

# Genetic contribution to renal function and electrolyte balance: a twin study

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## A B S T R A C T

A classical twin study was performed to assess the relative contributions of genetic and environmental factors to serum levels of calcium, phosphate and magnesium, urinary levels of calcium, sodium and potassium, and creatinine clearance. The subjects were 1747 adult female twin pairs: 539 monozygotic and 1208 dizygotic. The intraclass correlations were calculated, and maximum-likelihood model fitting was used to estimate genetic and environmental variance components. The intraclass correlations for all of the variables assessed were higher in monozygotic twin pairs. The heritabilities (with 95% confidence intervals) obtained from model fitting were: serum calcium, 33% (21–45%); serum phosphate, 58% (53–62%), serum magnesium, 27% (15–39%); 24 h urinary potassium, 40% (27–51%); 24 h urinary calcium, 52% (41–61%); 24 h urinary sodium, 43% (30–54%); fractional excretion of sodium, 52% (44–59%); serum creatinine, 37% (25–49%); calculated creatinine clearance, 63% (54–72%). This study provides evidence for the importance of genetic factors in determining urinary and blood levels of the major electrolytes involved in blood pressure regulation. Identifying heritability is the first step on the way to finding specific genes, which may improve our insight into the pathophysiology of the metabolism of these electrolytes, and thereby improve our understanding of the aetiology of complex diseases such as renal failure and hypertension.

## INTRODUCTION

Electrolyte balance and glomerular filtration are important determinants of the predisposition to developing hypertension. While a genetic influence has long been considered an important determinant of electrolyte balance and renal function, previous studies have lacked the power to quantify this contribution.

The endogenous production and excretion of creatinine maintains a nearly constant plasma concentration, which can be used to determine the glomerular filtration

rate [1]. Previous studies examining the heritability of serum creatinine levels or creatinine clearance have produced variable results in generally small numbers of twins [2–4].

Similarly inconsistent results have been produced for the heritability of the urinary handling of sodium and potassium [4–7]. A number of studies have demonstrated that hypertensive individuals have a lower fractional excretion of sodium [8–10]. There are no data available on whether this is mediated largely by genetic or environmental influences.

**Key words:** calcium, creatinine clearance, fractional sodium excretion, twin study.

**Abbreviations:** AIC, Akaike's information criterion; BMI, body mass index; CV, coefficient of variation; df, degrees of freedom; DZ, dizygotic; MZ, monozygotic; rMZ and rDZ, intraclass correlations for MZ and DZ twins respectively.

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Calcium, phosphate and magnesium have vital roles in a number of metabolic pathways. A previous study of serum calcium was based on small numbers, but suggested a large genetic influence [11]. More recently the calcium-sensing, G-protein-coupled cell-surface receptor has been suggested to account for 74% of the total variation in serum calcium [12]. Small studies have also looked at the heritability of phosphate and magnesium levels, and suggested a genetic influence [13].

In summary, the past literature suggests a possible genetic influence on a number of key electrolytes, although these studies were small, lacked consistency and did not compare the range of variables in a single study. Obtaining an accurate estimate of the relative contributions of genetic and environmental influences on the serum levels of common electrolytes and on renal function is the first step on the way to finding specific genes that may improve our understanding of the pathophysiology of disease. The aim of the present study was to determine the proportions of the variance of serum levels of calcium, phosphate, magnesium, creatinine clearance, and urinary excretion of sodium and potassium that are explained by genetic and environmental factors in a large sample of unselected twins.

## MATERIALS AND METHODS

### Study population

The subjects were 1747 female twin pairs (age range 18–72 years) from the St Thomas' UK Adult Twin Registry, a volunteer sample recruited through a national media campaign in the U.K. [14]. Zygosity is determined routinely by standardized questionnaire, and multiplex DNA fingerprinting with a variable number tandem repeats is used for confirmation where zygosity is uncertain following administration of the questionnaire [15].

Research was carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association, and was approved by the St Thomas' Hospital Ethical Committee. Consent was obtained from each subject after full explanation of the purpose, nature and risk of all procedures used.

### Data ascertainment

Demographic information was obtained by a standardized nurse-administered questionnaire. Height and weight were measured and body mass index (BMI; kg/m<sup>2</sup>) was calculated. Blood and second voided urine samples were collected in the early morning (at the same time and place for both twins of the pair) after an overnight fast, and stored at –20 °C until assayed. 24 h urine collection was commenced on the same day, and

returned the following day. All samples were measured in duplicate in the same assay.

### Assays

Assays for serum calcium (Ca), magnesium (Mg) and phosphate were performed using standard laboratory procedures. These tests were performed on a 950 Vitros analyser (Ortho-Clinical Diagnostics; Johnson and Johnson, Rochester, NY, U.S.A.). The analyser uses dry chemistry slide technology and reflection spectrophotometry. The interassay coefficients of variation (CVs) for serum Ca were 1.2% and 0.9% at 2.1 and 2.9 mmol/l respectively; interassay CVs for serum Mg were 1.3% and 1.2% at 1.0 and 2.0 mmol/l respectively; and interassay CVs for serum phosphate were 1.1 and 0.7% at 1.10 and 2.40 mmol/l respectively.

Serum albumin was assayed. The albumin–Bromocresol Green dye complex that forms during the incubation is measured by reflectance spectrophotometry at 630 nm. The interassay CVs for serum albumin were 1.3% and 1.5% at 28 and 44 g/l respectively.

Urinary sodium (Na) and potassium (K) were measured on a Corning 480 Flame photometer. The intra- and inter-assay CVs for sodium at 100 mmol/l and potassium at 80 mmol/l were less than 2%. 24 h urinary calcium (Ca) was measured using a standard assay (Vitros; Johnson and Johnson). The sensitivity of the calcium assay is 0.25 mmol/l, while the intra- and inter-assay CVs are below 3% and 4.3% respectively. The raw 24 h urine (Na, K and Ca) results are presented in Table 1, and were corrected for 24 h urinary creatinine in the analysis.

Plasma and urine creatinine levels were measured using an enzymic method (creatinine iminohydrolase) on the Vitros 950 analyser. The inter-assay CVs for plasma creatinine were 1.1% and 1.1% at 81 and 499 µmol/l respectively; interassay CVs for urine creatinine were 2.9% and 2.5% at 7.80 and 20.42 mmol/l.

Calculated creatinine clearance is calculated using the Cockcroft–Gault formula:

$$\text{Clearance} = \frac{\{(140 - \text{age}) \cdot \text{weight}/72\}}{\text{plasma creatinine}} f$$

where  $f = 1$  for males and  $f = 0.85$  for females. Measured creatinine clearance was calculated from UV/P for a 24 h urine collection (where U = urine creatinine concentration, V = urine volume and P = plasma creatinine concentration).

The fractional excretion of sodium (FENa) was calculated from a spot urine sodium concentration using the equation:

$$\text{FENa} = (\text{Pcr} \cdot \text{Una}/\text{Pna} \cdot \text{Ucr}) \cdot 100$$

where Pcr = plasma creatinine, Una = urinary sodium, Pna = plasma sodium and Ucr = urinary creatinine.

**Table 1** Characteristics of the MZ (*n* = 539) and DZ (*n* = 1208) twin pairs

Values are means (S.D.). Calculated creatinine clearance =  $\{[(140 - \text{age}) \cdot \text{weight}/72]/\text{plasma creatinine}\}f$  ( $f = 0.85$  for females). Measured creatinine clearance =  $UV/P$  for 24 h urine collection, where *U* = urine creatinine concentration, *V* = urine volume and *P* = plasma creatinine concentration. Fractional excretion of sodium (FENa) =  $(P_{\text{cr}} \cdot \text{Una}/P_{\text{na}} \cdot \text{Ucr}) \cdot 100$ , where *P*<sub>cr</sub> = plasma creatinine, *U*<sub>na</sub> = urinary sodium, *P*<sub>na</sub> = plasma sodium and *U*<sub>cr</sub> = urinary creatinine.

Parameter	MZ twins	DZ twins
Age (years)	48.0 (13.6)	46.8 (11.5)
Height (cm)	162.8 (6.6)	163.1 (6.6)
Weight (kg)	64.4 (11.0)	66.4 (12.8)
BMI (kg/m <sup>2</sup> )	24.3 (4.0)	25.0 (4.6)
Postmenopausal (%)	53	48
Serum calcium (mmol/l)	2.37 (0.10)	2.37 (0.10)
Albumin-corrected calcium (mmol/l)	2.31 (0.08)	2.30 (0.08)
Serum phosphate (mmol/l)	1.14 (0.15)	1.12 (0.17)
Serum magnesium (mmol/l)	0.81 (0.09)	0.79 (0.08)
24 h urinary K (mmol/24 h)	53.2 (16.3)	54.7 (17.6)
24 h urinary Na (mmol/24 h)	91.2 (37.3)	94.8 (42.7)
24 h urinary Ca (mmol/24 h)	3.01 (1.36)	2.88 (1.49)
Serum creatinine ( $\mu\text{mol/l}$ )	75.4 (11.7)	75.4 (13.6)
Calculated creatinine clearance (ml/min)	93.9 (22.3)	99.3 (27.0)
Measured creatinine clearance (ml/min)	119.9 (39.3)	119.4 (39.0)
FENa (%)	0.66 (0.55)	0.72 (0.61)

## Statistical analysis

### Background to twin analysis

The classical twin study makes use of the fact that monozygotic (MZ) twins share identical genotypes, whereas dizygotic (DZ) twins are no more alike genetically than siblings, sharing (on average) 50% of their segregating genes. If MZ twins show a greater resemblance for a specific trait than DZ twins, this is likely to be due to genetic factors. A higher intraclass correlation in MZ than in DZ twins provides a first indication of a genetic influence. Structural equation modelling allows a more extensive separation and quantification of the observed phenotypic variance into its genetic and environmental components: additive genetic variance, dominance genetic variance, shared (or common) environmental variance, and specific (or unique) environmental variance, which also contains measurement error. Heritability ( $h^2$ ) can be defined as the ratio of additive genetic variance to total phenotypic variance.

### Analytical approach

Preliminary data analysis was performed and intraclass correlations were estimated using STATA [16]. Where necessary, data were logarithmically transformed to obtain a normal distribution for all variables. We estimated genetic and environmental influences on all vari-

ables, after adjustment for age and BMI. Adjusted estimates were obtained by model fitting to trait residuals after removal of the effects of age and BMI by linear regression [17].

### Model-fitting procedure

The significance of additive genetic variance, dominance genetic variance and shared (or common) environmental variance is tested by removing them sequentially in specific submodels, leading eventually to a model that gives the most parsimonious fit to the data, i.e. a model in which the pattern of variances and covariance is explained by as few parameters as possible. Submodels were compared with the full model by hierarchical  $\chi^2$  tests. The difference between a submodel and the full model itself is distributed as  $\chi^2$ , with degrees of freedom (df) equal to the difference between the number of estimated parameters in the full model and the number of estimated parameters in the submodel. Akaike's information criterion (AIC;  $\chi^2 - 2df$ ) was also used to evaluate the fit of the genetic models. The model with the lowest AIC reflects the best balance between goodness of fit and parsimony. Estimates of variance components and their 95% confidence intervals were obtained from the best-fitting model. All quantitative genetic model fitting was carried out using the statistical modelling package Mx [18].

## RESULTS

The data in Table 1 show the general characteristics of the entire group of twin pairs studied (1747 pairs). Complete biochemical data were not available for the entire group (except for serum phosphate), but the twin characteristics of these subgroups did not differ significantly from those of the entire group. The MZ pairs were, on average, 1.2 years older, with a slightly higher proportion of pairs being postmenopausal. The DZ pairs were, on average, 2 kg heavier than the MZ pairs, reflected in a BMI that was 0.7 kg/m<sup>2</sup> higher. The mean values for all of the biochemical variables, however, did not differ significantly between the MZ and DZ groups.

The intraclass correlations for MZ twins (*r*<sub>MZ</sub>) and DZ twins (*r*<sub>DZ</sub>) are presented in Table 2. For all variables, *r*<sub>MZ</sub> was greater than *r*<sub>DZ</sub>, implying an important genetic influence, which was subsequently confirmed by model fitting to age- and BMI-adjusted residuals (Table 3). Dominant genetic effects did not contribute significantly to the explanation of the data, and could be removed from the model without a significant worsening of fit. Those parameters for which there was evidence of shared environmental effects include serum calcium, albumin-corrected calcium, serum magnesium, serum creatinine, and both calculated and measured creatinine clearance. A model specifying additive genetic and unique environ-

**Table 2** Intraclass correlation coefficients for electrolytes and creatinine clearance

95% confidence intervals are given in parentheses, and numbers of twin pairs are given in square brackets.

Parameter	rMZ	rDZ
Serum calcium	0.61 (0.56–0.66) [535]	0.45 (0.41–0.49) [1206]
Albumin-corrected calcium	0.72 (0.68–0.76) [435]	0.51 (0.46–0.56) [888]
Serum phosphate	0.54 (0.48–0.60) [539]	0.33 (0.28–0.38) [1208]
Serum magnesium	0.69 (0.65–0.73) [530]	0.49 (0.45–0.53) [1195]
24 h urine potassium	0.40 (0.27–0.53) [172]	0.19 (0.04–0.33) [164]
24 h urine sodium	0.40 (0.27–0.53) [172]	0.17 (0.02–0.32) [164]
24 h urine calcium	0.52 (0.48–0.56) [169]	0.35 (0.31–0.39) [161]
Serum creatinine	0.64 (0.59–0.69) [535]	0.47 (0.43–0.51) [1207]
Calculated creatinine clearance	0.77 (0.73–0.81) [516]	0.52 (0.48–0.56) [1157]
Measured creatinine clearance	0.47 (0.42–0.52) [70]	0.46 (0.43–0.49) [112]
Fractional sodium excretion	0.55 (0.47–0.63) [294]	0.34 (0.26–0.42) [454]

**Table 3** Variance component estimates for the best-fitting model (adjusted for age and BMI)95% confidence intervals are given in parentheses.  $a^2$  = additive genetic influence;  $c^2$  = common environmental variance;  $e^2$  = specific environmental influence.

Parameter	Variance components		
	$a^2$	$c^2$	$e^2$
Serum calcium	0.33 (0.21–0.45)	0.30 (0.21–0.39)	0.37 (0.33–0.42)
Albumin-corrected calcium	0.41 (0.28–0.54)	0.30 (0.20–0.40)	0.29 (0.24–0.34)
Serum phosphate	0.58 (0.53–0.62)	–	0.42 (0.38–0.47)
Serum magnesium	0.27 (0.15–0.39)	0.33 (0.24–0.43)	0.39 (0.35–0.44)
24 h urine potassium	0.40 (0.27–0.51)	–	0.60 (0.49–0.73)
24 h urine sodium	0.43 (0.30–0.54)	–	0.57 (0.46–0.70)
24 h urine calcium	0.52 (0.41–0.61)	–	0.48 (0.39–0.59)
Serum creatinine	0.37 (0.25–0.49)	0.26 (0.16–0.35)	0.37 (0.33–0.42)
Calculated creatinine clearance	0.63 (0.54–0.72)	0.18 (0.10–0.26)	0.19 (0.16–0.22)
Measured creatinine clearance	–	0.53 (0.33–0.64)	0.47 (0.36–0.59)
Fractional sodium excretion	0.52 (0.44–0.59)	–	0.48 (0.41–0.56)

mental variance components gave the best fit for the remainder of variables. A model specifying common environmental and unique environmental variance components gave the best fit for measured creatinine clearance. For the remaining variables, heritability estimates ranged from 27% for serum magnesium to 63% for creatinine clearance. Table 3 shows the genetic and environmental estimates for all of the variables adjusted for age and BMI. Estimates of genetic and environmental variance components before and after adjustment for age and BMI were virtually identical (results not shown).

## DISCUSSION

This study demonstrates that there is a large genetic contribution to the total variance in serum levels of calcium, phosphate and magnesium, calculated creatinine

clearance, and the urinary excretion of calcium, sodium and potassium.

Twin studies have been subject to theoretical criticism in two main areas: the assumption of equal environmental sharing of the two zygosity groups, and the generalizability of twins. In our data exploring a wide range of common diseases and risk factors, we have never found any excess sharing of environment that was strongly associated with the trait sufficient to alter the heritability estimates [17]. The mean values of the characteristics of MZ and DZ twins in the present study were very similar. Only age, weight and BMI showed slight differences between zygositys. Adjusting for age and BMI made no difference to the estimates of heritability in our analysis, providing reassurance that these estimates are a valid reflection of the genetic contribution to these measures.

The reported results are likely to be representative of the general population, because basic characteristics,

disease prevalence, and levels of blood and urine metabolites of the twins were similar to those in a population-based, age-matched sample of 1003 women participating in the Chingford cohort study, London [19]. We have studied only women because the major focus of our research is on osteoporosis and osteoarthritis, both of which are more prevalent in women. Our results cannot be directly extrapolated to males, although there is no clear reason to suggest major sex differences.

Extracellular calcium represents a very small percentage of the skeletal calcium content; however, this cation plays a number of vital physiological roles, and its level is maintained rigidly by the combined effects of parathyroid hormone, mediated by the calcium-sensing receptor, and vitamin D. Previous data, acquired with small numbers of subjects, have suggested a large genetic influence on the total variance of serum total calcium [11,20]. Our results suggest that approx. 41% of the entire variance of the albumin-corrected total calcium level is determined by genetic influences. There was no suggestion of a dominant gene effect. In addition, data published previously showed a genetic influence on calcium excretion, with a heritability of 52% for 24 h urine calcium excretion [21]. This finding is important, since current understanding of the regulation of extracellular calcium suggests that a large proportion of the total variance is determined by mechanisms that are themselves largely under genetic control [12], namely parathyroid hormone, vitamin D [21] and the calcium-sensing receptor [22]. Further work needs to be done to determine the exact proportion that each of these mechanisms contributes to the total variance of total calcium and ionized calcium.

Magnesium is the most abundant intracellular bivalent anion, and approx. 1% of total body magnesium is maintained in the extracellular compartment. Unlike calcium and phosphorus, there appears to be no important systemic or hormonal regulation of the magnesium concentration in the extracellular fluid, although ionized magnesium is tightly controlled by the tubular maximum or threshold for magnesium in the nephron [23]. Some genes that cause pathophysiological variations in measured values of magnesium have now been discovered. Mutations in the gene encoding paracellin 1 can give rise to familial hypomagnesaemia and hypercalciuria, due to defective absorption in the thick ascending limb [24,25]. Our present study suggests that approx. 27% of the variance of total serum magnesium is determined by genetic factors, which supports previous studies suggesting a modest genetic influence [13].

The serum phosphate concentration can vary quite widely throughout the day, and is influenced by a variety of factors, including age, sex, diet, pH and a variety of hormones. An adequate serum phosphate concentration is important for maintaining sufficient ion product for normal mineralization of bone. Our data suggest that

approx. 58% of the total variance of serum phosphate is determined by genetic mechanisms; as far as we are aware, this is the first study to explore the heritability of this inorganic bivalent anion.

A number of genetic models (twins, families of MZ twins, characterized hypertensives and normotensive first-degree relatives of essential hypertensives) have been used previously to explore the determinants of the urinary excretion of sodium and potassium [6,26,27]. It was found, using small numbers of subjects, that the urinary excretion of sodium and potassium have a genetic influence. Our present study extends these previous data by demonstrating a clear genetic (as opposed to familial environmental) effect, and is the first study to explore the heritability of the fractional excretion of sodium, an important risk factor in essential hypertension [9]. There is compelling evidence that essential hypertension results from a defect in the kidney, whereby there is an impaired ability to excrete salt (NaCl) at normal blood pressure [28,29]. This has led many investigators to search for a genetic basis for the defect in renal salt handling, such as alterations in the structure or regulation of sodium transport within the nephron. Recent research has shown a number of single gene defects in the renin-angiotensin axis, 11 $\beta$ -hydroxysteroid dehydrogenase or the epithelial sodium channel can give rise to defective renal sodium and potassium handling [30,31]. Our data provide support to these researchers seeking genetic mechanisms that influence sodium excretion.

Creatinine clearance is frequently used as a measure of glomerular filtration rate. Previous studies assessing the variance of creatinine levels and creatinine clearance have been conducted on small numbers, and conclusions have ranged widely, from predominantly environmental to predominantly genetically mediated influences [2-4,32]. Our present study demonstrates, in large numbers of female twins, that genetic influences explain 37% and 63% of the total variance of plasma creatinine and calculated creatinine clearance respectively. Measurement of the glomerular filtration rate following the injection of one of several suitable marker substances is frequently used to determine the severity of renal insufficiency, as well as its rate of progression. However, the expense of these procedures continues to restrict their use. The most widely used alternative is the determination of creatinine clearance from plasma creatinine. More importantly, a large (and growing) number of studies have demonstrated consistently that estimating glomerular filtration rate by creatinine clearance calculated from the Cockcroft-Gault formula is better than measuring creatinine clearance using 24 h urine collection [33]. While there was a strong correlation between calculated and measured creatinine clearance, there was a large contribution of common environmental variance for measured creatinine clearance. This may represent measurement error, as the numbers of twins for whom

this assay was carried out was small and the urine volumes were variable. The present study shows that calculated creatinine clearance is largely under genetic control.

In summary, the present study provides evidence for the importance of genetic factors in determining serum levels of calcium, phosphate and magnesium; urinary sodium, fractional sodium excretion and urinary potassium; and serum creatinine and calculated creatinine clearance. This should assist researchers attempting to find the genes responsible for these biochemical measures, and ultimately improve understanding of their pathophysiology. This information will be vitally important to researchers undertaking the genetic analysis of complex diseases such as hypertension and renal failure. Moreover, clarification of the inherent natural genetic variation that exists in electrolyte and renal biochemistry in healthy subjects should help to improve our understanding of physiology, and in interpreting the natural variation in normal ranges.

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