Chronic kidney disease

Thio, C. H. L.

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Familial aggregation of chronic kidney disease and heritability of renal biomarkers in the general population: The Lifelines Cohort Study

Jia Zhang, Chris HL Thio, Ron T Gansevoort, Harold Snieder

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CHAPTER

4

ABSTRACT

Introduction. Chronic kidney disease (CKD) is a major burden on patients and health resources, with a heritable component. We aimed to quantify familial aggregation of CKD in the general population, and assess the extent to which kidney traits can be explained by genetic or environmental factors.

Methods. This cross-sectional family study used baseline data from the Lifelines Cohort study, a sample of the general population of the Northern Netherlands with a unique three-generation design. CKD was defined by estimated glomerular filtration rate (eGFR) <60mL/min/1.73m^2 (1862 cases) and/or urinary albumin excretion (UAE) ≥30 mg/24hr (4127 cases). eGFR was calculated by CKD-EPI equation for serum creatinine (N=155,911). UAE was determined in 24h urine collections in a subsample (N=59,943). To quantify familial aggregation of CKD we calculated the recurrence risk ratio (RRR) with Cox proportional hazards models. Heritability of continuous kidney-related traits was estimated using linear mixed models. All models were adjusted for age, sex, and known renal risk factors.

Results. RRR of CKD in case of an affected first-degree relative was 3.05 (95%CI: 2.27-4.11), i.e. risk of CKD was 3.05 times higher compared to risk in the general population. In case of a spouse with CKD, the RRR was 1.61 (95% CI: 1.29-2.11), indicative of shared environmental factors and/or assortative mating. We report heritability estimates of eGFR (44%), UAE (20%), serum urea (31%), creatinine (37%) and uric acid (48%), and serum electrolytes (range 22%-28%).

Conclusions. In this large population-based family study, a positive family history was strongly associated with increased risk of CKD. We observed moderate to high heritability of renal traits and related biomarkers. These results indicate an important role of genetic factors in CKD risk and may inform preventive policies.

Keywords. familial aggregation; heritability; chronic kidney disease (CKD); kidney function; albuminuria

INTRODUCTION

Chronic kidney disease (CKD) is recognized as a global public health problem[1], with prevalence ranging between 3.3% and 17.3% in adult European populations[2]. Chronic kidney disease is defined by reduced estimated glomerular filtration rate (eGFR) and/or increased albuminuria, and is associated with an increased risk of cardiovascular disease and progression to end stage kidney disease (ESKD)[3-6].

Established risk factors for CKD, such as hypertension and diabetes, explain 50-70% of cases, and are the main targets of current risk prediction models for CKD[7]. Familial clustering of CKD and kidney related markers suggests that genetic factors or shared environmental factors are also important in the pathogenesis of this disease[8-12]. Support for a genetic component to CKD comes from recent genome-wide association studies (GWASs) on eGFR[13] and albuminuria[14], in which a large number of genetic loci have been reported. Despite the evidence for this genetic influence, the magnitude of the familial contribution to CKD susceptibility in the general population is poorly known.

One option of assessing the magnitude of the familial contribution to CKD is to examine its aggregation in families. Most familial aggregation studies on CKD focused on its later stages, i.e. end-stage kidney disease (ESKD) using medical records and registry data[8,9,12,15]. Focusing on early-stage CKD rather than ESKD may lead to more accurate estimates of familial recurrence risk of CKD, and may have added value for primary and secondary prevention strategies.

In addition to assessing familial recurrence risk, one can estimate the heritability of disease traits. Heritability quantifies the relative importance of genetic and environmental factors inexplaining the distribution of a trait or disease within a population[16]. Both kidney function and related blood biomarkers have been shown to be heritable[11,17,18]. Heritability estimates of eGFR range from 33% to 67.3%[11,18-23]. For the kidney related biomarkers, serum urea and uric acid, the heritability estimates range from 22% to 54%[18,21,24,25], and from 29% to 35%[21,25], respectively. Among subjects with type 2 diabetes, urinary albumin-creatinine ratio (UACR) shows evident familial clustering with a heritability estimate of 46%[26,27], which is higher than other population-based family studies (23% to 25%)[18,21]. The heritability of related biomarkers, such as serum electrolytes has been estimated to be moderate to high, varying from 33% to 61% for calcium[17,18,28], and 12% to 56% for

sodium and potassium[17,18,24,25,28]. To date, the heritability estimates for kidney traits have originated from twin studies[17,20,23,24,28], from family studies with relatively small sample size [11,18], or from studies in isolated founder or disease populations[21,25]. Due to sampling error, the results of these studies may have limited generalizability[16]. Therefore, heritability estimates from a large, representative sample of the general population are needed.

In this study, our aim was therefore to quantify the familial aggregation of CKD and to obtain heritability estimates of kidney traits and related biomarkers in the general population.

METHODS

Study design and population

In this cross-sectional family study, we used baseline data from the Lifelines Cohort Study and Biobank, a multidisciplinary prospective population-based cohort study of the Northern Netherlands with a unique three-generation design, and that included 167,548 subjects. It employs a broad range of investigative procedures in assessing the socio-demographic, biomedical, physical, behavioral and psychological factors which contribute to the health and disease of the general population, with a special focus on multi-morbidity and complex genetics. The overall design and rationale of this study have been described in detail elsewhere[29,30]. The recruitment of the Lifelines study was family-based by design. Eligible subjects between 20 and 50 years old were invited to participate through their general practitioner. Individuals were not invited when the participating general practitioner considered the patient not eligible, i.e. if they had severe psychiatric or physical illness, limited life expectancy or insufficient knowledge of the Dutch language. After the inclusion of these individuals their partner, children, parents and partner's parents were also invited to participate in the study. In addition, single individuals could register for participation online. In this way a three-generation family study was realized. We used the information on family members as well as information on (anonymized) names and birth dates of parents provided by all participants in questionnaires to define family relationships in Lifelines. After signing informed consent, participants received a baseline questionnaire and an invitation to a health assessment at one of the Lifelines research sites.

The Lifelines Cohort Study is conducted according to the Principles of the Declaration of Helsinki and in accordance with the research code of University Medical Center Groningen, and was approved by its medical ethical committee. All participants gave written informed consent.

Measurements

Kidney outcomes

Participants aged 8 years and older were invited to one of 12 local research sites in the north of The Netherlands for the physical examination. The baseline assessment consisted of two visits. During the first visit (duration 60 min) physical examinations were performed by a trained research nurse and containers for collection of a 24-h urine sample (age≥18 years) were handed out accompanied by oral and written instruction on how to collect this sample. Approximately 2 weeks after the first visit, a second visit (duration 10 min) was arranged to collect a fasting blood sample (age≥8 years) and hand in the collected 24-h urine.

Measurement of serum creatinine was performed by an IDMS-traceable enzymatic method on a Roche Modular analyzer using reagents and calibrators from Roche (Roche Diagnostics, Mannheim, Germany), with intra- and inter-assay coefficients of variation of 0.9% and 2.9%, respectively. Urinary albumin (UA) concentration was measured by nephelometry with a lower threshold of detection of 2.3 mg/l and intra- and inter-assay coefficient of variation of 2.2% and 2.6%, respectively (Dade Behring Diagnostic, Marburg, Germany). UA concentration was multiplied by urine volume to obtain a value of UA excretion (UAE) in milligram per 24 hours. Urinary albumin-creatinine ratio (UACR) was estimated by urinary albumin divided by urinary creatinine as measured in spot urine (age≥8 years). After addition of a constant of 1 to handle zero-values, UAE and UACR were transformed by their natural logarithm to approximate a normal distribution prior to statistical analyses.

CKD was defined as eGFR <60mL/min/1.73m^2 (CKD_{Scr}) in the complete sample. In a subsample where urinary albumin was available, we applied an additional definition of CKD according to Kidney Disease: Improving Global Outcomes (KDIGO) guidelines[31,32] (CKD_{KDIGO}, eGFR <60mL/min/1.73m^2 or UAE ≥30mg/24h, or UACR ≥30mg/g).

We calculated eGFR according to the 2012 Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation[33] for adults, and the Bedside Schwartz equation[34] for children (aged<18 years).

Kidney related biomarkers

The kidney related biomarkers (serum uric acid, urea and electrolytes) were measured using standard methods, i.e. for uric acid an enzymatic colorimetric assay, for urea an ultraviolet kinetic assay on a Roche Modular and for serum electrolytes (calcium, potassium, and sodium) using a Roche Modular P chemistry analyzer (Roche, Basel, Switzerland).

Covariates

Known CKD risk factors (body mass index [BMI], hypertension, type 2 diabetes, hypercholesterolemia, smoking status) were included as covariates and assessed at baseline. Blood pressure was measured ten times during 10 min with a Dinamap, PRO 100V2. The blood pressure registered was calculated by averaging the final three readings. Hypertension was defined as systolic blood pressure (SBP) ≥140 mmHg, and/or diastolic blood pressure (DBP) ≥90 mmHg, and/or self-reported prescribed use of antihypertensive drugs. Participants were categorized as having Type 2 diabetes mellitus (T2DM) if they had a measured fasting plasma glucose (FPG) ≥7.0 mmol/L, and/or a measured glycated hemoglobin (HbA1c) ≥6.5% (48 mmol/mol),[32] and/or self-reported T2DM in combination with self-reported medication use (i.e. ATC codes A10A and A10B). Hypercholesterolemia was defined as a total cholesterol of) ≥6.21 mmol/L or self-reported use of lipid-lowering drugs (ATC codes C10A, C10B). Smoking status was assessed by questionnaire and coded as smoker vs non-smoker.

Statistical analysis

Baseline characteristics

Baseline characteristics were examined for the total population. Multivariable linear regression, and multivariable logistic regression were used to examine age-adjusted differences between males and females (separately in children).

Recurrence risk ratio

The mean eGFR and prevalence of CKD were calculated for the general population and for individuals with affected first-degree relatives. Recurrence risk ratios (RRR) of CKD were calculated as the adjusted prevalence ratios between first-degree

relatives of an individual with CKD and the general population. The RRR estimated in this study is the recurrence risk ratio according to the Risch definition[35], which is the prevalence ratio between individuals with a specific type of affected relative and the general population. We used a Cox proportional hazards models, adapted according to Breslow[36], to estimate prevalence ratios in a cross-sectional study by applying an equal follow-up time for all subjects. This method has been proven to produce consistent estimates for prevalence ratios close to true limits.[37,38] A marginal proportional hazards model was used in this study to handle correlation between observations due to familial clustering. This model estimates the mean population hazard function and uses a robust sandwich method to estimate the confidence interval (CI)[39,40]. This approach has been applied and validated in previous studies on other diseases.[41-43] We calculated RRR for individuals with an affected first-degree relative of any kinship and also for individual kinship (parent, offspring, and sibling). To explore whether familial risk depends on type of kinship and sex of the affected relative, we created separate models based on type of kinship and sex of affected relatives (i.e. father, mother, son, daughter, brother, and sister). Additionally, we estimated RRR for individuals with an affected spouse (husband or wife) to quantify the effect of shared environment and/or assortative mating. In each model, we compared the risk for CKD in individuals with affected first-degree relatives or spouse with the risk in the general population. The RRR was adjusted for age, sex, BMI, hypertension, T2DM, hypercholesterolemia, and smoking. To examine the consistency of our estimates of familial recurrence risk for CKD_{SCr}, we compared results in the full sample with those in the subsample of approximately 60,000 individuals for this CKD definition.

Heritability estimates

For all continuous traits we estimated the heritability. Heritability in the narrow sense is defined as the ratio of the additive genetic variance, which reflects transmissible resemblance between relatives, to the total phenotypic variance. To estimate heritability, we used the Residual Maximum Likelihood-based variance decomposition method implemented in ASReml software[44], in which the overall phenotypic variance is decomposed into genetic and environmental components. We also included household or spousal effects in the model to estimate the influence of shared environment by using family id or spouse id as a proxy. This allowed us to distinguish between shared genes and shared environment as potential sources of familial resemblance. In addition we calculated spouse

correlations for all continuous traits. The significance of heritability was determined by using the likelihood ratio test. This test compares the likelihood of a model in which heritability is estimated, to that of a model in which heritability is constrained to zero. Age, age^2, sex, BMI, hypertension, T2DM, hypercholesterolemia, and smoking status were included as covariates in the model, irrespective of their statistical significance; we report the percentage of variance explained by these covariates (PVC). To assess the consistency of heritability estimates for eGFR, serum creatinine, and serum potassium, we compared results in the full sample with those in the subsample of approximately 60,000 participants for these traits.

All analyses were performed using ASReml (Release 4.1)[44] and R3.3.1[45]. Two-sided significance level for analyses were set at α=0.05.

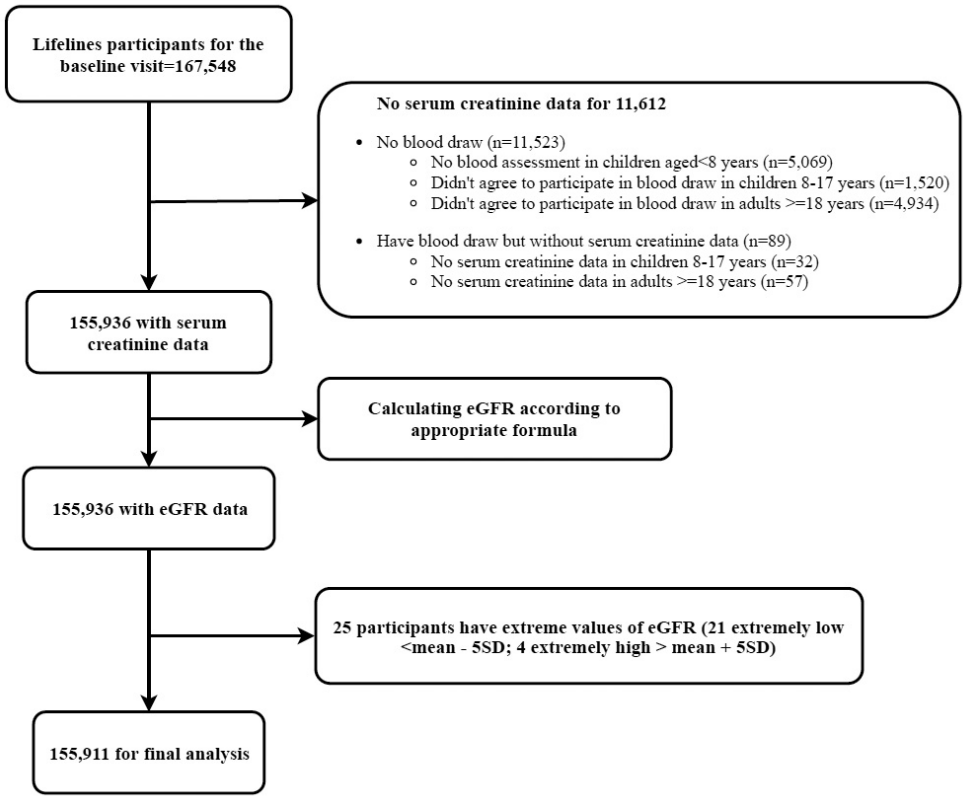
RESULTS

Baseline characteristics

We included 155,911 participants with serum creatinine and eGFR data during the baseline visit (**Figure 1**). In this full sample CKD was defined as an eGFR <60 mL/min/1.73 m^2 (CKD_{SCr}). In a subsample of approximately 60,000 participants, UAE or UACR were measured of whom 59,938 (including 743 children) had both eGFR and UACR, while 59,145 (only adults) had both eGFR and UAE data (**Supplementary Figure S1-S2**). In this subsample, CKD was defined as an eGFR <60 mL/min/1.73m^2, a UAE ≥30 mg/24h (or UACR≥ 30mg/g) or both, according to the 2011 revised Kidney Disease: Improving Global Outcomes guidelines (CKD_{KDIGO}). In the full sample there were 29,703 families (of size >=2) with an average family size of 3.92, and 39,836 singletons (i.e., individuals without any relative in the sample). The largest family consisted of 172 participants. Spouses without children were considered as a family of size 2. For the subsample, 11,477 families remained with an average family size of 3.39 and 18,537 singletons. The largest family in the subsample connected 75 participants.

Included were up to 155,911 participants (58.1% female; mean age ± SD: 43.1 ± 14.7 years) with a mean (SD) eGFR of 97.2 (15.7) mL/min/1.73 m^2 in the full sample. In the subsample of up to 59,943 subjects in which albuminuria was measured a median (interquartile range [IQR]) UAE of 3.86 (2.33-6.92) mg/24h, and a median (IQR) UACR of 2.72 (1.58-7.33) mg/g (**Table 1**) was observed. Sex stratified characteristics for adults and children revealed a slightly less favorable renal

Figure 1. Flowchart of eGFR analysis. Please note that 21 subjects with extremely low eGFR values (< 5SD from the mean) were considered CKD patients and retained in analyses of CKD. Abbreviations: estimated glomerular filtration rate, eGFR; standard deviation, SD.



risk profile for males compared to females (i.e. higher prevalence of smoking, hypertension, diabetes, and high cholesterol) but similar distributions in CKD risk and kidney markers (i.e. eGFR and UAE) (**Supplementary Table S1**). Distributions of age, sex and covariates in the subsample were similar to those in the full sample (**Supplementary Table S2**).

We identified 1862 CKD_{SCr} cases, which resulted in a crude prevalence of 1.19% (**Table 1**). A total of 2211 individuals had at least one first-degree relative with CKD_{SCr}: 1680 with at least one affected parent, 56 with at least one affected offspring, 499 with at least one affected sibling.

There was a steep increase in CKD_{SCr} prevalence after age 60. Mean eGFR was lower at higher age, and age-specific mean values of eGFR were lower among

Table 1. Baseline characteristics of adult and child participants

.	Adults (aged ≥18)	Children (aged <18)	Total
Total population, n	147715	8196	155911
Age (years)	44.83 (13.12)	12.21 (2.76)	43.11 (14.71)
Males (%)	61512 (41.64)	3891 (47.47)	65.403 (41.95)
BMI (kg/m²)	26.07 (4.33)	18.91 (3.18)	25.69 (4.56)
Current smoker (%)	31156 (21.38)	NA[a]	NA[a]
Hypertension (%)	38605 (26.13)	NA[a]	NA[a]
Diabetes (%)	5673 (3.84)	NA[a]	NA[a]
Hypercholesterolemia (%)	29888 (20.23)	NA[a]	NA[a]
Serum potassium (mEq/L)	3.86 (0.30)	3.84 (0.28)	3.86 (0.3)
Serum creatinine (mg/dL)	0.83 (0.14)	0.61 (0.13)	0.82 (0.15)
eGFR (mL/min/1.73 m²)	96.41 (15.29)	111.46 (16.81)	97.2 (15.74)
CKD_{SCr}: eGFR<60 (%)[b]	1858 (1.26)	4 (0.05)	1862 (1.19)
Subsample, n	59195	748	59943
Serum calcium (mg/dL)	9.14 (0.32)	9.50 (0.28)	9.14 (0.32)
Serum sodium (mmol/L)	141.74 (1.84)	141.63 (1.68)	141.73 (1.84)
Uric acid (mg/dL)	4.88 (1.18)	4.37 (1.01)	4.88 (1.18)
Serum urea (mg/dL)	14.48 (3.56)	12.41 (2.75)	14.45 (3.56)
UACR (mg/g)	2.72 (1.57-5.05)	2.93 (1.84-4.84)	2.72 (1.58-7.33)
UAE (mg/24h)	3.86 (2.33-6.92)	NA[a]	NA[a]
UACR ≥30	1622 (2.73)	20 (2.68)	1642 (2.73)
UAE ≥30	2431 (4.10)	NA[a]	NA[a]
CKD_{KDIGO}: eGFR<60 or UACR ≥30	3338 (5.52)	24 (3.20)	3362 (5.49)
CKD_{KDIGO}: eGFR<60 or UAE ≥30	4127 (6.83)	NA[a]	NA[a]

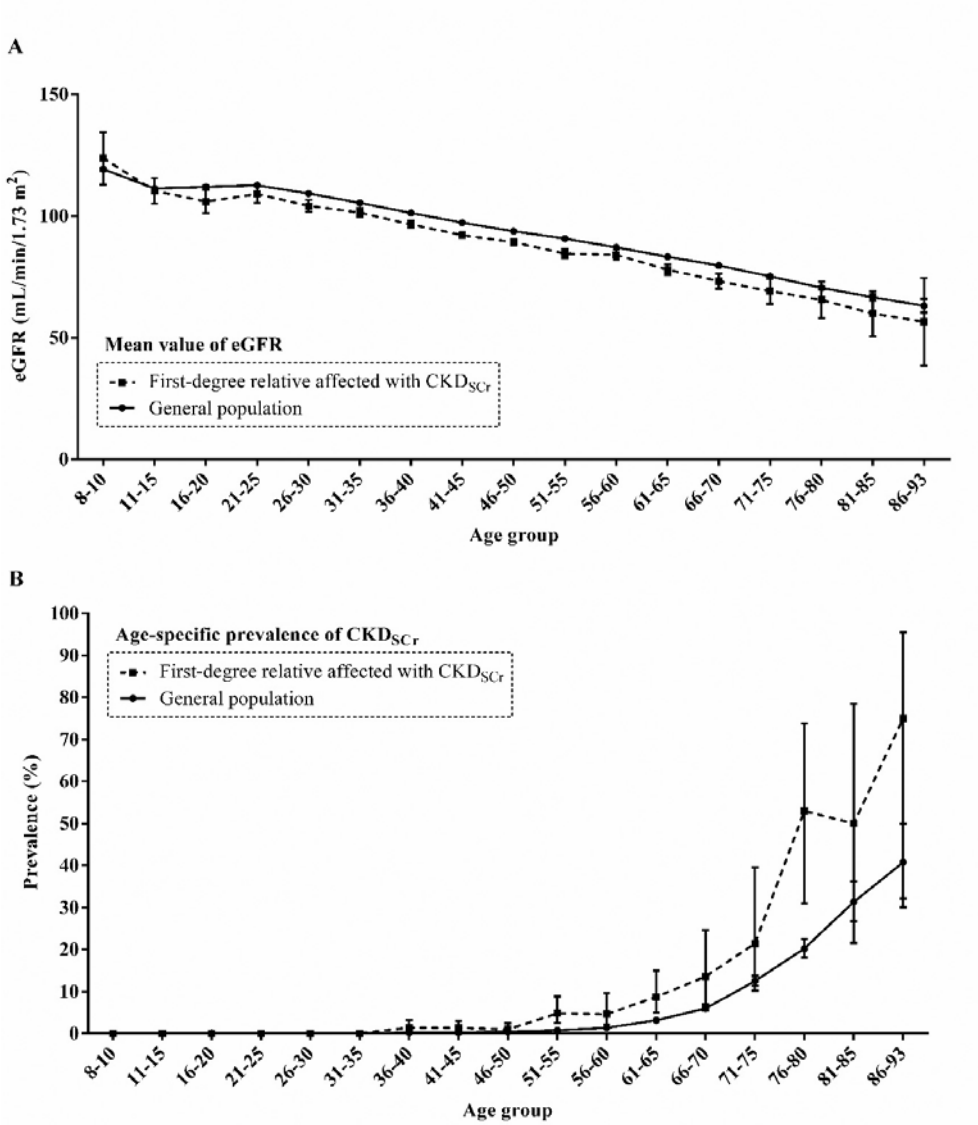
Data are presented as mean (SD), median (interquartile range) or number (%), where appropriate. Abbreviations and definitions: BMI, body mass index; SCr, serum creatinine; UACR, urinary albumin-creatinine ratio; UAE, urinary albumin excretion; eGFR, estimated glomerular filtration rate; CKD, chronic kidney disease. Conversion factors for calcium in mg/dL to mmol/L, ×0.2495; creatinine in mg/dL to µmol/L, ×88.4; uric acid in mg/dL to µmol/L, ×59.48; serum urea in mg/dL to mmol/L, ×0.357. [a]: data was not available for children. [b]: these include 21 subjects with extremely low eGFR values (< 5SD from the mean) were considered CKD patients and retained in analyses of CKD recurrence but excluded from heritability analyses

individuals with affected first-degree relatives compared to the general population (**Figure 2A**). Accordingly, the age-specific prevalence rates were also significantly higher in those with an affected first degree relative with CKD_{SCr} (**Figure 2B**). In the subsample, the crude prevalence of CKD_{KDIGO} was 5.5%-6.8% (depending on use of UACR or UAE, respectively, **Table 1**).

Recurrence risk ratio for CKD_{SCr} and CKD_{KDIGO} in individuals with affected first-degree relatives or spouses

Stratified analyses of the recurrence risk ratios (RRR) for CKD_{SCr} among individuals

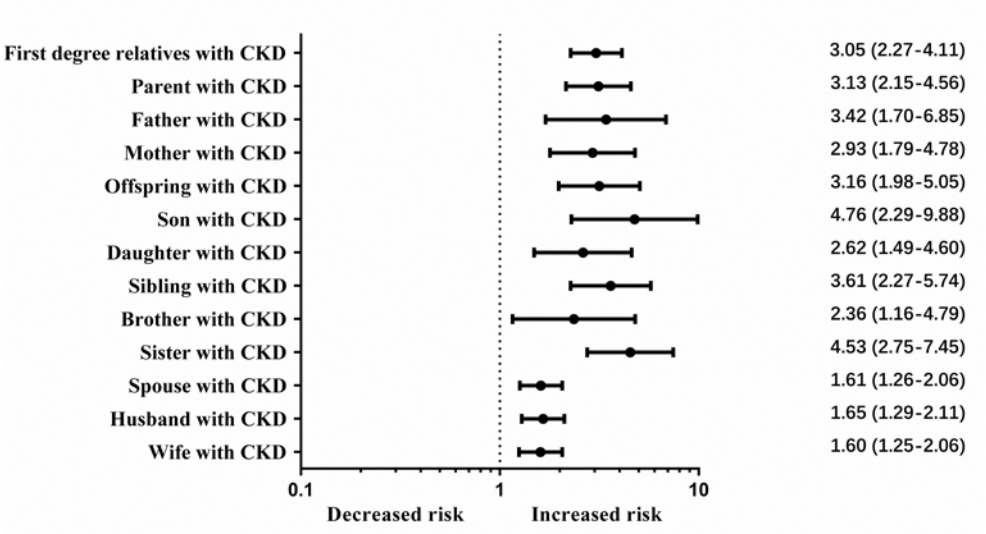
Figure 2. Comparisons of (A) age-specific mean values of eGFR and (B) age-specific prevalence of chronic kidney disease (CKD_{SCr}: eGFR<60 mL/min/1.73 m²) between individuals with affected first-degree relatives and the general population. Error bars indicate 95% confidence interval (CI).



with different affected first-degree relatives are shown in **Figure 3**. In general, having an affected first-degree relative with CKD_{SCr} was associated with an RRR of 3.05 (95% confidence interval CI: 2.27-4.11). The RRRs for CKD_{SCr} were 3.13 (95% CI: 2.15-4.56) for parents with disease, 3.16 (95% CI: 1.98-5.05) for offspring, and 3.61 (95% CI, 2.27-5.74) for siblings, respectively. Spouses of an affected individual were also at an increased risk compared to the general population (RRR = 1.61,

95% CI: 1.26-2.06). Familial recurrence showed no clear dependence on sex of the affected family member (**Figure 3**).

Figure 3. Recurrence risk ratios (adjusted for age, age2, sex, BMI, hypertension, diabetes, high cholesterol, and smoking status) for chronic kidney disease (CKD_{SCr}: eGFR<60 mL/min/1.73 m^2) in individuals with affected first degree relatives or spouse. Error bars indicate 95% confidence interval (CI).



RRRs for CKD_{SCr} in the subsample of ~60,000 participants in which albuminuria was measured showed a highly similar pattern, but values were slightly lower. The overall RRR for first degree relatives of patients with CKD_{SCr} reduced from 3.05 to 2.40.

In this subsample, prevalence of CKD_{KDIGO} was higher than that of CKD_{SCr} (**Supplementary Figure S3**). A trend was found of higher risk of CKD_{KDIGO} in first-degree relatives compared to risk in the general population, although this was less pronounced compared to the trend observed for CKD_{SCr} (**Supplementary Figure S4A and 4B**). In the subsample the RRR for first degree relatives for CKD_{KDIGO} was 1.38 (95% CI, 1.17-1.62), whereas for CKD_{SCr} this was 2.40 (95%CI, 1.78-3.23) as mentioned above (**Supplementary Figure S5**). Use of UACR instead of UAE as measure of albuminuria yielded highly similar results (**Supplementary Figures S3-S5**).

Heritability estimates

In **Table 2**, we report heritability estimates of the CKD defining traits, eGFR (44%), UAE (20%), and UACR (19%), for the kidney biomarkers serum urea (31%),

Table 2. Heritability of renal traits and related biomarkers

Traits	N	Model 1		Model 2	
		h^2 ± SE	PVC	h^2 ± SE	PVC
eGFR	155,911	0.435 ± 0.007	0.420	0.436 ± 0.007	0.423
ln(UAE) [a]	59,145	0.199 ± 0.014	0.026	0.193 ± 0.014	0.048
ln(UACR) [a]	59,938	0.185 ± 0.014	0.083	0.178 ± 0.014	0.103
Uric acid [a]	58,519	0.481 ± 0.013	0.338	0.497 ± 0.013	0.442
Serum creatinine	155,911	0.373 ± 0.007	0.374	0.379 ± 0.007	0.377
Serum urea [a]	58,481	0.307 ± 0.013	0.218	0.307 ± 0.013	0.219
Serum potassium	155,842	0.279 ± 0.007	0.041	0.278 ± 0.007	0.050
Serum calcium [a]	58,488	0.268 ± 0.013	0.059	0.266 ± 0.013	0.079
Serum sodium [a]	58,444	0.217 ± 0.013	0.066	0.221 ± 0.013	0.074

Model 1: adjusted for age, sex, age^2.
Model 2: adjusted for age, sex, age^2, body mass index, diabetes, hypertension, high cholesterol and smoking status.
Abbreviations and definitions: eGFR, estimated glomerular filtration rate; UAE, urinary albumin excretion; UACR, urinary albumin-creatinine ratio; h^2, heritability; SE, standard error; PVC, proportion of variance due to covariates.

[a] data was only available for a subsample of adult participants

serum creatinine (37%) and uric acid (48%), and finally for the serum electrolytes sodium (22%), potassium (28%), and calcium (27%). In the subsample of ~60,000 participants with available urinary albumin measurements, heritability estimates of eGFR, serum creatinine, and serum potassium were consistent with the estimates in the full sample but were less precise (**Supplementary Table S3**). Heritability estimates did not change when taking household or spousal effects into account as they explained less than 0.1% of the variance in all variables and confirmed by the small spousal correlations (**Supplementary Table S4**). Inclusion of additional covariates in model 2 did not substantially change the estimates of heritability. Around 42% of the phenotypic variance of eGFR could be explained by sex and age, whereas sex and age only explained 2.6% for UAE and 8.3% for UACR. Inclusion of additional covariates in model 2 only slightly increased the proportion of total phenotypic variance attributable to covariates (PVC) for most traits with the exception of uric acid which showed a substantial increase in PVC of 10.3%. The PVC for model 2 ranged from 4.6% for potassium to 44.1% for uric acid.

A number of traits (i.e. UAE, UACR, uric acid, serum urea, serum calcium, and serum sodium) were only available for the subsample, whereas eGFR, serum creatinine, and serum potassium were available for nearly all participants. To assess potential bias due to missingness, we repeated the heritability analysis for eGFR, serum creatinine, and serum potassium, in the subsample. Estimates were highly

comparable to those in the full sample, although the heritability estimate for serum creatinine was slightly higher in the subsample (**Supplementary Table S3**).

DISCUSSION

In this large population-based family study, we investigated the familial aggregation of CKD by comparing the risk of CKD in individuals with an affected first-degree relatives to that in the general population. Participants with an affected first-degree relative were observed to have a threefold higher risk of CKD when compared to the risk in the general population, independent of BMI, hypertension, type 2 diabetes, hypercholesterolemia, and smoking status. This may in part be explained by shared environmental factors and/or assortative mating, given that we observed a 1.6 fold higher risk in those with an affected spouse. Furthermore, we estimated the heritability of eGFR and albuminuria, as well as that of related biomarkers and electrolytes. The heritability estimate for eGFR was considerable (44%), whereas heritability of UAE was moderate (20%). Heritability of kidney related markers and serum electrolytes ranged between 20 and 50%. These results indicate an important role for genetic factors in modulating susceptibility to kidney disease in the general population.

In this study, a threefold higher risk of CKD was observed for participants with an affected first-degree relative. Previous studies that examined familial aggregation of CKD focused on its end-stage, i.e. ESKD. In African-Americans, the presence of a first-degree relative with ESKD conveyed a nine-fold increase in risk of ESKD[46], while in Taiwanese Han-Chinese, there was a 2.5-fold increase in risk[12]. In the US, a multi ancestry (African and European) population-based case-control study conducted by Lei et al. demonstrated familial aggregation of ESKD, with estimates for recurrence risk ranging from a 1.3-fold to over a tenfold increase, depending on number of family members affected[8]. In a large registry-based study among Norwegians, individuals with an affected first-degree were at a 7.2-fold higher relative risk of ESKD[15]. Among incident dialysis patients in the ESKD Network 6, 23% of subjects have close relatives with ESKD[47], and individuals with family history of ESKD are at increased risk for CKD[48]. These studies focused on ESKD as determined through registry data. Early stages of CKD remain unrecognized in such data, leading to potential misestimation of familial clustering of CKD. The present study is unique in that it included a large population and that it is based on objective laboratory

measurements of eGFR and UAE in three-generational data. Our study is therefore more sensitive to non-symptomatic, early-stage CKD in multiple family members.

The RRR of CKD_{KDIGO} in those with an affected relative was statistically significant, but considerably lower than that of CKD_{Scr}. This may be explained by our observation that measures of albuminuria were only moderately heritable, i.e. genetic factors contribute relatively little to between-individual variation in urinary albumin excretion.

Spouses of those affected by CKD were at a 1.6 times higher risk of CKD, and in addition, kidney traits showed weak but significant positive correlations between spouses. As spouses are unrelated, the increased risk of CKD in spouses and weak spousal correlations of kidney traits may reflect effects of shared environmental factors or assortative mating. To further assess the effects of shared environment on the continuous kidney traits, we examined family and spouse effects as variance components in our heritability models. As these effects were negligible, the elevated risk in spouses seems therefore more related to assortative mating, i.e. partner selection based on phenotypes that convey higher risk of CKD. In literature, strong evidence of assortment exists for factors such as substance use (e.g. smoking, alcohol use)[49], anthropometrics (e.g. height, BMI, waist-to-hip ratio), and educational attainment[50], each a potential determinant of CKD risk and progression. In the present study however, spousal correlations of eGFR and UAE did not diminish after adjustment for renal risk factors (including BMI and smoking status). Thus, assortment likely occurs on factors other than those that select for currently known CKD determinants. Future study in spousal pairs may further investigate the mechanisms and the impact of assortative mating in CKD risk.

Between-study comparison of heritability estimates is not straightforward, as phenotypic variance and contribution of genetic factors depend on population, ethnicity, environment, measurement methods, and sampling error. Some inconsistency in estimates can therefore be expected. To date, the heritability of eGFR has been described in several twin studies and a few community-based studies. In the present large-scale study, we observed a heritability of 44% for eGFR (estimated by CKD-EPI equation for serum creatinine), corroborating estimates from previous, relatively small-scale, population-based studies, such as from

Switzerland (N=1128, 46%)[18] and from South-Tyrol (N=4373, 39%)[13]. Inconsistencies in heritability estimates for eGFR can be observed with studies in specific populations or that applied different methods. A previous analysis, in pedigree data (N=1224) from the population-based Framingham Heart Study, reported a lower heritability estimate for eGFR (33%)[51]. MacCluer et al. also found a lower heritability estimate of eGFR (33%) among Zuni Indians[21]. The lower estimates in these two studies are possibly due to differences between populations, random sampling error, or use of older, less accurate eGFR estimating methods[52]. As generally observed for most traits, also for eGFR, twin studies (50%-67.3%)[20,22,23] yielded somewhat higher heritability estimates than family-based studies (33%-46%)[13,18,19,21].

The heritability estimate of urinary albumin (i.e. UAE and UACR) in the present study (20%) was similar to that in a previous Swiss population-based study by Moulin et al (23%),[18] a study that was also based on 24-hour urine collections. The heritability of UACR was 21% in Pima Indians[45], and 25% in Zuni Indians[21]. In a twin study, the heritability of UACR was 45.2±7.4%[53]. Previous studies in diabetic patients have reported highly variable heritability estimates of albuminuria measured in spot urine samples, ranging from 21% to 46%[26,27,54-56]. Finally, we observed a 22%-28% heritability for the serum electrolytes, potassium, calcium, and sodium, which confirms the potential for identifying genetic variants involved in electrolyte homeostasis in the general population.

The heritability estimates in the present study provide an upper bound to the amount of phenotypic variance that can be attributed to genetic factors. A popular method of identifying genetic factors associated with disease and disease traits is the genome-wide association study (GWAS). Large-scale GWASs have thus far identified 306 common (i.e. with minor allele frequency >1%) single nucleotide polymorphisms (SNPs) for eGFRcrea explaining 7.1% of phenotypic variance[13], whereas the present study estimates the heritability of eGFRcrea to be 44%. Similarly, the 59 SNPs thus far identified in GWASs on UACR explained 0.7%, which is modest compared to our heritability estimate of 20%[14]. Thus, much of the heritability of kidney traits remains to be discovered. Potentially, future whole genome sequencing study that focus on rare variants (i.e. SNPs with a minor allele frequency <1%) may unveil a large proportion of this missing heritability[57,58].
The present study is by far the largest family-based study on kidney traits that

uses laboratory defined CKD, whereas other similar studies relied on medical records or health insurance data on ESKD that are not sensitive to earlier stage CKD. Leveraging these laboratory data, the present study is the first to quantify the familial clustering of CKD including early (i.e. non-ESKD) stages of CKD. Furthermore, Lifelines is representative with regards to its source population (i.e. the general population of the northern Netherlands), which facilitated precise estimation of heritability. In addition, albuminuria was determined not only in spot urine samples, but also in 24h urine collections, that are considered the gold standard to assess albuminuria. There are only very few large scale epidemiological studies that have 24h urine collections available. .

Several limitations need to be addressed. First, although gold standard 24h albuminuria measurements were available, this was only true for a subsample of approximately 60,000 participants. However, this substantial sample size still offers ample statistical power and reliable results. Furthermore, missingness was likely random as age, sex and covariate distributions were highly similar between sub- and full sample, and therefore unlikely to have seriously biased our results. This is supported by the consistency of the heritability estimates of eGFR, serum creatinine, and serum potassium between the total sample and the subsample. Second, GFR was not measured directly but estimated from serum creatinine. Therefore, some bias is possible due to creatinine metabolism. In addition, eGFR estimating equations are known to be less precise in the higher ranges (>60 mL/min/1.73m^2)[59-61]. These measurement errors may have caused downward bias in our heritability estimates. Third, no kidney biopsy data was available, nor could we exclude Mendelian forms of inherited kidney disease; we therefore could not distinguish between the different etiologies of CKD. Fourth, potential preferential missingness of data from non-participating affected family members may have led to underestimation of recurrence risk ratios. Finally, 98% of Lifelines participants are of European ancestry[62]; we therefore cannot generalize our results to other ancestries.

The results of this study may have several implications in addition to those previously mentioned. First, the data on familial recurrence of CKD may guide clinical decision-making with regards to CKD diagnosis and prevention. Further study is warranted to assess the added value of family history in risk stratification of CKD, and to investigate the potential impact of specifically targeting family members of CKD patients for screening and prevention strategies. Second, the

heritability estimates provide an upper bound to how much variance of a trait can be explained by genetic factors. Future studies, e.g. GWAS, may focus on identifying these genetic factors.

In summary, we demonstrate that CKD clusters in families in the general population, given that risk of CKD was strongly elevated in those with an affected relative. Considerable heritability (20-50%) of kidney traits was observed. Therefore, much of the familial clustering may be attributed to genetic factors. The data presented in this study inform future work on risk stratification based on family history, and provide a step forward in disentangling genetic and environmental risk factors in CKD.

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Author Contributions: Study conception and design: HS, CHLT, JZ; data analysis: CHLT, JZ; data interpretation and literature review: JZ, CHLT, HS, RTG; research mentorship and supervision: HS, RTG. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the author's own contribution, and agrees to ensure that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

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REFERENCES

1. Jha V, Garcia-Garcia G, Iseki K, et al. Chronic kidney disease: Global dimension and perspectives. *The Lancet*. 2013;382(9888):260-272.
2. Bruck K, Stel VS, Gambaro G, et al. CKD prevalence varies across the European general population. *J Am Soc Nephrol*. 2016;27(7):2135-2147.
3. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med*. 2004;351(13):1296-1305.
4. Kahn MR, Robbins MJ, Kim MC, Fuster V. Management of cardiovascular disease in patients with kidney disease. *Nat Rev Cardiol*. 2013;10(5):261.
5. Gansevoort RT, Correa-Rotter R, Hemmelgarn BR, et al. Chronic kidney disease and cardiovascular risk: Epidemiology, mechanisms, and prevention. *The Lancet*. 2013;382(9889):339-352.
6. Nitsch D, Grams M, Sang Y, et al. Associations of estimated glomerular filtration rate and albuminuria with mortality and renal failure by sex: A meta-analysis. *BMJ*. 2013;346:f324.
7. Echouffo-Tcheugui JB, Kengne AP. Risk models to predict chronic kidney disease and its progression: A systematic review. *PLoS Med*. 2012;9(11):e1001344. Epub 2012 Nov 20.
8. Lei HH, Perneger TV, Klag MJ, Whelton PK, Coresh J. Familial aggregation of renal disease in a population-based case-control study. *J Am Soc Nephrol*. 1998;9(7):1270-1276.
9. Queiroz Madeira EP, da Rosa Santos O, Ferreira Santos SF, Alonso da Silva L, MacIntyre Innocenzi A, Santoro-Lopes G. Familial aggregation of end-stage kidney disease in Brazil. *Nephron*. 2002;91(4):666-670.
10. Satko SG, Freedman BI. The familial clustering of renal disease and related phenotypes. *Med Clin North Am*. 2005;89(3):447-456.
11. Fava C, Montagnana M, Burri P, et al. Determinants of kidney function in swedish families: Role of heritable factors. *J Hypertens*. 2008;26(9):1773-1779.
12. Wu HH, Kuo CF, Li IJ, et al. Family aggregation and heritability of ESRD in Taiwan: A population-based study. *Am J Kidney Dis*. 2017;70(5):619-626.
13. Wuttke M, Li Y, Li M, et al. A catalog of genetic loci associated with kidney function from analyses of a million individuals. *Nat Genet*. 2019;51(6):957-972.
14. Teumer A, Li Y, Ghasemi S, et al. Genome-wide association meta-analyses and fine-mapping elucidate pathways influencing albuminuria. *Nat Commun*. 2019;10(1):1-19.
15. Skrunes R, Svarstad E, Reisaeter AV, Vikse BE. Familial clustering of ESRD in the Norwegian population. *Clin J Am Soc Nephrol*. 2014;9(10):1692-1700.
16. Visscher PM, Hill WG, Wray NR. Heritability in the genomics era - concepts and misconceptions. *Nat Rev Genet*. 2008;9(4):255-266.
17. Hunter DJ, Lange M, Snieder H, et al. Genetic contribution to renal function and electrolyte balance: A twin study. *Clin Sci (Lond)*. 2002;103(3):259-265.
18. Moulin F, Ponte B, Pruijm M, et al. A population-based approach to assess the heritability and distribution of renal handling of electrolytes. *Kidney Int*. 2017;92(6):1536-1543.
19. Bochud M, Elston RC, Maillard M, et al. Heritability of renal function in hypertensive families of African descent in the Seychelles (Indian ocean). *Kidney Int*. 2005;67(1):61-69.
20. Raggi P, Su S, Karohl C, Veledar E, Rojas-Campos E, Vaccarino V. Heritability of renal function and inflammatory markers in adult male twins. *Am J Nephrol*. 2010;32(4):317-323.
21. MacCluer JW, Scavini M, Shah VO, et al. Heritability of measures of kidney disease among Zuni Indians: The Zuni kidney project. *Am J K Dis*. 2010;56(2):289-302.
22. Pasha DN, Davis JT, Rao F, et al. Heritable influence of DBH on adrenergic and renal function: Twin and disease studies. *PloS one*. 2013;8(12):e82956.

23. Arpegard J, Viktorin A, Chang Z, de Faire U, Magnusson PK, Svensson P. Comparison of heritability of cystatin C- and creatinine-based estimates of kidney function and their relation to heritability of cardiovascular disease. *J Am Heart Assoc*. 2015;4(1):e001467.

24. Bathum L, Fagnani C, Christiansen L, Christensen K. Heritability of biochemical kidney markers and relation to survival in the elderly—results from a Danish population-based twin study. *Clin Chim Acta*. 2004;349(1-2):143-150.

25. Pilia G, Chen W, Scuteri A, et al. Heritability of cardiovascular and personality traits in 6,148 Sardinians. *PLoS Genet*. 2006;2(8):e132.

26. Forsblom CM, Kanninen T, Lehtovirta M, Saloranta C, Groop LC. Heritability of albumin excretion rate in families of patients with type II diabetes. *Diabetologia*. 1999;42(11):1359-1366.

27. Langefeld CD, Beck SR, Bowden DW, Rich SS, Wagenknecht LE, Freedman BI. Heritability of GFR and albuminuria in caucasians with type 2 diabetes mellitus. *Am J Kidney Dis*. 2004;43(5):796-800.

28. Whitfield J, Martin N. The effects of inheritance on constituents of plasma: A twin study on some biochemical variables. *Ann Clin Biochem*. 1984;21(3):176-183.

29. Stolk RP, Rosmalen JG, Postma DS, et al. Universal risk factors for multifactorial diseases. *Eur J Epidemiol*. 2008;23(1):67-74.

30. Scholtens S, Smidt N, Swertz MA, et al. Cohort profile: LifeLines, a three-generation cohort study and biobank. *Int J Epidemiol*. 2015 Aug;44(4):1172-80.

31. Levey AS, de Jong PE, Coresh J, et al. The definition, classification, and prognosis of chronic kidney disease: A KDIGO controversies conference report. *Kidney Int*. 2011;80(1):17-28.

32. Levin A, Stevens PE, Bilous RW, et al. Kidney disease: Improving global outcomes (KDIGO) CKD work group. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. *Kidney Int Supp*. 2013;3(1):1-150.

33. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150(9):604-612.

34. Schwartz GJ, Munoz A, Schneider MF, et al. New equations to estimate GFR in children with CKD. *J Am Soc Nephrol*. 2009;20(3):629-637.

35. Risch N. Linkage strategies for genetically complex traits. I. multilocus models. *Am J Hum Genet*. 1990;46(2):222-228.

36. Breslow N. Covariance analysis of censored survival data. *Biometrics*. 1974:89-99.

37. Skov T, Deddens J, Petersen MR, Endahl L. Prevalence proportion ratios: Estimation and hypothesis testing. *Int J Epidemiol*. 1998;27(1):91-95.

38. Barros AJ, Hirakata VN. Alternatives for logistic regression in cross-sectional studies: An empirical comparison of models that directly estimate the prevalence ratio. *BMC medical research methodology*. 2003;3(1):21.

39. Lin D. Cox regression analysis of multivariate failure time data: The marginal approach. *Stat Med*. 1994;13(21):2233-2247.

40. Biswas A, Datta S, Fine JP, Segal MR. *Statistical advances in the biomedical science*. Wiley Online Library; 2007.

41. Lichtenstein P, Yip BH, Björk C, et al. Common genetic determinants of schizophrenia and bipolar disorder in swedish families: A population-based study. *The Lancet*. 2009;373(9659):234-239.

42. Kuo C, Grainge MJ, Valdes AM, et al. Familial risk of Sjögren's syndrome and co-aggregation of autoimmune diseases in affected families: A nationwide population study. *Arthritis & Rheumatology*. 2015;67(7):1904-1912.

43. Kuo C, Grainge MJ, Valdes AM, et al. Familial aggregation of systemic lupus erythematosus and coaggregation of autoimmune diseases in affected families. *JAMA Int Med*. 2015;175(9):1518-1526.

44. Gilmour A, Gogel B, Cullis B, Welham S, Thompson R. ASReml user guide release 4.1 structural specification. *Hemel hempstead: VSN international ltd*. 2015.

45. R Core Team. R: A language and environment for statistical computing. R foundation for statistical computing, vienna, Austria.

46. Freedman BI, Spray BJ, Tuttle AB, Buckalew Jr VM. The familial risk of end-stage renal disease in african americans. *Am J Kidney Dis*. 1993;21(4):387-393.

47. Freedman BI, Volkova NV, Satko SG, et al. Population-based screening for family history of end-stage renal disease among incident dialysis patients. *Am J Nephrol*. 2005;25(6):529-535.

48. McClellan WM, Satko SG, Gladstone E, Krisher JO, Narva AS, Freedman BI. Individuals with a family history of ESRD are a high-risk population for CKD: Implications for targeted surveillance and intervention activities. *Am J Kidney Dis*. 2009;53(3):S100-S106.

49. Agrawal A, Heath AC, Grant JD, et al. Assortative mating for cigarette smoking and for alcohol consumption in female Australian twins and their spouses. *Behav Genet*. 2006;36(4):553-566.

50. Robinson MR, Kleinman A, Graff M, et al. Genetic evidence of assortative mating in humans. *Nat Hum Behaviour*. 2017;1(1):0016.

51. Fox CS, Yang Q, Cupples LA, et al. Genomewide linkage analysis to serum creatinine, GFR, and creatinine clearance in a community-based population: The Framingham heart study. *J Am Soc Nephrol*. 2004;15(9):2457-2461.

52. Michels WM, Grootendorst DC, Verduijn M, Elliott EG, Dekker FW, Krediet RT. Performance of the cockcroft-gault, MDRD, and new CKD-EPI formulas in relation to GFR, age, and body size. *Clin J Am Soc Nephrol*. 2010;5(6):1003-1009.

53. Rao F, Wessel J, Wen G, et al. Renal albumin excretion: Twin studies identify influences of heredity, environment, and adrenergic pathway polymorphism. *Hypertension*. 2007;49(5):1015-1031.

54. Fogarty DG, Rich SS, Hanna L, Warram JH, Krolewski AS. Urinary albumin excretion in families with type 2 diabetes is heritable and genetically correlated to blood pressure. *Kidney Int*. 2000;57(1):250-257.

55. Imperatore G, Knowler WC, Pettitt DJ, Kobes S, Bennett PH, Hanson RL. Segregation analysis of diabetic nephropathy in Pima Indians. *Diabetes*. 2000;49(6):1049-1056.

56. Krolewski AS, Poznik G, Placha G, et al. A genome-wide linkage scan for genes controlling variation in urinary albumin excretion in type II diabetes. *Kidney Int*. 2006;69(1):129-136.

57. Nolte IM, Tropf FC, Snieder H. Missing heritability of complex traits and diseases. *eLS*. 2001:1-9.

58. Wainschtein P, Jain DP, Yengo L, et al. Recovery of trait heritability from whole genome sequence data. *bioRxiv*. 2019:588020.

59. Stevens LA, Coresh J, Greene T, Levey AS. Assessing kidney function—measured and estimated glomerular filtration rate. *N Engl J Med*. 2006;354(23):2473-2483.

60. Earley A, Miskulin D, Lamb EJ, Levey AS, Uhlig K. Estimating equations for glomerular filtration rate in the era of creatinine standardization: A systematic review. *Ann Intern Med*. 2012;156(11):785-795.

61. Levey AS, Inker LA, Coresh J. GFR estimation: From physiology to public health. *Am J Kidney Dis*. 2014;63(5):820-834.

62. van der Ende, M Yldau, Hartman MH, Hagemeijer Y, et al. The LifeLines cohort study: Prevalence and treatment of cardiovascular disease and risk factors. *Int J Cardiol*. 2017;228:495-500.

Supplementary Material

CHAPTER 4

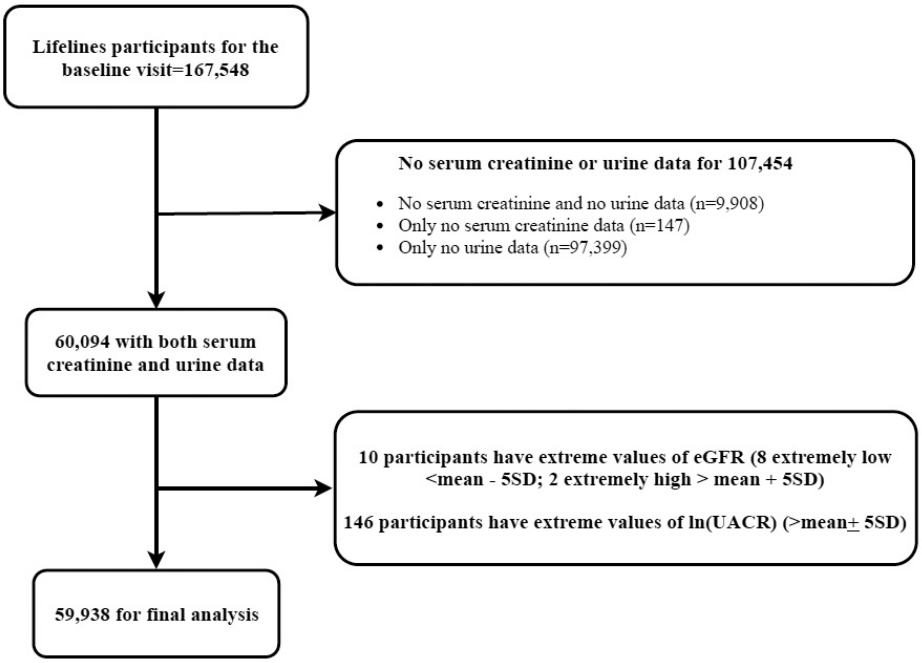

Figure S1. Flowchart of eGFR and UACR analysis. Abbreviations: estimated glomerular filtration rate, eGFR; standard deviation, SD; urinary albumin-creatinine ratio, UACR.

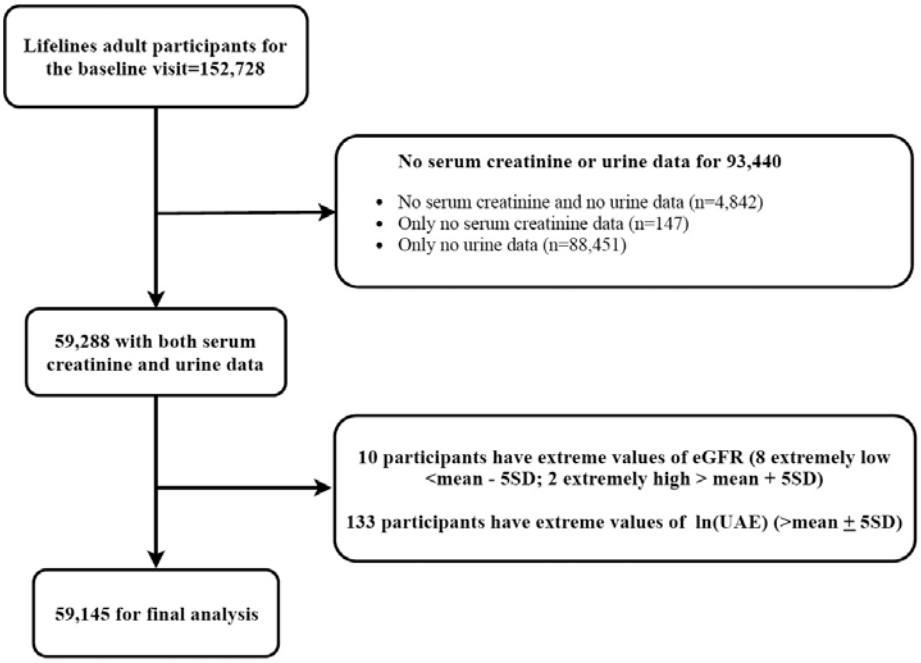

Figure S2. Flowchart of eGFR and UAE analysis. Abbreviations: estimated glomerular filtration rate, eGFR; standard deviation, SD; urinary albumin excretion, UAE.

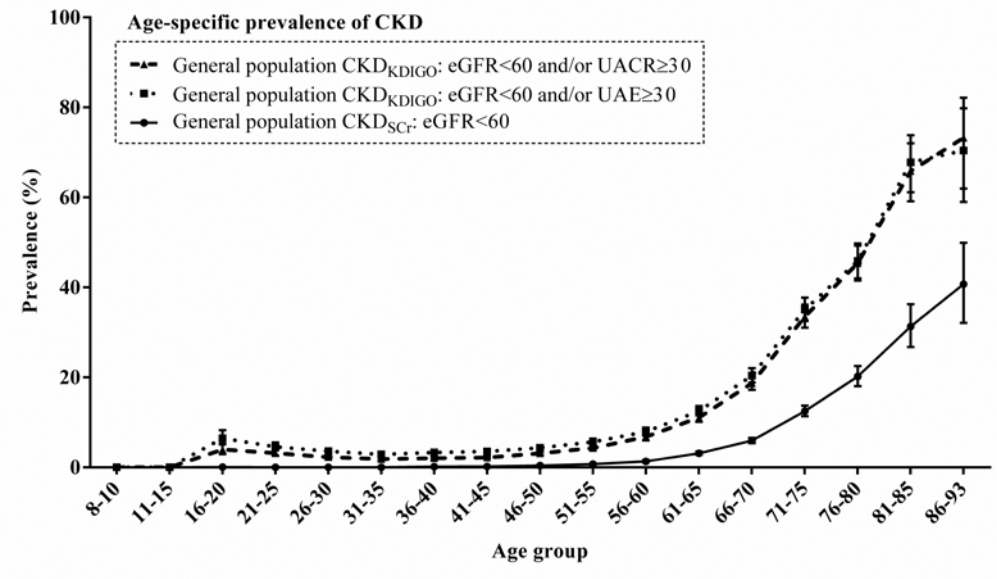

Figure S3. Age-specific prevalence of chronic kidney disease (CKD_{SCr}: eGFR<60 mL/min/1.73 m²; CKDKDIGO: eGFR < 60 mL/min/1.73 m² and/or UAE (UACR) ≥ 30 mg/24 hours (mg/g)) in the general population diagnosed by different criteria. Error bars indicate 95% confidence interval (CI). Abbreviations: estimated glomerular filtration rate, eGFR; urinary albumin excretion, UAE; urinary albumin-creatinine ratio, UACR.

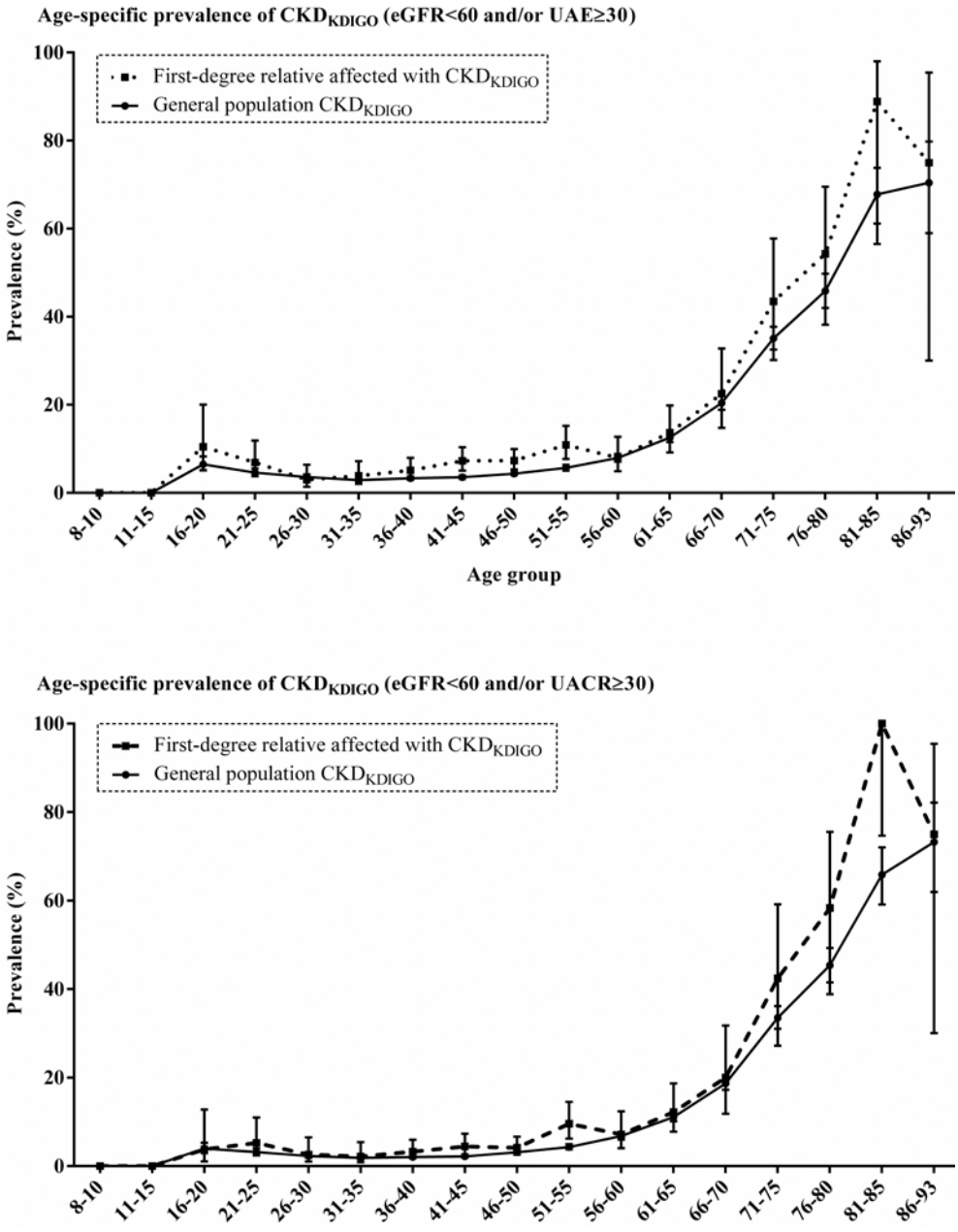

Figure S4. Comparisons of age-specific prevalence of chronic kidney disease (CKD_{KDIGO}: eGFR < 60 mL/min/1.73 m² and/or UAE (UACR) ≥ 30 mg/24 hours (mg/g)) between individuals with affected first-degree relatives and the general population. Error bars indicate 95% confidence interval (CI). Abbreviations: estimated glomerular filtration rate, eGFR; urinary albumin excretion, UAE; urinary albumin-creatinine ratio, UACR.

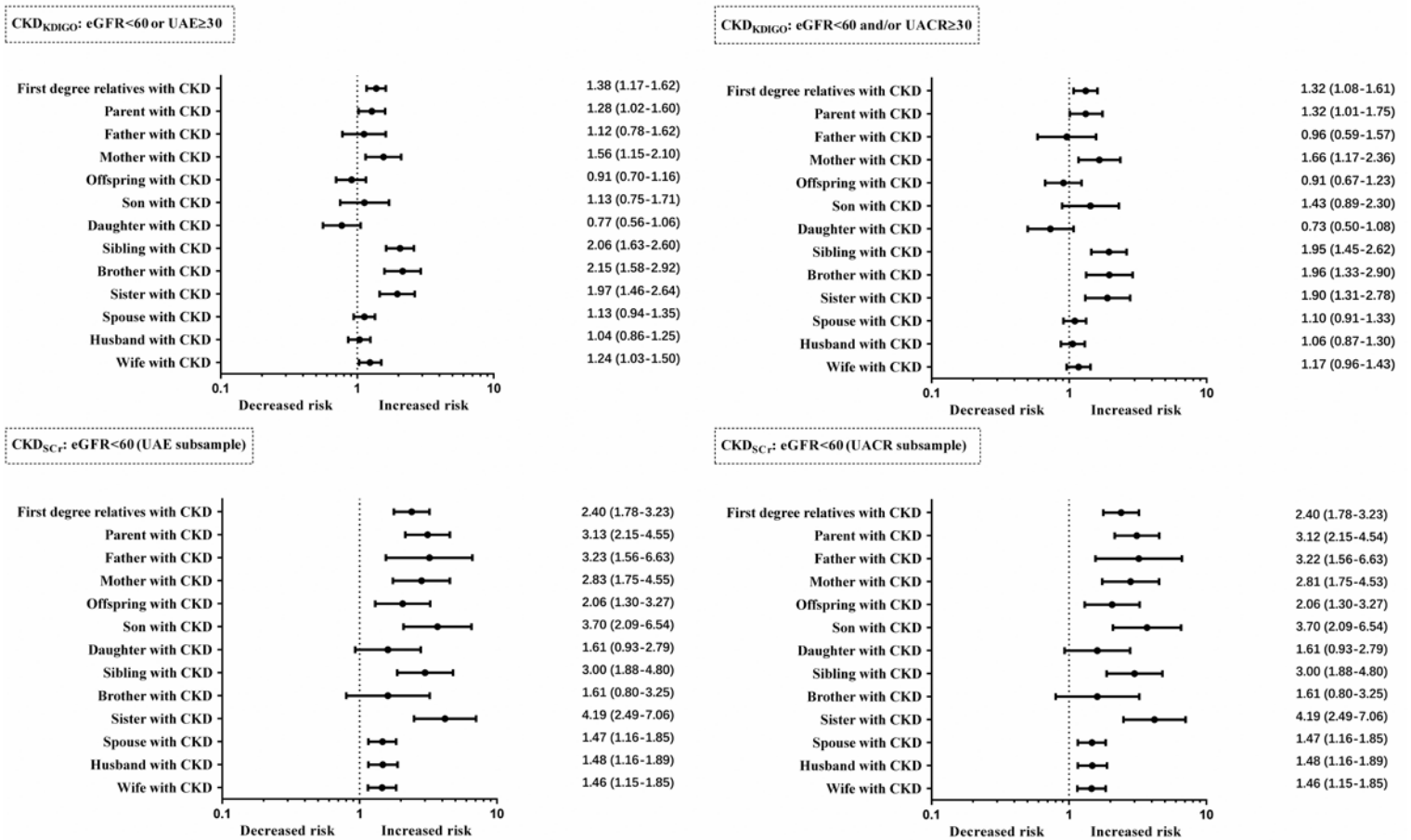

Figure S5. Recurrence risk ratios (adjusted for age, age², sex, BMI, hypertension, diabetes, high cholesterol, and smoking status) for chronic kidney disease (CKD) in individuals with affected first degree relatives or spouse. Error bars indicate 95% confidence interval (CI). Abbreviations: estimated glomerular filtration rate, eGFR; urinary albumin excretion, UAE; urinary albumin-creatinine ratio, UACR.

Table S1. Baseline characteristics of adult and child participants stratified by sex and age

Characteristics	Adults (aged ≥18)				Children (aged <18)				Total
	Total	Males	Females	P[a]	Total	Males	Females	P[a]	
Total population, n (%)	147715	61512 (41.64)	86203 (58.36)		8196	3891 (47.47)	4305 (52.53)		155911
Age (years)	44.83 (13.12)	45.46 (13.18)	44.38 (13.05)	<0.001	12.21 (2.76)	12.05 (2.72)	12.35 (2.79)	<0.001	43.11 (14.71)
BMI (kg/m²)	26.07 (4.33)	26.37 (3.68)	25.85 (4.72)	<0.001	18.91 (3.18)	18.61 (2.99)	19.19 (3.31)	<0.001	25.69 (4.56)
Current smoker (%)	31156 (21.38)	14245 (23.39)	17085 (19.94)	<0.001	NA[c]	NA[c]	NA[c]	NA[c]	NA[c]
Hypertension (%)	38605 (26.13)	19876 (32.31)	18729 (21.73)	<0.001	NA[c]	NA[c]	NA[c]	NA[c]	NA[c]
Diabetes (%)	5673 (3.84)	2805 (4.56)	2868 (3.33)	<0.001	NA[c]	NA[c]	NA[c]	NA[c]	NA[c]
Hypercholesterolemia (%)	29888 (20.23)	14361 (23.35)	15527 (18.01)	<0.001	NA[c]	NA[c]	NA[c]	NA[c]	NA[c]
Serum potassium (mEq/L)	3.86 (0.30)	3.89 (0.33)	3.84 (0.29)	<0.001	3.84 (0.28)	3.85 (0.28)	3.83 (0.28)	<0.001	3.86 (0.3)
Serum creatinine (mg/dL)	0.83 (0.14)	0.93 (0.13)	0.76 (0.11)	<0.001	0.61 (0.13)	0.61 (0.14)	0.60 (0.12)	<0.001	0.82 (0.15)
eGFR (mL/min/1.73 m²)	96.41 (15.29)	97.34 (15.22)	95.74 (15.31)	<0.001	111.46 (16.81)	111.35 (16.84)	111.56 (16.78)	<0.001	97.2 (15.74)
CKD_{SCr}; eGFR<60 (%)[d]	1858 (1.26)	806 (1.31)	1052 (1.22)	0.0352	4 (0.05)	1	3	NA[b]	1862 (1.19)
Subsample, n (%)	59195	24517 (41.42)	34678 (58.58)		748	356 (47.59)	392 (52.41)		59943
Age (years)	44.94 (12.52)	45.46 (12.60)	44.57 (12.45)	<0.001	12.27 (2.78)	12.20 (2.73)	12.34 (2.82)	0.4748	44.53 (12.96)
Serum calcium (mg/dL)	9.14 (0.32)	9.22 (0.32)	9.10 (0.32)	<0.001	9.50 (0.28)	9.54 (0.28)	9.46 (0.28)	0.0019	9.14 (0.32)
Serum sodium (mEq/L)	141.74 (1.84)	142.23 (1.76)	141.39 (1.85)	<0.001	141.63 (1.68)	141.69 (1.82)	141.57 (1.54)	0.287	141.73 (1.84)
Uric acid (mg/dL)	4.88 (1.18)	5.72 (1.01)	4.37 (1.01)	<0.001	4.37 (1.01)	4.54 (1.01)	4.20 (0.84)	<0.001	4.88 (1.18)
Serum urea (mg/dL)	14.48 (3.56)	15.77 (3.61)	13.61 (3.33)	<0.001	12.41 (2.75)	12.97 (2.69)	11.88 (2.72)	<0.001	14.45 (3.56)
UACR (mg/g)	2.72 (1.57-5.05)	2.00 (1.20-3.70)	3.29 (2.00-5.85)	<0.001	2.93 (1.84-4.84)	2.52 (1.64-4.52)	3.12 (1.97-5.41)	<0.001	2.72 (1.58-7.33)
UAE (mg/24h)	3.86 (2.33-6.92)	3.68 (2.18-6.78)	4.00 (2.44-7.01)	<0.001	NA[c]	NA[c]	NA[c]	NA[c]	NA[c]
UACR ≥30	1622 (2.73)	688 (2.80)	934 (2.69)	0.883	20 (2.68)	5 (1.41)	15 (3.85)	0.053	1642 (2.73)
UAE ≥30	2431 (4.10)	1254 (5.10)	1177 (3.39)	<0.001	NA[c]	NA[c]	NA[c]	NA[c]	NA[c]
CKD_{KDIGO}; eGFR<60 or UACR ≥30	3338 (5.52)	1400 (5.58)	1938 (5.48)	0.0325	24 (3.20)	6 (1.69)	18 (4.58)	0.0354	3362 (5.49)
CKD_{KDIGO}; eGFR<60 or UAE ≥30	4127 (6.83)	1949 (7.78)	2178 (6.16)	<0.001	NA[c]	NA[c]	NA[c]	NA[c]	NA[c]

Data are presented as mean (SD), median (interquartile range) or number (%), where appropriate. Abbreviations and definitions: BMI, body mass index; SCr, serum creatinine; UACR, urinary albumin-creatinine ratio; UAE, urinary albumin excretion; eGFR, estimated glomerular filtration rate; CKD, chronic kidney disease. Conversion factors for calcium in mg/dL to mmol/L, ×0.2495; creatinine in mg/dL to µmol/L, ×88.4; uric acid in mg/dL to µmol/L, ×59.48; serum urea in mg/dL to mmol/L, ×0.357. [a]: P-values were adjusted for age using multiple linear regression, and multiple logistic regression where appropriate. [b]: sample size was too small to calculate P-value. [c]: data was not available for children. [d]: 21 subjects with extremely low eGFR values (< 5SD from the mean) were considered CKD patients and retained in analyses of CKD

Table S2. Baseline characteristics of adult and child participants among subsample stratified by sex and age

Characteristics	Adults (aged ≥18)				Children (aged <18)				Total
	Total	Males	Females	P[a]	Total	Males	Females	P[a]	
Subsample, n (%)	59195	24517 (41.42)	34678 (58.58)		748	356 (47.59)	392 (52.41)		59943
Age (years)	44.94 (12.52)	45.46 (12.60)	44.57 (12.45)	<0.001	12.27 (2.78)	12.20 (2.73)	12.34 (2.82)	0.474	44.53 (12.96)
BMI (kg/m²)	26.08 (4.31)	26.42 (3.65)	25.84 (4.70)	<0.001	19.11 (3.17)	18.71 (2.71)	19.48 (3.50)	<0.001	25.99 (4.37)
Current smoker (%)	13090 (22.55%)	5893 (24.53)	7197 (21.16)	<0.001	NA[c]	NA[c]	NA[c]	NA[c]	NA[c]
Hypertension (%)	15951 (26.95)	8302 (33.86)	7649 (22.06)	<0.001	NA[c]	NA[c]	NA[c]	NA[c]	NA[c]
Diabetes (%)	2324 (3.93)	1162 (4.74)	1162 (3.35)	<0.001	NA[c]	NA[c]	NA[c]	NA[c]	NA[c]
Hypercholesterolemia (%)	11397 (19.25)	5444 (22.21)	5953 (17.17)	<0.001	NA[c]	NA[c]	NA[c]	NA[c]	NA[c]
Serum potassium (mEq/L)	3.90 (0.30)	3.92 (0.31)	3.88 (0.29)	<0.001	3.95 (0.29)	3.97 (0.29)	3.93 (0.28)	0.087	3.90 (0.30)
Serum creatinine (mg/dL)	0.84 (0.14)	0.94 (0.12)	0.77 (0.11)	<0.001	0.62 (0.13)	0.63 (0.14)	0.61 (0.11)	0.002	0.84 (0.14)
eGFR (mL/min/1.73 m²)	95.33 (14.92)	96.55 (14.77)	94.47 (14.97)	<0.001	108.55 (15.23)	108.31 (15.52)	108.77 (14.98)	0.377	95.49 (15.00)
CKD_{Scr}: eGFR<60 (%)[d]	709 (1.20)	306 (1.25)	403 (1.16)	0.482	0	0	0	NA[b]	709 (1.18)

Data are presented as mean (SD), median (interquartile range) or number (%), where appropriate. Abbreviations and definitions: BMI, body mass index; SCr, serum creatinine; eGFR, estimated glomerular filtration rate; CKD, chronic kidney disease. Conversion factors for creatinine in mg/dL to μmol/L, ×88.4. [a]: P-values were adjusted for age using multiple linear regression, and multiple logistic regression where appropriate. [b]: sample size was too small to calculate P-value. [c]: data was not available for children. [d]: 21 subjects with extremely low eGFR values (< 5SD from the mean) were considered CKD patients and retained in analyses of CKD

Table S3. Heritability of eGFR, serum creatinine and potassium in subsample participants only

Traits	N	Model 1		Model 2	
		h^2 ± SE	PVC	h^2 ± SE	PVC
eGFR	59.943	0.456 ± 0.013	0.387	0.458 ± 0.013	0.388
Serum creatinine	59.943	0.433 ± 0.012	0.361	0.436 ± 0.012	0.363
Serum potassium	59.943	0.262 ± 0.013	0.034	0.263 ± 0.013	0.041

Model 1: adjusted for age, sex, age^2.
Model 2: adjusted for age, sex, age^2, body mass index, diabetes, hypertension, high cholesterol, and smoking status.

Abbreviations and definitions: eGFR, estimated glomerular filtration rate; UAE, urinary albumin excretion; UACR, urinary albumin-creatinine ratio; h^2, heritability; SE, standard error; PVC, proportion of variance due to covariates.

Table S4. Spousal correlations

Renal traits	N (Pairs)	model1		model2	
		Pearson correlation	P value	Pearson correlation	P value
eGFR	29356	0.069	<0.001	0.067	<0.001
serum creatinine	29356	0.074	<0.001	0.073	<0.001
ln(UAE)	9935	0.035	<0.001	0.034	<0.001
ln(UACR)	9951	0.032	<0.001	0.034	<0.001
serum urea	9933	0.116	<0.001	0.115	<0.001
uric acid	9948	0.082	<0.001	0.050	<0.001
serum potassium	29325	0.167	<0.001	0.166	<0.001
serum calcium	9934	0.034	<0.001	0.035	<0.001
serum sodium	9913	0.077	<0.001	0.069	<0.001
Chronic Kidney Disease (CKD)	N (Pairs)	Phi coefficient		P value	
CKD_{SCr}: $eGFR_{SCr}$<60 (%)	29356	0.12		<0.001	
CKD_{KDIGO}: $eGFR_{SCr}$<60 or UACR ≥30	9951	0.15		<0.001	
CKD_{KDIGO}: $eGFR_{SCr}$<60 or UAE ≥30	9935	0.12		<0.001	

Model 1: adjusted for age, age^2, and sex
Model 2: adjusted for age, age^2, sex, BMI, hypertension, diabetes, high cholesterol, and smoking status

Abbreviations and definitions: eGFR, estimated glomerular filtration rate; UAE, urinary albumin excretion; UACR, urinary albumin-creatinine ratio

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