Blood HER2 and Uromodulin as Causal Mediators of CKD

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ABSTRACT

Many biomarkers have been epidemiologically linked with CKD; however, the possibility that such associations are due to reverse causation or confounding limits the utility of these biomarkers. To overcome this limitation, we used a Mendelian randomization (MR) approach to identify causal mediators of CKD. We performed MR by first identifying genetic determinants of 227 serum protein biomarkers assayed in 4147 participants of the Outcome Reduction with Initial Glargine Intervention (ORIGIN) trial who had early or prediabetes, and assessing the effects of these biomarkers on CKD in the CKD genetics consortium (n = 117,165; 12,385 cases) using the inverse-variance weighted (fixed-effects) method. We then estimated the relationship between the serum concentration of each biomarker identified and incident CKD in ORIGIN participants. MR identified uromodulin (UMOD) and human EGF receptor 2 (HER2) as novel, causal mediators of CKD (UMOD: odds ratio [OR], 1.30 per SD; 95% confidence interval [95% CI], 1.25 to 1.35; P = 5.3 × 10⁻²; HER2: OR, 1.30 per SD; 95% CI, 1.14 to 1.48; P = 8.0 × 10⁻²). Consistent with these findings, blood HER2 concentration associated with CKD in ORIGIN participants (OR, 1.07 per SD; 95% CI, 1.01 to 1.13; P = 0.01). Additional exploratory MR analyses identified angiotensin-converting enzyme (ACE) as a regulator of HER2 levels (β = 0.13 per SD; 95% CI, 0.08 to 0.16; P = 2.5 × 10⁻²). This finding was corroborated by an inverse relationship between ACE inhibitor use and HER2 levels. Thus, UMOD and HER2 are independent causal mediators of CKD in humans, and serum HER2 levels are regulated in part by ACE. These biomarkers are potential therapeutic targets for CKD prevention.


CKD is a growing public health problem that increases the risk of cardiovascular disease, kidney failure, and other complications.1 Whereas glucose lowering, BP lowering, and therapies that target the renin-angiotensin-aldosterone system (RAAS)2 can slow progression of CKD, the mechanism(s) by which they work are not fully understood. Elucidation of these and other CKD-related mechanisms may identify novel therapies, and the identification of causal biomarkers for CKD represents one promising approach. Unfortunately, candidate biomarkers identified using traditional epidemiologic approaches may be confounded with, or caused by, other unmeasured biomarkers or mechanisms. Under a strict set of assumptions,
Mendelian randomization (MR) can overcome these problems.3

MR is a powerful genetic methodology that is on the basis of the principle that genetic variants are inherited randomly and independently of other risk factors for disease. If the levels of a particular biomarker are affected by the presence of a genetic variant, and if that variant also affects the incidence of a disease, the variant may be causing the disease by modulating the levels of the risk factor. The random distribution of the genetic variant at birth minimizes the possibility of confounding or reverse causation as explanations for the link between the biomarker and disease, in the same way that the random allocation of a therapy in a randomized, controlled trial minimizes this possibility.4 These principles have been successful in identifying risk factors that are causal for CVD, and biomarkers identified using MR have been subsequently validated in randomized trials of therapeutic agents.5 Specifically, MR techniques have confirmed LDL cholesterol, IL-6 receptor, and lipoprotein(a) as causal biomarkers of coronary artery disease.6–8 Whereas there are fewer examples of MR in the field of nephrology, this approach has recently identified a causal effect of lower iron and ferritin levels on decreased kidney function and has ruled out a causal relationship between fetuin-A and mortality in patients on dialysis.9,10

MR methodology has traditionally been used to determine whether a candidate biomarker is causally related to a clinical outcome (clinicaltrials.gov; NCT 00069784). However, when combined with a large panel of biomarkers measured in a prospective study which accrued many clinical outcomes, it can also be used to identify new, unsuspected biomarkers that are likely to be causally related to the clinical outcome. We therefore sought to identify such CKD biomarkers by applying MR techniques to a comprehensive panel of 237 biomarkers covering cardiovascular, metabolic, and inflammatory processes within the recently completed Outcome Reduction with an Initial Glargine Intervention (ORIGIN) trial that was performed in people with type 2 diabetes or prediabetes.11

RESULTS

Identification of CKD Biomarkers Using MR
Two hundred twenty-seven serum biomarkers were tested for an association with CKD using an MR approach. After removing biomarkers without any significant cis single nucleotide polymorphism (SNP) associations and with minor allele frequency<0.05 (according to CKD genetics consortium [CKDGen]), 197 biomarkers were retained for downstream analysis. After MR analysis, two biomarkers were found to be significantly associated with CKD after Bonferroni correction for multiple hypothesis testing (P<0.05/197), namely uromodulin (UMOD, also known as Tamm–Horsfall glycoprotein) and human EGF receptor 2 (HER2). As noted in Figure 1, the MR analysis suggested a deleterious effect of UMOD (odds ratio [OR], 1.30 per SD; 95% confidence interval [95% CI], 1.25 to 1.35; P<5×10−20, number of SNPs=17) and HER2 (OR, 1.30 per SD; 95% CI, 1.14 to 1.48; P=8.0×10−5, number of SNPs=5) on CKD. All SNPs used in the MR models had P<0.01 and FDR<0.05 for the SNP-biomarker associations (Supplemental Tables 1 and 2). Regional plots of SNP associations with serum UMOD and HER2 at the UMOD and ERBB2 loci, respectively, are depicted in Supplemental Figure 1. To assess for the presence of unmeasured horizontal pleiotropy, we utilized the MR–Egger12 method where the y intercept is allowed to float, rather than be fixed at zero. We found no evidence of pleiotropy as determined by the significance of the y intercept (P>0.05). As a sensitivity analysis, we use a leave-one-out strategy in which MR analyses were repeated, excluding one variant at a time, and consistent estimates were obtained for each SNP excluded (see Supplemental Tables 3 and 4 for full results).

Association of UMOD and HER2 Concentration with CKD in ORIGIN
The MR-generated hypothesis that these biomarkers promoted CKD was then tested using the ORIGIN data. We therefore assessed the epidemiologic relationship of baseline UMOD and HER2 with incident CKD. We found that increased levels of blood UMOD were significantly associated with decreased risk of CKD, whereas increased levels of blood HER2 were associated with an increased risk of incident CKD in models adjusting for age, sex, and ethnicity (UMOD: OR, 0.83 per SD; 95% CI, 0.78 to 0.88; P<0.001 and HER2: OR, 1.07 per SD; 95% CI, 1.01 to 1.13; P=0.01). Consistent results were also observed in models fully adjusted for CKD risk factors and are provided in the Supplemental Material. We performed subgroup analyses to assess the consistency of the association of UMOD and HER2 concentration with CKD. No significant interaction across subgroups was observed after adjustment for multiple hypothesis testing (Figure 2).

Association of UMOD with Kidney Mass in Healthy Patients Undergoing Nephrectomy
We assessed for the possibility that reverse causation could play a role in the apparent discrepant epidemiologic association of UMOD with CKD (OR, 0.83; 95% CI, 0.78 to 0.88) and the evidence for UMOD as a CKD risk factor found in the MR (OR, 1.30; 95% CI, 1.25 to 1.35). Because UMOD is exclusively synthesized in the kidney, we hypothesized that UMOD is...
Figure 1. UMOD and HER2 identified as novel markers of CKD using MR. Forest plots depict a summary of the MR results for (A) UMOD and (B) HER2 at the UMOD and ERBB2 locus, respectively. A single SNP MR was conducted for each independent SNP (pairwise $R^2<0.1$). ORs were determined by the IVW method by regressing the effect estimates from the CKD association (from CKDGen) on the biomarker association (from ORIGIN). A two-tailed $P$-value was calculated using a z-test from 100,000 random simulations. IVW, inverse-variance weighted.
linked to a reduced kidney mass, and therefore lower UMOD expression, in patients with CKD. Therefore, the hypothesis that UMOD concentration is a marker of kidney mass, rather than CKD progression, was explored in ten healthy kidney donors. Briefly, participants had to meet clinical criteria for kidney donation; namely, normal BP, nonsmoker, eGFR > 80 ml/min, and absence of major chronic disease. Mean age was 49.9 years and 30% were male (see Supplemental Material for further details). Indeed, UMOD blood levels were almost halved after uninephrectomy as compared with the presurgery period (m = 217.7 ng/ml, SD = 75.6 versus m = 129.5, SD = 39.1; P = 7.6 × 10^{-2}, paired samples t test) (see Figure 3). We also tested UMOD levels in urine (indexed to creatinine) and found a significant, positive correlation between blood and urine levels (R^2 = 0.29, P = 0.02).

Identification of Regulators of UMOD and HER2 Using MR

To explore the mechanism by which UMOD and HER2 exert their effect on CKD, we tested for a causal effect of all other biomarkers on UMOD and HER2 levels using MR. Specifically, we investigated whether any of the other biomarkers play a causal role in the regulation of UMOD and HER2 through a similar MR analysis using cis SNPs for the biomarker under study (where possible) as instrumental variables. Significant cis SNPs (P < 0.001) were identified for 169 biomarkers (decreased from 197 due to the more stringent threshold applied for IV selection to the one sample MR design) and were thus tested for their effect on UMOD and HER2. No significant biomarkers were found as regulators of UMOD (P > 0.05/169). However, after adjusting for multiple hypothesis testing (P < 0.05/169), angiotensin-converting enzyme (ACE) was identified as a causal regulator of serum HER2 levels (β = 0.13 per SD; 95% CI, 0.08 to 0.16; P = 2.5 × 10^{-7}). We identified 16 independent cis ACE SNPs which were associated with ACE levels at P < 0.001 to be used as instrumental variables in the MR analysis. We also tested for pleiotropic effects of UMOD and HER2 with other CV traits using MR, but found no significant associations after multiple hypothesis testing (data not shown).

After the identification of ACE as a causal mediator of HER2, we decided to further explore this relationship by investigating the effect of different classes of BP-lowering medications on HER2 and ACE concentration in ORIGIN. The following medications were assessed as dichotomous (yes/no) variables: ACE inhibitors or angiotensin II receptor blockers (ARBs), diuretics (grouped as one variable), aldosterone inhibitors, β-blockers, and calcium channel blockers. Specifically, a linear model was used, with HER2 or ACE concentration as the dependent variable, and use of medication (yes/no) as the independent variable (Table 1). The models were adjusted for age, sex, ethnicity, hypertension diagnosis, and prior renal disease; HER2 models were further adjusted for serum ACE levels. We identified a significant association between use of ACE inhibitors/ARBs and HER2 concentration, indicating lower levels of HER2 in patients using ACE inhibitors or ARBs (β = 0.25 SD decrease with ACE inhibition; 95% CI, −0.30 to −0.20; P < 5 × 10^{-16}), consistent with our MR findings indicating ACE increases HER2 levels. Conversely, no other BP
medication was associated with lower levels of ACE after adjusting for multiple hypothesis testing ($P<0.05/5$); in fact, diuretics showed a marginal increase in HER2 levels consistent with an activation of the renin-angiotensin system with diuretics. Additionally, ACE inhibitors/ARBs, diuretics, and β-blockers were associated with increased levels of ACE, consistent with the RAAS inhibitory feedback loop. Aldosterone blockers and calcium channel blockers showed no effect on ACE levels after adjusting accounting for test multiplicity ($P<0.05/5$). A summary of ACE/HER2 findings and their effect on CKD risk can be seen in Figure 4.

**DISCUSSION**

Using MR to screen a comprehensive panel of 227 blood biomarkers, we identified blood UMOD and HER2 as causal mediators of CKD. UMOD is a kidney-specific protein ubiquitously expressed by the epithelial cells of the thick ascending loop of Henle. Under normal physiologic conditions, UMOD is the most abundant protein found in urine. Despite much research after its discovery over 50 years ago, the role of UMOD in renal physiology remains unclear. Clinical and experimental studies implicated UMOD in several forms of bacteria clearance from the urinary tract and in inflammatory kidney disease, although these findings were inconsistent. With the advent of large genome-wide association studies, UMOD has emerged as an important locus in both CKD and hypertension. A meta-analysis by Olden et al. identified an SNP in the UMOD promoter to be strongly associated with UMOD urinary levels, confirming the role of variants at the UMOD locus in UMOD excretion. Furthermore, this same allele was previously identified to be associated with increased CKD risk. These results indicate a positive relationship between urinary UMOD and CKD, consistent with our MR

![Figure 3.](image-url)
findings in blood. Notably, Trudu et al.\textsuperscript{19} have demonstrated that UMOD overexpression in transgenic mice led to salt-sensitive hypertension and activation of the renal sodium cotransporter NKCC2; this is consistent with their data in humans where pharmacologic inhibition of NKCC2 was found to be more effective in lowering BP in patients homozygous for UMOD risk variants. These findings establish a link between UMOD, hypertension, and CKD. Together with our results, these data point to UMOD as a therapeutic target for lowering BP and preserving renal function. However, it should be noted that it is impossible to know from these results if UMOD is acting through urine, blood, or possibly an intracellular mechanism to exert its risk on CKD. In our epidemiologic analysis, however, increased UMOD levels were associated with a decreased risk of incident CKD, consistent with other studies.\textsuperscript{20–22} Possible explanations for the divergent direction of effect between the MR and epidemiologic association include confounding and reverse causation. Although adjustment for relevant risk factors did not alter conclusions, our analysis in healthy patients undergoing nephrectomy revealed almost halving of UMOD blood levels after uninephrectomy in healthy donors, consistent with previous studies.\textsuperscript{23} Although we cannot rule out other biologic and statistical explanations, including residual confounding, these results suggest the protective epidemiologic finding may be a result of reverse causation, reflective of loss of nephron mass in progressive kidney disease.

HER2 is a member of the human EGF receptor (EGFR) family which are key regulators of cellular proliferation. The EGFR family has been implicated in CKD previously because EGFR signaling is involved in renal physiology through nephrogenesis, tissue repair, and electrolyte balance. Numerous experimental studies have shown that pharmacologic and genetic blockade of the EGFR system inhibits renal deterioration and fibrosis in animal models of kidney damage.\textsuperscript{24} Additionally, in three independent cohorts of patients with CKD, low urine excretion of EGF predicted accelerated loss of kidney function.\textsuperscript{25} The authors suggested that low urine EGF excretion reflects reserved concentration in the tubules and represents a key factor in CKD progression. EGFR has also been shown to play a role in hypertensive and diabetic nephropathy.\textsuperscript{26,27} For instance, EGFR expression is increased in the kidneys of hypertensive rats. Similarly, administration of gefitinib, an EGFR–tyrosine kinase inhibitor, improves renal function in rats with hypertension-induced renal disease.\textsuperscript{28} Furthermore, significant reduction of diabetes-associated glomerular hypertrophy and renal enlargement has been seen upon blockade of EGFR signaling.\textsuperscript{29} These animal studies are consistent with our MR and with epidemiologic findings suggesting a causal effect of HER2 on CKD progression and development. Currently, HER2 inhibitors are used clinically in EGFR-mediated cancers.\textsuperscript{30,31} Our data suggest to explore those drugs in models of kidney disease, as others have suggested.\textsuperscript{32}

We identified ACE as a positive regulator of HER2 levels, consistent with the observation of increased EGFR activity in hypertensive rats. Furthermore, we identified a lower concentration of HER2 in patients on ACE inhibitors or ARBs versus those not on these medications, consistent with the hypothesis that ACE does increase HER2 levels. Moreover, as medication control we found no evidence of other BP-lowering medications to have an effect on lowering HER2 levels. Together, these results implicate HER2 as a mediator by which ACE inhibitors and ARBs exert their protective effect on patients with CKD, beyond that of other classes of antihypertensive drugs.\textsuperscript{33,34} These results signify that blockade of RAAS through ACE inhibitors and ARBs does indeed reduce HER2, and also suggest that HER2 levels may be able to guide RAAS inhibition. Finally, as noted previously, HER2 inhibitors are commonly used to treat EGFR-mediated cancers. Therefore, given these findings, it is possible that inhibition of ACE would decrease cancer risk by decreasing HER2 levels. Indeed, a very large, cross-sectional cohort study, of nearly 300,000 individuals, identified a lower cancer risk in individuals on ACE inhibitors and ARBs as compared with those not on these medications.\textsuperscript{35} Although these results should be cautiously interpreted and may be confounded, they do suggest a novel application of ACE inhibitors which should be explored in future studies.

These findings are limited by the two-sample MR study design, where genetic estimates were obtained from independent populations. In such a design, weak instrumental variables lead to estimates which are biased toward the null hypothesis, which reduced the likelihood of type I error but decreased power.\textsuperscript{36} Thus not all of the causal biomarkers may have been identified. Additionally, the genetic variants studied may have other effects beyond their effect on the biomarker being studied (i.e., genetic pleiotropy). We mitigated this source of bias by limiting our investigation to variants at or near the gene coding for the biomarker of interest. Furthermore, associated loci were individually inspected for proximity to other potential genes and we did not identify any genes near the UMOD and ERBB2 loci that were plausible sources of pleiotropy.

Identification of CKD risk factors is instrumental to further our understanding of the disease, evaluate its risk, and guide treatment. Using MR, we have investigated a comprehensive

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**Table 1. Epidemiologic association of BP medications on HER2 serum levels**

<table>
<thead>
<tr>
<th>Medication</th>
<th>No. on Medication</th>
<th>β (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE inhibitors/ARBs</td>
<td>5641</td>
<td>−0.25 (−0.30 to −0.20)</td>
<td>&lt;5×10\textsuperscript{−16}</td>
</tr>
<tr>
<td>Diuretics</td>
<td>1309</td>
<td>0.08 (0.02 to 0.13)</td>
<td>0.01</td>
</tr>
<tr>
<td>Aldosterone antagonists</td>
<td>275</td>
<td>0.12 (0.01 to 0.24)</td>
<td>0.04</td>
</tr>
<tr>
<td>β blockers</td>
<td>4426</td>
<td>−0.04 (−0.09 to 0.01)</td>
<td>0.05</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>1561</td>
<td>−0.02 (−0.07 to 0.04)</td>
<td>0.54</td>
</tr>
</tbody>
</table>

Estimates are given for medication use (yes/no) using HER2 concentration as a dependent variable. Models were adjusted for age, sex, ethnicity, hypertension, prior renal disease, and blood ACE levels. Estimates are given for medication use (yes/no) using HER2 concentration as a dependent variable.
Our study presents the first MR analysis of blood UMOD and identified HER2 as a novel causal mediator of CKD, consistent with previous model systems and the known biologic role of these biomarkers. We also found compelling evidence to suggest HER2 as a mediator through which ACE inhibitors and ARBs protect against CKD progression and development. Increased serum UMOD and HER2 concentrations represent independent mechanisms leading to CKD, which can be assessed through a simple blood test. These findings pave the way for risk stratification and therapeutic interventions and provide important insights into the pathophysiology of CKD, and its relation to the current clinical practice of ACE inhibitors and ARBs in CKD treatment. Future research should be aimed at identifying the causal mechanisms and whether interventions targeted at reducing UMOD and HER2 levels can reduce CKD.

CONCISE METHODS

Study Population—ORIGIN

The design and findings of the ORIGIN trial have been described in detail.\textsuperscript{37,38} Briefly 12,537 people with established cardiovascular risk factors who also had diabetes, impaired glucose tolerance, or impaired fasting glucose were studied. After random allocation to two therapies using a factorial design (basal insulin glargine versus standard care and omega 3 fatty acid supplements versus placebo), they were followed for a median of 6.2 years for cardiovascular events and other health outcomes. The ethics committee at each participating site approved the trial, and all participants provided written informed consent. As previously described,\textsuperscript{38} a subset of 8401 participants from the ORIGIN trial consented to further biologic analysis and were therefore included in the biomarker ORIGIN substudy. Biomarker levels were analyzed using the serum that was drawn at the beginning of the study (a detailed description of biomarker measurement and quality control is found in the Supplemental Material and a complete list of biomarkers analyzed is found in Supplemental Table 5).

A further subset of the 8401 participants in the biomarker substudy also consented to genetic analyses and were genotyped on Illumina’s HumanCore Exome chip, comprising 5078 ORIGIN individuals. Standard quality control measures were assessed. After quality control, the sample consisted of 4147 participants and 284,024 SNPs from two ethnic groups (European and Latin American). Imputation was then performed on the post-QC data to predict unobserved genotypes in the study population. Over 30 million SNPs were imputed, allowing for comprehensive coverage of known genetic variants (a detailed description of quality control and imputation procedures is in the Supplemental Material). Key clinical characteristics of the study populations are shown in Supplemental Table 6.

CKDGen Consortium Data

Genetic data on SNP associations with CKD (defined as eGFR\textsubscript{crea} < 60 ml min\textsuperscript{-1} per 1.73 m\textsuperscript{2}) were obtained from the CKDGen database and downloaded from https://www.nhlbi.nih.gov/research/intramural/researchers/ckdgen. Specifically, we used the most recent meta-analysis (released in 2015) of 43 genome-wide association studies with up to 117,165 individuals for CKD (12,385 cases), of European descent.\textsuperscript{39}

SNP Association with Biomarkers and CKD

The analysis was restricted to biomarkers directly encoded by a gene(s) on autosomal chromosomes (i.e., chromosomes 1–22). Thus, removal of five biomarkers because they were products of genes on the X chromosome, and five biomarkers because they were not a direct gene product (e.g., cortisol), left 227 biomarkers for analysis.

SNP selection was carried out in four steps. First, as noted in Supplemental Figure 2, we restricted our analysis for each of the 227 biomarkers to SNPs within 300 kb of the gene(s) encoding the corresponding protein or protein component, hereafter referred to as \textit{cis} associations. This process identified 1,067,955 SNP/biomarker

\textbf{Figure 4.} Proposed mechanism by which HER2 mediates the effects of ACE on CKD risk. Flow chart shows summary of ACE/HER2 results, red lines depict epidemiological associations, and blue lines depict MR associations. Black lines indicate previously known relationships.
cis pairs (note that some SNPs are in cis with multiple biomarkers). Second, after removing SNPs not found in the CKDGen database and those with a minor allele frequency < 0.05 according to CKDGen, we estimated the relationship between the remaining SNPs and their corresponding cis biomarker(s) in ORIGIN, by regressing each SNP against the concentration of its cis biomarker (with biomarker concentration as the dependent variable and SNP dosage as the independent variable). In other words, for each biomarker we only tested SNPs near the respective encoding gene(s). The regression models were first computed in each ethnic group separately, adjusting for age, sex, and the first five principal components, using SNPassist.40 The ethnic-specific models were then meta-analyzed across the two ethnicities using fixed-effects models to minimize the risk of confounding caused by population stratification. Third, cis SNPs with a biomarker association of P < 0.01 were selected. Finally, SNPs were pruned for linkage disequilibrium at a stringent threshold of r² < 0.1 using the 1000 Genomes data (Europeans) to ensure associations retained for MR analysis were nonredundant. SNPs were selectively prioritized on the basis of the significance of the association with their biomarker. For each biomarker, the cis SNP with the most significant association with the biomarker was first retained and all SNPs in linkage (r² > 0.1) with that SNP removed. This process was then repeated for any remaining SNPs. Thus, 1307 cis SNP/biomarker associations remained after pruning. SNP filtering was performed in R (version 3.0.1) and PLINK was used to calculate R² statistics in 1000 genomes. A summary of the SNP and biomarker selection can be found in Supplemental Figure 3.

Identification of Blood Mediators of CKD Using MR

A two-sample MR was performed on the 197 biomarkers which had at least one significant cis SNP (P < 0.01) and that were also found in the CKDGen data. Input variables for the MR analysis for each biomarker were (1) the β coefficients of the SNPs on their cis biomarker that were estimated using the regression models above, and (2) the β coefficients of the SNPs on CKD that were estimated from the CKDGen (Supplemental Figure 4). MR associations were performed using the inverse-variance weighted (IVW) method by regressing genetic effect estimates for CKD (dependent variable) on genetic effect estimates of biomarkers. To determine significance, a bootstrap method was used under the null hypothesis of no effect between CKD and biomarkers. Predicted effects on CKD were sampled from a normal distribution with mean and SDs as determined from CKDGen. A two-tailed P value was calculated using a z-test from 100,000 random simulations. In other words, CKD estimates for each SNP were sampled 100,000 times and regressed onto the corresponding β estimates from ORIGIN (this procedure is equivalent to the IVW fixed effect method). Biomarkers were deemed significant after adjusting for multiple testing hypothesis (P < 0.05/197).

Association of Biomarker Levels with Incident CKD in ORIGIN

Once significant biomarkers were identified by the MR analysis, we tested whether biomarker levels showed a consistent association with incident CKD in 8197 ORIGIN participants with ethnicity information and biomarker levels. CKD was defined as the composite of either doubling of serum creatinine, worsening in albuminuria category, RRT, or death due to end stage renal failure. This was assessed using logistic models with incident CKD as the dependent variable and biomarker concentration as the independent variable of interest. Models were adjusted for age, sex, and ethnicity to remain consistent with MR model adjustment. We also tested models after further adjusting for prior type 2 diabetes, prior renal disease, BMI, current smoker, diagnosis of hypertension, baseline eGFR, and LDL. Subgroup analyses were performed to test for heterogeneity between groups using models adjusted for age, sex, and ethnicity (where appropriate).

Identification of Regulators of CKD Biomarkers Using MR

To gain further biologic insights regarding the novel CKD biomarkers, a second set of MR analyses were then performed to explore whether the levels of the novel CKD-causing biomarkers were determined by any of the other biomarkers. Specifically, we tested all biomarkers for an effect on both UMOD and HER2 levels (i.e., the novel biomarkers identified in the CKD MR), where the input variables for each MR were (1) the effect of SNPs on their cis biomarker as the independent variable (where possible), and (2) the effect of the same set of SNPs on the novel CKD biomarkers as the dependent variable. Because of the fact that both of these estimates were obtained from ORIGIN (i.e., one-sample), a more conservative significance threshold of P < 0.001 (or F > 10) was applied for the inclusion of SNPs into the MR model because weak instruments can bias results toward false-positives.42 Statistical analyses were performed using R (version 3.0.1), unless stated otherwise. A summary of the analysis plan can be seen in Figure 5.

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Figure 5. Flow chart depicts a summary of the four analyses conducted and their respective sample sizes.
J.S., H.G., and G.P. designed the study, planned the analyses, interpreted the results, and wrote the first draft of the report. J.S. performed the statistical and bioinformatics analyses. All authors contributed to the critical reading and revision of the manuscript.

DISCLOSURES

H.G. has received consulting fees from Sanofi, Novo Nordisk, Lilly, AstraZeneca, Boehringer Ingelheim, and GlaxoSmithKline and support for research or continuing education through his institution from Sanofi, Lilly, Takeda, Novo Nordisk, Boehringer, Ingelheim, and AstraZeneca. G.P. has received consulting fees from Sanofi, Bristol Myers Squibb, Lexicomp, and Amgen and support for research through his institution from Sanofi. S.H. is an employee of Sanofi, J.F.E.M. has received consulting fees from Novo Nordisk, AstraZeneca, Amgen, Braun, ACI, Fresenius, Celgene, Gambauro, Abibbie, Roche, Sandoz, Lanthio, Sanifit, Relypsam, and ZS Pharma; and grants from the European Union, McMaster University Canada, Abibbie, Medice, Novo Nordisk, Roche, and Sandoz. J.S., D.T., and M.W. report no conflicts.

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