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Glomerular Filtration Rate and Electrolyte Handling in Response to Sodium Loading and Depletion

A Twin Study

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Possible genetic influences on glomerular filtration rate and electrolyte excretion were investigated in 55 (37 monozygotic, 18 dizygotic) young adult white twin pairs. Subjects were studied during a five-day hospitalization involving sodium loading and sodium depletion. No evidence of genetic variability was found in the control levels of serum or urine sodium and potassium. Following a saline infusion it was possible to detect genetic influence in electrolyte handling. Creatinine clearance, used as a measure of glomerular filtration rate, did not appear to be genetically mediated. The results indicate that genetic factors are important in sodium handling in normal individuals and that this is independent of glomerular filtration rate.

Key words: Blood pressure, Glomerular filtration, Electrolyte excretion, Sodium handling, Genetic factors, Twin study

INTRODUCTION

Elevated blood pressure is an established risk factor in cardiovascular disorders. Aggregation of blood pressure levels within families has been shown [14], and more recent studies have established that this aggregation is present in early childhood [17] and does not diminish with age [18]. Such evidence alone does not establish that the blood pressure level is genetically determined, since common environmental influence cannot be ruled out. However, several studies of twins in different populations have supported the presence of significant genetic variance in blood pressure levels [2, 13]. A unique analysis utilizing the families of identical adult twins from our laboratory has shown that genetic variance remains significant when body size effects are removed and common environment is taken into account [16].

Studies of societies at low levels of acculturation have shown that trends of blood pressure in relation to age were correlated with dietary differences, particularly salt

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intake [15]. We have previously shown correlation with several estimates of sodium intake and blood pressure in normal subjects [11], although correction for body size removes this effect. Thus, one factor which, from an epidemiologic point of view, is important in blood pressure levels is salt intake. Studies by Dahl have shown that in rats, strains can be selectively bred for sensitivity and resistance to the blood pressure-raising effects of salt [7, 8]. These studies provide precedent for considering genetic factors in sodium handling in humans. The ultimate influence of salt on blood pressure is dependent on the ability of the kidneys to excrete sodium. In another genetic form of hypertension, in the Milan rat, some prime factor in the kidney's ability to handle salt is involved [1]. Evidence of a genetic influence on kidney function in normal man has not been reported. Since the first factor that determines sodium excretion is glomerular filtration rate, it seemed reasonable to investigate whether glomerular filtration rate has genetic variance. We have utilized a study of twins to investigate a possible genetic influence on creatinine clearance and sodium handling.

METHODS

Population

White twin pairs were recruited from the existing twin panel in the Department of Medical Genetics, Indiana University School of Medicine, from the college registration records of Indiana University and, from responses to news media publicity. Subjects were paid $100.00 plus travel expenses as an additional incentive to participate. Zygosity was determined by extensive genotyping and dermatoglyphic analysis. Of the 55 total twin pairs available for analysis, 37 were monozygotic (MZ: 21 male, 16 female) and 18 were dizygotic (DZ: 13 male, 5 female). The age range of the participants was 14 to 27 years.

Participants were preselected for a previous history of a normal blood pressure and for the absence of drug ingestion. Females were excluded if they had taken oral contraceptives in the preceding three months, since these have been shown to raise blood pressure in some people. Females were hospitalized during day 5 to day 15 of the menstrual cycle, in order to minimize variance due to cyclic hormonal changes.

Study Protocol

Subjects were admitted to the Indiana University Clinical Research Center for the five days of the study. The research protocol was approved by the Indiana University Committee for the Protection of Human Subjects and informed consent was obtained. The basic purpose of this protocol was to investigate humoral and renal factors that may be important in blood pressure control [10].

Outpatient day. The subjects began a 24-hour urine collection at 8:00 AM. They were admitted to the research unit in the afternoon or evening.

Inpatient day. The subjects ate an ad lib hospital diet. A second 24-hour urine collection was made, and control blood samples were obtained.

Sodium loading day. The subjects arose at 6:00 AM and were upright until 8:00 AM. Venous blood was sampled, and the subjects then received a normal saline infusion (500 cc/hr) from 8:00 AM to noon, at which time blood was again taken. Urine on this day was divided into three aliquots. Urine excreted from 8:00 AM to noon was called four-hour urine; urine collected from noon until bedtime was termed awake urine; urine passed from bedtime to 6:00 AM was termed sleep urine.

Sodium depletion day. The subjects had blood sampled at 6:00 AM and 8:00 AM. At 10:00 AM, 2:00 PM, and 6:00 PM they received 40 mg of furosemide orally. The diet on this day was restricted to 10 mEq sodium.

After sodium depletion day. Blood was again sampled at 6:00 AM and 8:00 AM.

Laboratory Methods

Creatinine was determined by the Technicon picric acid method. Creatinine clearance was calculated by the formula:

$$\text{Creatinine clearance (cc/min)} = \frac{\text{Urinary creatinine excreted/min}}{\text{Serum creatinine concentration}}$$

Serum and urinary sodium and potassium were measured by standard flame photometry.

Data Analysis

The statistical methods involved in the analysis of twin data have been recently reviewed by Christian [6]. Despite recent controversy surrounding the analysis of twin data [9], the classic comparison of MZ and DZ twins remains, in our opinion, the most efficient method for the initial investigation of heritable components in quantitative traits.

In the present analysis, tests of the basic assumptions of the twin model were performed whenever possible. Program TWNAN, developed for this purpose by the Department of Medical Genetics, systematically tests for the significance of differences between the means of MZ and DZ twins [5], and also for differences in the total variance of the two types of twins [12]. Several estimates of genetic variance have been proposed and the current analysis utilizes the criteria established by Christian et al [3]. The additional test of the equality of environmental covariance in the two types of twins in also performed by the program [4].

RESULTS AND DISCUSSION

On the Inpatient Day, the 24-hour urine collection yielded no evidence of genetic variance for sodium or potassium excretion, nor did control levels of serum sodium or potassium. Table 1 reports the mean values for these variables. This suggests that dietary salt preference is not genetically mediated nor are the factors controlling serum sodium and potassium on an unrestricted diet.

However, the excretion of creatinine and the serum creatinine concentration on this day were highly heritable. Since creatinine production and excretion are equal in the steady state, and since creatinine production is directly related to muscle mass and body weight ($r = 0.51$, $P < 0.001$), this heritability was attributed to the inheritance of body size. In this sample the inheritance of body size, as reflected by both body weight and standing height, was significant, using the within-pairs F test.

While the control levels (before saline) of serum and urine electrolytes showed little evidence of genetic variance, the results following the four-hour saline infusion were very different (Table 2) and showed definite evidence of genetic variation in sodium handling *following* a salt load. Means and total variance were not different in the two types of twins for the variables reported in Table 3. Although not shown in Table 3, serum K+ at 8:00 AM on the morning following the salt load appeared to be genetically mediated (F

TABLE 1. Control Electrolyte Values – Inpatient Day

Variable	MZ	DZ
Sodium excretion (mEq/24 hr)	163.8	150.5
Potassium excretion (mEq/24 hr)	65.6	68.4
Creatinine excretion (gm/24 hr)	1.7	1.9
Serum sodium (mEq/liter)	140.1	141.7
Serum potassium (mEq/liter)	4.2	4.4
Serum creatinine (mg/100 ml)	1.0	1.0

TABLE 2. Electrolyte Handling on the Salt Load Day

Variable	F = MSWDZ/MSWMZ	P
Serum NA^+		
Before saline 8:00 AM	0.168	0.99
After saline 12:00 noon	4.405	0.00
24-Hour urine		
Total Na^+	1.890	0.05
Total K^+	2.202	0.02
Sleep urine		
Total Na^+	2.031	0.04
Total K^+	2.716	0.01
Awake urine		
Total Na^+	1.414	0.19
Total K^+	1.164	0.34
Four-hour urine		
Total Na^+	1.000	0.48
Totak K^+	1.223	0.29

TABLE 3. Intraclass Correlations – Electrolyte Excretion Following Saline Challenge

Variable	MZ	DZ
Total Na^+ excretion	0.78	0.63
Total K^+ excretion	0.88	0.78
Sleep Na^+ excretion	0.65	0.17
Sleep K^+ excretion	0.71	0.40
Awake Na^+ excretion	0.73	0.71
Awake K^+ excretion	0.87	0.87
Four-hour Na^+ excretion	0.65	0.52
Four-hour K^+ excretion	0.70	0.63
Noon serum Na^+	0.85	0.64

[within pairs] = 3.496, $P < 0.001$). Plasma and urinary electrolytes during and after salt depletion with furosemide did not give evidence of significant genetic variation.

It thus appeared that the day-to-day variability in sodium intake would hinder any attempts to investigate genetic variability of sodium metabolism. However, when normal twins were given a predetermined sodium load, it was possible to detect significant genetic variability in electrolyte handling. Particularly intriguing was significant genetic variance of the serum sodium level immediately following the saline infusion. Since excretion during the infusion period was not genetically mediated, the distribution of the infused sodium would seem to be genetically determined. The physiologic mediation of this finding could be genetic control of antidiuretic hormone levels.

Although the 24-hour excretion of sodium on the day of the saline infusion showed evidence of genetic variance, the fractionation of this urine into several time periods illustrated further the importance of careful standardization of conditions for such studies. Thus, neither the sodium nor potassium excreted during or immediately after the

saline infusion appeared heritable. However, the excretion during sleep did show such variance.

Table 3 presents the intraclass correlation coefficients for the relevant variables. The similarities and differences in these correlations for the two types of twins are intriguing in view of the fact that twins received equivalent diets and the same volume of saline during the infusion. These results merely reinforce conclusions based on the analysis of variance that some genetic factor is influencing sodium excretion during sleep.

The dominant factor controlling sodium excretion is creatinine clearance (CCR). However, our data showed no evidence of heritability, even following the saline infusion. Since the awake sample collection following saline would have come at the period of greatest volume challenge, if it existed, one might expect to find evidence of genetic variation. This was not the case. When CCR was calculated per square meter of body surface area, again no evidence of heritability of glomerular filtration rate as determined by creatinine clearance was found. Finally, creatinine clearance was not correlated with blood pressure.

Since this study failed to show evidence of heritability of glomerular filtration rate as reflected in creatinine clearance rates in normal man, it seems reasonable to suggest that the heritability of blood pressure cannot be accounted for by the heritability of this first factor controlling sodium excretion.

Previous data leave little doubt that blood pressure is to a degree genetically influenced and that there are relationships between hypertension and sodium metabolism. This study suggests that genetic factors are closely related to sodium handling but not glomerular filtration rate. Further investigation may lead to identification of physiologic mediators and eventually to genes in man responsible for sodium retention and elevated blood pressure.

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