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Genetic variants associated with adult blood pressure and kidney function do not affect fetal kidney volume. The Generation R Study

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ABSTRACT

Background: Smaller kidneys with reduced number of nephrons in early life lead to impaired kidney function and risk for hypertension and chronic kidney disease. These associations might be partly explained by common genetic variation.
Aims: To assess the associations between common genetic variants, which have recently shown to be associated with blood pressure or kidney function, with fetal kidney volume.
Study design: A prospective population based cohort study in Rotterdam, The Netherlands.
Subjects: 855 children, followed from early fetal life onwards (born 2003–2005).
Predictor: Common genetic variants previously associated with blood pressure or kidney function.
Outcome measures: Combined third trimester fetal kidney volume.
Results: After taking into account multiple testing, only rs12940887 (near *ZNF652*) was significantly associated with fetal kidney volume (β: 0.88 (95% CI: 0.40; 1.37) cm^3 per minor allele, P-value<0.001), but the effect showed the opposite direction as expected. The remaining common genetic variants were not associated with fetal kidney volume. We also did not find associations of genetic variants previously shown to affect newborn kidney volume, with third trimester fetal kidney volume.
Conclusions: Our results suggest that common genetic variants, associated with kidney function or disease and blood pressure, do not affect the third trimester fetal kidney volume. Further studies are needed to elucidate the mechanisms underlying the associations between small kidney size and increased risks of hypertension and impaired kidney function in adulthood.

1. Introduction

Many studies have shown associations of low birth weight with cardiovascular disease and chronic renal failure [1,2]. Low birth weight is also associated with impaired renal growth, raised blood pressure and impaired kidney function in later life [1–3]. The hyperfiltration hypothesis suggests that smaller kidneys with lower numbers of nephrons lead to hyperfiltration in the remnant nephrons, eventually resulting in glomerular sclerosis [4,5]. This may predispose the individual to renal damage and development of higher blood pressure, impaired kidney function and end stage kidney disease in adulthood [4]. A previous study showed associations of kidney size and low nephron number with hypertension [6]. Nephron number has been shown to vary widely between individuals, ranging from 250,000 to 2,000,000 nephrons per kidney [7,8]. A strong relationship between newborn kidney volume and nephron number was shown in fifteen infants who died before three months of age, in whom an ultrasound was performed in the first two days of life [9]. Several other post-mortem studies in humans, who died in the perinatal period, showed consistent associations between renal size and glomerular number [8,10]. Therefore, kidney volume seems to be a valid marker for nephron number. Kidney growth and development is complex and influenced by many genetic and environmental factors [11,12]. Multiple genes are involved in kidney development, e.g. in regulating the branching process of the ureteric bud. Mutations in these genes are known to cause agenesis or dysgenesis of the kidney [11]. It seems likely that also common genetic variants account for part of the normal variation in nephron endowment. Thus far, only a few genetic variants have been shown to affect kidney volume [9,13,14]. It might be that common genetic variants, previously associated with blood pressure or kidney function, are also associated with kidney volume. These common genetic variants are identified in genome wide association studies conducted in thousands of

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individuals and explain ~1–2% of the variation in these phenotypes [15–21].

We hypothesized that common genetic variants underlie part of the associations of smaller kidney size in early life, as marker for a lower nephron number, with higher blood pressure and impaired kidney function in later life. Therefore we assessed in a population-based cohort study among 855 subjects, the associations of 58 common genetic variants, previously shown to be related to blood pressure or kidney function in adult life, with fetal kidney volume. We expected that a blood pressure increasing allele or kidney function decreasing risk allele would be associated with a smaller kidney volume in early life. Also, we attempted to replicate the associations of four common genetic variants and one haplotype with fetal kidney volume [9,13,14].

2. Patients and methods

2.1. Design

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood in Rotterdam, The Netherlands [22,23]. Detailed assessments of fetal and postnatal growth and development have been conducted in a randomly selected subgroup of Dutch children and their parents. Mothers, who were already participating during pregnancy, were asked to participate in additional detailed renal and cardiovascular measurements. These women were all enrolled before a gestational age of 24 weeks. In total 80% of the approached mothers were willing to participate in these additional studies. Fetal kidney ultrasounds were performed in the third trimester of pregnancy (median age: 30.4 weeks of gestation (90% range 28.8–32.1 weeks)). In total 1232 women were enrolled in the subgroup cohort. Twin pregnancies (n = 15) and pregnancies leading to perinatal death (n = 2) were excluded from the analysis, leading to 1215 singleton live births. No renal or ureterovesical anomalies other then mild pyelectasis over 10 mm (n = 3) were present in our study. Kidney ultrasounds were successfully performed in 95% (n = 1158) of these subjects. DNA was available in 855 (74%) of these subjects. The study was approved by the Medical Ethics Committee of the Erasmus MC, Rotterdam. Written informed consent was obtained from all participants [22,23].

2.2. Genotyping

Cord blood for DNA isolation was available in 74% of all live-born participating children. Sex-mismatch rate between genome based sex and midwife-record based sex was low (<0.5%), indicating that possible contamination of maternal DNA was extremely low. Missing cord blood samples were mainly due to logistical constraints at the delivery. Individual genotype data were extracted from the genome-wide Illumina 610 Quad Array. If SNPs were not directly genotyped, we used MACH (version 1.0.15) software to impute genotypes using the HapMap II CEU (release 22) as reference set.

2.3. Selection of common genetic variants

The PubMed database was searched with 'genome wide association study', combined with 'blood pressure' or 'kidney function' as search criteria. We selected genome wide association studies, since these provide robust evidence of association, conducted in samples of European ancestry. Seven genome wide association studies were found; four on blood pressure and hypertension, three on kidney function and kidney disease [15–21]. If identical study populations were used in subsequent genome wide association studies, we selected common genetic variants from the genome wide association study with the largest sample size. Furthermore, we selected SNPs to assess in our study if the P-value of the association was $<5.0\times10^{-8}$. If SNPs were in high linkage disequilibrium (R^2 HapMap CEU ≥ 0.5) with each other, we selected the SNP with the strongest association reported, unless these SNPs were associated with different phenotypes. In total, 30 SNPs related to blood pressure and 28 SNPs related to kidney function or disease, were selected for this study. Next, the PubMed database was searched with 'common variant' and 'kidney size' or 'kidney volume' as search criteria, to identify the studies which assessed associations between common genetic variants and kidney size or volume. We identified four studies investigating genes in the branching pathway; the PAX-gene, the RET-gene, the ALDH1A2-gene and the GDNF-gene. We selected SNPs in our study if the P-value of the association in previous studies was <0.05 [9,13,14,24].

2.4. Kidney measurements

Fetal left and right kidneys were measured in the third trimester of pregnancy with an ATL-Philips HDI 5000 instrument (Seattle, WA, USA) equipped with a 2.0–5.0 MHz curved array transducer. In a sagittal plane the maximum longitudinal kidney length was measured, with the calipers placed on the outer edges of the caudal and cranial sides. Antero-posterior (kidney width) and transverse (kidney depth) diameters were measured perpendicular to each other, from the one outer edge to the other, in an axial plane. Values of maximum bipolar kidney length, width and depth were obtained from both the left and right kidneys. Kidney width and depth were measured at the level of the kidney hilum. Fetal kidney volume was calculated, using the equation of an ellipsoid: volume (cm^3) = (0.523 × length (mm) × width (mm) × depth (mm))/1000. Left and right kidney volumes were added for the combined kidney volume (cm^3) [25,26]. Fetal growth characteristics (head circumference (HC), abdomen circumference (AC) and femur length were measured at the same visit, and fetal weight was estimated [27]. Two well-trained, experienced sonographers performed all measurements. Quality checks were frequently carried out and feedback was provided to minimize interoperator differences. Fetal growth measurements were shown to be measured reliably. The intra- and interobserver interclass correlation coefficients were all higher than 0.98, indicating good reproducibility [28]. The estimated fetal weight (EFW) is calculated by the formula: EFW (grams) = 10 * (1.326 − 0.00326 * AC * FL + 0.0107 * HC + 0.0438 * AC + 0.158 * FL) [29].

2.5. Statistical methods

Associations of common genetic variants and fetal kidney volume were assessed using linear regression, assuming an additive model. The model was adjusted for sex, gestational age at measurement and estimated fetal weight. We adjusted for these variables because of their relation with kidney volume [30]. The results did not differ materially between analyses with and without adjustment. In order to assess the combined effects of the common genetic variants, we calculated a risk allele score, by summing the risk alleles (common genetic variants previously associated with higher adult blood pressure or impaired kidney function) per individual, and analyzed the association of the number of risk alleles with fetal kidney volume. To take into account multiple testing, we applied a Bonferroni correction and considered a P-value lower than 8.6×10^{-4} (0.05/58) as statistically significant. Controlling for the false discovery rate, a less conservative approach [31,32], did not change the results materially. All statistical analyses were performed using the Statistical Package for the Social Science version 17.0.2 for Windows (SPSS Inc, Chicago, IL, USA).

3. Results

Table 1 presents the maternal and fetal subject characteristics, including all measured kidney characteristics. The combined kidney

Table 1
Population characteristics.

Maternal characteristics (n = 855)	
Age	31.9 (21.9–39.4)
Height	171.1 (6.4)
Weight	71.4 (12.8)
Body mass index	23.4 (18.9–34.6)
Parity (%) ≥ 1	40.6
Fetal characteristics (n = 855)	
Sex (males %)	53.3
Gestational age at measurement	30.4 (28.5–32.7)
Estimated fetal weight (g)	1639 (268)
Right kidney structures	
Length (mm)	39.0 (32.1–46.0)
Width (mm)	23.0 (18.0–29.7)
Depth (mm)	22.0 (17.0–28.0)
Volume (cm^3)	10.3 (5.8–17.9)
Left kidney structures	
Length (mm)	39.0 (32.7–47.0)
Width (mm)	22.0 (17.0–28.0)
Depth (mm)	21.0 (16.9–26.9)
Volume (cm^3)	9.6 (5.4–16.1)
Combined kidney volume (cm^3)	20.6 (5.6)
Kidney volume/EFW (cm^3/kg)	12.7 (3.1)

Values are means (sd) or medians (95% range).
Estimated fetal weight is the estimated weight at measurement.

volume, as well as the relative kidney volume was higher in boys than in girls (P<0.01).

Table 2 gives the associations of the selected common genetic variants, known to be associated with blood pressure or kidney function, with fetal kidney volume. Out of 30 genetic variants known to be associated with blood pressure, only rs12940887 (near *ZNF652*) was significantly associated with fetal kidney volume (β: 0.88 (95% CI: 0.40; 1.37) cm^3 per minor allele, P<0.001), but the effect of this variant did not show the expected direction. Overall, eighteen common variants (60%) previously associated with blood pressure, showed the expected direction of the association with fetal kidney volume, but these associations were not significant. Of the common variants known to be associated with kidney function or kidney disease, four (rs11959928 (in *DAB2*), rs10109414 (in *STC1*), rs12460876 (in *SLC7A9*) and rs4805834 (near *SLC7A9*)) out of 28 variants (14.3%) showed evidence of association with fetal kidney volume (P<0.05), though they did not reach the significance threshold after adjustment for multiple testing. The expected direction of the associations was found in two (rs11959928 and 4805834) of these common variants. Overall, thirteen (46.4%) common genetic variants showed the expected direction of the association with fetal kidney volume. A risk allele score summing all risk alleles of the common genetic variants associated with adult blood pressure and kidney function, was not associated with fetal kidney volume (β -0.04 (95% CI: -0.11 , 0.03) cm^3 per risk allele, P = 0.25).

We did not find evidence of associations between common genetic variants in *PAX2* (rs4244341 and rs11592735), *RET* (rs1800860) and *ALDH1A2* (rs7169289) and fetal kidney volume (Table 3). The previously described AAA-haplotype in *PAX2* (rs11190688, rs11190702 and 11599825) [13] was not significantly associated with fetal kidney volume.

4. Discussion

Results from this population-based prospective cohort study suggest that common genetic variants, which have previously shown to be associated with blood pressure or kidney function [15–20], not explain the associations of smaller kidneys with higher blood pressure and impaired kidney function in later life. Also, previously found associations of common genetic variants involved in the branching pathway with neonatal kidney size [9,13,14], were not confirmed in this study.

Previous studies showed the association of low birth weight with smaller kidneys and a lower number of nephrons [7,8,10]. Smaller kidneys, with a reduced nephron number, may lead to hyperfiltration in the remaining nephrons, resulting in glomerular sclerosis. Subsequently this may predispose an individual to the development of higher blood pressure, impaired kidney function and chronic kidney disease [4,10,33–35]. Postmortem studies in humans showed that a lower nephron number is associated with low birth weight and hypertension [6,36]. A recent study showed an association between newborn kidney volume and nephron number in fifteen infants, who died before three months of age, in whom an ultrasound was performed in the first two days of life. There was a strong relationship between kidney mass and nephron number [9]. This association is supported by study by Hinchliffe et al. demonstrating a strong correlation between renal volume and glomerular number up to 40 weeks of gestation, in eleven spontaneously aborted fetuses [37]. Several other post-mortem studies in humans, who died in the perinatal period, showed consistent associations between renal size and glomerular number [8,10]. Low birth weight has also been shown to be associated with low nephron number [8,10,33,36]. Ultrasound studies in fetuses and children have shown that low birth weight is associated with smaller kidney size [35,38–40]. Previously we have shown that kidney characteristics track from third trimester of pregnancy to the postnatal age of two years [30]. Therefore, fetal kidney volume also seems to be a good surrogate for nephron number. However, differences in fetal kidney volume might be smaller and therefore more difficult to detect. This could have affected the power of our study to establish associations.

The nephron number varies widely between individuals ranging from 250,000 to 2,000,000 nephrons per kidney [7,8]. The fetal kidney develops forms two components, the metanephric mesenchyme and the Wolffian duct. The ureteric bud forms as an outgrowth of the Wolffian duct and reciprocal induction between the metanephric mesenchyme and the ureteric bud results in branching of the ureteric bud. Through various complicated processes, metanephric tubules and glomerular components are formed. Kidney and nephron growth and development are influenced by genetic and various environmental factors [11,12,41–44]. Many molecular mechanisms are required for different aspects of nephrogenesis, such as the ureteric bud outgrowth and branching [11,12]. Ureteric branching is a very important part of nephrogenesis and thought to be a major determinant of nephron endowment [11,12]. Several genes, such as PAX2, RET and GDNF, have been suggested to be involved in these steps in nephrogenesis. Mutations in these genes seem to cause kidney agenesis or dysgenesis and fewer nephrons in animals and humans [11,45]. Adverse fetal environmental exposures could also affect kidney development, although information on specific adverse fetal exposures affecting kidney development is limited. It has been shown that continued smoking during pregnancy of more than ten cigarettes per day is associated with smaller kidneys in fetal life [41] and higher blood pressure in childhood [46]. Also, other environmental exposures, such as nutrition, folic acid supplementation, and placental dysfunction might affect kidney development. Several animal studies have shown that low maternal protein intake and vitamin A deficiency during pregnancy lead to smaller kidneys and increased blood pressure in the offspring [42–44].

It seems likely that genetic factors partly explain variation of nephron number, and thus kidney size, in the general population [11]. There have been no heritability studies performed on kidney volume in healthy populations, but a study in families with polycystic kidney disease showed that kidney volume has a heritability of 0.42 [47]. The association between smaller kidneys and higher blood pressure, impaired kidney function and kidney disease in adulthood, might be partly explained by common genetic variation, resulting in

Table 2
Common genetic variants known to be associated with blood pressure or kidney function and their association with fetal kidney volume.

SNP	Chr.	Position	Minor allele	Minor allele frequency	Gene (in/near)	Previously reported effect on blood pressure	Effect estimate for fetal kidney volume (cm^3)	P-value	Direction as expected
Blood pressure									
rs2932538	1	113018066	A	0.26	MOV10	SBP ↓; DBP ↓[21]	−0.38 (−0.89; 0.13)	0.15	No
rs17367504	1	11785365	G	0.15	MTHFR-NPPB	SBP ↓; DBP ↓; hypertension ↓[21]	0.12 (−0.52; 0.76)	0.71	Yes
rs13082711	3	27512913	C	0.23	SLC4A7	DBP ↑[21]	−0.52 (−1.08; 0.04)	0.07	Yes
rs3774372	3	41852418	C	0.15	ULK4	DBP ↑ [21]	−0.17 (−0.84; 0.50)	0.63	Yes
rs419076	3	170583580	T	0.47	MECOM	SBP ↑; DBP ↑[21]	−0.35 (−0.82; 0.11)	0.14	Yes
rs1458038	4	81383747	A	0.30	FGF5	SBP ↑ ; DBP ↑ [21]	0.43 (−0.07; 0.93)	0.09	No
rs13107325	4	103407732	T	0.05	SLC39A8	SBP ↓; DBP ↓[21]	0.79 (−0.47; 2.04)	0.22	Yes
rs13139571	4	156864963	A	0.22	GUCY1A3-GUCY1B3	DBP ↓[21]	0.36 (−0.28; 0.94)	0.22	Yes
rs1173771	5	32850785	A	0.41	NPR3-C5orf23	SBP ↓; DBP ↓; hypertension ↓[21]	−0.19 (−0.66; 0.27)	0.41	No
rs11953630	5	157777980	T	0.37	EBF1	SBP ↓; DBP ↓[21]	0.30 (−0.20; 0.79)	0.24	Yes
rs1799945	6	26199158	G	0.14	HFE	SBP ↑; DBP ↑; hypertension ↑[21]	−0.14 (−0.80; 0.53)	0.69	Yes
rs805303	6	31724345	A	0.38	BAT2-BAT5	SBP ↓; DBP ↓; hypertension ↓ [21]	0.10 (−0.38; 0.59)	0.68	Yes
rs4373814	10	18459978	C	0.42	CACNB2 (5′)	SBP ↑; DBP ↑; hypertension ↑[21]	0.00 (−0.47; 0.47)	1.00	-
rs1813353	10	18747454	C	0.31	CACNB2 (3′)	SBP ↓; DBP ↓; hypertension ↓[21]	−0.59 (−1.11; −0.07)	**0.03**	No
rs4590817	10	63137559	C	0.15	C10orf107	SBP ↓; DBP ↓; hypertension ↓[21]	0.20 (−0.45; 0.84)	0.55	Yes
rs932764	10	95885930	G	0.44	PLCE1	SBP ↑; hypertension ↑[21]	−0.35 (−0.82; 0.12)	0.15	Yes
rs11191548	10	104836168	C	0.08	CYP17A1-NT5C2	SBP ↓; DBP ↓[21]	0.47 (−0.44; 1.39)	0.31	Yes
rs381815	11	16858844	T	0.24	PLEKHA7	SBP ↑; DBP ↑ [21]	−0.34 (−0.88; 0.20)	0.22	Yes
rs7129220	11	10307114	A	0.09	ADM	SBP ↑; DBP ↑[21]	−0.59 (−1.39; 0.20)	0.14	Yes
rs633185	11	100098748	G	0.28	FLJ32810-TMEM133	SBP ↓; DBP ↓; hypertension ↓[21]	−0.03 (−0.52; 0.47)	0.99	No
rs3184504	12	110368991	T	0.47	SH2B3	SBP ↑; DBP ↑ [17,21]	−0.19 (−0.66; 0.29)	0.44	Yes
rs10850411	12	113872179	C	0.31	TBX5-TBX3	SBP ↓; DBP ↓[21]	−0.10 (−0.59; 0.39)	0.68	No
rs17249754	12	88584717	A	0.16	ATP2B1	SBP ↓; DBP ↓; hypertension ↓[21]	−0.64 (−1.28; 0.00)	0.05	No
rs1378942	15	72864420	C	0.35	CYP1A1-ULK3	SBP ↑; DBP ↑ [21]	0.15 (−0.34; 0.64)	0.54	No
rs2521501	15	89238392	T	0.31	FURIN-FES	SBP ↑; DBP ↑[21]	−0.44 (−0.94; 0.05)	0.08	Yes
rs13333226	16	20273155	G	0.18	UMOD	Hypertension ↓[15]	−0.03 (−0.66; 0.59)	0.92	No
rs17608766	17	42368270	C	0.15	GOSR2	SBP ↑[21]	−0.20 (−0.84; 0.45)	0.55	Yes
rs12940887	17	44757806	T	0.38	ZNF652	SBP ↑; DBP ↑ [21]	0.88 (0.40; 1.37)	**0.00035**	No
rs1327235	20	10917030	G	0.46	JAG1	DBP ↑ [21]	0.13 (−0.34; 0.60)	0.58	No
rs6015450	20	57184512	G	0.12	GNAS-EDN3	SBP ↑; DBP ↑; hypertension ↑[21]	−0.51 (−1.18; 0.17)	0.14	Yes
Kidney function									
rs267734	1	149218101	C	0.21	ANXA9	eGFRcrea ↑[19]	0.29 (−0.29; 0.86)	0.33	Yes
rs7422339	2	211248752	A	0.32	CPS1	eGFRcrea ↓ [19]	0.41 (−0.08; 0.90)	0.10	No
rs1260326	2	27584444	T	0.39	GCKR	eGFRcrea ↑ [19]	0.17 (−0.31; 0.64)	0.50	Yes
rs13538	2	73721836	G	0.21	NAT8-ALMS1	eGFRcrea ↑ [19]	0.17 (−0.41; 0.75)	0.56	Yes
rs10206899	2	73754408	C	0.21	NAT8-ALMS1	serum creat ↑ [16]	0.22 (−0.36; 0.80)	0.46	No
rs347685	3	143289827	C	0.28	TFDP2	eGFRcrea ↑ [19]	0.01 (−0.53; 0.53)	0.99	Yes
rs17319721	4	77587871	A	0.45	SHROOM3	eGFRcrea ↓ [19]	0.11 (−0.37; 0.58)	0.66	No
rs6420094	5	176750242	G	0.31	SLC34A1	eGFRcrea ↓ [19]	−0.48 (−0.97; 0.01)	0.06	Yes
rs11959928	5	39432889	A	0.44	DAB2	eGFRcrea ↓ [19]	−0.50 (−0.97; −0.04)	**0.03**	Yes
rs2279463	6	160588379	G	0.12	SLC22A2	eGFRcrea ↓ [19]	0.17 (−0.56; 0.91)	0.64	No
rs3127573	6	160601383	G	0.12	SLC22A2	serum creat ↑[16]	0.06 (−0.66; 0.79)	0.87	No
rs881858	6	43914587	G	0.29	CVEGFA	eGFRcrea ↑ [19]	0.03 (−0.48; 0.54)	0.90	Yes
rs7805747	7	151038734	A	0.26	PRKAG2	eGFRcrea ↓ [19]	0.05 (−0.47; 0.57)	0.85	No
rs6465825	7	77254375	C	0.39	TMEM60	eGFRcrea ↓ [19]	−0.16 (−0.63; 0.31)	0.50	Yes
rs10109414	8	23807096	T	0.42	STC1	eGFRcrea↓ [19]	−0.65 (−1.11; −0.18)	**0.007**	No
rs4744712	9	70624527	A	0.41	PIP5K1B-FAM122A	eGFRcrea ↓ [19]	−0.12 (−0.60; 0.37)	0.64	Yes
rs10774021	12	219559	C	0.35	SLC6A13	eGFRcrea ↑ [19]	−0.27 (−0.77; 0.22)	0.28	No
rs653178	12	110492139	C	0.47	ATXN2	eGFRcys ↑ [19]	−0.18 (−0.65; 0.30)	0.47	Yes
rs626277	13	71245697	C	0.41	DACH1	eGFRcrea ↑ [19]	−0.03 (−0.50; 0.43)	0.89	No
rs2453533	15	43428517	A	0.38	GATM, SPATA5L1	eGFRcrea ↓[19]	0.14 (−0.35; 0.64)	0.57	Yes
rs491567	15	51733885	C	0.24	WDR72	eGFRcrea ↑ [19]	−0.15 (−0.70; 0.41)	0.61	No
rs1394125	15	73946038	A	0.35	UBE2Q2-FBXO22	eGFRcrea ↓ [19]	0.16 (−0.32; 0.65)	0.51	No
rs12917707	16	20275191	T	0.18	UMOD	eGFRcrea ↑; CKD ↓ [19]	−0.05 (−0.69; 0.59)	0.88	No
rs9895661	17	56811371	C	0.19	TBX2-BCAS3	eGFRcrea ↓ [19]	−0.37 (−1.01; 0.26)	0.25	Yes
rs8068318	17	56838548	C	0.28	TBX2-BCAS3	Serum creat ↑ [16]	0.17 (−0.37; 0.70)	0.54	No
rs12460876	19	38048731	C	0.37	SLC7A9	eGFRcrea ↑ [19]	−0.59 (−1.07; −0.12)	**0.02**	No
rs4805834	19	38145499	T	0.14	SLC7A9	Serum creat ↑ [16]	−0.99 (−1.68; −0.31)	**0.004**	Yes
rs911119	20	23560737	C	0.21	CST3-CST4, CST9	eGFRcys↑[19]	0.38 (−0.21; 0.97)	0.21	No

Effect estimates are regression coefficients (95% CI) and reflect the difference in kidney volume per minor allele. All regression models were adjusted for fetal sex, gestational age at measurement and estimated fetal weight. We assumed an additive model.
Chr, chromosome; SBP, systolic blood pressure; DBP, diastolic blood pressure.
Expected direction: We expected that a blood pressure increasing risk allele or a risk allele, known to be associated with impaired kidney function is associated with decreased kidney volume.

lower nephron endowment in individuals. In our study, we only found rs12946454, near *ZNF652*, to be significantly associated with a larger kidney volume. *ZNF652* has been implicated in tumor genesis [48]. Another gene in this region, *PHB*, is shown to be involved in angiogenesis [49]. It might be that this variant influences angiogenesis which may subsequently lead to a larger kidney volume. Further research is needed to identify underlying mechanisms and to evaluate whether carrying kidney volume increasing alleles of this variant also leads to better kidney function and lower blood pressure.

We did not observe any associations in the expected direction of previously identified common variants affecting blood pressure and kidney function in adulthood [15–21], with fetal kidney volume. We

Table 3
Common genetic variants and their association with fetal kidney volume.

SNP	Chr.	Position	Minor allele	Minor allele frequency	Gene	Previously reported effect on kidney volume	Effect estimate for fetal kidney volume (cm^3)	P-value	Direction as expected
rs11190688	10	102514460	Haplotype: AAA		PAX2	↓[11]	0.19 (−0.52; 0.89)	0.60	No
rs11190702		102532515							
rs11599825		102510165							
rs4244341	10	102498567	T	0.22	PAX2	↓[11]	−0.37 (−0.94; 0.20)	0.21	Yes
rs11592735	10	102518253	A	0.04	PAX2	↓[11]	−0.43 (−1.73; 0.87)	0.52	Yes
rs1800860	10	42926693	A	0.30	RET	↓[9]	0.31 (−0.21; 0.82)	0.25	No
rs7169289	15	56030975	G	0.17	ALDH1A2	↑[12]	0.30 (−0.38; 0.98)	0.39	Yes

Effect estimates are regression coefficients (95% CI) and reflect the difference in kidney volume per minor allele. All regression models were adjusted for fetal sex, gestational age at measurement and estimated fetal weight. We assumed an additive model.
Chr, chromosome.
Expected direction: We expected that the common genetic variants, known to be associated with kidney volume, showed the same direction as described before in literature.

expected that a blood pressure increasing allele or a kidney function decreasing allele would be associated with a smaller kidney volume in fetal life. This suggests that these variants do not underlie the association between smaller kidneys and increased blood pressure and impaired kidney function in later life. It might be that we cannot identify associations, because of the small differences in kidney size in fetal life. It also could indicate that there might be other genes underlying this association. Combining the information of all common genetic variants, by calculating a risk allele count, also did not show evidence for association of these variants with fetal kidney volume. Genome wide association studies including much larger sample sizes than published so far, might result in common genetic variants that do affect kidney volume. These studies might be powerful enough to detect small differences in blood pressure or kidney function possibly caused by slightly smaller or larger kidneys and could provide evidence for a genetic basis of the hyperfiltration hypothesis.

Other interesting genes could be genes involved in the kidney branching morphogenesis. Mutations in genes such as *PAX2* and *RET* lead to hypoplasia or agenesis of the kidney [9,11,13]. Both *PAX2* and *RET* play a role in the outgrowth of the ureteric bud and are mainly involved in first phase kidney development [11].To determine whether common genetic variants in these genes also reduce kidney volume, several studies were conducted in a Canadian newborn population [9,13,14]. Variants in *PAX2*, *RET* and *ALDH1A2* have been shown to affect kidney volume [9,13,14]. Subjects carrying a specific *PAX2*-haplotype or the adverse allele of rs1800860 in the *RET* gene had a 10% smaller kidney volume than subjects not carrying these variants [9,13]. Also rs4244341 and rs11592735 in the *PAX2* gene were associated with a smaller kidney volume [13]. Homozygosity for the minor allele of rs7169289 in *ALDH1A2* was associated with a 22% larger newborn kidney volume [14].

In our study, we did not find any association of these variants with fetal kidney volume. The absolute effects on fetal kidney volume might be smaller and more difficult to establish, as compared to a newborn population. This study (n = 855) includes a larger number of subjects, as compared to the previous studies [9,13,14]. However, it could also be that these variants exert their effect later in pregnancy. Also, the variants in *PAX2*, *RET* and *ALDH1A2* have not been associated with increased blood pressure or impaired kidney function in adulthood as far as we know. These findings do not support the hypothesis that genetic factors partly underlie the association between smaller kidneys and increased blood pressure or impaired kidney function.

The lack of evidence for a genetic basis of the hyperfiltration hypothesis could indicate that an adverse fetal environment, such as fetal nicotine exposure, is more important than genetic factors in explaining this hypothesis. It seems likely that genetic factors only cause small differences in kidney volume, which could be difficult to detect and might be of little clinical interest, but combined could add to the hyperfiltration hypothesis. Further research is necessary to identify specific adverse fetal exposures and genetic factors that underlie the association of kidney volume with blood pressure and kidney function.

5. Conclusion

Our results suggest that common genetic variants, previously associated with adult blood pressure and kidney function, do not underlie the associations of smaller kidneys with a reduced nephron endowment in early life with higher blood pressure or impaired kidney function in later life.

Conflict of interest

All the authors declared no competing interests.

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References

[1] Eriksson JG, Forsen T, Tuomilehto J, Osmond C, Barker DJ. Early growth and coronary heart disease in later life: longitudinal study. BMJ 2001;322(7292):949–53.
[2] 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(10):1472–6.
[3] Keijzer-Veen MG, Schrevel M, Finken MJ, Dekker FW, Nauta J, Hille ET, et al. Microalbuminuria and lower glomerular filtration rate at young adult age in subjects born very premature and after intrauterine growth retardation. J Am Soc Nephrol 2005;16(9):2762–8.
[4] Brenner BM, Chertow GM. Congenital oligonephropathy and the etiology of adult hypertension and progressive renal injury. Am J Kidney Dis 1994;23(2):171–5.
[5] Brenner BM, Garcia DL, Anderson S. Glomeruli and blood pressure. Less of one, more the other? Am J Hypertens 1988;1(4 Pt 1):335–47.
[6] Keller G, Zimmer G, Mall G, Ritz E, Amann K. Nephron number in patients with primary hypertension. N Engl J Med 2003;348(2):101–8.
[7] Nyengaard JR, Bendtsen TF. Glomerular number and size in relation to age, kidney weight, and body surface in normal man. Anat Rec 1992;232(2):194–201.
[8] Hughson M, Farris III AB, Douglas-Denton R, Hoy WE, Bertram JF. Glomerular number and size in autopsy kidneys: the relationship to birth weight. Kidney Int 2003;63(6):2113–22.

[9] Zhang Z, Quinlan J, Hoy W, Hughson MD, Lemire M, Hudson T, et al. A common RET variant is associated with reduced newborn kidney size and function. J Am Soc Nephrol 2008;19(10):2027–34.
[10] Manalich R, Reyes L, Herrera M, Melendi C, Fundora I. Relationship between weight at birth and the number and size of renal glomeruli in humans: a histomorphometric study. Kidney Int 2000;58(2):770–3.
[11] Shah MM, Sampogna RV, Sakurai H, Bush KT, Nigam SK. Branching morphogenesis and kidney disease. Development 2004;131(7):1449–62.
[12] Nigam SK, Shah MM. How does the ureteric bud branch? J Am Soc Nephrol 2009;20(7):1465–9.
[13] Quinlan J, Lemire M, Hudson T, Qu H, Benjamin A, Roy A, et al. A common variant of the PAX2 gene is associated with reduced newborn kidney size. J Am Soc Nephrol 2007;18(6):1915–21.
[14] El Kares R, Manolescu DC, Lakhal-Chaieb L, Montpetit A, Zhang Z, Bhat PV, et al. A human ALDH1A2 gene variant is associated with increased newborn kidney size and serum retinoic acid. Kidney Int 2010;78(1):96–102.
[15] Padmanabhan S, Melander O, Johnson T, Di Blasio AM, Lee WK, Gentilini D, et al. Genome-wide association study of blood pressure extremes identifies variant near UMOD associated with hypertension. PLoS Genet 2010;6(10):e1001177.
[16] Chambers JC, Zhang W, Lord GM, van der Harst P, Lawlor DA, Sehmi JS, et al. Genetic loci influencing kidney function and chronic kidney disease. Nat Genet 2010;42(5):373–5.
[17] Levy D, Ehret GB, Rice K, Verwoert GC, Launer LJ, Dehghan A, et al. Genome-wide association study of blood pressure and hypertension. Nat Genet 2009;41(6):677–87.
[18] Newton-Cheh C, Johnson T, Gateva V, Tobin MD, Bochud M, Coin L, et al. Genome-wide association study identifies eight loci associated with blood pressure. Nat Genet 2009;41(6):666–76.
[19] Kottgen A, Pattaro C, Boger CA, Fuchsberger C, Olden M, Glazer NL, et al. New loci associated with kidney function and chronic kidney disease. Nat Genet 2010;42(5):376–84.
[20] Kottgen A, Glazer NL, Dehghan A, Hwang SJ, Katz R, Li M, et al. Multiple loci associated with indices of renal function and chronic kidney disease. Nat Genet 2009;41(6):712–7.
[21] Ehret GB, Munroe PB, Rice KM, Bochud M, Johnson AD, Chasman DI, et al. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. Nature 2011;478(7367):103–9.
[22] Jaddoe VW, Bakker R, van Duijn CM, van der Heijden AJ, Lindemans J, Mackenbach JP, et al. The Generation R Study Biobank: a resource for epidemiological studies in children and their parents. Eur J Epidemiol 2007;22(12):917–23.
[23] Jaddoe VW, van Duijn CM, van der Heijden AJ, Mackenbach JP, Moll HA, Steegers EA, et al. The Generation R Study: design and cohort update 2010. Eur J Epidemiol 2010;25(11):823–41.
[24] Zhang Z, Quinlan J, Grote D, Lemire M, Hudson T, Benjamin A, et al. Common variants of the glial cell-derived neurotrophic factor gene do not influence kidney size of the healthy newborn. Pediatr Nephrol 2009;24(6):1151–7.
[25] Gupta AK, Anand NK, Lamba IM. Ultrasound evaluation of kidney dimensions in neonates. Indian Pediatr 1993;30(3):319–24.
[26] Konje JC, Okaro CI, Bell SC, de Chazal R, Taylor DJ. A cross-sectional study of changes in fetal renal size with gestation in appropriate- and small-for-gestational-age fetuses. Ultrasound Obstet Gynecol 1997;10(1):22–6.
[27] Geelhoed JJ, Taal HR, Steegers EA, Arends LR, Lequin M, Moll HA, 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(2):289–98.
[28] Verburg BO, Mulder PG, Hofman A, Jaddoe VW, Witteman JC, Steegers EA. Intra- and interobserver reproducibility study of early fetal growth parameters. Prenat Diagn 2008;28(4):323–31.
[29] Hadlock FP, Harrist RB, Carpenter RJ, Deter RL, Park SK. Sonographic estimation of fetal weight. The value of femur length in addition to head and abdomen measurements. Radiology 1984;150(2):535–40.
[30] Geelhoed JJ, Verburg BO, Nauta J, Lequin M, Hofman A, Moll HA, et al. Tracking and determinants of kidney size from fetal life until the age of 2 years: the Generation R Study. Am J Kidney Dis 2009;53(2):248–58.
[31] Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc B Methodol 1995;57(1):289–300.
[32] Benjamini Y, Yekutieli D. Quantitative trait Loci analysis using the false discovery rate. Genetics 2005;171(2):783–90.
[33] Hinchliffe SA, Lynch MR, Sargent PH, Howard CV, Van Velzen D. The effect of intrauterine growth retardation on the development of renal nephrons. Br J Obstet Gynaecol 1992;99(4):296–301.
[34] Brenner BM, Lawler EV, Mackenzie HS. The hyperfiltration theory: a paradigm shift in nephrology. Kidney Int 1996;49(6):1774–7.
[35] Silver LE, Decamps PJ, Korst LM, Platt LD, Castro LC. Intrauterine growth restriction is accompanied by decreased renal volume in the human fetus. Am J Obstet Gynecol 2003;188(5):1320–5.
[36] Hughson MD, Douglas-Denton R, Bertram JF, Hoy WE. Hypertension, glomerular number, and birth weight in African Americans and white subjects in the southeastern United States. Kidney Int 2006;69(4):671–8.
[37] Hinchliffe SA, Sargent PH, Howard CV, Chan YF, van Velzen D. Human intrauterine renal growth expressed in absolute number of glomeruli assessed by the disector method and Cavalieri principle. Lab Invest 1991;64(6):777–84.
[38] Spencer J, Wang Z, Hoy W. Low birth weight and reduced renal volume in Aboriginal children. Am J Kidney Dis 2001;37(5):915–20.
[39] Schmidt IM, Main KM, Damgaard IN, Mau C, Haavisto AM, Chellakooty M, et al. Kidney growth in 717 healthy children aged 0–18 months: a longitudinal cohort study. Pediatr Nephrol 2004;19(9):992–1003.
[40] Verburg BO, Geelhoed JJ, Steegers EA, Hofman A, Moll HA, Witteman JC, et al. Fetal kidney volume and its association with growth and blood flow in fetal life: the Generation R Study. Kidney Int 2007;72(6):754–61.
[41] Taal HR, Geelhoed JJ, Steegers EA, Hofman A, Moll HA, Lequin M, et al. Maternal smoking during pregnancy and kidney volume in the offspring: the Generation R Study. Pediatr Nephrol 2011;26(8):1275–83.
[42] Langley-Evans SC, Welham SJ, Jackson AA. Fetal exposure to a maternal low protein diet impairs nephrogenesis and promotes hypertension in the rat. Life Sci 1999;64(11):965–74.
[43] Lelievre-Pegorier M, Vilar J, Ferrier ML, Moreau E, Freund N, Gilbert T, et al. Mild vitamin A deficiency leads to inborn nephron deficit in the rat. Kidney Int 1998;54(5):1455–62.
[44] Celsi G, Kistner A, Aizman R, Eklof AC, Ceccatelli S, de Santiago A, et al. Prenatal dexamethasone causes oligonephronia, sodium retention, and higher blood pressure in the offspring. Pediatr Res 1998;44(3):317–22.
[45] Abitbol CL, Ingelfinger JR. Nephron mass and cardiovascular and renal disease risks. Semin Nephrol 2009;29(4):445–54.
[46] Brion MJ, Leary SD, Lawlor DA, Smith GD, Ness AR. Modifiable maternal exposures and offspring blood pressure: a review of epidemiological studies of maternal age, diet, and smoking. Pediatr Res 2008;63(6):593–8.
[47] Fain PR, McFann KK, Taylor MR, Tison M, Johnson AM, Reed B, et al. Modifier genes play a significant role in the phenotypic expression of PKD1. Kidney Int 2005;67(4):1256–67.
[48] Kumar R, Cheney KM, McKirdy R, Neilsen PM, Schulz RB, Lee J, et al. CBFA2T3-ZNF652 corepressor complex regulates transcription of the E-box gene HEB. J Biol Chem 2008;283(27):19026–38.
[49] White JJ, Ledbetter DH, Eddy Jr RL, Shows TB, Stewart DA, Nuell MJ, et al. Assignment of the human prohibitin gene (PHB) to chromosome 17 and identification of a DNA polymorphism. Genomics 1991;11(1):228–30.