risk would seriously decrease the applicability of small molecule disruptors of the glucokinase-GKRP complex as new antidiabetic drug. Unfortunately, there were too few studies that were specifically conducted in type 2 diabetes to formally investigate such an interaction in the current meta-analysis.

This study has several strengths and limitations. First, the meta-analysis of the association of GCKR with CAD depends to a large extent on the combined UK Biobank, CARDioGRAMplusC4D 1000 genomes-based GWAS, and Myocardial Infarction Genetics and CARDioGRAM Exome dataset, which is actually a meta-analysis by itself [31]. In subsequent sensitivity analyses we replaced this large dataset by other CARDioGRAMplusC4D-based studies that—despite a substantial overlap with the original study—included a large number of independent samples [32–34]. Although similar effect sizes were observed, statistical significance was not reached. The positive association between the GCKR effect allele and CAD in the primary analysis should therefore be interpreted with some caution.

Second, the definition of CKD was only based on eGFR—not the presence of albuminuria—in all of the included studies. Both factors are part of the classification of CKD as defined by the Kidney Disease Improving Global Outcomes (KDIGO) [53]. The CKD Genetics Consortium recently reported that the GCKR variant that protects from deterioration of renal function is associated with an increased urine albumin-creatinine ratio [51]. These findings emphasize the need for further research on the pathophysiological mechanisms relating GKRP to the kidney.

Third, it is not entirely clear whether the effects of genetic variants in GCKR and small molecule disruptors of the glucokinase-GKRP complex are truly comparable. This is one of the
general limitations of the Mendelian randomization approach in which genetic variants are used as an instrument to study the effects of a specific drug of interest. However, previous experimental studies have shown that both the product of the \textit{GCKR} minor allele and glucokinase-GKRP disruptors cause an increased translocation of glucokinase from the nucleus towards the cytosolic space in the liver [9, 17]. This explains the reduced plasma glucose levels that have been associated with both the \textit{GCKR} minor allele and treatment with glucokinase-GKRP disruptors [9, 54].

Another aspect that deserves consideration is the moderate-to-high heterogeneity that was observed in some of the meta-analyses. This could be the result of genotyping errors or difference in methodology, such as discrepancies in outcome measures (particularly for CAD) or study populations (e.g. population-based versus hospital-based). Although ancestry did not account for the moderate-to-high heterogeneity, the number of studies was too small to make strong inferences. Furthermore, differences in diet could contribute to the observed heterogeneity given the previously reported \textit{GCKR}-diet interaction on plasma triglycerides levels [55, 56]. It is, however, unlikely that these factors truly account for the opposing effect sizes that were present in the individual studies, e.g. \textit{GCKR} seemed to protect from CKD in one Japanese cohort [36, 41] whereas a predisposing effect appeared to be present in one other [40]. These opposing effects could simply be the consequence of chance, especially in small-sized studies with few events. Alternatively, \textit{GCKR} could theoretically be in linkage disequilibrium with a gene that exerts an opposing effect on cardiorenal risk in certain but not all populations. These opposing effects could have important therapeutic implications if they would be inherent to GKRP function and therefore deserve further attention.

| Author, Year | CKD   | Control | Odds Ratio [95% CI] |
|--------------|-------|---------|---------------------|
| Hishida, 2014| 578   | 2746    | 1.12 [ 0.98 , 1.27 ]|
| Pattaro, 2016| 12385 | 104780  | 0.98 [ 0.95 , 1.01 ]|
| Sveinbjörnsson, 2014 | 15594 | 291428  | 0.96 [ 0.93 , 0.99 ]|
| Yamada, 2013 | 3085  | 9478    | 0.96 [ 0.90 , 1.02 ]|

Model (Q = 5.54, df = 3, p = 0.14; \( I^2 = 45.9\% \))

0.80 0.90 1.00 1.10 1.20 1.30

Odds ratio

Fig 3. Meta-analysis of the relationship between the \textit{GCKR} effect allele and chronic kidney disease (CKD).

https://doi.org/10.1371/journal.pone.0206174.g003
A final limitation was that we were forced to exclude a considerable amount of studies, and hence a substantial number of subjects, from the meta-analyses because of partial overlap of individual study cohorts. Yet, we were still able to include a high number of individuals, ranging from ~200,000 to 400,000 in the three meta-analyses, which can be attributed to our search strategy that was not confined to studies specifically reporting on GCKR. We correctly assumed that GWAS were likely to include our variants of interest without reporting in the manuscript’s title or abstract.

**Conclusions**

The present study extends our knowledge on the systemic effects of enhanced disruption of the glucokinase-GKRP complex by demonstrating that the GCKR effect allele is associated with a better eGFR. A disadvantageous effect on CAD risk can, however, not be ruled out. These findings question the benefits and applicability of small molecule disruptors of the glucokinase-GKRP complex as a potential new class of antidiabetic drugs. Further studies are warranted to identify the factor that mediates the renoprotective effects of enhanced disruption of the glucokinase-GKRP complex.

**Supporting information**

S1 Table. Search strategy for CAD. (DOCX)

S2 Table. Search strategy for eGFR and CKD. (DOCX)

S3 Table. Overview of the excluded CAD studies with duplicate cohorts. (DOCX)

S4 Table. Quality assessment of the CAD studies based on the Newcastle-Ottawa Scale. (DOCX)

S5 Table. Overview of the excluded eGFR and CKD studies with duplicate cohorts. (DOCX)

S6 Table. Quality assessment of the eGFR and CKD studies based on the Newcastle-Ottawa Scale. (DOCX)

S1 Fig. Flowchart of the systematic review on CAD. (DOCX)

S2 Fig. Flowchart of the systematic review on eGFR and CKD. (DOCX)

S3 Fig. Forest plot of the meta-analysis on CAD–sensitivity analysis. (DOCX)

S4 Fig. Forest plot of the meta-analysis on CAD–stratified by ancestry. (DOCX)

S5 Fig. Forest plot of the meta-analysis on CKD–stratified by ancestry. (DOCX)

S1 Checklist. (DOCX)
Author Contributions
Conceptualization: Nynke Simons, Martijn C. G. J. Brouwers.
Formal analysis: Nynke Simons, Martijn C. G. J. Brouwers.
Funding acquisition: Martijn C. G. J. Brouwers.
Investigation: Pomme I. H. G. Simons, Nynke Simons, Martijn C. G. J. Brouwers.
Methodology: Nynke Simons, Martijn C. G. J. Brouwers.
Supervision: Coen D. A. Stehouwer, Casper G. Schalkwijk, Nicolaas C. Schaper, Martijn C. G. J. Brouwers.
Validation: Nynke Simons, Martijn C. G. J. Brouwers.
Writing – original draft: Pomme I. H. G. Simons, Nynke Simons, Martijn C. G. J. Brouwers.
Writing – review & editing: Coen D. A. Stehouwer, Casper G. Schalkwijk, Nicolaas C. Schaper, Martijn C. G. J. Brouwers.

References
1. Meining er GE, Scott R, Alba M, Shentu Y, Luo E, Amin H, et al. Effects of MK-0941, a novel glucokinase activator, on glycemic control in insulin-treated patients with type 2 diabetes. Diabetes Care. 2011; 34 (12):2560–6. https://doi.org/10.2337/dc11-1200 PMID: 21994424; PubMed Central PMCID: PMC3220852.
2. Denney WS, Denham DS, Riggs MR, Amin NB. Glycemic Effect and Safety of a Systemic, Partial Glucokinase Activator, PF-04937319, in Patients With Type 2 Diabetes Mellitus Inadequately Controlled on Metformin-A Randomized, Crossover, Active-Controlled Study. Clinical pharmacology in drug development. 2016; 5(6):517–27. https://doi.org/10.1002/cpdd.261 PMID: 27870481.
3. Sarabu R, Bizzarro FT, Corbett WL, Dvorozniak MT, Geng W, Grippo JF, et al. Discovery of piragliatin—first glucokinase activator studied in type 2 diabetic patients. Journal of medicinal chemistry. 2012; 55 (16):1694–702. https://doi.org/10.1021/jm3008689 PMID: 22809456.
4. Johnson D, Shepherd RM, Gill D, Gorman T, Smith DM, Dunne MJ. Glucose-dependent modulation of insulin secretion and intracellular calcium ions by GKA50, a glucokinase activator. Diabetes. 2007; 56 (6):1694–702. https://doi.org/10.2337/db07-0026 PMID: 17360975.
5. Agius L. Glucokinase and molecular aspects of liver glycogen metabolism. Biochem J. 2008; 414(1):1–18. Epub 2008/07/25. https://doi.org/10.1042/BJ20080595 PMID: 18651836.
6. Pfefferkorn JA, Guzman-Perez A, Litchfield J, Aiello R, Treadway JL, Pettersen J, et al. Discovery of (S)-6-(3-cyclopentyl-2-(4-(trifluoromethyl)-1H-imidazol-1-yl)propanamido)nicotinic acid as a hepatoselective glucokinase activator clinical candidate for treating type 2 diabetes mellitus. Journal of medicinal chemistry. 2012; 55(3):1318–33. https://doi.org/10.1021/jm2014887 PMID: 22196621.
7. de la Iglesia N, Veiga-da-Cunha M, Van Schaftingen E, Guinovart JJ, Ferrer JC. Glucokinase regulatory protein is essential for the proper subcellular localisation of liver glucokinase. FEBS letters. 1999; 456 (2):332–8. PMID: 10456334.
8. van Schaftingen E, Vandercammen A, Detheux M, Davies DR. The regulatory protein of liver glucokinase. Adv Enzyme Regul. 1992; 32:133–48. Epub 1992/01/01. PMID: 1496915.
9. Lloyd DJ, St Jean DJ Jr., Kurzeja RJ, Wahl RC, Michelsen K, Cupples R, et al. Antidiabetic effects of glucokinase regulatory protein small-molecule disruptors. Nature. 2013; 504(7480):437–40. Epub 2013/11/15. https://doi.org/10.1038/nature12724 PMID: 24226772.
10. Walker VM, Davey Smith G, Davies NM, Martin RM. Mendelian randomization: a novel approach for the prediction of adverse drug events and drug repurposing opportunities. Int J Epidemiol. 2017; 46 (6):2078–89. Epub 2017/10/19. https://doi.org/10.1093/ije/dyw207 PMID: 29040597; PubMed Central PMCID: PMCPMC5837479.
11. Brouwers MC, Jacobs C, Bast A, Stehouwer CD, Schaper NC. Modulation of Glucokinase Regulatory Protein: A Double-Edged Sword? Trends Mol Med. 2015; 21(10):583–94. https://doi.org/10.1016/j.trendsmmolmed.2015.08.004 PMID: 26432016.
12. Orho-Melander M, Melander O, Guiducci C, Perez-Martinez P, Corella D, Roos C, et al. Common missense variant in the glucokinase regulatory protein gene is associated with increased plasma triglyceride and C-reactive protein but lower fasting glucose concentrations. Diabetes. 2008; 57(11):3112–21.
13. Speliotes EK, Yerges-Armstrong LM, Wu J, Hernaez R, Kim LJ, Palmer CD, et al. Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits. PLoS Genet. 2011; 7(3):e1001324. Epub 2011/03/23. https://doi.org/10.1371/journal.pgen.1001324 PMID: 21423719; PubMed Central PMCID: PMCPMC3035321.

14. Matsuo H, Yamamoto K, Nakaoka H, Nakayama A, Sakiyama M, Chiba T, et al. Genome-wide association study of clinically defined gout identifies multiple risk loci and its association with clinical subtypes. Annals of the rheumatic diseases. 2016; 75(4):652–9. https://doi.org/10.1136/annrheumdis-2014-206191 PMID: 25646370; PubMed Central PMCID: PMCPMC3053321.

15. Matsuo H, Yamamoto K, Nakaoka H, Nakayama A, Sakiyama M, Chiba T, et al. Genome-wide association study of clinically defined gout identifies multiple risk loci and its association with clinical subtypes. Annals of the rheumatic diseases. 2016; 75(4):652–9. https://doi.org/10.1136/annrheumdis-2014-206191 PMID: 25646370; PubMed Central PMCID: PMCPMC3053321.

16. Kottgen A, Pattaro C, Boger CA, Fuchsberger C, Olden M, Glazer NL, et al. New loci associated with kidney function and chronic kidney disease. Nat Genet. 2010; 42(5):376–84. Epub 2010/04/13. https://doi.org/10.1038/ng.568 PMID: 20383146; PubMed Central PMCID: PMCPMC2997674.

17. Beer NL, Tribble ND, McCulloch LJ, Roos C, Johnson PR, Orho-Melander M, et al. The P446L variant in GCKR associated with fasting plasma glucose and triglyceride levels exerts its effect through increased glucokinase activity in liver. Hum Mol Genet. 2009; 18(21):4081–8. Epub 2009/08/01. https://doi.org/10.1093/hmg/ddp357 PMID: 19643913; PubMed Central PMCID: PMCPMC2758140.

18. Rees MG, Wincovitch S, Schultz J, Waterstradt R, Beer NL, Baltrusch S, et al. Cellular characterisation of the GCKR P446L variant associated with type 2 diabetes risk. Diabetologia. 2012; 55(1):70–7. https://doi.org/10.1007/s00125-011-2348-5 PMID: 22038520; PubMed Central PMCID: PMCPMC3278843.

19. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. The BMJ. 2009; 339:b2535. https://doi.org/10.1136/bmj.b2535 PMID: 19622551.

20. Wells GA, Shea B, O’Connell D, Peterson J, Welch V, Losos M, et al. The Newcastle–Ottawa Scale (NOS) for Assessing the Quality of Non-Randomized Studies in Meta-Analysis. 2010; 50(4):1088–10.

21. Higgins JP, White IR, Anzures-Cabrera J. Meta-analysis of skewed data: combining results reported on log-transformed or raw scales. Stat Med. 2008; 27(29):6072–92. https://doi.org/10.1002/sim.3427 PMID: 18903942; PubMed Central PMCID: PMC2978323.

22. Viechtbauer W. Conducting meta-analyses in R with the metafor package. Journal of Statistical Software. 2010; 36:1–48.

23. Lian J, Guo J, Chen Z, Jiang Q, Ye H, Huang X, et al. Positive association between GCKR rs780093 polymorphism and coronary heart disease in the aged Han Chinese. Dis Markers. 2013; 35(6):863–8. Epub 2014/01/05. https://doi.org/10.1155/2013/215407 PMID: 24385677; PubMed Central PMCID: PMCPMC3671702.

24. Nelson CP, Goel A, Butterworth AS, Kanoni S, Webb TR, Marouli E, et al. Association analyses based on false discovery rate implicate new loci for coronary artery disease. Nat Genet. 2017; 49(9):1385–91. Epub 2017/07/18. https://doi.org/10.1038/ng.3913 PMID: 28714975.

25. Raffield LM, Cox AJ, Carr JJ, Freedman BI, Hicks PJ, Langefeld CD, et al. Analysis of a cardiovascular disease genetic risk score in the Diabetes Heart Study. Acta Diabetol. 2015; 52(4):743–51. Epub 2015/02/24. https://doi.org/10.1007/s00592-015-0720-5 PMID: 25700702; PubMed Central PMCID: PMCPMC4506855.

26. Takeuchi F, Isono M, Katsuya T, Yokota M, Yamamoto K, Nabika T, et al. Association of genetic variants influencing lipid levels with coronary artery disease in Japanese individuals. PLoS One. 2012; 7(9):e46385. Epub 2012/10/11. https://doi.org/10.1371/journal.pone.0046385 PMID: 23050023; PubMed Central PMCID: PMCPMC3458872.

27. Zhou YJ, Hong SC, Yin RX, Yang Q, Cao XL, Chen WX. Polymorphisms in the GCKR are associated with serum lipid traits, the risk of coronary artery disease and ischemic stroke. Int J Clin Exp Med. 2015; 8(7):10678–86. Epub 2015/09/18. PMID: 26379859; PubMed Central PMCID: PMCPMC4565242.

28. Sterne JA, Sutton AJ, Ioannidis JP, Terrin N, Jones DR, Lau J, et al. Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. BMJ. 2011; 343:d4002. Epub 2011/07/26. https://doi.org/10.1136/bmj.d4002 PMID: 21784880.
30. Higgins JPT, Green S, (editors). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2011. Available from http://handbook.cochrane.org.

31. Nelson CP, Goel A, Butterworth AS, Kanoni S, Webb TR, Marouli E, et al. Association analyses based on false discovery rate implicate new loci for coronary artery disease. Nat Genet. 2017; 49(10):1450–7. https://doi.org/10.1038/ng.3943 PMID: 28869590; PubMed Central PMCID: PMC5844224.

32. Deloukas P, Kanoni S, Willenborg C, Farrall M, Assimes TL, Thompson JR, et al. Large-scale association analysis identifies new risk loci for coronary artery disease. Nature Genetics. 2013; 45(1);25–33. https://doi.org/10.1038/ng.2480 PMID: 23202125.

33. Schunkert H, Konig IR, Kathiresan S, Reilly MP, Assimes TL, Holm H, et al. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. Nat Genet. 2011; 43(4);333–8. Epub 2011/03/08. https://doi.org/10.1038/ng.784 PMID: 21378990; PubMed Central PMCID: PMCPMC3119261.

34. Yamada Y, Nishida T, Ichihara S, Kato K, Fujimaki T, Oguri M, et al. Identification of chromosome 3q28 and ALPK1 as susceptibility loci for chronic kidney disease in Japanese individuals by a genome-wide association study. J Med Genet. 2013; 50(6);410–8. Epub 2013/03/30. https://doi.org/10.1136/jmedgenet-2013-101518 PMID: 23539754.

35. Sveinbjörnsson G, Mikaelsson E, Palsson R, Indridason OS, Holm H, Jonasdottir A, et al. Rare mutations associating with serum creatinine and chronic kidney disease. Hum Mol Genet. 2014; 23(25);6935–43. Epub 2014/08/02. https://doi.org/10.1093/hmg/ddu399 PMID: 25082825.

36. Bonetti S, Trombetta M, Boselli ML, Turrini F, Malerba G, Trabetti E, et al. Variants of GCKR affect both beta-cell and kidney function in patients with newly diagnosed type 2 diabetes: the Verona newly diagnosed type 2 diabetes study 2. Diabetes Care. 2011; 34(5);1205–10. Epub 2011/03/18. https://doi.org/10.2337/dc10-2218 PMID: 21411509; PubMed Central PMCID: PMCPMC3114499.

37. Deshmukh HA, Palmer CN, Morris AD, Colhoun HM. Investigation of known estimated glomerular filtration rate loci in patients with type 2 diabetes. Diabet Med. 2013; 30(10);1230–5. Epub 2013/04/17. https://doi.org/10.1111/dme.12211 PMID: 23586973; PubMed Central PMCID: PMCPMC4204276.

38. Hishida A, Takashima N, Turin TC, Kawai S, Wakai K, Hamajima N, et al. GCK, GCKR polymorphisms and risk of chronic kidney disease in Japanese individuals: data from the J-MICC Study. J Nephrol. 2014; 27(2);143–9. Epub 2014/02/19. https://doi.org/10.1007/s40620-013-0025-0 PMID: 24535998.

39. Okada Y, Sim X, Go MJ, Wu JY, Gu D, Takeuchi F, et al. Meta-analysis identifies multiple loci associated with kidney function-related traits in east Asian populations. Nat Genet. 2012; 44(8);904–9. Epub 2012/07/17. https://doi.org/10.1038/ng.2352 PMID: 22797727; PubMed Central PMCID: PMCPMC4737645.

40. Pattaro C, Teumer A, Gorski M, Chu AY, Li M, Mijatovic V, et al. Genetic associations at 53 loci highlight cell types and biological pathways relevant for kidney function. Nat Commun. 2016; 7;10023. Epub 2016/02/03. https://doi.org/10.1038/ncomms10023 PMID: 26831199; PubMed Central PMCID: PMCPMC4735748.

41. Chambers JC, Zhang W, Sehmi J, Li X, Wass MN, Van der Harst P, et al. Genome-wide association study identifies loci influencing concentrations of liver enzymes in plasma. Nat Genet. 2011; 43(11);1311–8. Epub 2011/10/18. https://doi.org/10.1038/ng.970 PMID: 22001757; PubMed Central PMCID: PMCPMC3482372.

42. Kozian DH, Barthel A, Cousin E, Brunhofer R, Anderka O, Marz W, et al. Glucokinase-activating GCKR polymorphisms increase plasma levels of triglycerides and free fatty acids, but do not elevate cardiovascular risk in the Ludwigsfahnen Risk and Cardiovascular Health Study. Hormone and metabolic research = Hormon- und Stoffwechselfororschung = Hormones et metabolisme. 2010; 42(7);502–6. https://doi.org/10.1055/s-0030-1249637 PMID: 20352598.

43. Do R, Willer CJ, Schmidt EM, Sengupta S, Gao C, Peloso GM, et al. Common variants associated with plasma triglycerides and risk for coronary artery disease. Nat Genet. 2013; 45(11);1345–52. https://doi.org/10.1038/ng.2795 PMID: 24097064; PubMed Central PMCID: PMCPMC3904346.
46. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu C-y. Chronic Kidney Disease and the Risks of Death, Cardiovascular Events, and Hospitalization. New England Journal of Medicine. 2004; 351(13):1296–305. https://doi.org/10.1056/NEJMoa041031 PMID: 15385656.

47. Gu Y, Mao Y, Li H, Zhao S, Yang Y, Gao H, et al. Long-term renal changes in the liver-specific glucokinase knockout mouse: implications for renal disease in MODY2. Translational research: the journal of laboratory and clinical medicine. 2011; 157(3):111–6. https://doi.org/10.1016/j.trsl.2010.11.003 PMC3654931. PMID: 21316027.

48. Kolz M, Johnson T, Sanna S, Teumer A, Vitart V, Perola M, et al. Meta-analysis of 28,141 individuals identifies common variants within five new loci that influence uric acid concentrations. PLoS Genet. 2009; 5(6). Epub 2009/06/09. https://doi.org/10.1371/journal.pgen.1000504 PMID: 19503597.

49. Musso G, Gambino R, Tabibian JH, Ekstedt M, Kechagias S, Hamaguchi M, et al. Association of Non-alcoholic Fatty Liver Disease with Chronic Kidney Disease: A Systematic Review and Meta-analysis. PLOS Medicine. 2014; 11(7):e1001680. https://doi.org/10.1371/journal.pmed.1001680 PMID: 25050550.

50. De Cosmo S, Viazzi F, Pacilli A, Giorda C, Ceriello A, Gentile S, et al. Serum Uric Acid and Risk of CKD in Type 2 Diabetes. Clinical journal of the American Society of Nephrology: CJASN. 2015; 10(11):1921–9. Epub 2015/09/06. https://doi.org/10.2215/CJN.03140315 PMID: 26342044; PubMed Central PMCID: PMC4633786.

51. Lanktree MB, Theriault S, Walsh M, Pare G, HDL Cholesterol, LDL Cholesterol, and Triglycerides as Risk Factors for CKD: A Mendelian Randomization Study. Am J Kidney Dis. 2017. https://doi.org/10.1053/ajkd.2017.06.011 PMID: 28754456.

52. Simons N, Dekker JM, van Greevenbroek MM, Nipels G, t Hart LM, van der Kallen CJ, et al. A Common Gene Variant in Glucokinase Regulatory Protein Interacts With Glucose Metabolism on Diabetic Dyslipidemia: the Combined CODAM and Hoorn Studies. Diabetes Care. 2016; 39(10):1811–7. Epub 2016/09/24. https://doi.org/10.2337/dc16-0153 PMID: 27660121.

53. Ketteler M, Block GA, Evenepoel P, Fukagawa M, Herzog CA, McCann L, et al. Executive summary of the 2017 KDIGO Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD) Guideline Update: what’s changed and why it matters. Kidney international. 2017; 92(1):26–36. https://doi.org/10.1016/j.kint.2017.04.006 PMID: 28646995.

54. Dupuis J, Langenberg C, Prokopenko I, Saxena R, Soranzo N, Jackson AU, et al. New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. Nat Genet. 2010; 42 (2):105–16. Epub 2010/01/19. ng.520 [pii] https://doi.org/10.1038/ng.520 PMID: 20081858.

55. Tam CH, Wang Y, Lee HM, Luk AO, Tong PC, Chan MH, et al. Early gene-diet interaction between glucokinase regulatory protein (GCKR) polymorphism, vegetable and fish intakes in modulating triglyceride levels in healthy adolescents. Nutr Metab Cardiovasc Dis. 2015; 25(10):951–8. https://doi.org/10.1016/j.numecd.2015.06.011 PMID: 26234566.

56. Rousseaux J, Duhamel A, Dumont J, Dallongeville J, Molnar D, Widhalm K, et al. The n-3 long-chain PUFAs modulate the impact of the GCKR Pro446Leu polymorphism on triglycerides in adolescents. J Lipid Res. 2015; 56(9):1774–80. https://doi.org/10.1194/jlr.M057570 PMID: 26136510; PubMed Central PMCID: PMC4548781.