Novel biomarkers of inflammation, kidney function and chronic kidney disease in the general population

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Running title: Inflammation and kidney function

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ABSTRACT

Background. Inflammatory processes have been implicated in the development of chronic kidney disease (CKD). We investigated the association of a large panel of inflammatory biomarkers reflecting aspects of immunity with kidney function and CKD incidence.

Methods. We used data from two independent population-based studies, KORA F4 (discovery, n=1,110, mean age 70.3 years, 48.7% male) and ESTHER (replication, n=1,672, mean age 61.9 years, 43.6% male). Serum levels of biomarkers were measured using proximity extension assay technology. The association of biomarkers with estimated glomerular filtration rate (eGFR) at baseline and with incident CKD was investigated using linear and logistic regression models adjusted for cardiorenal risk factors. Independent results from prospective analyses of both studies were pooled. The significance level was corrected for multiple testing by false-discovery rate ($P_{FDR} < 0.05$.)

Results. In the KORA F4 discovery study, 52 out of 71 inflammatory biomarkers were inversely associated with eGFR estimated based on serum creatinine. Top biomarkers included CD40, TNFRSF9 and IL10RB. Forty-two of these 52 biomarkers were replicated in the ESTHER study. Nine of the 42 biomarkers were associated with incident CKD independently of cardiorenal risk factors in the meta-analysis of the KORA (n=142, mean follow-up of 6.5 years) and ESTHER (n=103, mean follow-up of 8 years) studies. Pathway analysis revealed the involvement of inflammatory and immunomodulatory processes reflecting cross-communication of innate and adaptive immune cells.

Conclusions. Novel and known biomarkers of inflammation were reproducibly associated with kidney function. Future studies should investigate their clinical utility and underlying molecular mechanisms in independent cohorts.

Keywords: chronic kidney disease, glomerular filtration rate, inflammation, population cohort, proteomics

KEY LEARNING POINTS

What is already known about this subject?

- In clinical practice, the glomerular filtration rate (GFR) remains to be one of the most important markers used to evaluate chronic kidney disease (CKD) diagnosis and prognosis. However, GFR represents functional information without pathophysiological insights underlying kidney impairment processes.
- There is an increasing necessity to identify novel biomarkers for early kidney impairment and deliver better insight into pathophysiological pathways.

What this study adds?

- In extension to previous studies that have been limited by small sample size, cross-sectional study design and restrictions to patients with diabetes or advanced CKD stages, we examined a relatively large number of participants from two population-based cohorts to identify novel protein biomarkers for early kidney impairment and CKD development.
- We identified 42 inflammatory biomarkers to be inversely associated with GFR estimated based on serum creatinine in both the discovery and validation study. Nine out of these were nominally significantly associated with CKD incidence.
- Pathway analysis revealed the involvement of inflammatory and immunomodulatory processes reflecting cross-communication of innate and adaptive immune cells.

What impact this may have on practice or policy?

- The application of proteomics in easily accessible tissues is of particular interest in population-based settings, where early screening interventions could facilitate identifying high-risk individuals of CKD.

INTRODUCTION

Chronic kidney disease (CKD) affects between 8% to 16% of the population in high-income countries and the prevalence continues to increase, accelerated by risk factors such as diabetes, hypertension and obesity [1-3]. In the setting of an aging population, 35% of those older than 70 years are diagnosed with an early stage of CKD [3, 4]. In turn, kidney impairment is an independent risk factor for cardiovascular morbidity, mortality and decreased quality of life, which is expected to put a high burden on health care systems [5, 6].

In clinical practice, the glomerular filtration rate (GFR) based on creatinine remains to be one of the most important markers used to evaluate CKD diagnosis and prognosis. However, this marker represents functional information without pathophysiological insights underlying kidney impairment processes. Also, no targeted therapies exist for CKD beyond the management of traditional cardiometabolic risk factors. Therefore, there is an increasing necessity in kidney research to identify novel biomarkers for early kidney impairment and deliver better insight into pathophysiological pathways, a knowledge that can be used to design possible drug targets.

Several pathways have been hypothesized to play an important role in CKD development, with systemic inflammation being one of the most prominent [7]. However, the molecular mechanisms are largely unknown. Previous evidence suggests that, for example, tumor necrosis factor receptor superfamily members predict progression to end-stage renal disease in individuals with diabetes [8]. Other efforts have attempted to identify markers of renal function but they have been characterized by limited sample size, cross-sectional study design or they were performed in subpopulations of people with diabetes or advanced CKD

stages [9-12]. Investigations in the general population with a longitudinal design in relation to CKD development are rare [13].

High-throughput proteomics approaches developed in recent years allow the simultaneous deeper characterization of immune pathways in easily accessible tissues such as blood that might be implicated in inflammatory processes in CKD [14, 15].

Therefore, our study aimed to investigate and validate associations between a multiplex assay panel of biomarkers of inflammation and kidney function in two population-based cohorts of middle-aged and older adults in a discovery/replication design. Biological pathways of significant biomarkers for kidney function were further explored. A prospective analysis was performed to identify novel biomarkers of CKD development, independent of established cardiorenal risk factors.

MATERIALS AND METHODS

Study population

The Cooperative Health Research in the Region of Augsburg (KORA) Study

The KORA F4 (2006–2008) and FF4 (2013-3014) studies are both follow-up examinations of the population-representative KORA S4 study (1999–2001), conducted in Augsburg (Germany) and two surrounding counties [16]. The studies were carried out in accordance with the Declaration of Helsinki, including written informed consent from all participants, and were approved by the ethics committee of the Bavarian Chamber of Physicians (Munich, Germany). The KORA F4 study was used as a discovery sample. From 1,161 KORA F4 participants aged 62-81 years, after excluding participants as shown in the flow chart of **Supplementary Figure 1.A**, the final baseline study population resulted in 1,110 participants

with available proteomics data. For the prospective analysis, there were 576 participants without CKD at baseline. The study design and other standard physical and medical examinations have previously been described in detail [17, 18]. A drop-out analysis comparing participants and non-participants in KORA FF4 was published before [16].

ESTHER Study

For the baseline examination (2000–2002) of the ESTHER study (German study name: **E**pidemiologische **S**tudie zu Chancen der Verhütung, Früherkennung und optimierten **TH**erapie chronischer **ER**krankungen in der älteren Bevölkerung), 9,940, individuals aged 50–74 years were recruited by their general practitioners (GPs) during a routine health check-up in the German federal state of Saarland. The study design has previously been described in detail [19]. The cohort study is ongoing and was followed up for incident chronic diseases after 2, 5, 8, 11, and 14 years by standardized questionnaires sent to study participants and their GPs. The ESTHER study has been approved by the ethics committees of the Medical Faculty of the University of Heidelberg and the Medical Association of Saarland and has been conducted in accordance with the Declaration of Helsinki. The ESTHER study was used as a replication sample using the baseline visit and the 8-year follow-up visit (to approximate a similar follow-up time as in KORA F4/FF4). **Supplementary Figure 1.B** describes the study population selection for the present study. Biomarkers were measured in a randomly selected subsample of n=1,750 study participants due to limited funding availability. After exclusion of serum samples, which did not pass quality control or missed a serum creatinine measurement, 1,672 individuals were available at baseline with both clinical and proteomics assay data. The prospective analysis resulted in 675 participants, after excluding CKD cases at baseline and individuals lost to follow-up.

Measurement of outcomes

Renal function was defined based on estimated GFR from standard creatinine (eGFRcr). Standard creatinine was calculated using the following equation: standard creatinine= 0.95 × calibrated creatinine [20]. eGFRcr was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI) [21]. CKD cases were defined as having eGFRcr < 60 ml/min per 1.73m^2. Additionally, estimated GFR from cystatin C (eGFRcys) and urine albumin to creatinine ratio (UACR) were also calculated. For further details on the outcomes, please refer to **Supplementary Text 1**.

Measurement of biomarkers of inflammation

Biomarkers of inflammation from KORA and ESTHER participants were measured in serum samples of fasting participants using the OLINK Inflammation multiplex immunoassay (OLINK Proteomics, Uppsala, Sweden). The OLINK Inflammation panel covers 92 protein biomarkers including pro-and anti-inflammatory cytokines, chemokines, growth factors and factors involved in acute inflammatory and immune responses, angiogenesis, fibrosis and endothelial activation. More information on the biomarkers can be found in a previous publication of our group [22]. This immunoassay is based on the proximity extension assay (PEA) technology, measuring the relative abundance of the proteins [23, 24]. After exclusion of some of the biomarkers (**Supplementary Text 1**), 71 markers were used for analysis in KORA.

Individual information of the OLINK and CKD related measurements in respective studies can be found in **Supplementary Text 1** and **Supplementary Table 1**.

Statistical analysis

Continuous variables are reported as mean ± SD for normally distributed data and as median [interquartile range (IQR)] for skewed data. Categorical variables are presented as total numbers with the corresponding percentage. We log-transformed all variables that did not follow a normal distribution. All biomarkers of inflammation were analyzed per unit change in standard deviation (SD).

In the cross-sectional part of the study, linear regression models were performed to assess associations between biomarkers of inflammation and eGFRcr. Model 1 was age- and sex-adjusted. In both studies, a multivariable model (model 2) was compiled, including a wide range of established cardiorenal risk factors such as age, sex, body mass index (BMI), systolic blood pressure, use of antihypertensive medication, triglycerides (naturally log-transformed), high-density lipoprotein cholesterol, use of lipid-lowering medication, smoking, physical activity, alcohol intake, prevalent diabetes, use of glucose-lowering medication and prevalent cardiovascular disease. For the ESTHER analysis, the multivariable model accounted for plate effects as well because samples were measured at two distinct time points. Correlations between biomarkers of inflammation that showed significant associations with kidney function were estimated using Pearson's correlation coefficients. Linear regression models were also used to assess associations between biomarkers of inflammation and UACR in participants of the F4 study, adjusting for all covariates in the above model 2 and baseline eGFR.

The significance level was corrected for multiple testing by a false-discovery rate (FDR) method using the Benjamini-Hochberg procedure [25]. A FDR-adjusted p-value (P_{FDR}) < 0.05 was considered statistically significant.

To investigate the prospective association of biomarkers of inflammation with CKD incidence, logistic regression analysis was performed in a multivariable model in both cohorts with participants without CKD at baseline. Additional adjustment in the multivariable model for baseline eGFRcr levels was performed in this analysis. To account for individual follow-up times, a Cox proportional hazards model would have been most appropriate; however, given our population-based setting in both studies, we did not know the exact times when the events happened. Furthermore, especially the exclusion of participants who died during the follow-up period could have introduced some selection bias of healthier participants. The results of the discovery and replication sample were pooled in an inverse-variance weighted fixed-effects meta-analysis to maximize the statistical power. The significance level was set to a nominal p-value < 0.05. Data analysis was performed with R-Studio v. 1.2.1335. Except for HDL-cholesterol levels in the ESTHER study which had 40% missing values, all other covariates in both studies had a relatively low percentage of missing values (~10%). In order to retain as much data from the study participants and maximize power, missing data were imputed by multiple imputations. Afterwards, the results from five datasets were pooled to obtain the final estimates.

Ingenuity Pathway Analysis (IPA) software (QIAGEN, Hilden, Germany) was used to identify biological pathways that are enriched for biomarkers of inflammation associated with kidney function in the fully adjusted model.

RESULTS

Characteristics of the study population

Table 1 summarizes the baseline characteristics for the participants from the KORA and ESTHER studies. The largest differences between the two cohorts were observed for age, systolic blood pressure, LDL cholesterol, prevalence of diabetes, use of glucose-lowering and lipid-lowering medication. Mean baseline eGFRcr levels were lower in KORA than in ESTHER participants. Nevertheless, ESTHER study participants had a higher prevalence of CKD at baseline (17.2% vs 14.1% in KORA). Clinical and renal characteristics stratified by incident CKD status are presented in **Supplementary Table 2**.

Association of biomarkers of inflammation with kidney function

A flowchart of the study analysis is shown in **Figure 1**. First, we investigated the multivariable-adjusted associations of biomarkers of inflammation with eGFRcr in 1,110, participants of the KORA study. Fifty-two out of 71 inflammatory markers were (P_{FDR} < 0.05) and inversely associated with eGFRcr (**Table 2**). The five biomarkers with the lowest P_{FDR} value for the association with eGFRcr were CD40 (P_{FDR} < 3.34E-40), TNFRSF9 (P_{FDR} < 6.34E-32), IL10RB (P_{FDR} < 1.59E-31), CST5 (P_{FDR} < 2.42E-27) and CX3CL1 (P_{FDR} < 2.42E-27). Pairwise correlations of the 52 biomarkers with significant associations with eGFRcr are shown in **Figure 2**. The biomarkers were mostly positively correlated with a mean correlation coefficient of 0.28 (SD 0.16, range -0.10 to 0.8). Additionally, we investigated the association of these biomarkers with eGFR based on cystatin C (mean (SD), 75.71 (17.93) ml/min/1.73 m^2). All but one biomarker were found to be also significantly associated with eGFRcys, reaffirming that the identified biomarkers robustly represent renal filtration. **Supplementary Figure 2** represents a scatter plot of beta estimates of the association of biomarkers of

inflammation with eGFRcr against those of eGFRcys (r=0.69, P <2E-16). Beta estimates for associations of all individual biomarkers of inflammation with eGFRcr and eGFRcys are presented in **Supplementary Table 3** (KORA F4). Beta estimates of the association of 63 biomarkers with eGFRcr at baseline for ESTHER are presented in **Supplementary Table 4.**

Forty-five out of 52 discovery markers were available in ESTHER. In this replication cohort, we found 42 out of these 45 biomarkers to be significantly and inversely associated with eGFRcr in the multivariable model (threshold: $P_{FDR} < 0.05$) (**Table 2**).

Association with incident CKD

During a mean follow-up time of 6.5 years in 576 participants free of CKD at baseline, we identified 141 cases with incident CKD in the KORA study (incidence rate, 3.7 per 100 person-years). For the ESTHER study, during a mean follow-up time of 8 years in 675 participants free of CKD at baseline, 103 incident cases were identified with CKD (incidence rate, 2.1 per 100 persons-years). To increase the power of our investigation, we meta-analyzed the prospective results of CKD incidence (n=244 cases) from both KORA and ESTHER. All replicated 42 biomarkers were investigated using multivariable models further adjusted for baseline eGFRcr levels. Nine out of the 42 biomarkers (IL8, MCP3, EN_RAGE, MCP1, MCP4, CD5, MMP10, TNFRSF9, and OSM) were nominally significantly associated with CKD incidence. The odds ratios of associations between these biomarkers and incident CKD ranged from 1.22 (e.g. for OSM and MMP10, P=0.042 and P=0.032, respectively) to 1.36 (e.g. for IL8, P=0.001). **Supplementary Table 5** shows the association of replicated biomarkers with CKD in KORA and ESTHER, separately. Adjusting the models for the difference in time (years) between measurements of eGFRcr at baseline and follow-up did not change the results.

Moreover, when investing the association of the biomarkers with UACR, a marker of kidney damage, more than half of the 42 replicated markers were nominally significant among participants of the KORA F4 study **(Supplementary Table 6).**

Pathway analysis

Ingenuity pathway analysis revealed 15 canonical pathways (P_{FDR}<0.01), which were enriched for 42 biomarkers of inflammation associated and replicated with kidney function in the fully adjusted model in both studies **(Table 3)**. Top pathways involved inflammatory responses related to innate immunity such as granulocyte adhesion and diapedesis, cell signalling, cell differentiation or maturation; other pathways related to adaptive immunity with the involvement of T cell signalling and activation. These pathways suggest communication between innate and adaptive immune cells. The IPA analysis revealed several processes related to conditions such as atherosclerosis, (rheumatoid) arthritis, allergy, influenza and other hepatic components.

DISCUSSION

In this study, we identified a panel of biomarkers of inflammation associated with kidney function in two population-based studies: the KORA and the ESTHER study. All biomarkers (n=42) were inversely associated with eGFRcr and were involved in processes related to a variety of inflammatory and immune-modulatory responses linked to kidney disease. Higher serum concentrations of 9 of these biomarkers (IL8, MCP3, EN_RAGE, MCP1, MCP4, CD5, MMP10, OSM, and TNFRSF9) were found to be associated with increased risk of incident CKD independent of other cardiorenal risk factors.

While most of the identified biomarkers of inflammation represent novel findings in relation to kidney function, some of them have been previously reported to play an important role in kidney pathophysiology. A previous study investigated the association of another panel of 92 biomarkers related to cardiovascular diseases with eGFR decline in a discovery sample of 687 participants and replicated the top biomarkers in a sample of 360 men [13]. This panel included several biomarkers also measured in the present study. They found 20 markers to be nominally significantly associated with eGFR decline, three of which were identified also in our cross-sectional analysis: CD40 receptor, monocyte chemotactic protein 1 (MCP-1) and fibroblast growth factor 23 (FGF-23). These biomarkers have been previously reported to be involved in progressive kidney disease [26-28]. In the community-based InCHIANTI, IL-18 did not show any significant association with kidney function and incident CKD as opposed in our analysis, in which the biomarker was inversely associated with eGFR [9].

Nine biomarkers of inflammation from our panel were associated with CKD development after adjustment for cardiorenal risk factors. Some of them have been previously related to kidney pathology. For example, among the three chemokines, MCP3, MCP4, and MCP1, the latter is involved in promoting inflammation, renal injury and fibrosis in diabetic nephropathy [29, 30]. EN_RAGE represents a calcium-binding proinflammatory protein mainly secreted by granulocytes and has been hypothesized to link CKD and CVD through pathways related to vascular calcification and endothelial dysfunction [31]. TNFRSF9 is a member of the TNF receptor superfamily involved in biological mechanisms such as cell survival, proliferation and death together with other immunomodulatory responses linked to kidney disease. A previous study used an aptamer-based approach to measure a panel of 194 proteins that included many of the circulating inflammatory proteins known in the literature. The study identified a kidney risk inflammatory signature (KRIS) comprised of 17 inflammatory

proteins enriched for TNF-R superfamily members to be significantly associated with a 10-year risk of end-stage renal disease among patients with diabetes [8]. In line with our pathway analysis, the study supported the involvement of both innate and adaptive immune response. The study also found no correlation between the majority of the KRIS proteins and their corresponding gene expression in kidney biopsy specimens in 56 participants. These results suggest that non-kidney sources (such as leukocytes) may play an important role in generating these KRIS proteins.

Another longitudinal study in individuals with type 2 diabetes (PROVALID) investigated 17 plasma biomarkers in relation to kidney function decline and found among others two of our identified biomarkers, FGF23 and HGF, to be associated with eGFRcr decline over time in the multivariable analysis [32]. The authors reported that baseline eGFR exhibited the largest explained variability of kidney decline, whereas the identified markers explained only a small percentage. In our prospective analysis, nine out of forty-two markers were significantly associated with CKD incidence. In the individual studies, most of our associations between biomarkers and CKD were also explained by baseline eGFR levels. These findings suggest that inflammation may to some extent be a consequence rather than a cause of CKD. Integration of proteomics with genetic data might allow Mendelian randomization-based approaches to test whether markers lie in causal pathways to kidney impairment or CKD development [33].

When investigating the associations of the biomarkers with UACR, more than half of the 42 replicated biomarkers associated with eGFR at baseline and 6 out of the 9 biomarkers associated with incident CKD were found to be nominally significant. UACR is often used in clinical studies as a risk marker for CKD progression, with slightly raised UACR levels

indicating early-stage kidney disease and very high levels indicating more severe kidney disease. The partial overlap of biomarkers (e.g., IL8, EN_RAGE, and TNFRSF9) between different outcomes (i.e., baseline eGFR, baseline UACR, incident CKD) in our study hints that some of the inflammatory biomarkers may play a role in pathophysiological mechanisms involved in the development of CKD (through glomerular filtration rate), but also albuminuria. Along the same line, a recent cross-sectional study reported that urinary IL8 and EN_RAGE levels were positively associated with urinary albumin in 90 participants of the Uppsala Seniors Study [34]. Another study, conducted among 200 individuals with advanced CKD, found that plasma EN_RAGE levels were higher in CKD stage 5 patients compared with CKD stage 3-4 patients and healthy controls [35]. Additionally, EN_RAGE was associated with increased mortality risk in stage 5 CKD patients. Of note, evidence from in vivo studies has suggested that the involvement of IL8 in the pathology of urinary albumin excretion might be mediated through the metabolic alterations of compounds located in the glomerular basement membrane [36]. In case of our population-based setting, with most of the participants having low to moderate risk of disease progression, we did not have the ideal study design to investigate the role of inflammation in advanced stages. Therefore, there is a need for future prospective studies to reassess the association of these biomarkers with CKD, and in particular, their association with advanced stages of CKD.

It is noteworthy that the identified biomarkers for incident CKD are unlikely to be specific. We and others have shown that proteins such as EN_RAGE, IL8 or TNFRSF9 are related to several clinical outcomes such as incident polyneuropathy, diabetes or cardiovascular disease in population-based settings [22, 37, 38].

Ongoing investigations in the proteomics field provide an opportunity not only to shed light on new pathophysiological mechanisms, but also to discover biomarkers that could improve risk prediction for CKD. Promising results have been shown using a urine protein-based classifier consisting of 273 CKD biomarkers, which improved prediction accuracy more than standard measures for detection and prediction of CKD [39, 40]. The application of proteomics in easily accessible tissues is of particular interest in population-based settings, where early screening interventions could facilitate identifying high-risk individuals.

Strengths of our study include the discovery/replication design, sample size, the prospective design and the use of the same proteomics platform in both studies. Moreover, we were able to adjust for a wide range of cardiorenal risk factors including use of medication and comorbidities. Given that most of the biomarkers are novel, we used non-conservative methods of adjustments such as P_{FDR} for kidney function and nominal ($P<0.05$) significance for CKD incidence to avoid missing any potentially important biomarkers.

We are also aware of the limitations of our study. Although we adjusted our models for potential confounders, particularly baseline eGFR, some residual confounding might still be present. Protein levels appear to be highly dynamic and influenced by environmental stimuli. We had only one time-point of biomarker measurements in our studies. In the future, longitudinal trajectories of these biomarkers should be analyzed to better understand the directionality of associations. We combined our CKD analysis to optimize the power, but other larger studies are encouraged to replicate the findings individually, preferably using time to event modelling (if data allow). Pathway analysis represents selected results based on the biomarkers under study; therefore, we do not exclude other inflammatory pathways involved in kidney function impairment. The average age of the population-based KORA

study was relatively high resulting in considerable loss to follow-up. However, this is an inherent characteristic of population-based studies in particular in the middle-aged and elderly. The generalizability of our results is limited to a middle-aged and elderly population of European ancestry.

CONCLUSION

Multiple biomarkers of inflammation were associated with kidney function and CKD incidence in two population-based studies, highlighting the role of inflammation with the possible involvement of both innate and adaptive immunity in kidney function impairment. Future investigations are required to replicate these findings in independent cohorts and potentially integrate the results with other omics technologies and kidney tissue-based investigations.

ACKNOWLEDGEMENTS

JSL would like to thank the China Scholarship Council (CSC) for the financial support (No. 202008440343).

CONFLICT OF INTEREST STATEMENT

CHe reports grants from Sanofi-Aventis, outside the submitted work; MR reports personal fees from Eli Lilly, personal fees from Boehringer Ingelheim Pharma, personal fees from Terra Firma, personal fees from Sanofi US, personal fees from Novo Nordisk, personal fees from Target Pharmasolutions, outside the submitted work; WK reports personal fees from AstraZeneca, personal fees from Novartis, personal fees from Pfizer, personal fees from The Medicines Company, personal fees from DalCor, personal fees from Kowa, personal fees from Amgen, personal fees from Corvidia, personal fees from Daiichi-Sankyo, personal fees from Genentech, personal fees from Novo Nordisk, personal fees from Esperion, personal

fees from OMEICOS, personal fees from Berlin-Chemie, personal fees from Sanofi, personal fees from Bristol-Myers Squibb, grants and non-financial support from Abbott, grants and non-financial support from Roche Diagnostics, grants and non-financial support from Beckmann, grants and non-financial support from Singulex, outside the submitted work.

AUTHORS' CONTRIBUTIONS

JN drafted the analysis plan, performed the statistical analysis and wrote the manuscript. BT, CHe designed the study, drafted the analysis plan, and interpreted data. BT, CHe, BS, HB, AP, WK, MR, CM contributed data. All authors reviewed and edited the manuscript and approved its submission. JN is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

FUNDING

The study was funded by grants from the German Center for Diabetes Research (DZD; to C. Herder and B. Thorand). The KORA study was initiated and financed by the Helmholtz Zentrum München – German Research Center for Environmental Health, which is funded by the German Federal Ministry of Education and Research (BMBF) and by the State of Bavaria. Furthermore, KORA research was supported within the Munich Center of Health Sciences (MC-Health), Ludwig-Maximilians-Universität, as part of LMUinnovativ. This work was further supported by the Ministry of Culture and Science of the State of North Rhine-Westphalia and the German Federal Ministry of Health. This study was supported in part by a grant from the German Federal Ministry of Education and Research (BMBF) to the German Center for Diabetes Research (DZD). The funders of the study had no role in study design, data collection, analysis, interpretation, or writing of the report.

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Table 1. Characteristics of the two independent study cohorts at baseline

	KORA	ESTHER
Clinical Characteristics		
N	1110	1672
Age (years)	70.3 (5.4)	61.9 (6.6)
Sex, N (% male)	541 (48.7)	729 (43.6)
Body mass index (kg/m^2)	28.7 (4.5)	27.8 (4.3)
Systolic blood pressure (mmHg)	128.6 (19.8)	140.6 (19.6)
Triglycerides (mg/dl)	112.0 (83.0, 158.7)	115.8 (80.7, 170.3)
High-density lipoprotein cholesterol (mg/dl)	55.6 (14.1)	53.6 (14.5)
Low-density lipoprotein cholesterol (mg/dl)	139.9 (35.9)	148.9 (39.3)
Smoking status, N (%)		
Never smoker	539 (48.7)	869 (52.0)
Former smoker	486 (43.9)	526 (31.5)
Current smoker	82 (7.4)	277 (16.6)
Alcohol intake (g/day)	5.7 (0.0, 20.0)	3.39 (0.0, 12.8)
Physically active, N (%)	556 (50.2)	
Physical activity, N (%)		
Medium or high: ≥ 2 h of vigorous and ≥ 2 h of light physical activity/week		545 (32.6)
Low: other		764 (45.7)
Inactive: < 1 h of physical activity/week		363 (21.7)
Type 2 diabetes, N (%)	230 (21.2)	230 (13.8)
Cardiovascular disease, N (%)	177 (16.0)	288 (17.2)
Use of antidiabetic medication, N (%)	121 (10.9)	113 (6.8)
Use of antihypertensive medication, N (%)	472 (42.6)	736 (44.0)
Use of lipid-lowering medication, N (%)	272 (24.6)	189 (11.3)
Renal characteristics		
eGFRcr (ml/min/1.73 m^2)	75.8 (14.8)	79.3 (19.6)
CKD, N (%)	157 (14.1)	288 (17.2)
eGFRcr >30 - ≤60 (ml/min/1.73 m^2), N (%)	151 (13.6)	279 (16.7)
eGFRcr ≤30 (ml/min/1.73 m^2), N (%)	6 (0.5)	9 (0.5)

Continues measures are summarized as mean (SD) or median (25th, 75th percentiles); categorical variables are given as N (percentages). For the KORA study, physical activity represents the number and % of participants who were physically active, defined as participating in sports in summer and in winter and reporting at least more than 1 h sports per week in at least one of the seasons. Cardiovascular disease was defined based on previous history of angina pectoris, myocardial infarction or stroke. For ESTHER, cardiovascular disease included prevalent coronary heart disease/angina pectoris, stroke and myocardial infarction.
Abbreviations: CKD, chronic kidney disease; eGFRcr, estimate glomerular filtration rate from creatinine

Table 2. Association estimates between biomarkers of inflammation and eGFR (cross-sectional results in the discovery KORA F4 study and in the replication ESTHER study) and CKD incidence (prospective pooled results from both studies).
Only biomarkers with statistically significant eGFR results in the discovery study (KORA F4) are shown. The biomarkers are sorted by PFDR in the discovery study.

	Cross-sectional association with eGFR KORA F4 (discovery)				**Cross-sectional association with eGFR ESTHER (replication)**				**Prospective analysis with CKD incidence Meta-analysis (KORA F4 and ESTHER)**			
Marker	**Beta**	**SE**	**P**	**P_{FDR}**	**Beta**	**SE**	**P**	**P_{FDR}**	**OR**	**(95% CI)**	**P**	**I^2**
CD40	-5.57	0.39	4.70E-42	**3.34E-40**	-8.87	0.57	2.28E-51	**2.05E-50**	1.22	(0.99, 1.52)	6.40E-02	0.77
TNFRSF9	-5.17	0.41	1.79E-33	**6.34E-32**	-6.26	0.60	1.66E-24	**6.97E-24**	**1.25**	**(1.01, 1.55)**	**4.00E-02**	0.00
IL10RB	-4.92	0.40	6.72E-33	**1.59E-31**	-4.02	0.56	7.12E-13	**1.60E-12**	1.18	(0.97, 1.43)	9.90E-02	0.00
CST5	-4.43	0.39	1.63E-28	**2.42E-27**	-3.76	0.46	1.14E-15	**2.76E-15**	1.13	(0.95, 1.36)	1.66E-01	0.50
CX3CL1	-4.51	0.40	1.71E-28	**2.42E-27**	-1.35	0.56	1.57E-02	**1.83E-02**	1.08	(0.89, 1.31)	4.43E-01	0.80
CCL23	-4.21	0.39	1.05E-25	**1.07E-24**	-3.13	0.47	2.57E-11	**5.06E-11**	1.11	(0.94, 1.32)	2.32E-01	0.00
FGF23	-4.47	0.42	9.22E-26	**1.07E-24**	NA				NA			
PD_L1	-4.30	0.41	6.97E-25	**6.19E-24**	-3.56	0.81	1.02E-05	**1.57E-05**	1.18	(0.94, 1.48)	1.58E-01	0.79
CSF1	-4.01	0.41	8.10E-22	**6.39E-21**	-5.28	0.77	1.06E-11	**2.15E-11**	1.09	(0.88, 1.34)	4.41E-01	0.70
CD5	-3.95	0.42	1.66E-20	**1.18E-19**	-6.25	0.61	1.05E-23	**3.68E-23**	**1.26**	**(1.03, 1.54)**	**2.70E-02**	0.00
IL15RA	-3.55	0.41	1.71E-17	**1.10E-16**	NA				NA			
CCL25	-3.39	0.40	8.10E-17	**4.79E-16**	-1.63	0.55	3.05E-03	**3.76E-03**	1.02	(0.84, 1.23)	8.51E-01	0.78
4E_BP1	-3.62	0.44	6.84E-16	**3.74E-15**	-4.77	0.52	1.18E-19	**3.39E-19**	1.08	(0.88, 1.33)	4.42E-01	0.33
FGF5	-3.29	0.40	1.07E-15	**5.41E-15**	NA				NA			
IL12B	-3.31	0.42	1.03E-14	**4.88E-14**	-3.71	0.70	1.23E-07	**1.98E-07**	1.03	(0.83, 1.28)	8.01E-01	0.80
FGF21	-3.42	0.44	2.30E-14	**1.02E-13**	1.26	0.54	1.84E-02	**2.07E-02**	1.03	(0.84, 1.27)	7.61E-01	0.00
CXCL9	-3.24	0.43	1.31E-13	**5.48E-13**	-3.49	0.49	2.43E-12	**5.10E-12**	1.04	(0.87, 1.25)	6.66E-01	0.00
MMP10	-2.73	0.41	4.56E-11	**1.80E-10**	-3.09	0.57	5.84E-08	**9.95E-08**	**1.22**	**(1.02, 1.46)**	**3.20E-02**	0.00
MIP1A	-2.59	0.41	5.46E-10	**2.04E-09**	NA				NA			
VEGFA	-2.50	0.40	7.40E-10	**2.63E-09**	-5.95	0.52	1.01E-29	**4.88E-29**	1.19	(0.98, 1.43)	7.50E-02	0.74
Beta_NGF	-2.46	0.41	2.28E-09	**7.69E-09**	-1.01	0.57	7.91E-02	8.31E-02	1.17	(0.96, 1.41)	1.13E-01	0.79
SCF	-2.52	0.42	3.51E-09	**1.13E-08**	-3.85	0.54	1.28E-12	**2.78E-12**	1.00	(0.83, 1.21)	9.79E-01	0.00

IL17C	-2.38	0.41	7.49E-09	**2.31E-08**	NA				NA			
TGF_alpha	-2.27	0.41	2.53E-08	**7.48E-08**	-4.82	0.59	5.63E-16	**1.42E-15**	1.07	(0.89, 1.30)	4.64E-01	0.88
MCP3	-2.08	0.41	4.38E-07	**1.24E-06**	-10.48	0.40	2.69E-126	**8.47E-125**	**1.34**	**(1.11, 1.62)**	**2.00E-03**	0.72
GDNF	-2.08	0.41	4.67E-07	**1.27E-06**	NA				NA			
SLAMF1	-1.87	0.44	2.63E-05	**6.92E-05**	NA				NA			
IL10	-1.72	0.42	3.59E-05	**9.10E-05**	-5.06	0.62	4.57E-16	**1.20E-15**	1.21	(0.98, 1.50)	7.20E-02	0.57
OPG	-1.78	0.43	4.48E-05	**1.10E-04**	-2.12	0.51	3.25E-05	**4.77E-05**	1.18	(0.98, 1.42)	7.90E-02	0.00
FGF19	-1.64	0.41	5.98E-05	**1.42E-04**	-1.32	0.50	8.37E-03	**9.95E-03**	1.03	(0.87, 1.23)	7.04E-01	0.47
UPA	-1.61	0.41	8.46E-05	**1.94E-04**	-5.99	0.49	1.96E-32	**1.12E-31**	1.03	(0.87, 1.23)	7.15E-01	0.63
STAMBP	-1.56	0.41	1.61E-04	**3.57E-04**	-6.24	0.65	2.86E-21	**8.58E-21**	1.09	(0.89, 1.35)	3.97E-01	0.00
CD244	-1.54	0.41	1.72E-04	**3.71E-04**	-6.07	0.60	2.63E-23	**8.28E-23**	1.14	(0.94, 1.37)	1.88E-01	0.48
ADA	-1.50	0.40	2.03E-04	**4.24E-04**	-7.36	0.73	1.62E-23	**5.36E-23**	1.04	(0.86, 1.26)	6.93E-01	0.28
CCL19	-1.54	0.42	2.87E-04	**5.83E-04**	0.36	0.49	4.65E-01	4.65E-01	1.10	(0.92, 1.31)	3.09E-01	0.00
HGF	-1.53	0.44	5.40E-04	**1.04E-03**	-5.65	0.52	2.37E-26	**1.07E-25**	1.13	(0.93, 1.37)	2.14E-01	0.53
LIFR	-1.46	0.42	5.26E-04	**1.04E-03**	-2.26	0.72	1.65E-03	**2.08E-03**	1.02	(0.82, 1.25)	8.90E-01	0.71
EN_RAGE	-1.32	0.41	1.27E-03	**2.37E-03**	-7.46	0.51	5.13E-45	**3.59E-44**	**1.30**	**(1.08, 1.57)**	**5.00E-03**	0.74
TRAIL	-1.31	0.41	1.57E-03	**2.86E-03**	-1.19	0.67	7.44E-02	7.94E-02	0.98	(0.80, 1.20)	8.69E-01	0.64
MCP1	-1.22	0.40	2.66E-03	**4.73E-03**	-8.06	0.49	4.23E-56	**4.44E-55**	**1.27**	**(1.07, 1.52)**	**7.00E-03**	0.00
MMP1	-1.19	0.40	3.33E-03	**5.76E-03**	-1.52	0.64	1.70E-02	**1.94E-02**	1.03	(0.86, 1.25)	7.35E-01	0.00
IL18	-1.23	0.42	3.43E-03	**5.81E-03**	-3.27	0.54	1.93E-09	**3.58E-09**	1.12	(0.94, 1.34)	2.02E-01	0.00
SIRT2	-1.17	0.41	4.88E-03	**8.06E-03**	-5.66	0.55	5.48E-24	**2.16E-23**	1.07	(0.88, 1.30)	5.08E-01	0.30
MCP4	-1.09	0.40	7.02E-03	**1.13E-02**	-2.41	0.47	3.21E-07	**5.06E-07**	**1.23**	**(1.03, 1.46)**	**2.10E-02**	0.00
OSM	-1.07	0.41	8.63E-03	**1.36E-02**	-10.05	0.42	1.01E-109	**2.13E-108**	**1.22**	**(1.01, 1.47)**	**4.20E-02**	0.88
CDCP1	-1.13	0.43	9.34E-03	**1.44E-02**	-1.38	0.61	2.32E-02	**2.57E-02**	1.11	(0.91, 1.35)	2.99E-01	0.00
CXCL10	-1.07	0.42	1.09E-02	**1.65E-02**	-2.26	0.55	4.62E-05	**6.47E-05**	1.05	(0.89, 1.25)	5.60E-01	0.00
CCL20	-1.05	0.42	1.16E-02	**1.72E-02**	-5.03	0.49	6.04E-24	**2.24E-23**	1.08	(0.89, 1.31)	4.25E-01	0.00
CXCL6	-1.04	0.42	1.30E-02	**1.89E-02**	-2.98	0.53	1.94E-08	**3.39E-08**	1.01	(0.83, 1.22)	9.62E-01	0.87
FLT3L	-0.96	0.41	1.88E-02	**2.68E-02**	-2.11	0.51	4.47E-05	**6.40E-05**	1.04	(0.87, 1.24)	6.78E-01	0.61
IL8	-0.92	0.41	2.55E-02	**3.55E-02**	-10.56	0.40	1.47E-126	**8.47E-125**	**1.36**	**(1.13, 1.65)**	**1.00E-03**	0.77
CCL11	-0.87	0.41	3.30E-02	**4.51E-02**	-3.03	0.51	2.65E-09	**4.76E-09**	1.20	(1.00, 1.44)	5.20E-02	0.00

The estimates are for model 2 (multivariable model; See methods section). For the meta-analysis results, model 2 was additionally adjusted for baseline eGFRcr. Association estimates refer to 1-SD increase in biomarker concentrations. Study-significant results are printed in bold.
Abbreviations: CI, confidence interval; eGFR, estimated glomerular filtration rate; FDR, false discovery rate; I^2, the percentage of variance that is attributable to heterogeneity between study-specific association estimates; NA, not available; OR, odds ratio; P, p value; 95% CI, 95% confidence interval. Full names of the biomarkers can be found in Supplementary Table 1.

Table 3. Canonical pathways enriched for biomarkers of kidney function (IPA). The table shows all canonical pathways 42 significant and replicated associations between biomarkers of inflammation and kidney function in the multivariable model.

#	Ingenuity Canonical Pathways	Biomarkers	P_{FDR}
1	Granulocyte Adhesion and Diapedesis	CCL11, CCL13, CCL2, CCL20, CCL23, CCL25, CCL7, CX3CL1, CXCL10, CXCL6, CXCL8, CXCL9, IL18, MMP1, MMP10, TNFRSF11B	2.12E-08
2	Agranulocyte Adhesion and Diapedesis	CCL11, CCL13, CCL2, CCL20, CCL23, CCL25, CCL7, CX3CL1, CXCL10, CXCL6, CXCL8, CXCL9, IL18, MMP1, MMP10	1.17E-07
3	Hepatic Fibrosis / Hepatic Stellate Cell Activation	CCL2, CD40, CSF1, CXCL8, CXCL9, HGF, IL10, MMP1, TGFA, TNFRSF11B, VEGFA	6.99E-05
4	Role of IL-17A in Arthritis	CCL2, CCL20, CCL7, CXCL6, CXCL8, MMP1	4.10E-03
5	Atherosclerosis Signaling	CCL11, CCL2, CD40, CSF1, CXCL8, IL18, MMP1	4.95E-03
6	T Helper Cell Differentiation	CD40, IL10, IL10RB, IL12B, IL18, TNFRSF11B	4.95E-03
7	Role of Macrophages, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis	CCL2, CSF1, CXCL8, IL10, IL18, MMP1, OSM, TNFRSF11B, VEGFA	4.95E-03
8	TREM1 Signaling	CCL2, CCL7, CD40, CXCL8, IL10, IL18	4.95E-03
9	Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza	CCL2, CXCL10, CXCL8, IL12B, IL18	6.51E-03
10	Role of IL-17F in Allergic Inflammatory Airway Diseases	CCL2, CCL7, CXCL10, CXCL6, CXCL8	6.51E-03
11	Communication between Innate and Adaptive Immune Cells	CD40, CXCL10, CXCL8, IL10, IL12B, IL18	6.51E-03
12	Bladder Cancer Signaling	CXCL8, FGF19, FGF21, MMP1, MMP10, VEGFA	6.51E-03
13	Hematopoiesis from Pluripotent Stem Cells	CSF1, CXCL8, IL10, IL12B, KITLG	6.64E-03
14	Neuroinflammation Signaling Pathway	CCL2, CD40, CX3CL1, CXCL10, CXCL8, IL10, IL12B, IL18	8.84E-03
15	Th1 Pathway	CD274, CD40, IL10, IL10RB, IL12B, IL18	9.49E-03

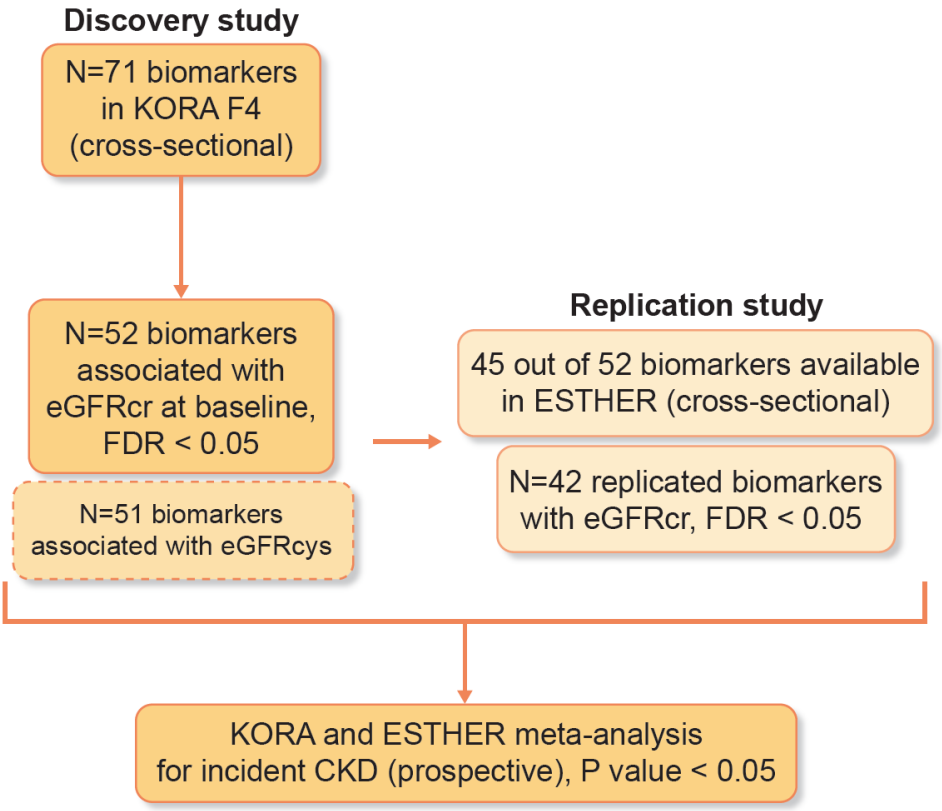

Figure 1. Flowchart of study analysis. (Abbreviations: eGFR, estimated glomerular filtration rate based on creatinine (eGFRcr) or cystatin C (eGFRcys); FDR, false discovery rate)

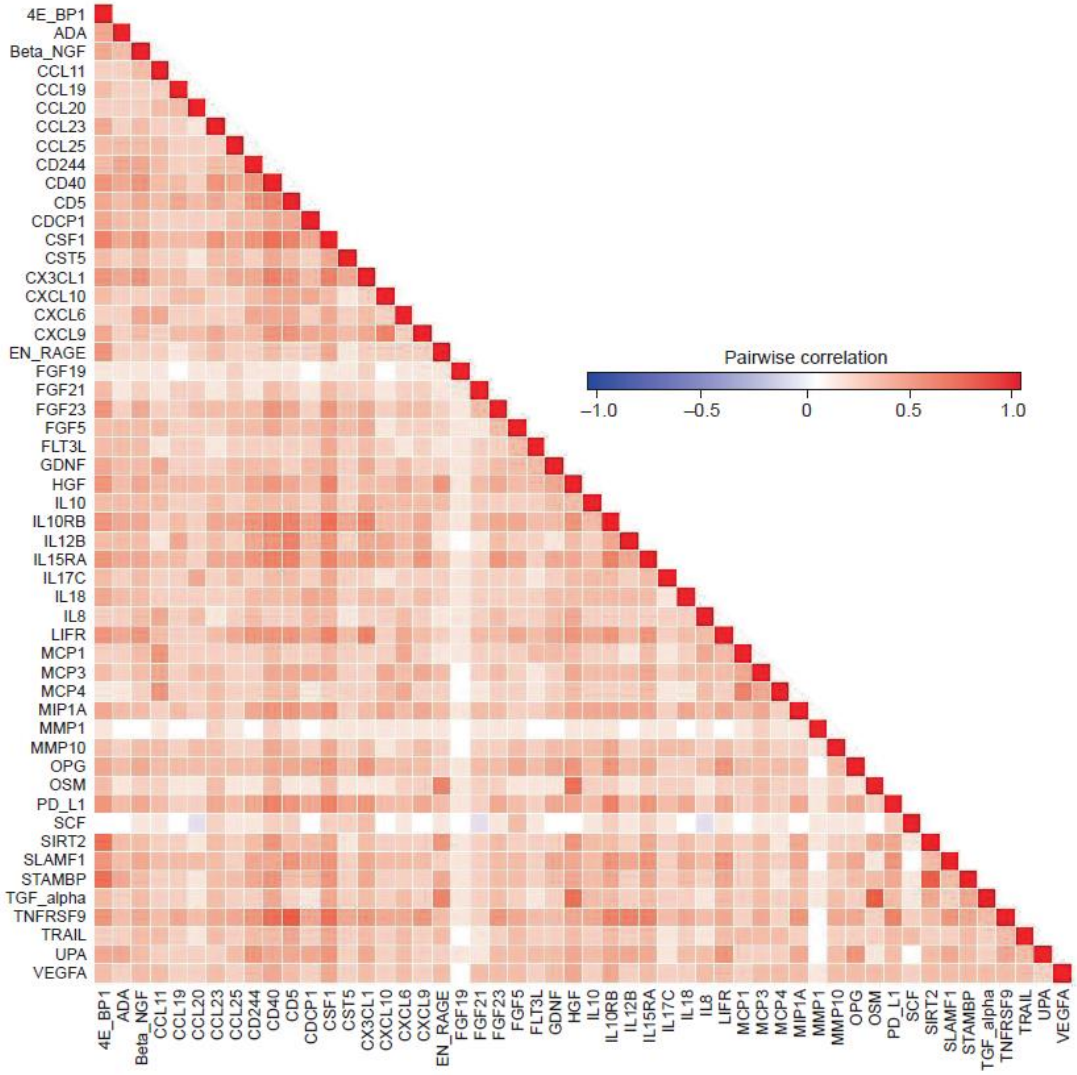

Figure 2. Pairwise correlation matrix for the 52 biomarkers of inflammation significantly associated with eGFR in the discovery study (KORA F4). Full names of the markers are given in Supplementary Table 1.