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# Influence of common genetic variants on childhood kidney outcomes

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**BACKGROUND:** Kidney measures in early life are associated with kidney disease in later life. We hypothesized that these associations are partly explained by common genetic variants that lead to both smaller kidneys with lower kidney function in early childhood and kidney disease in adulthood.

**METHODS:** We examined in a population-based prospective cohort study among 4,119 children the associations of a weighted genetic risk score combining 20 previously identified common genetic variants related to adult $eGFR_{creat}$ with kidney outcomes in children aged 6.0 years (95% range 5.7–7.8). Childhood kidney outcomes included combined kidney volume, glomerular filtration rate (eGFR) based on creatinine levels, and microalbuminuria based on albumin and creatinine urine levels.

**RESULTS:** We observed that the genetic risk score based on variants related to impaired kidney function in adults was associated with a smaller combined kidney volume (*P* value $3.0\times10^{-3}$) and with a lower eGFR (*P* value $4.0\times10^{-4}$) in children. The genetic risk score was not associated with microalbuminuria.

**CONCLUSION:** Common genetic variants related to impaired kidney function in adults already lead to subclinical changes in childhood kidney outcomes. The well-known associations of kidney measures in early life with kidney disease in later life may at least be partly explained by common genetic variants.

End-stage kidney disease seems to partly originate in early life (1,2). Smaller kidneys in early life with a reduced number of nephrons lead to glomerular hyperfiltration and sclerosis and predispose to kidney disease in adulthood (3,4). Also, risk factors for impaired kidney function track from childhood to adulthood (5,6). Early kidney growth and development is a complex developmental process and is influenced by many environmental factors (7,8). Various nutritional and environmental exposures have been suggested to affect early kidney development and may subsequently influence the risk of kidney disease in later life (9,10).

However, the associations between early life kidney characteristics and kidney disease in later life may also be explained by common genetic variants. These variants may affect early kidney development and function and thereby predispose individuals to kidney disease in later life. Several genes are known to be involved in kidney development (8). Rare mutations in these genes can cause severe anomalies, such as agenesis or dysgenesis of the kidney (7). These mutations do not explain variation in kidney development within normal range. However, large genome-wide association studies (GWAS) in adults have identified 30 common genetic variants associated with impaired kidney function (11–13). For this study, we hypothesized that these genetic variants also affect kidney growth and function in early childhood, and thereby partly explain the well-known associations of early life kidney measures with kidney disease in later life.

To test this hypothesis, we examined, in a population-based prospective cohort study among 4,119 children, the associations of genetic risk scores combining previously identified common genetic variants related to impaired kidney function in adults with kidney outcomes in school-age children.

## RESULTS

### Participant Characteristics

The flowchart of study participants is given in the **Figure 1**. **Table 1** shows the characteristics of the participants. In the full group, the mean combined kidney volume and $eGFR_{creat}$ were 120 $cm^3$ (SD ± 23.3) and 119 ml/min/1.73 $m^2$ (SD ± 16), respectively. Of all the children, 7.9% had microalbuminuria and 0.07% had an eGFR <60 ml/min/1.73 $m^2$. The characteristics of the subgroup of European children only were similar to those of the full group (**Supplementary Table S1** online).

### Individual Genetic Variants and Childhood Kidney Outcomes

Results for the associations of the individual single-nucleotide polymorphisms (SNPs) with childhood kidney outcomes are given in the **Supplementary Material** online. Of the 20 available SNPs known to be associated with $eGFR_{creat}$ and the single SNP associated with microalbuminuria in adults, none were individually significantly associated with kidney volume, $eGFR_{creat}$, $eGFR_{cystC}$, and microalbuminuria, in the full group

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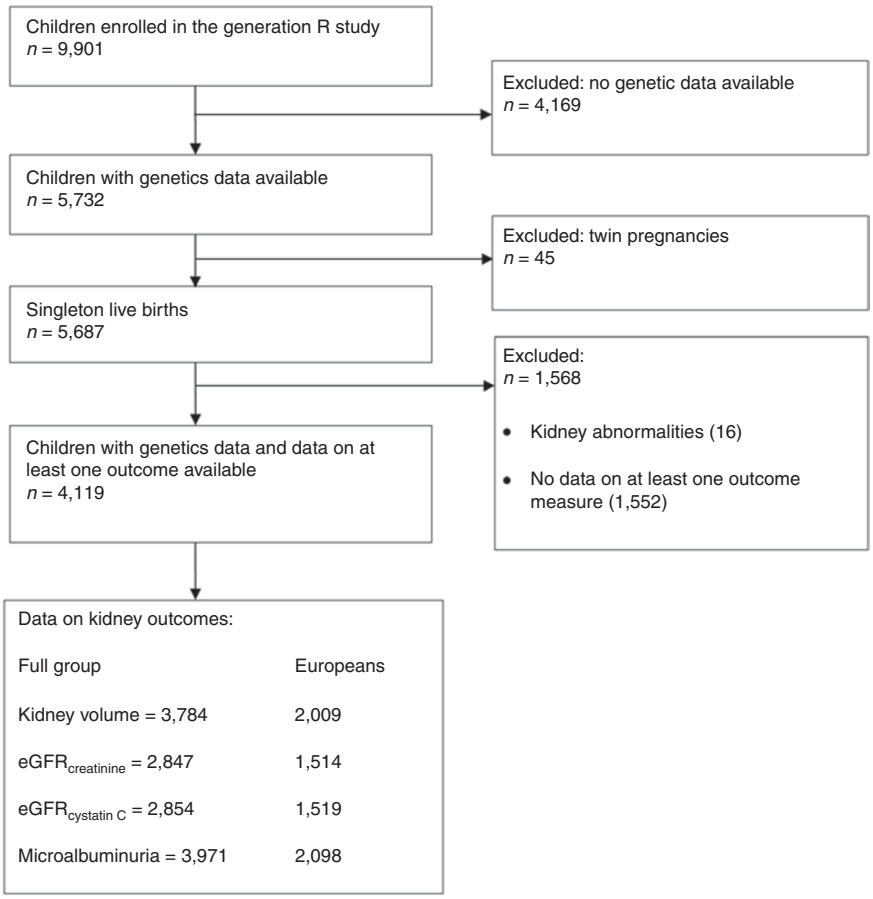


**Figure 1** Flow chart of the study participants.

**Table 1.** Subject characteristics ($N = 4,119$)

| | |
|---|---|
| Girls (%) | 49.7 |
| Gestational age at birth (weeks) | 40.1 (36.4–42.3) |
| Birth weight (g) | 3,464 (515) |
| Age at kidney measurements (y) | 6.0 (5.7–7.8) |
| Height at kidney measurements (m) | 1.20 (0.06) |
| Weight at kidney measurements (kg) | 23.3 (4.2) |
| Combined kidney volume ($cm^3$) | 120.0 (23.3) |
| Creatinine (μmol/l) | 37.4 (5.7) |
| Cystatin C (μg/l) | 783 (83) |
| $eGFR_{creat}$ (ml/min/1.73 $m^2$) | 119 (16) |
| $eGFR_{cystC}$ (ml/min/1.73 $m^2$) | 103 (15) |
| Microalbuminuria (%) | 7.9 |

Values are valid percentages for categorical variables, means (SD) for continuous variables with a normal distribution, or medians (95% range) for continuous variables with a skewed distribution.
$eGFR_{creat}$, estimated glomerular filtration rate calculated based on creatinine blood levels; $eGFR_{cystC}$, estimated glomerular filtration rate calculated based on cystatin C blood levels.

(**Supplementary Table S2** online). In both, the full group and the European ancestry subgroup, rs3925584, located upstream of MPPED2, was associated with childhood creatinine blood levels ($P$ values $1.8 \times 10^{-3}$ and $5.6 \times 10^{-4}$, respectively). In addition, in the European ancestry subgroup, rs11078902 in CDK12 was associated with childhood creatinine blood levels and with $eGFR_{creat}$ ($P$ values $1.1 \times 10^{-3}$ and $2.3 \times 10^{-3}$, respectively) (**Supplementary Tables S3–S5** online). The 20 adult kidney function SNPs together explained 1.3, 1.4, and 0.6% of the variance in combined kidney volume, $eGFR_{creat}$, and $eGFR_{cystC}$ in childhood, respectively.

### Genetic Risk Score and Childhood Kidney Outcomes

Table 2 presents the associations of the weighted genetic risk score based on 20 SNPs known to be associated with impaired adult $eGFR_{creat}$ with childhood kidney outcomes. The weighted risk score ranged from 13 to 33 with a mean of 22.8 (SD 2.8) and was significantly associated with childhood combined kidney volume ($P$ value $3.0 \times 10^{-3}$). For each additional average risk allele, combined kidney volume was −0.39 $cm^3$ (95% confidence interval (CI): −0.64, −0.13) smaller. The difference in combined kidney volume between the lowest and highest risk categories (≤ 22 and ≥ 33 risk alleles, respectively) was 2.62 $cm^3$ (Figure 2a), which corresponds with a 2.2% difference. A higher weighted genetic risk score was also associated with a lower $eGFR_{creat}$ ($P$ value $4.0 \times 10^{-4}$). For each additional average risk allele, $eGFR_{creat}$ was −0.38 ml/min/1.73$m^2$ (95% CI: −0.60; −0.17) lower. The difference in mean $eGFR_{creat}$ between

**Table 2.** Associations of the genetic risk score based on adult kidney function with childhood kidney outcomes ($N = 4,119$)[a]

| | Genetic risk score | | | | | |
|---|---|---|---|---|---|---|
| | Weighted | | | Unweighted | | |
| Childhood kidney outcomes (*N*) | Difference (95% confidence interval) | *P* value | % of explained variance | Difference (95% confidence interval) | *P* value | % of explained variance |
| Kidney volume ($cm^3$)[b] (3,784) | −0.39 (−0.64; −0.13) | $\mathbf{3.0\times10^{-3}}$ | 0.3 | −0.38 (−0.63; −0.13) | $\mathbf{3.2\times10^{-3}}$ | 0.3 |
| $eGFR_{creat}$ (ml/min/1.73 $m^2$)[b] (2,847) | −0.38 (−0.60; −0.17) | $\mathbf{4.0\times10^{-4}}$ | 0.4 | −0.40 (−0.61; −0.20) | $\mathbf{1.7\times10^{-4}}$ | 0.7 |
| $eGFR_{cystC}$ (ml/min/1.73 $m^2$)[b] (2,854) | −0.27 (−0.47; −0.07) | $\mathbf{8.0\times10^{-3}}$ | 0.2 | −0.28 (−0.48; −0.09) | $\mathbf{5.0\times10^{-3}}$ | 0.3 |

[a]Analyses were performed in children with complete data on genetics, at least one outcome under study, and covariates. [b]Values are β coefficients and 95% confidence interval from linear regression models adjusted for child age, sex and the first four principal components from the genetic data.
$eGFR_{creat}$, estimated glomerular filtration rate calculated based on creatinine blood levels; $eGFR_{cystC}$, estimated glomerular filtration rate calculated based on cystatin C blood levels.
*P* value for the significant associations **< 0.05** (bold). The percentage of variance explained by the genetic risk score, was calculated by comparing the unadjusted $R^2$ between a model including the risk score and the covariates and a model including only the covariates, which were sex, age at measurements, and the first four principal components.

the lowest and highest risk categories was 4.46 ml/min/1.73 $m^2$ (**Figure 2b**), which corresponds with a 3.7% difference. Similarly, per additional average risk allele of the adult risk score, childhood $eGFR_{cystC}$ was −0.27 ml/min/1.73 $m^2$ (95% CI: −0.47; −0.07) lower. The difference in mean $eGFR_{cystC}$ between the lowest and highest risk categories was 3.38 ml/min/1.73$m^2$ (**Figure 2c**). We did not observe an association of the weighted risk score with the risk of childhood microalbuminuria (data not shown).

Results from the unweighted risk score (mean 21.8, SD 2.8) were similar to those of the weighted score (**Table 2**). **Supplementary Table S6** online shows that higher genetic risk scores were also associated with higher childhood creatinine blood levels and cystatin C blood levels (*P* values < 0.05). Results in the subgroup of only European children tended to be similar to those in the full group, although the association of both risk scores with $eGFR_{cystC}$ lost its significance and the unweighted risk score lost its significance with combined kidney volume in this group (**Supplementary Table S7** online). The effect of the genetic risk score on combined kidney volume did not change after adjusting our model additionally for child height (**Supplementary Table S8** online).

The genetic risk scores including 27 SNPs, with 7 additional SNPs related to creatinine metabolism in adults, and the genetic risk score including 11 SNPs which may have a known function related to kidney development, revealed similar results as our main risk score. Results are shown in **Supplementary Tables S9 and S10** online. In the **Supplementary Table S11** online, the effect of the unweighted risk score which includes the additional 2 SNPs related to $eGFR_{cystC}$ is shown. The results did not change, but the effect estimates on $eGFR_{cystC}$ and cystatin C were larger.

## DISCUSSION

We observed in a large population-based prospective cohort study, that a higher genetic risk score combining previously identified common genetic variants related to lower kidney function in adults was also associated with a smaller combined kidney volume and lower $eGFR_{creat}$ and $eGFR_{cystC}$ in childhood.

Previous GWAS have identified many common genetic variants related to impaired kidney function in adults (11–13). Of the 20 available SNPs known to be associated with adult $eGFR_{creat}$ and the single SNP associated with adult microalbuminuria, only 2 were individually associated with childhood kidney outcomes. First, we observed an association of rs3925584, located upstream of *MPPED2*, with creatinine blood levels both in the full group and in the subgroup of European children only. In zebrafish, knockdown of *MPPED2* caused abnormal podocyte anatomy (11). Rs3925584 has also been associated with magnesium levels in a large GWAS (14). Rs3925584 is also located close to *DCDC5*, which has been associated with bone mineral density (15). Second, rs11078902 in *CDK12* was associated with $eGFR_{creat}$ and creatinine blood levels in the subgroup of European children only (11). *CDK12*, cyclin-dependent kinase 12, regulates the expression of genes involved in DNA repair and is required for the maintenance of genomic stability. *CDK* inhibitors have been described to play a role in human glomerular disease (16). All these associations were directionally consistent with the results reported in previous GWAS among adults (11,13). None of the included common genetic variants were associated with childhood microalbuminuria. The results for the associations of individual SNPs with childhood kidney outcomes should be interpreted carefully. The original GWAS meta-analysis discovery studies in adults were performed in much larger samples than the current study and our negative results may have occurred due to a lack of power.

In the current study, the weighted genetic risk score related to impaired kidney function in adults was associated with smaller combined kidney volume and lower eGFR in childhood. For an average child in our population, the observed effect estimates correspond with a 0.3% smaller combined kidney volume, a 0.3% lower $eGFR_{creat}$, and a 0.3% lower $eGFR_{cystC}$ per each additional risk allele. We did not observe an association of the genetic risk score with microalbuminuria. We found similar results for the weighted and unweighted risk genetic scores, suggesting that the adult weights used in this score did not strongly influence the observed associations. Adding to the risk score 7 SNPs previously reported to play a role in creatinine production and secretion revealed similar results. Restricting the genetic risk score to the 11 SNPs with a likely or known function related to kidney development revealed similar results with slightly stronger effect estimates. This restricted genetic risk score may be more specific to our outcomes because of the young age range. Including in the unweighted risk score two additional SNPs known to be

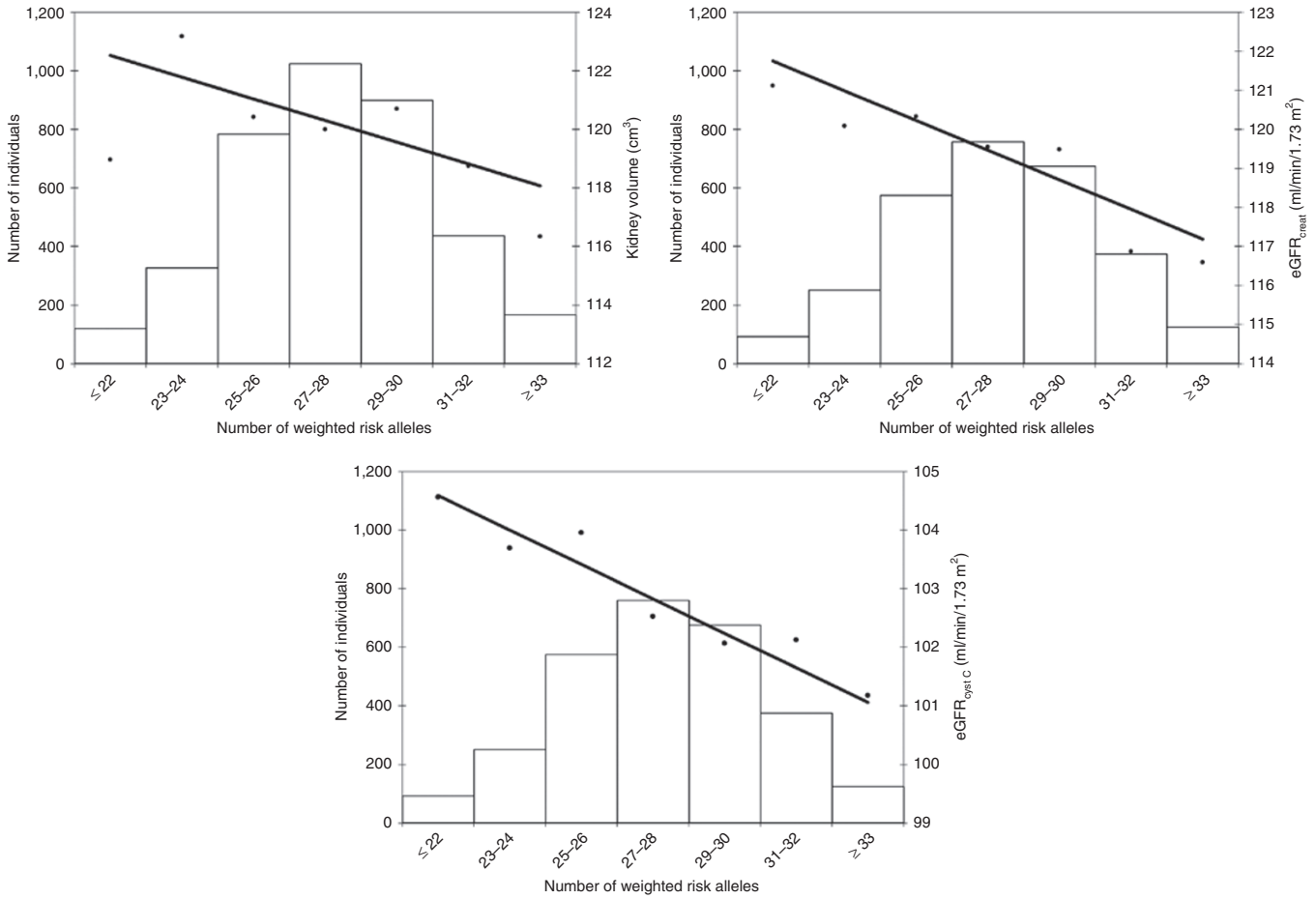


**Figure 2** Effect of the weighted adult $eGFR_{creat}$ genetic risk score on childhood kidney outcomes ($N = 4,119$). (**a**–**c**) Effect of the weighted adult $eGFR_{creat}$ genetic risk score on childhood kidney outcomes ($N = 4,119$). (**a**) Effect of adult eGFR creatinine-based genetic risk score on childhood combined kidney volume: The *x*-axis gives the categories of the risk score (overall sum of risk alleles, weighted by previously reported effect sizes, rescaled and rounded to the nearest integer). The left y-axis gives the number of individuals in each risk-score category, whereas the right y-axis gives the mean combined kidney volume. The histogram reflects the observed number of individuals in each risk-score category. The dots and regression line reflect the estimated mean combined kidney volume for each risk-score category. *P* value is based on the continuous risk score, as presented in Table 2. ***P* value $3.0 \times 10^{-3}$. (**b**) Effect of adult eGFR creatinine-based genetic risk score on childhood eGFR creatinine-based: The *x*-axis gives the categories of the risk score (overall sum of risk alleles, weighted by previously reported effect sizes, rescaled and rounded to the nearest integer). The left y-axis gives the number of individuals in each risk-score category, whereas the right y-axis gives the mean $eGFR_{creat}$. The histogram reflects the observed number of individuals in each risk-score category. The dots and regression line reflect the estimated mean $eGFR_{creat}$ for each risk-score category. *P* value is based on the continuous risk score, as presented in Table 2. ***P* value $4.0 \times 10^{-4}$. (**c**) Effect of adult eGFR creatinine-based genetic risk score on childhood eGFR cystatin C-based: The *x*-axis gives the categories of the risk score (overall sum of risk alleles, weighted by previously reported effect sizes, rescaled and rounded to the nearest integer). The left y-axis gives the number of individuals in each risk-score category, whereas the right y-axis gives the mean $eGFR_{cystC}$. The histogram reflects the observed number of individuals in each risk-score category. The dots and regression line reflect the estimated mean $eGFR_{cystC}$ for each risk-score category. *P* value is based on the continuous risk score, as presented in Table 2. ***P* value $8.0 \times 10^{-3}$.

associated with $eGFR_{cystC}$ (13,17), showed similar associations, with stronger effect estimates on $eGFR_{cystC}$ and cystatin C, suggesting a strong influence of the two added SNPs on these two markers. Ideally, the weights in the genetic risk score would be child-specific, as these best reflect the relative importance of the variants in children. For this, we would need results from large-scale GWAS with independent replication samples on childhood kidney function outcomes. However, a GWAS focused on kidney function among healthy children is not available yet. A recent paper described a GWAS to identify common genetic variants influencing renal function in children with chronic kidney disease (CKD). Although no genome-wide significant associations were found, a number of potentially interesting sub-threshold SNPs were identified (18). This underlines the need for large-scale GWAS efforts to discover the genetic background of kidney function in children, both with and without CKD.

We hypothesized that common genetic variants may partly explain the associations of early life kidney measures with kidney function in later life. The observed associations of the genetic risk scores related to kidney function in adults with kidney outcomes in childhood support this hypothesis. However, confirmation of our findings in other children cohorts and further functional studies are required to establish the causal genes and the mechanisms underlying the associations of these variants with kidney function in childhood and disease in adulthood. In the same cohort as the current study, we have reported associations of smaller kidneys with lower kidney function (19). To explore if the associations of the genetic risks score

with kidney function outcomes were explained by kidney volume, we additionally adjusted the kidney function measures for combined kidney volume. However, the results did not change. Also, adding childhood height to the models associating the genetic risk score with combined kidney volume did not materially change the results. The observed effect estimates in the present study are small, but important from an etiological perspective. They provide further insights into pathways leading to changes in kidney function from the earliest phase of life. Whether common genetic variants related to kidney outcomes in childhood and disease in adulthood are clinically useful for identification of groups at risk needs further study.

Some methodological issues need to be considered. Major strengths of the current study are the detailed phenotypes and the relatively large number of subjects. Still, our sample size was too small to identify many of the associations of individual common genetic variants with childhood kidney outcomes, assuming similar effect sizes as in adults. It has been shown that kidney function and the prevalence of chronic kidney disease vary across ethnic groups in adults (20). Our study population was multi-ethnic, but a sensitivity analysis in European children only, as the largest ethnic subgroup, revealed similar results as in the full group. The other ethnic subgroups were too small to analyze individually. Of all children with genetic information, data on kidney outcomes was available in 72%. As compared to children with kidney follow-up measurements, those without these measurements had a lower birth weight ($P$ value < 0.05) (data not shown). A selective loss to follow-up may have reduced variation in birth weight and early kidney size and therefore reduced the power to detect differences. We performed detailed measurements of childhood kidney outcomes. Kidney size correlates with the number of glomeruli and can be used as a measure of kidney development (21). In children the estimation of GFR is challenging. Blood creatinine is most commonly used to calculate eGFR. In addition to blood creatinine levels, we also calculated eGFR based on cystatin C levels using Zappitelli's formula (22,23). It has been suggested that blood cystatin C levels might be a better biomarker to estimate GFR because the production rate is constant, it is freely filtered, and less dependent on child weight, height, and sex compared to creatinine (24,25). Finally, in our study, we have captured the vast majority of, but not all, known SNPs related to kidney function, as not all SNPs were available in the GWAS dataset.

### Conclusion

Our results suggest that common genetic variants related to kidney function in adults could influence kidney structure and function from early childhood onwards. The previously observed associations of early life kidney measures with kidney disease in later life seem to be partly explained by common genetic variants.

## METHODS

### Design and Study Population

This study was embedded in the Generation R Study, a multiethnic population-based prospective cohort study from fetal life onwards in Rotterdam, the Netherlands, which has been described in detail previously (26). The study has been approved by Medical Ethical Committee of Erasmus MC, University Medical Center Rotterdam. All children were born between April 2002 and January 2006 and form a largely prenatally enrolled birth cohort that is currently being followed until young adulthood. Written consent was obtained for every participant. A genome-wide association screen was available in 5,732 children (see below for details). The present analyses were performed among 4,119 singleton live births for whom we have detailed information on kidney outcomes at the median age of 6 y (95% range 5.7, 7.8). The flowchart of study participants is given in the **Figure 1**.

### Genetic Variants

DNA was isolated from cord blood samples. If DNA samples from cord blood were missing (in 6.3% of the participants), DNA was isolated from blood samples at follow-up measurements. Genome-wide association arrays were run using the Illumina 610 Quad and 660 platforms (27). A stringent process of quality control was applied. Individuals with low sample call rates or sex mismatches were excluded. Before imputation, SNPs were excluded in case of high levels of missing data (SNP call rate <98%), highly significant departures from Hardy-Weinberg equilibrium ($P < 1 \times 10^{-6}$), or low minor allele frequencies (< 1%) (26). MACH software was used to impute genotypes to the cosmopolitan panel of HapMap II (release 22) (28,29). Three SNPs (rs6431731, rs2453580, and rs881858) had imputation qualities between 0.68 and 0.9, indicating reasonable imputation. All other SNPs had imputation qualities above 0.9, indicating good imputation. Based on previous studies, we identified 30 SNPs robustly associated with adult kidney function: 29 for adult $eGFR_{creat}$ and 1 for adult microalbuminuria (11–13). Of these 30 SNPs, information on 26 was available in the GWAS. Information on rs11078903, rs7805747, rs2279463, and rs7422339, all previously associated with adult $eGFR_{creat}$, was not available. However, rs3798156 was used as a perfect proxy ($R^2 = 1$) for rs2279463 and rs11078902 was used as a perfect proxy for rs11078903. No perfect proxies were available in the GWAS dataset for the other two SNPs. As the focus of this study was to examine SNPs related to estimated glomerular filtration rate and not to creatinine metabolism, 20 out of these 28 SNPs were used for the genetic risk score analyses. The individual SNPs used are shown in **Supplementary Table S2** online.

### Childhood Kidney Outcomes

Kidney outcomes were assessed at a dedicated research center in the Erasmus MC—Sophia Children's Hospital in Rotterdam by well-trained staff (30). We measured kidney volume with ultrasound, using an ATL-Philips HDI 5000 instrument (Seattle, WA), with a 2.0–5.0 MHz curved array transducer. We identified the left and right kidneys in the sagittal plane along its longitudinal axis. Also, we performed measurements of maximal bipolar kidney length, width, and depth. Kidney width and depth were measured at the level of the hilum. The cross-sectional area in which the kidney appeared symmetrically round at its maximum width was used. Kidney volume was calculated using the equation for a prolate ellipsoid: volume ($cm^3$) = 0.523 × length (cm) × width (cm) × depth (cm) (31). Combined kidney volume was calculated by summing right and left kidney volume. We previously reported good intraobserver and interobserver correlation coefficients (32).

Nonfasting blood samples were drawn by antecubital venipuncture. Creatinine levels were measured with enzymatic methods and cystatin C levels with a particle-enhanced immunoturbidimetric assay (using Cobas 8000 analyzers, Roche, Almere, the Netherlands). Quality control samples demonstrated intra-assay and interassay coefficients of variation of 0.51% for creatinine and 1.65% for cystatin C, and 1.37% for creatinine and 1.13% for cystatin C, respectively. We estimated glomerular filtration rate (eGFR) according to the revised Schwartz 2009 formula: $eGFR_{creat} = 36.5 \times$ (height (cm)/serum creatinine (μmol/l)) (33). Additionally, we estimated the glomerular filtration rate using Zappitelli's formula based on cystatin C levels: $eGFR_{cystC} = 75.94/[CysC^{1.17}]$ (22).

Urine creatinine (mmol/l) and urine albumin (mg/l) levels were determined with a Beckman Coulter AU analyser, creatinine levels were measured with the Jaffe reaction. We also calculated the albumin-creatinine ratio. In line with clinical cut-offs, microalbuminuria

was defined as an albumin-creatinine ratio >2.5 mg/mmol for boys and > 3.5 mg/mmol for girls (34).

### Statistical Analysis

First, we performed multiple linear and logistic regression analyses, adjusted for sex, age at measurements and the first four principal components, to examine the associations of the 21 SNPs (20 SNPs for kidney function per se and 1 for microalbuminuria) individually with combined kidney volume, creatinine and cystatin C levels, $eGFR_{creat}$ and $eGFR_{cystC}$ and the risk of microalbuminuria in childhood, assuming additive genetic effects. To adjust for multiple testing in the analyses of the individual SNPs, we applied Bonferroni correction for the number of SNPs tested. A $P$ value of $< 2.4 \times 10^{-3}$ (0.05/21) was considered statistically significant. Second, for each outcome, we calculated the percentage of variance explained by all SNPs combined by deducting the unadjusted $R^2$ of the model including only the covariates from the unadjusted $R^2$ of the full model including all SNPs and the covariates, which were sex, age at measurements, and the first four principal components from our genome wide data. Third, we combined the 20 SNPs related to adult $eGFR_{creat}$ in a genetic risk score that summed the number of $eGFR_{creat}$-decreasing alleles weighted by their previously reported effect sizes in adults. As for every SNP, the number of effect alleles can be 0, 1, or 2, the weighted risk score was rescaled to a score ranging from 0 to 40, the maximum number of effect alleles, and rounded to the nearest integer. Additionally, we computed an unweighted genetic risk score based on the 20 adult $eGFR_{creat}$ SNPs to see whether the results were independent of the weight based on adults findings, by adding the number of risk alleles for the 20 SNPs. Linear and logistic regression analyses were performed to examine the association of these risk scores with combined kidney volume, creatinine and cystatin C levels, $eGFR_{creat}$, $eGFR_{cystC}$, and the risk of microalbuminuria in childhood, adjusted for the same covariates as mentioned above. All analyses were performed first in the full group and subsequently repeated as sensitivity analyses in the subgroup of children of European ethnicity, the largest ethnic subgroup in our population. A child was classified as European if he/she was within four standard deviations from the HapMap CEU panel mean value for all first four principal components, based on the genetic data. To assess whether the associations were different by child sex we evaluated the statistical interaction by adding the product term of the child sex and individual SNPs to the models. However, no significant interactions were observed. We also aimed to explore whether child height affected the associations of the genetic risk score with combined kidney volume. We performed a sensitivity analysis to explore whether adding 7 SNPs related to creatinine metabolism (rs10774021, rs10794720, rs491567, rs6465825, rs9895661, rs2453533, rs3798156) to the genetic risk score would affect the results (11). In addition, we explored the association of a genetic risk score including only 11 of the 20 SNPs, which may have a role related to kidney developmental outcomes (nephrogenesis (rs881858, VEGFA; rs626277, DACH1); glomerular filtration barrier and podocyte function (rs11959928, DAB2; rs3925584, MPPED2; rs13538, NAT8), metabolic function of the kidney (rs1260326, GCKR; rs10109414, STC1), solute transport (rs12460876, SLC7A9; rs6420094, SLC34A1) and renal cell structure development (rs12917707, UMOD; rs17319721, SHROOM3)) (13,17,35,36). Furthermore, we explored the effect of 2 SNPs known to be associated with $eGFR_{cystC}$, by adding them to the unweighted risk score. All analyses were performed using the Statistical Package for the Social Sciences version 21.0 for Windows (SPSS IBM, Chicago, IL).

### SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/pr

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### AUTHOR CONTRIBUTIONS

K.M., J.F.F., and V.W.V.J. conceptualized and designed the study, carried out the analyses, drafted the initial manuscript, and approved the final manuscript as submitted. K.M. and S.V. analyzed the data. S.V. critically reviewed and revised the manuscript, and approved the final manuscript as submitted. A.H. and O.H.F. contributed to the data collection, critically reviewed the manuscript, and approved the final manuscript as submitted. K.M., J.F.F., and V.W.V.J., 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.

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### REFERENCES

1. Lackland DT, Bendall HE, Osmond C, Egan BM, Barker DJ. Low birth weights contribute to high rates of early-onset chronic renal failure in the Southeastern United States. Arch Intern Med 2000;160:1472–6.
2. White SL, Perkovic V, Cass A, et al. Is low birth weight an antecedent of CKD in later life? A systematic review of observational studies. Am J Kidney Dis 2009;54:248–61.
3. Brenner BM, Lawler EV, Mackenzie HS. The hyperfiltration theory: a paradigm shift in nephrology. Kidney Int 1996;49:1774–7.
4. Luyckx VA, Brenner BM. Birth weight, malnutrition and kidney-associated outcomes–a global concern. Nat Rev Nephrol 2015;11:135–49.
5. Singh A, Satchell SC. Microalbuminuria: causes and implications. Pediatr Nephrol 2011;26:1957–65.
6. Chen X, Wang Y. Tracking of blood pressure from childhood to adulthood: a systematic review and meta-regression analysis. Circulation 2008;117:3171–80.
7. Shah MM, Sampogna RV, Sakurai H, Bush KT, Nigam SK. Branching morphogenesis and kidney disease. Development 2004;131:1449–62.
8. Nigam SK, Shah MM. How does the ureteric bud branch? J Am Soc Nephrol 2009;20:1465–9.
9. Brenner BM, Chertow GM. Congenital oligonephropathy and the etiology of adult hypertension and progressive renal injury. Am J Kidney Dis 1994;23:171–5.
10. Miliku K, Voortman T, Bakker H, Hofman A, Franco OH, Jaddoe VW. Infant Breastfeeding and Kidney Function in School-Aged Children. Am J Kidney Dis 2015;66:421–8.
11. Pattaro C, Köttgen A, Teumer A, et al.; CARDIoGRAM Consortium; ICBP Consortium; CARe Consortium; Wellcome Trust Case Control Consortium 2 (WTCCC2). Genome-wide association and functional follow-up reveals new loci for kidney function. PLoS Genet 2012;8:e1002584.

12. Böger CA, Chen MH, Tin A, et al.; CKDGen Consortium. CUBN is a gene locus for albuminuria. J Am Soc Nephrol 2011;22:555–70.
13. Köttgen A, Pattaro C, Böger CA, et al. New loci associated with kidney function and chronic kidney disease. Nat Genet 2010;42:376–84.
14. Meyer TE, Verwoert GC, Hwang SJ, et al.; Genetic Factors for Osteoporosis Consortium; Meta Analysis of Glucose and Insulin Related Traits Consortium. Genome-wide association studies of serum magnesium, potassium, and sodium concentrations identify six Loci influencing serum magnesium levels. PLoS Genet 2010;6:.
15. Estrada K, Styrkarsdottir U, Evangelou E, et al. Genome-wide meta-analysis identifies 56 bone mineral density loci and reveals 14 loci associated with risk of fracture. Nat Genet 2012;44:491–501.
16. Shankland SJ, Eitner F, Hudkins KL, Goodpaster T, D'Agati V, Alpers CE. Differential expression of cyclin-dependent kinase inhibitors in human glomerular disease: role in podocyte proliferation and maturation. Kidney Int 2000;58:674–83.
17. Köttgen A, Glazer NL, Dehghan A, et al. Multiple loci associated with indices of renal function and chronic kidney disease. Nat Genet 2009;41:712–7.
18. Wuttke M, Wong CS, Wühl E, et al.; CKDGen Consortium. Genetic loci associated with renal function measures and chronic kidney disease in children: the Pediatric Investigation for Genetic Factors Linked with Renal Progression Consortium. Nephrol Dial Transplant 2016;31:262–9.
19. Bakker H, Gaillard R, Franco OH, et al. Fetal and infant growth patterns and kidney function at school age. J Am Soc Nephrol 2014;25:2607–15.
20. Kramer H, Palmas W, Kestenbaum B, et al. Chronic kidney disease prevalence estimates among racial/ethnic groups: the Multi-Ethnic Study of Atherosclerosis. Clin J Am Soc Nephrol 2008;3:1391–7.
21. Luyckx VA, Brenner BM. The clinical importance of nephron mass. J Am Soc Nephrol 2010;21:898–910.
22. Zappitelli M, Parvex P, Joseph L, et al. Derivation and validation of cystatin C-based prediction equations for GFR in children. Am J Kidney Dis 2006;48:221–30.
23. Bacchetta J, Cochat P, Rognant N, Ranchin B, Hadj-Aissa A, Dubourg L. Which creatinine and cystatin C equations can be reliably used in children? Clin J Am Soc Nephrol 2011;6:552–60.
24. Filler G, Bökenkamp A, Hofmann W, Le Bricon T, Martínez-Brú C, Grubb A. Cystatin C as a marker of GFR–history, indications, and future research. Clin Biochem 2005;38:1–8.
25. Groesbeck D, Köttgen A, Parekh R, et al. Age, gender, and race effects on cystatin C levels in US adolescents. Clin J Am Soc Nephrol 2008;3:1777–85.
26. Jaddoe VW, van Duijn CM, Franco OH, et al. The Generation R Study: design and cohort update 2012. Eur J Epidemiol 2012;27:739–56.
27. Kruithof CJ, Kooijman MN, van Duijn CM, et al. The Generation R Study: Biobank update 2015. Eur J Epidemiol 2014;29:911–27.
28. Li Y, Willer C, Sanna S, Abecasis G. Genotype imputation. Annu Rev Genomics Hum Genet 2009;10:387–406.
29. Li Y, Willer CJ, Ding J, Scheet P, Abecasis GR. MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. Genet Epidemiol 2010;34:816–34.
30. Bakker H, Kooijman MN, van der Heijden AJ, et al. Kidney size and function in a multi-ethnic population-based cohort of school-age children. Pediatr Nephrol 2014;29:1589–98.
31. Geelhoed JJ, Taal HR, Steegers EA, et al. Kidney growth curves in healthy children from the third trimester of pregnancy until the age of two years. The Generation R Study. Pediatr Nephrol 2010;25:289–98.
32. Geelhoed JJ, Kleyburg-Linkers VE, Snijders SP, et al. Reliability of renal ultrasound measurements in children. Pediatr Nephrol 2009;24:1345–53.
33. Schwartz GJ, Muñoz A, Schneider MF, et al. New equations to estimate GFR in children with CKD. J Am Soc Nephrol 2009;20:629–37.
34. Donaghue KC, Chiarelli F, Trotta D, Allgrove J, Dahl-Jorgensen K. Microvascular and macrovascular complications. Pediatr Diabetes 2007;8:163–70.
35. Benetti E, Caridi G, Vella MD, et al. Immature renal structures associated with a novel UMOD sequence variant. Am J Kidney Dis 2009;53:327–31.
36. Chambers JC, Zhang W, Lord GM, et al. Genetic loci influencing kidney function and chronic kidney disease. Nat Genet 2010;42:373–5.