

Heritability of renal function in hypertensive families of African descent in the Seychelles (Indian Ocean)

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Background. We estimated the heritability of three measures of glomerular filtration rate (GFR) in hypertensive families of African descent in the Seychelles (Indian Ocean).

Methods. Families with at least two hypertensive siblings and an average of two normotensive siblings were identified through a national hypertension register. Using the ASSOC program in SAGE (Statistical Analysis in Genetic Epidemiology), the age- and gender-adjusted narrow sense heritability of GFR was estimated by maximum likelihood assuming multivariate normality after power transformation. ASSOC can calculate the additive polygenic component of the variance of a trait from pedigree data in the presence of other familial correlations. The effects of body mass index (BMI), blood pressure, natriuresis, along with sodium to potassium ratio in urine and diabetes, were also tested as covariates.

Results. Inulin clearance, 24-hour creatinine clearance, and GFR based on the Cockcroft-Gault formula were available for 348 persons from 66 pedigrees. The age- and gender-adjusted correlations (\pm SE) were 0.51 (\pm 0.04) between inulin clearance and creatinine clearance, 0.53 (\pm 0.04) between inulin clearance and Cockcroft-Gault formula and 0.66 (\pm 0.03) between creatinine clearance and Cockcroft-Gault formula. The age- and gender-adjusted heritabilities (\pm SE) of GFR were 0.41 (\pm 0.10) for inulin clearance, 0.52 (\pm 0.13) for creatinine clearance, and 0.82 (\pm 0.09) for Cockcroft-Gault formula. Adjustment for BMI slightly lowered the correlations and heritabilities for all measurements whereas adjustment for blood pressure had virtually no effect.

Conclusion. The significant heritability estimates of GFR in our sample of families of African descent confirm the familial

aggregation of this trait and justify further analyses aimed at discovering genetic determinants of GFR.

High blood pressure is a strong and independent risk factor for end-stage renal disease (ESRD) in industrialized countries [1, 2]. For example, men with severe hypertension (systolic pressure \geq 210 mm Hg or diastolic pressure \geq 120 mm Hg) had a 22-fold increased risk of developing ESRD over men with optimal blood pressure levels (systolic pressure <120 mm Hg and diastolic pressure <80 mm Hg) in the MRFIT study [1]. Although few data are available, hypertension appears to be an important public health problem and a major cause of ESRD in African countries as well, with hypertension affecting about 20% of the adult population [3]. Unravelling genes controlling renal function is particularly important among individuals of African ancestry with, or susceptible to, essential hypertension because of their known propensity to develop ESRD [4–10].

Techniques available to determine glomerular filtration rate (GFR) have been described previously [11, 12]. Although the inulin clearance is the accepted gold standard, the endogenous creatinine clearance, formulas such as the Cockcroft-Gault [13] or the Modification of Diet in Renal Disease (MDRD) [14] formulas are more commonly used in clinical practice.

With one exception [15], several previous studies have suggested an important role of genetic factors in determining GFR [16–18]. We are not aware of any study reporting the heritability of inulin clearance, the gold standard, in a large population. Moreover, the heritability of renal function has never been assessed in African pedigrees. Therefore, the purpose of this study was to investigate and compare the heritabilities of three measures of GFR (inulin clearance, creatinine clearance, and

Key words: heritability, blood pressure, glomerular filtration rate, genetics, humans, renal function.

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Cockcroft-Gault formula) in families of African descent in the Seychelles.

METHODS

Population and sampling

This report is of a secondary study conducted on a sample of families collected prospectively for the primary purpose of a candidate gene study of hypertension. The study took place in the Seychelles Islands, which lie approximately 1000 km east of Kenya and 1000 km north of Madagascar and Mauritius. The Seychelles Islands, which were first colonized in the late 1700s, are populated predominantly by individuals of East African descent. Two previous population surveys showed a high prevalence of hypertension in the adult population [19, 20]. Health care is delivered through a national health system. Medical care and treatment are free of charge to all inhabitants. Families were selected from an ongoing national register that includes all patients with hypertension who attend primary health care centers. We used a convenience sampling strategy. The identification of eligible families relied on the presence of two or more persons with the same family name in the register as well as on information about eligible persons as provided by nurses working in the health centers attended by these eligible individuals. Families were selected if there were two or more full siblings with hypertension (defined as being on current antihypertensive treatment or having a systolic and/or diastolic blood pressure $\geq 140/90$ mm Hg based on the average of six office readings obtained on a single day) and two or more other first-degree relatives (full siblings or parents), irrespective of their hypertension status. Participants were recruited from July 1999 until January 2002. Four hundred and ninety-four subjects participated in the primary study and 357 had all three GFR measures available. Three of the 357 subjects had missing off-treatment office blood pressure values. We also excluded six individuals with extreme outlier values for GFR (i.e., observations lying three interquartile ranges beyond the first and third quartiles) from the remaining 354, leaving 348 individuals for the analyses. There were 66 one- or two-generation families with GFR measures (i.e., 348 participants from 77 sibships). Forty-seven families comprised one generation and 19 comprised two generations. The average sibship size (\pm SD) was 4.3 (\pm 1.7), the average number of normotensive siblings was 2.0 (\pm 1.8), and the average number of parents with data per sibship was 0.35 (\pm 0.05). We also conducted additional analyses on participants with available daytime ambulatory blood pressure (i.e., 330 participants) on the 314 nondiabetic participants and on participants above the gender-specific 20th percentile of urinary creatinine excretion (i.e., 280 participants with a creatininuria >0.115 mmol/kg/24-hour for men and >0.112 mmol/kg/24-hour for women) as a

proxy for ensuring completeness of the urinary collection. All participants were aged 18 years or older, were born in the Seychelles Islands, and gave their written informed consent. The study protocol was approved by the Ethical Committees of the Ministry of Health in the Seychelles and of the Faculty of Medicine of the University of Lausanne (Switzerland).

Body mass index (BMI) and blood pressure

Weight was measured using an electronic scale calibrated to the nearest 0.1 kg on participants without shoes and only wearing underwear, and height was measured to the nearest 0.5 cm using a wall-mounted stadiometer. BMI was calculated as weight (kg) divided by squared height (m^2). Daytime ambulatory blood pressure was measured using Diasys devices (Diasys Integra, Novacor SA, Rueil-Malmaison, France) placed on the left arm with an appropriately sized cuff. Measurements were based on the auscultatory mode, relayed by the oscillometric mode in case of failure of the auscultatory mode, and were recorded every 20 minutes. Daytime ambulatory blood pressure in this study is the average of the first ten valid readings between 7:00 a.m. and the patient's self-reported bedtime. We considered as invalid values: systolic blood pressure <50 mm Hg or >250 mmHg; diastolic blood pressure <30 mm Hg or >150 mmHg; pulse pressure (systolic blood pressure – diastolic blood pressure) <10 mm Hg or >150 mmHg; and pulse <35 beats/min or >250 beats/min. In total, 0.6% of the ambulatory blood pressure values were invalid and discarded. Office blood pressure was measured in a sitting position using a standard mercury sphygmomanometer with a cuff that automatically adapts the bladder to arm circumference (TriCuff, Stockholm, Sweden) in subjects who had been quiet for at least 10 minutes. Triplicate measurements on two consecutive mornings after a 2-week washout period off-treatment were averaged. The mean ambulatory and office blood pressure was defined as $(2/3)$ diastolic blood pressure + $(1/3)$ systolic blood pressure.

Clearance procedures and laboratory measurements

Antihypertensive therapy, if any, was stopped for 2 weeks before starting the protocol. After this washout period, participants were requested to collect urine by voluntarily voiding during 24 hours while on their usual diet, to measure sodium, potassium, and creatinine excretions. Creatinine clearance was based on this 24-hour urine collection. After overnight fasting, on the day following the 24-urine collection clearance protocols began between 7:00 a.m. and 8:00 a.m. in a quiet room with the subject lying on a bed throughout the procedure except for active voiding. Two intravenous catheters were inserted into antecubital veins, one for the infusion of inulin and the second into the contralateral forearm for

blood drawing. Fasting blood samples were collected first. After an oral water load of 200 mL, a bolus and a following sustained infusion of inulin, which were adapted to the participant's height, weight, and gender, were given to ensure a stable plasma concentration as described previously [21]. Participants received 400 mL of oral water at time 60 minutes and thereafter 200 mL every hour. After a 2-hour equilibration period, two 1-hour inulin clearances were obtained to measure GFR. The inulin and 24-hour creatinine clearances (C_x) were calculated with the formula $C_x = U_x \cdot V / P_x$, where U_x and P_x are urinary and plasma concentrations of the x solute, and V is the urine flow rate in mL/min. We also calculated GFR using the Cockcroft-Gault formula: creatinine clearance = $\{[(140 - \text{age}[\text{yr}]) \times \text{weight}[\text{kg}]/72] / \text{plasma creatinine} [\text{mg/dL}]\} \times s$, where $s = 1$ for males and 0.85 for females [13].

Urinary and plasma sodium and potassium concentrations were measured by flame photometry (IL-943) (Instrumentation Laboratory, Milano, Italy) and creatinine concentration was measured by the picric acid method (Cobas-Mira; Roche, Basel, Switzerland). Urinary and plasma concentrations of inulin were determined by photometry (Autoanalyzer II-Technicon; Bran & Luebbe, Norderstedt, Germany). Participants on antidiabetic treatment during the preceding month or with fasting blood glucose ≥ 7.0 mmol/L (measured using a Glycotronic[®] C reflectometer; Macherey-Nagel, Düren, Germany) were considered as diabetics. Untreated participants were considered as diabetic only if a second test confirmed the first result of a fasting blood glucose > 7 mmol/L. None of the 15 newly diagnosed (hence untreated) out of a total of 34 diabetic participants had a second test below 7.1 mmol/L.

Statistical analyses

Heritability is a measure of familial resemblance which rests on the assumption that the total phenotypic variance of a quantitative trait can be partitioned into independent genetic and environmental components. In turn, the genetic variance can be divided into an additive genetic variance, a dominance variance and an epistatic variance. Additive variance represents the average effects of individual alleles on the trait and reflects transmissible resemblance between relatives. Dominance variance represents the nonlinear interaction effects between alleles at the same locus while epistatic variance represents interaction effects between alleles at different loci. Heritability in the narrow sense is defined as the ratio of the additive genetic variance to the total phenotypic variance [22]. In this paper we refer to "heritability in the narrow sense" simply as heritability. Estimating the heritability of a trait is one of the first steps in the gene-finding process. To estimate heritability we used a linear regression model in which the total residual variance is partitioned, after regressing on covariates, into the sum

of an additive polygenic component, a sibling component and an individual-specific random component. Heritability was estimated as the polygenic component divided by the total residual variance. We estimated the heritability of the three GFR measures, inulin clearance, creatinine clearance, and Cockcroft-Gault formula, as well as of plasma creatinine for comparison with Cockcroft-Gault formula. Age, gender, BMI, diabetes status, along with diabetes duration, 24-hour natriuresis and urinary sodium to potassium ratio (Na/K) and mean blood pressure (both office and ambulatory) were used as covariates. Continuous covariates were standardized for the analyses. We used the ASSOC program in Statistical Analysis in Genetic Epidemiology (SAGE), so named because it estimates from family data association parameters (regression coefficients). ASSOC implements maximum likelihood estimation of both the components of variance and covariate coefficients, on the assumption of multivariate normality of the residuals but allowing for a Box-Cox transformation [23] of both sides of the regression equation [24], simultaneously estimating the power parameter. Allowing for a transformation relaxes the usual strict assumption of normality, and transformation of both sides results in median-unbiased estimates of the covariate coefficients on the original scale of measurement. All models were analyzed with and without a common sibship component, which can represent either a dominance component or a common sibship environmental component. Correlation coefficients between inulin clearance, creatinine clearance, and Cockcroft-Gault formula (each with covariate adjustments calculated from the coefficients estimated using ASSOC) were consistently estimated using all pairs of relatives of a particular type, as implemented in the Family CORrelations (FCOR) program in SAGE, with their standard errors calculated allowing for all pedigree relationships. This program does not make any distributional assumptions, but computes standard errors using only the large sample properties of the sums of squares and cross-products [25]. The genetic (polygenic) correlations between pairs of GFR measurements (i.e., inulin clearance-creatinine clearance, inulin clearance-Cockcroft-Gault formula, and creatinine clearance-Cockcroft-Gault formula) were estimated using the formula:

$$\rho_{\text{polygenic}} = \frac{\text{Cov}(X_{\text{adj}}, Y_{\text{adj}})}{\sqrt{V_p(X_{\text{adj}})V_p(Y_{\text{adj}})}}$$

where $V_p(X_{\text{adj}})$ and $V_p(Y_{\text{adj}})$ represent the polygenic variances of the adjusted residuals of traits X and Y and $\text{Cov}(X_{\text{adj}}, Y_{\text{adj}})$ is the polygenic covariance.

RESULTS

The characteristics of the participants are listed in Table 1. Overall, the average creatinine clearance was

Table 1. Participant characteristics

	All (N = 348) estimate (SE)	Complete UC (N = 280) estimate (SE)	Not complete UC (N = 68) estimate (SE)	P value ^a
Gender % female	0.56 (0.03)	0.56 (0.03)	0.56 (0.06)	0.98
Age years	46.2 (0.9)	45.0 (0.7)	51.1 (1.5)	0.0001
Body mass index kg/m ²	27.8 (0.3)	27.4 (0.3)	29.7 (0.7)	0.001
Office blood pressure (off-treatment) mm Hg				
Systolic	133.3 (1.0)	132.4 (1.1)	137.4 (1.1)	0.06
Diastolic	85.4 (0.6)	84.9 (0.7)	87.3 (1.4)	0.14
Mean	101.4 (0.7)	100.7 (0.8)	104.0 (1.7)	0.08
Daytime ambulatory blood pressure ^b mm Hg				
Systolic	134.4 (0.9)	133.7 (1.0)	137.1 (2.4)	0.17
Diastolic	88.1 (0.7)	87.5 (0.7)	90.4 (1.5)	0.09
Mean	103.5 (0.7)	102.9 (0.8)	106.0 (1.7)	0.10
Fasting blood glucose mmol/L	4.7 (0.1)	4.6 (0.1)	5.3 (0.3)	0.01
Diabetes ^c % diabetics	9.8 (1.6)	8.9 (1.7)	13.2 (4.1)	0.28
Diabetes duration ^d years	4.6 (0.9)	4.7 (1.1)	4.7 (1.1)	0.77
Plasma mmol/L				
Sodium	140.1 (0.2)	140.1 (0.2)	140.1 (0.5)	0.95
Potassium	3.75 (0.02)	3.74 (0.01)	3.75 (0.04)	0.85
Creatinine	76.8 (0.9)	77.1 (1.1)	75.3 (1.9)	0.41
Urinary (24-hour) mmol/24 hours				
Sodium	107 (3)	114 (3)	79 (5)	<0.0001
Potassium	44 (1)	46 (1)	34 (3)	<0.0001
Creatinine	12.4 (0.2)	13.2 (0.2)	8.9 (0.3)	<0.0001
Inulin clearance	116 (2)	115 (2)	118 (5)	0.60
Creatinine clearance (24-hour) mL/min	113 (2)	120 (2)	83 (3)	<0.0001
GFR calculated using Cockcroft-Gault formula mL/min	109 (2)	108 (2)	112 (5)	0.36

Abbreviations are: GFR, glomerular filtration rate; Complete UC, urinary creatinine excretion above the gender-specific 20th percentile (0.154 mmol/kg/24 hours for men and 0.112 mmol/kg/24 hours for women).

^aP values for the differences between the creatininuria groups were calculated using a *t* test for continuous variables and a chi-square test for categorical variables.

^bAverage of the 10 first valid measures.

^cOn antidiabetic treatment or having a fasting blood glucose >7 mmol/L.

^dFor diabetic participants only. Results are given as mean (SE) unless stated otherwise.

slightly lower than the inulin clearance, but if the analysis was restricted to the 280 participants with a 24-hour creatininuria above the 20th percentile, which suggests a more complete 24-hour urine collection, the average creatinine clearance was 4% higher than the average inulin clearance. Women (not shown) had significantly higher BMI than men (28.8 vs. 26.6 kg/m²) ($P = 0.0002$), longer duration of diabetes (6.7 vs. 3.1 years) ($P = 0.04$), lower plasma potassium (3.7 vs. 3.8 mmol/L) ($P = 0.001$), lower creatinine (70.4 vs. 84.8 μmol/L) ($P < 0.0001$), lower urinary creatinine excretion (10.4 vs. 14.9 mmol/24-hour) ($P < 0.0001$), lower inulin clearance (109 vs. 123 mL/min) ($P = 0.0003$), and lower creatinine clearance (105 vs. 123 mL/min) ($P < 0.0001$). Urinary electrolyte excretions and Cockcroft-Gault formula were similar for men and women.

Unadjusted and adjusted (for various covariates) correlations are presented in Table 2. Overall correlations were moderate, ranging from 0.44 to 0.81. The highest correlations were seen between creatinine clearance and Cockcroft-Gault formula, while both creatinine clearance and Cockcroft-Gault formula correlated less strongly with inulin clearance. Adjustment for age and gender slightly lowered inulin clearance-Cockcroft-Gault formula and creatinine clearance-Cockcroft-Gault formula correlations, but not the inulin clearance-creatinine

clearance correlation. The other adjustments made only small changes to the correlations. Inulin clearance-creatinine clearance correlations as well as creatinine clearance-Cockcroft-Gault formula correlations were one to three standard errors higher for those participants with a complete urine collection as compared to the whole sample, whereas inulin clearance-Cockcroft-Gault formula correlations are about one standard error lower for this subgroup. The age- and gender-adjusted correlations (se) between plasma creatinine and inulin clearance, creatinine clearance, and Cockcroft-Gault formula were 0.08 (0.06), -0.03 (0.06), and -0.29 (0.06), respectively.

The parent-offspring correlations ranged from 0.19 to 0.25 for inulin clearance, from 0.03 to 0.08 for creatinine clearance, and from 0.06 to 0.19 for Cockcroft-Gault formula (Table 3). The sibling correlations ranged from 0.07 to 0.13 for inulin clearance, from 0.12 to 0.21 for creatinine clearance, and from 0.26 to 0.42 for Cockcroft-Gault formula. The sibling correlations restricted to the 280 individuals with a more complete urine collection were one standard error higher than for the whole sample for creatinine clearance but not for either inulin clearance or Cockcroft-Gault formula. Because of their large standard errors (due to the small number of parent-offspring pairs in our sample), the parent-offspring correlations are of limited usefulness.

Table 2. Unadjusted and adjusted correlations between glomerular filtration rate (GFR) measures for the whole sample and the subgroup with complete urine collection

Adjustment	Inulin clearance-creatinine clearance		Inulin clearance-Cockcroft-Gault formula		Creatinine clearance-Cockcroft-Gault formula	
	All	Complete UC	All	Complete UC	All	Complete UC
Unadjusted	0.49 (0.05)	0.54 (0.05)	0.56 (0.04)	0.53 (0.05)	0.73 (0.03)	0.81 (0.03)
Age and gender	0.51 (0.04)	0.56 (0.04)	0.53 (0.04)	0.49 (0.05)	0.66 (0.03)	0.75 (0.03)
Age, gender, and BMI	0.49 (0.04)	0.52 (0.04)	0.51 (0.04)	0.44 (0.05)	0.67 (0.03)	0.75 (0.03)
Age, gender, and diabetes ^a	0.47 (0.04)	0.53 (0.05)	0.50 (0.04)	0.46 (0.05)	0.65 (0.04)	0.73 (0.03)
Age, gender, and office blood pressure ^b	0.48 (0.04)	0.54 (0.04)	0.51 (0.04)	0.47 (0.05)	0.65 (0.03)	0.74 (0.03)
Age, gender, natriuresis, and urine Na/K	0.51 (0.04)	0.56 (0.04)	0.53 (0.04)	0.49 (0.05)	0.66 (0.03)	0.74 (0.03)

BMI is body mass index.

^aDiabetes includes both an indicator variable for diabetes (1) versus no diabetes (0), and a variable for the duration of diabetes.

^bOff-treatment office blood pressure is used. Results are expressed as correlations (SE).

Table 3. Familial correlations for three glomerular filtration rate (GFR) measures

	Covariates				
	None	Body mass index	Diabetes	Office mean arterial pressure	Natriuresis and urine Na/K
Parent-offspring (all)					
IC	0.25 (0.11)	0.19 (0.10)	0.20 (0.12)	0.25 (0.11)	0.25 (0.11)
CC	0.08 (0.10)	0.08 (0.09)	0.03 (0.11)	0.08 (0.10)	0.08 (0.10)
CGF	0.19 (0.14)	0.06 (0.12)	0.16 (0.14)	0.18 (0.14)	0.18 (0.14)
Parent-offspring (complete urine)					
IC	0.26 (0.13)	0.18 (0.14)	0.19 (0.11)	0.25 (0.14)	0.26 (0.13)
CC	0.11 (0.15)	0.03 (0.13)	0.03 (0.14)	0.10 (0.15)	0.10 (0.15)
CGF	0.16 (0.16)	-0.02 (0.14)	0.10 (0.15)	0.15 (0.15)	0.15 (0.16)
Sibling (all)					
IC	0.12 (0.06)	0.07 (0.05)	0.11 (0.05)	0.13 (0.06)	0.12 (0.06)
CC	0.20 (0.06)	0.12 (0.06)	0.21 (0.06)	0.20 (0.06)	0.20 (0.06)
CGF	0.41 (0.07)	0.26 (0.07)	0.42 (0.07)	0.41 (0.07)	0.40 (0.07)
Sibling (complete urine)					
IC	0.15 (0.07)	0.10 (0.06)	0.14 (0.07)	0.14 (0.07)	0.15 (0.07)
CC	0.27 (0.08)	0.18 (0.07)	0.31 (0.08)	0.27 (0.08)	0.28 (0.08)
CGF	0.37 (0.08)	0.24 (0.07)	0.37 (0.08)	0.37 (0.08)	0.36 (0.08)

Abbreviations are: IC, inulin clearance; CC, 24-hour creatinine clearance; CGF, GFR calculated using the Cockcroft-Gault formula. All traits were adjusted for age and gender. Results are expressed as correlations (SE).

Age- and gender-adjusted heritabilities for all three GFR traits, without additional covariates, were significant at least at the 0.01 level, whether or not a sibling component of variance was included in the model (Table 4). Heritabilities for inulin clearance (ranging from 0.31 to 0.42) and creatinine clearance (ranging from 0.42 to 0.52) were moderate, whereas Cockcroft-Gault formula heritability (ranging from 0.52 to 0.85) was higher. Allowing for an additional sibling correlation (i.e., a variance component that is due to either a dominance component or a common sibship environmental component) did not substantially modify the heritability estimates for inulin clearance or creatinine clearance because the sibship component of variance was either estimated to be zero or was not significant. However, it slightly decreased Cockcroft-Gault formula heritability, without being significant at the 0.05 level. Unlike office blood pressure that did not affect heritabilities when added as a covariate, BMI slightly lowered heritabilities for all three GFR traits (but not for creatinine alone). Analyses using daytime ambulatory mean

blood pressure as a covariate yielded results similar to those of office blood pressure (results not shown). The inclusion of natriuresis along with urine Na/K as covariates had little impact on the estimated heritabilities. The inclusion of diabetes [using both a dichotomous (diabetes status) and a continuous (diabetes duration) variable] in the model barely changed creatinine clearance heritabilities but somewhat decreased inulin clearance and Cockcroft-Gault formula heritabilities. Analyses restricted to non-diabetic participants yielded heritabilities ranging from 0.24 to 0.32 for inulin clearance (i.e., up to one standard error lower than for the whole sample), from 0.43 to 0.54 for creatinine clearance (i.e., from 0% to 15% of one standard error lower than for the whole sample), and from 0.69 to 0.85 for Cockcroft-Gault formula (i.e., from 9% to 50% of one standard error higher than for the whole sample). Models without a sibship component using plasma creatinine instead of Cockcroft-Gault formula gave lower heritabilities as compared to Cockcroft-Gault formula, whereas the plasma creatinine models that

Table 4. Heritabilities of three measures of glomerular filtration rate (GFR)

		Covariates									
		None		Body mass index		Diabetes ^a		Office mean arterial pressure		Urine Na and Na/K	
		h ² (SE)	λ_1^b	h ² (SE)[Int]	λ_1	h ² (SE) [Int]	λ_1	h ² (SE) [Int]	λ_1	h ² (se) [Int]	λ_1
IC	With sibship ^c	0.40 (0.13)	0.03	0.35 (0.11)	0.03	0.30 [0.31 ^{e,g}] (0.13)	0.07	0.39 (0.14)	0.05	0.36 (0.11)	0.06
	Without sibship ^c	0.41 (0.10)	0.03	0.35 [0.36 ^e] (0.11)	0.03	0.34 [0.34 ^{e,g}] (0.10)	0.07	0.42 (0.11)	0.06	0.37 [0.36 ^{e,h}] (0.10)	0.07
CC	With sibship	0.52 (0.13)	0.22	0.42 (0.13)	0.29	0.50 (0.12)	0.23	0.52 (0.13)	0.24	0.48 (0.13)	0.15
	Without sibship	0.52 (0.13)	0.22	0.42 [0.42 ^d] (0.13)	0.29	0.50 (0.12)	0.23	0.54 [0.52 ^f] (0.13)	0.25	0.48 (0.13)	0.15
CGF	With sibship	0.72 (0.17)	-0.11	0.36 [0.52 ^d] (0.26)	-0.02	0.56 (0.25)	-0.12	0.71 [0.72 ^f] (0.18)	-0.10	0.68 [0.81 ^h] (0.18)	-0.10
	Without sibship	0.82 (0.09)	-0.09	0.66 [0.70 ^d] (0.10)	-0.01	0.79 (0.09)	-0.08	0.83 [0.82 ^f] (0.09)	-0.08	0.85 (0.10)	-0.09
Creatinine	With sibship	0.33 (0.34)	0.23	0.36 (0.35)	0.25	0.69 [0.68 ^e] (0.23)	0.13	0.37 (0.33)	0.25	0.36 (0.32)	0.23
	Without sibship	0.65 (0.10)	0.25	0.66 (0.11)	0.26	0.75 [0.74 ^e] (0.11)	0.13	0.65 (0.10)	0.25	0.65 (0.11)	0.26

Abbreviations are: [Int], terms in square brackets are heritability estimates from models with interaction terms; IC, inulin clearance; CC, 24-hour creatinine clearance; CGF, GFR calculated using the Cockcroft-Gault formula. Results are expressed as heritabilities (SE). All traits were adjusted for age and sex.

^aDiabetes includes both an indicator variable for diabetes (1) versus no diabetes (0), and a variable for the duration of diabetes.

^b λ_1 is power parameter in the generalized modulus power transformation.

^cWith/without sibship, including/not including in the model a sibship correlation over and above that expected for polygenic inheritance.

^dSignificant age \times body mass index interaction.

^eSignificant age \times gender interaction.

^fSignificant age \times office blood pressure interaction.

^gSignificant age \times diabetes status interaction.

^hSignificant age \times (urine Na), gender \times (urine Na), age \times (urine Na/K), and gender \times (urine Na/K) interactions. Cut-off *P* value for significant interactions: 0.05. λ_1 and standard errors with and without interaction terms are similar.

included a sibship component did not maximize optimally (i.e., the first derivatives of the log likelihoods were not as close to zero as for the other models).

We analyzed all two-way interactions and found, in particular, significant age \times gender and age \times BMI interactions. All interactions significant at the 0.05 level were kept in the models and are listed in the notes for Table 4. The only major change due to the inclusion of an interaction term was a 40% increase in Cockcroft-Gault formula heritability due to a significant age \times BMI interaction. The power transformation values (λ_1) (Table 4) indicate that the appropriate transformation to help induce the assumed normality of the residuals is roughly logarithmic for inulin clearance and Cockcroft-Gault formula and a fourth root for creatinine clearance.

The age- and gender-adjusted genetic correlations were 0.56 for inulin clearance-creatinine clearance, 0.14 for inulin clearance-Cockcroft-Gault formula, and 0.48 for creatinine clearance-Cockcroft-Gault formula. The age-, gender- and BMI-adjusted genetic correlations were estimated to be -0.14 for inulin clearance-creatinine clearance, -0.04 for inulin clearance-Cockcroft-Gault formula, and 0.61 for creatinine clearance-Cockcroft-Gault formula. The age-, gender- and diabetes-adjusted genetic correlations were 0.55 for inulin clearance-creatinine clearance, 0.88 for inulin clearance-Cockcroft-Gault formula, and 0.18 for creatinine clearance-Cockcroft-Gault formula. The age-, gender-, and off-treatment office

blood pressure-adjusted genetic correlations were 0.24 for inulin clearance-creatinine clearance, 0.23 for inulin clearance-Cockcroft-Gault formula, and 0.24 for creatinine clearance-Cockcroft-Gault formula. The age- and gender-adjusted genetic correlations between plasma creatinine and inulin clearance, creatinine clearance, and Cockcroft-Gault formula were -0.75, -0.69, and -0.80, respectively. That these correlations are high in magnitude indicates that to a certain extent any one of the three GFR measures can be used to study the genes involved in creatinine control.

DISCUSSION

We have demonstrated significant heritability estimates for three methods of determining GFR, ranging from 31% to 85%, in a sample of East African families each containing at least two hypertensive members. Considering inulin clearance as the gold standard, both creatinine clearance and Cockcroft-Gault formula overestimate GFR heritability. Our study demonstrates that there exists a familial aggregation of GFR in Black African pedigrees, as was reported previously in Caucasians and African Americans [16–18] but our results are the first to demonstrate such an association with the use of inulin clearance. Our results also confirm the high heritability of GFR (63%) found in female twin data in the United Kingdom (presumably from a

predominantly Caucasian sample) when measured with the Cockcroft-Gault formula [16] and the moderate heritabilities (ranging from 27% to 56%) of the creatinine clearance found in 49 Utah pedigrees ascertained through either early coronary heart disease death, stroke death, or hypertension for a Caucasian population [18]. Although our results do not provide information about which genes or how many genes might be involved, they justify conducting additional analyses to look for genes involved in GFR control.

We have compared GFR heritabilities using three different measures, each of which suffers from different limitations. It is well recognized that creatinine is not the ideal marker to measure since creatinine is secreted into the urine and this secretion increases as renal failure progresses. Nonetheless, these approaches are those commonly used in clinical trials and epidemiologic studies. In addition, the fact that creatinine is not the ideal marker, use of the 24-hour creatinine clearance is also limited by the high risk of incomplete urine collection. In our study, only a single 24-hour urinary collection was obtained from each participant. In contrast to the clearance techniques, the calculation of the Cockcroft-Gault formula does not rest on urine measurements. Thus, the formula avoids the problem of incomplete urine collections. However, the Cockcroft-Gault formula relies very much on body weight and acute weight gains or losses can lead to erroneous estimation of GFR.

The impact of the limitations of each technique are illustrated by the moderate correlations between the three proxies used, particularly inulin clearance-creatinine clearance and inulin clearance-Cockcroft-Gault formula. The correlations between inulin clearance-creatinine clearance and inulin clearance-Cockcroft-Gault formula remained low even if we only considered participants for whom very similar inulin clearances were obtained, and for whom therefore a high quality result can be assumed, which suggests that the true correlations are indeed low in our sample. Analyses restricted to individuals with a higher creatininuria yielded slightly higher correlations involving creatinine clearance, which suggests that incomplete urine collection affects, albeit modestly, these correlations. A large discrepancy in terms of correlations between various GFR measurements can also be found in the literature and our correlations are comparable with those of other studies [26–32].

Because all three measures indicate a significant GFR heritability, we are confident that genetic factors involved in GFR control can be identified in our sample. The addition of covariates tended to lower heritabilities, the largest and most consistent effect across all three GFR traits being observed with BMI. Although such a finding might suggest a common genetic control between GFR and BMI, further evidence of this is needed, as this effect is only modest for inulin clearance, the gold standard. In

addition, the maximum effect is observed for Cockcroft-Gault formula (and no effect is observed for creatinine alone) and one might argue that controlling Cockcroft-Gault formula for BMI is debatable because body weight is part of both composite variables. The genetic correlations were often low and varied a lot according to which covariates were included in the model. These results suggest that the three GFR traits only modestly share genetic factors in common. For instance, the near-zero estimates for the genetic correlations between inulin clearance and Cockcroft-Gault formula and between inulin clearance and creatinine clearance, when adjusted for BMI, suggest that studies designed to identify loci influencing GFR are likely to detect different loci if these surrogate measures are used instead of inulin clearance. In view of these results, even though inulin clearance has a lower heritability, it is a purer measure of GFR and we would recommend using inulin clearance for a genetic study on GFR.

Our sampling scheme via individuals with high blood pressure is not recommended to estimate a population heritability. However, the fact that the heritability estimates hardly changed after adjusting for off-treatment office or ambulatory blood pressure, the trait on which the families were ascertained, suggests that these estimates are nevertheless appropriate for the general Seychelles population. We acknowledge that, in terms of generalizability of the findings, such a correction is an imperfect proxy for random sampling from the general population or for conditioning the likelihood on the exact ascertainment event, which would have been very difficult, given the criteria of at least two hypertensive siblings (i.e., multiplex ascertainment).

The fact that inulin clearance and creatinine clearance heritabilities were similar, with or without the inclusion of a sibship component in the model, is compatible with no significant departure from an additive genetic model. On the other hand, the effect of the sibship component on heritabilities was slightly more pronounced for Cockcroft-Gault formula and highest for plasma creatinine. As plasma creatinine models with a sibship component did not maximize adequately, we suspect that the lower Cockcroft-Gault formula heritabilities when a sibship component was estimated are due to the creatinine included in the Cockcroft-Gault formula. Because we adjusted for some of the covariates that are part of the Cockcroft-Gault formula, we also analyzed plasma creatinine to facilitate interpreting the results. Note that the sibship effect can, however, also partly be due to either a dominance component, a common sibship environmental component, or both.

Several caveats about the concept of heritability deserve mention. First, as in any ratio, factors that influence either the numerator or the denominator will change heritability. Second, heritability being a population concept,

our estimates only apply to the Seychelles Islands. Third, several assumptions underlie the concept of heritability, such as random mating and the absence of genotype-environment interaction. Statistical interaction between two variables can depend on their scale of measurement and can often be removed by an appropriate transformation. Although we cannot exclude genotype-environment interactions, their effects are minimized by the choice of an appropriate scale of measurement, which we achieved by simultaneously estimating that scale (the power transformation) under a model that assumed no such interactions. As mentioned above, the similar heritability estimates obtained for inulin clearance and creatinine clearance, regardless of the presence of a sibship correlation, point toward no genetic dominance variance of GFR. Finally, the individual-specific random component of variance includes measurement error in addition to other more strictly environmental influences. The low intra-trait correlations obtained between traits that measure the same entity illustrate the potential degree of measurement error for the determination of GFR, but do not allow quantifying this error. Keeping all these limitations in mind, the presence of a nonzero heritability remains a necessary, although not sufficient, condition for the detection of genes controlling GFR.

Although heritability has often been estimated from samples of twins, the twin method is based on several assumptions, the most fundamental of which are that monozygotic and dizygotic twin pairs differ in their similarities as a result of only genetic causes, and that both types of twins and singletons have the same genotypic and environmental population variances [33]. There is good evidence that the former assumption often does not hold, as monozygotic twins have been shown, for instance, to be more similar than dizygotic twins for several demographic and lifestyle factors [34]. With regard to the latter assumption, the majority of monozygotic twins are mono-chorionic, who have on average a lower birth weight and a higher perinatal mortality than dichorionic dizygotic twins [35, 36]. In addition, mono-chorionic monozygotic twins are characterized by placental anastomoses, which can lead (in up to 30% of cases) to twin-twin transfusion syndrome and subsequent renal tubular dysgenesis [37]. Although we did not identify any study evaluating the impact of chorionicity on long-term renal function, the impact of low birth weight, which preferentially affects mono-chorionic monozygotic twin, as a risk factor for high blood pressure, has been extensively described [38, 39]. Such findings raise the question of how heritability, in general, and heritability of blood pressure-related phenotypes such as GFR, in particular, calculated in twins can be generalized to singletons. We therefore consider our family-based approach to be a more appropriate and more valid way to estimate GFR heritability than a twin-based method.

CONCLUSION

The significant heritability estimates of GFR, as measured by inulin clearance, creatinine clearance, and Cockcroft-Gault formula, in our sample of families of African descent with at least two hypertensive siblings, confirm the familial aggregation of this trait and justify further analyses aimed at discovering genetic determinants of GFR.

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REFERENCES

1. KLAG MJ, WHELTON PK, RANDALL BL, et al: Blood pressure and end-stage renal disease in men. *N Engl J Med* 334:13-18, 1996
2. TOZAWA M, ISEKI K, ISEKI C, et al: Blood pressure predicts risk of developing end-stage renal disease in men and women. *Hypertension* 41:1341-1345, 2003
3. NAICKER S: End-stage renal disease in sub-Saharan and South Africa. *Kidney Int* 63 (Suppl 83):S119-S122, 2003
4. FOGO AB: Hypertensive risk factors in kidney disease in African Americans. *Kidney Int* 63 (Suppl 83):S17-S21, 2003
5. KLAG MJ, WHELTON PK, RANDALL BL, et al: End-stage renal disease in African-American and white men. 16 year MRFIT findings. *JAMA* 277:1293-1298, 1997
6. SEEDAT YK, NAICKER S, RAWAT R, et al: Racial differences in the causes of end-stage renal failure in Natal. *South Afr Med J* 65:956-958, 1984
7. FRASSINETTI FERNANDES P, ELLIS PA, CAIRNS HS, et al: Causes of end-stage renal failure in black patients starting renal replacement therapy. *Am J Kidney Dis* 36:301-309, 2000
8. PAZIANAS M, EASTWOOD JB, MACRAE KD, et al: Racial origin and primary renal diagnosis in 771 patients with end-stage renal disease. *Nephrol Dial Transplant* 6:931-935, 1991
9. CLARK TJ, RICHARDS NT, ADU D, et al: Increased prevalence of dialysis-dependent renal failure in ethnic minorities in the west Midlands. *Nephrol Dial Transplant* 8:146-148, 1993
10. KOTCHEN TA, PIERING AW, COWLEY AW, et al: Glomerular hyperfiltration in hypertensive African Americans. *Hypertension* 35:822-826, 2000
11. CORESH J, TOTO RD, KIRK KA, et al: Creatinine clearance as a measure of GFR in screenees for the African-American Study of Kidney Disease and Hypertension Pilot Study. *Am J Kidney Dis* 32:32-42, 1998
12. WALSER M: Assessing renal function from creatinine measurements in adults with chronic renal failure. *Am J Kidney Dis* 32:23-31, 1998
13. COCKCROFT DW, GAULT MH: Prediction of creatinine clearance from serum creatinine. *Nephron* 16:31-41, 1976
14. LEVEY AS, BOSCH JP, LEWIS JB, et al: A more accurate method to estimate glomerular filtration rate from serum creatinine: A new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* 130:461-470, 1999
15. GRIM CE, MILLER JZ, CHRISTIAN JC: Glomerular filtration rate and electrolyte handling in response to sodium loading and depletion. A twin study. *Acta Genet Med Gemellol (Roma)* 28:149-154, 1979

16. HUNTER DJ, DE LANGE M, SNIEDER H, et al: Genetic contribution to renal function and electrolyte balance: A twin study. *Clin Sci (Lond)* 103:259–265, 2002
17. DEWAN AT, ARNETT DK, ATWOOD LD, et al: A genome scan for renal function among hypertensives: The HyperGEN Study. *Am J Hum Genet* 68:136–144, 2001
18. HUNT SC, HASSTEDT SJ, COON H, et al: Linkage of creatinine clearance to chromosome 10 in Utah pedigrees replicates a locus for end-stage renal disease in humans and renal failure in the fawn-hooded rat. *Kidney Int* 62:1143–1148, 2002
19. BOVET P, SHAMLAYE C, KITUA A, et al: High prevalence of cardiovascular risk factors in the Seychelles (Indian Ocean). *Arterioscler Thromb* 11:1730–1736, 1991
20. BOVET P, PERRET F, SHAMLAYE C: The Seychelles Heart Study II: Methods and basic findings. *Seychelles Med Dent J* 5:8–24, 1997
21. BURNIER M, RUTSCHMAN B, NUSSBERGER J, et al: Salt dependent renal effects of an angiotensin II antagonist in healthy subjects. *Hypertension* 22:339–347, 1993
22. VOGEL F, MOTULSKY AG: *Human Genetics. Problems and Approaches*, 3rd ed., Berlin, Springer-Verlag, 1997
23. BOX GEP, COX DR: An analysis of transformations. *J R Stat Soc Ser B Method* 26: 211–243, 1964
24. CARROLL RJ, RUPPERT D: Power transformations when fitting theoretical models to data. *J Am Stat Assoc* 79:321–328, 1984
25. KEEN KJ, ELSTON RC: Robust asymptotic sampling theory for correlations in pedigrees. *Stat Med* 22:3229–3247, 2003
26. MPIO I, LAVILLE M, HADI-AÏSSA A, et al: Predicted creatinine clearance to evaluate glomerular filtration rate in black Caribbean subjects. *Nephrol Dial Transplant* 18:1307–1310, 2003
27. LEVEY AS, BERG RL, GASSMAN JJ, et al: Creatinine filtration, secretion and excretion during progressive renal disease. Modification of Diet in Renal Disease (MDRD) Study Group. *Kidney Int* 36:S73–S80, 1989
28. LUKE DR, HALSTENSON CE, OPSAHL JA, et al: Validity of creatinine clearance estimates in the assessment of renal function. *Clin Pharmacol Ther* 48:503–508, 1990
29. PIERRAT A, GRAVIER E, SAUNDERS C, et al: Predicting GFR in children and adults: A comparison of the Cockcroft-Gault, Schwartz, and Modification of Diet in Renal Disease formulas. *Kidney Int* 64:1425–1436, 2003
30. BARACSKAY D, JARJOURA D, CUGINO A, et al: Geriatric renal function: Estimating glomerular filtration in an ambulatory elderly population. *Clin Nephrol* 47:222–228, 1997
31. FRIEDMAN JR, NORMAN DC, YOSHIKAWA TT: Correlation of estimated renal function parameters versus 24-hour creatinine clearance in ambulatory elderly. *J Am Geriatr Soc* 37:145–149, 1989
32. MARKANTONIS SL, AGATHOKLEOUS-KIOUPAKA E: Can two-, four- or eight-hour urine collections after voluntary voiding be used instead of twenty-four-hour collections for the estimation of creatinine clearance in healthy subjects? *Pharm World Sci* 20:258–263, 1998
33. ELSTON RC, BOKLAGE CE: An examination of fundamental assumptions of the twin method. *Prog Clin Biol Res* 24A:189–199, 1978
34. HELLER RF, O'CONNEL DL, ROBERTS DCK, et al: Lifestyle factors in monozygotic and dizygotic twins. *Genet Epidemiol* 5:311–321, 1988
35. DUBE J, DODDS L, ARMSON BA: Does chorionicity or zygosity predict adverse perinatal outcomes in twins? *Am J Obstet Gynecol* 186:579–583, 2002
36. HATKAR PA, BHITE AG: Perinatal outcome of twins in relation to chorionicity. *J Postgrad Med* 45:33–37, 1999
37. BARR M, SEDMAN AB, HEIDELBERGER KP: Renal tubular dysgenesis in twins. *Pediatr Nephrol* 12:408–413, 1998
38. MZAYEK F, SHERWIN R, FONSECA V, et al: Differential association of birth weight with cardiovascular risk variables in African-Americans and Whites: The Bogalusa heart study. *Ann Epidemiol* 14:258–264, 2004
39. ERIKSSON M, WALLANDER MA, KRAKAU I, et al: Birth weight and cardiovascular risk factors in a cohort followed until 80 years of age: The study of men born in 1913. *J Intern Med* 255:236–246, 2004