Genetic Influences on the Urinary Excretion of Aldosterone in Children

Amita K. Manatunga, Teresa K. Reister, Judy Z. Miller, and J. Howard Pratt

Several studies have shown an inverse relation between blood pressure and plasma aldosterone levels. Since blood pressure is in part genetically regulated, we looked for evidence that genetic factors might also affect aldosterone production. The nocturnal urinary excretion rate was used to estimate aldosterone production, and electrolyte excretion rates were used to estimate sodium and potassium intakes. Studies were carried out in monozygotic (MZ) (n=37 pairs) and dizygotic (DZ) (n=26 pairs) twins, aged 6–17 years. Both groups of twins were white. The intraclass correlation coefficient for aldosterone excretion was 0.686 (p=0.0001) for MZ twins, and 0.290 (p=0.079) for DZ twins, indicating high heritability for the aldosterone excretion rate. In a second study, we looked for a racial effect on the genetic regulation of aldosterone excretion. Siblings from both black and white families (72 black siblings and 157 white siblings) were selected from an ongoing longitudinal study. Mean values for nocturnal aldosterone excretion rates measured every 6 months over 1.5–3.5 years, were used in the analysis. The intraclass correlation coefficient for aldosterone excretion, adjusted for sodium and potassium excretion, was 0.510 (p=0.001) for black siblings and 0.087 (p=0.228) for white siblings, indicating a strong familial aggregation for aldosterone excretion in black children. In conclusion, studies in twins showed that regulation of urinary aldosterone excretion in children is determined partially by genetic factors. A familial component affecting the aldosterone excretion rate appears to be much stronger in blacks than in whites. (Hypertension 1992;19:192–197)

Essential hypertension is a common genetic disease.\(^1\) A genetic basis for the pathophysiology that leads to higher blood pressures has not been defined nor has a biochemical marker been associated with a mechanism for hypertension. Several studies have shown that plasma aldosterone concentrations were inversely related to levels of blood pressure in normotensive subjects\(^2\)–\(^4\) as well as patients with hypertension.\(^2\) In addition, plasma aldosterone concentrations were found to be lower in children whose parents had hypertension.\(^5\) In other studies, black children, who typically have higher blood pressures\(^6\)–\(^7\) and are known to be more likely to develop hypertension,\(^8\) were found to have lower plasma aldosterone levels and lower urinary aldosterone excretion rates than white children.\(^7\) These observations suggest that aldosterone production may be related to a mechanism for regulation of blood pressure, a mechanism that could be genetic in origin. The principal stimuli of aldosterone production, angiotensin II (Ang II)\(^9\) and the extracellular potassium concentration,\(^10\) mediate the known environmental influences on aldosterone production: those derived from the dietary intakes of sodium and potassium. Whether aldosterone production, like blood pressure, is affected by genetic factors is not known. Conceivably, a genetic influence on aldosterone production has relevance to a mechanism for control of blood pressure.

The current study sought evidence for genetic influences on the aldosterone excretion rate. Presented are results from two separate studies. In the first, the question of heritability of aldosterone excretion was addressed by using monozygotic (MZ) and dizygotic (DZ) twins. MZ twins are genetically identical, whereas DZ twins, like non-twin siblings, share half of their genes on average. The degree to which a quantitative measurement, such as the aldosterone excretion rate, shows similarity in MZ as compared with DZ twins, can be used to estimate the heritability of the quantitative trait. A second study was conducted to look for a race-related genetic influence on aldosterone excretion. For this study, full siblings from both black and white families were used. These data can be used to measure familial aggregation within both black and white families.

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Methods

Studies in Twins

There were 37 MZ and 26 DZ twin pairs recruited from the Indiana University twin panel who participated in the present study. All twins were white, and they were on an ad libitum dietary intake of sodium and potassium. In the outpatient facility of the General Clinical Research Center, blood pressures were recorded with a random zero sphygmomanometer (Hawksley and Sons, Lansing, England) in the right arm with the subject in the sitting position. The first and fifth Korotkoff sounds were used to designate systolic and diastolic blood pressures, respectively, and the average of the second and third readings was used as the final blood pressure measurement. Weight was also recorded for each individual. Urine samples were collected overnight in each subject's home, and the urinary excretion of sodium and potassium served as an estimate of dietary intake of electrolytes. The excretion of aldosterone was used to estimate aldosterone production. The nocturnal excretion of aldosterone and electrolytes has been shown to correlate with 24-hour excretion rates.

Studies in Siblings

In the second study, sibling pairs from 34 black families and 72 white families participated. These children were recruited from an ongoing longitudinal study of blood pressure control in children in which subjects were seen every 6 months for 1.5–3.5 years. Blood pressures were measured as in the twin study, but in contrast to the previous study, subjects were visited at school. Each sibling collected a urine sample overnight at home and returned it the next day to the school for measurement of urinary sodium, potassium, and aldosterone excretion. Since each individual had multiple measurements over the period of the longitudinal study, a mean blood pressure and a mean value for each of the excretion rates were calculated and used in the analyses.

In both twin and sibling studies, informed consent was obtained from each subject as well as from his or her parents or a legal guardian. Both studies were approved by the institutional review board of Indiana University–Purdue University of Indianapolis. Subjects with a history of renal or cardiac disease or diabetes mellitus and those taking medication that could affect aldosterone production or blood pressure were excluded.

Assay Procedures

Urinary aldosterone was measured by radioimmunoassay after acid hydrolysis overnight; aldosterone antiserum was from Diagnostic Products Corp., Los Angeles, Calif. Urinary sodium and potassium were measured with an IL 943 flame photometer (Instrumentation Laboratory, Lexington, Mass.). Aldosterone and electrolyte excretion rates were expressed per milligram of urinary creatinine. Creatinine was measured using a Beckman-2 creatinine analyzer (Beckman Instruments, Inc., Fullerton, Calif.).

Statistical Analyses

Subjects' characteristics are expressed as the mean ± SEM. Log transformations were used to normalize the distribution of aldosterone excretion values. Although similar results were obtained without the transformation, the log transformed values better met the assumptions of various statistical analyses. Since aldosterone excretion is regulated by the dietary intake of sodium and potassium, regression techniques were used to adjust for potassium and sodium excretion. In addition, all excretion rates, since they were expressed per milligram of creatinine, were adjusted for weight because of the positive relation of body weight to urinary creatinine excretion. Similarly, since blood pressure increases with body weight, blood pressures were adjusted for weight. Statistical analysis of differences in group means between blacks and whites were performed using the random-effect mixed model, which allows for a within-family correlation.

As a measure of familial aggregation for the aldosterone excretion rate and blood pressure, intraclass correlation coefficients were calculated for twins and siblings from both black and white families. The intraclass correlation coefficient measures familial aggregation, which includes both genetic and common environmental effects. Therefore, the intraclass correlation is a suitable estimate of heritability only when there are no common environmental effects.

Many approaches have been used to estimate heritability. For the purposes of this study, twins heritability (H^2) was calculated according to the formula

\[ H^2 = 2(r_{MZ} - r_{DZ}) \]

where \( r_{MZ} \) and \( r_{DZ} \) are the intraclass correlation coefficients for MZ and DZ twins. The formula for \( H^2 \) estimates the proportion of the total population variance attributed to genetic sources if several assumptions are met, including: 1) equal total variances for MZ and DZ groups, 2) no interaction between or among genetic loci, and 3) equal effects on the MZ and DZ groups due to a common environment. Estimates of \( H^2 \) were not calculated for black and white siblings because it is not possible to evaluate common environmental effects by sibling data alone. Thus, for example, if an intraclass correlation for a trait of 0.5 is observed for siblings, it is unclear how much of the sibling resemblance is due to common genes or to common diets, exercise patterns, or some other common environmental effect.

Results

Studies in Twins

Descriptive characteristics for MZ and DZ twin populations are shown in Table 1. There were more female MZ twins and more male DZ twins; MZ twins...
were slightly older \((p=0.026)\) and heavier \((p=0.008)\) than DZ twins. The age range for both types of twins was 6–17 years. Both MZ and DZ twin groups were similar in terms of electrolyte excretion rates. Systolic blood pressure for DZ twins was higher than for MZ twins \((p=0.0025)\), whereas diastolic blood pressures were not significantly different for both groups.

To measure heritability for aldosterone excretion and blood pressure, we calculated the respective intraclass correlation coefficients for MZ and DZ twins, with results presented in Table 2. The intraclass correlation coefficient for aldosterone excretion was highly significant for MZ twins \(r_{MZ}=0.686, p=0.0001\) and not significant for DZ twins \(r_{DZ}=0.290, p=0.079\). This gave a high H\(^2\) estimate of 0.792. The intraclass correlation coefficients for systolic and diastolic blood pressures adjusted for body weight were also significant for both MZ and DZ twins, with high H\(^2\) estimates for both (0.412 and 0.316 for systolic and diastolic blood pressures, respectively). These findings were consistent with known genetic influences on blood pressure.

As a test of twin model assumptions, a test for the equality of total variance for MZ and DZ twin groups was conducted for the adjusted aldosterone excretion rate and blood pressure. A null hypothesis of equal variance was not rejected for either variable.

Because there was a predominance of girls in the MZ twin group and a predominance of boys in the DZ twin group, estimates of H\(^2\) were performed within each gender group to assess for an effect of gender imbalance. The intraclass correlation coefficient for aldosterone excretion in MZ female twins was 0.527 \((n=26\) pairs, \(p=0.002)\) and 0.0353 in DZ female twins \((n=8\) pairs, \(p=0.500)\). In boys, the intraclass correlation coefficient for aldosterone excretion in MZ twins was 0.839 \((n=11\) pairs, \(p=0.000)\), and in DZ twins it was close to zero \((n=15\) pairs). These results suggested that the significance of the H\(^2\) of aldosterone excretion is preserved within gender groups. Similar results were obtained from an analysis of blood pressure adjusted for weight: the intraclass correlation coefficients for systolic blood pressure in MZ twins were 0.619 \((p=0.000)\) and 0.698 \((p=0.004)\) in girls and boys, respectively. The intraclass correlation coefficients for diastolic blood pressure in MZ twins were 0.517 \((p=0.003)\) and 0.802 \((p=0.005)\) in girls and boys, respectively. On the other hand, the intraclass correlation coefficient for blood pressure in DZ twins was not significant in any gender group with the exception of diastolic blood pressure in the DZ male twins, in which it was 0.473 \((p=0.028)\). This lack of significance was probably due to the smaller sample sizes \((n=8\) pairs for girls and \(n=15\) pairs for boys).

We also examined familial aggregation of blood pressure without adjusting for weight. No appreciable difference in results was observed: for systolic blood pressure, intraclass correlation coefficients were 0.614 \((p=0.000)\) and 0.350 \((p=0.036)\) for MZ and DZ twins, respectively; for diastolic blood pressure, intraclass correlation coefficients were 0.580 \((p=0.000)\) and 0.480 \((p=0.0056)\) in MZ and DZ twins, respectively.

**Studies in Siblings**

These studies were conducted to look for racial differences in the familial aggregation of aldosterone excretion. Siblings ranged in age from 5 to 13 years at the outset of the study. Their characteristics are shown in Table 3. Blacks and whites were of similar age, but as has been reported previously, black
Children were heavier \((p=0.003)\). Systolic blood pressures were higher in blacks than in whites before adjusting for weight \((p=0.006)\) but not significant with the adjustment. Urinary excretion of potassium and aldosterone was lower in blacks \((p=0.013\) and \(p=0.000\) for potassium and aldosterone excretion, respectively). After adjusting aldosterone excretion for body weight and excretion of sodium and potassium, aldosterone excretion rates remained significantly lower in black children \((p=0.013)\). Diastolic blood pressures and urinary sodium excretion rates were not significantly different in either blacks or whites.

The intraclass correlation coefficient for aldosterone excretion was highly significant for black children \((0.510, p=0.001)\) (Table 4) but not significant for white children. A subgroup analysis of the sibling data, looking at each gender group separately, revealed a high intraclass correlation coefficient for aldosterone excretion in black girls, 0.475 \((n=24\) families, \(p=0.033)\). For black boys, the intraclass correlation coefficient for aldosterone excretion was 0.125 \((n=18\) families, \(p=0.385)\). Thus, the high intraclass correlation coefficient for aldosterone excretion in blacks appeared to reside primarily with black girls. The intraclass correlation coefficient for white boys and girls, as in the previous analysis, was not significant.

The intraclass correlation coefficient for blood pressure after adjusting for weight was significant only for systolic blood pressure in white children.

### Table 4. Intraclass Correlation Coefficients for Aldosterone Excretion Rates and Blood Pressures in Both Black and White Siblings

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Blacks</th>
<th>Whites</th>
</tr>
</thead>
<tbody>
<tr>
<td>log Aldosterone excretion</td>
<td>0.510</td>
<td>0.087</td>
</tr>
<tr>
<td>((p=0.0014))</td>
<td>((p=0.228))</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>-0.096</td>
<td>0.326</td>
</tr>
<tr>
<td>((p=0.706))</td>
<td>((p=0.002))</td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.089</td>
<td>-0.024</td>
</tr>
<tr>
<td>((p=0.305))</td>
<td>((p=0.580))</td>
<td></td>
</tr>
</tbody>
</table>

Siblings were from 34 black families and 72 white families. Values of \(p\) correspond to the test of null hypothesis that the intraclass correlation coefficient is equal to zero.

When unadjusted blood pressure values were used in the analysis, significant intraclass correlation coefficients for systolic blood pressure in whites and diastolic blood pressure in blacks were obtained: 0.179 \((p=0.050)\) and 0.338 \((p=0.023)\) in the two groups, respectively. Similar results were found after adjusting for weight and age. A subgroup analysis of sibling data in which groups were separated by gender revealed a significant intraclass correlation coefficient only for systolic blood pressure in white girls, a value of 0.349 \((p=0.039)\).

### Discussion

The present study was undertaken to look for evidence that genetic factors participate in regulating the secretion of aldosterone. We found in an analysis of MZ and DZ twins that aldosterone excretion rates, adjusted for sodium and potassium intake, showed a high estimate of \(H^2\). This pointed to a significant genetic influence on aldosterone excretion.

A genetically derived influence on aldosterone excretion may have been mediated through an alteration in the generation of Ang II. Renin released from the kidney acts enzymatically on angiotensinogen to form the decapeptide angiotensin I, which is then cleaved by angiotensin converting enzyme (ACE) to the octapeptide Ang II.\(^{15}\) Renin, angiotensinogen, and ACE, as well as the Ang II receptor, are products of gene-directed protein synthesis. Different alleles for any of these substances could result in a different degree of Ang II–stimulated aldosterone production. In support of a genetic influence on renin secretion were the studies by Grim et al\(^{16}\) in which, under various states of sodium balance, plasma renin activity (PRA) showed significant genetic effects. In these same studies, the plasma aldosterone concentration under specific conditions of sodium balance was also heritable.

Aldosterone produced by an adrenal tumor increases blood pressure by expanding the blood volume.\(^{17,18}\) On the other hand, several investigative groups have now shown that nonautonomous aldosterone production is inversely related to levels of blood pressure.\(^{2-4}\) Although a reduction in stimulation by Ang II might explain the lower aldosterone...
production, it is less clear why a lower level of renin-Ang II activity would be associated with a higher blood pressure. There is, however, ample precedent for an association of low renin activity with increases in blood pressure. Approximately a quarter of all patients with essential hypertension have low PRA, and in blacks, in whom hypertension is common, PRA is often decreased. The presumption has been that PRA was suppressed by a greater total plasma volume. However, an encoding of renin by a variant renin allele could also result in a lower level of PRA.

Studies in genetic animal models of hypertension support the concept of a primary gene abnormality in low-renin hypertensive states. In the hypertensive salt-sensitive Dahl rat (SS/Jr), in which PRA and aldosterone levels are lower than in the Dahl normotensive salt-resistant rat (SR/Jr), Rapp et al showed in cross-breedings of SS/Jr and SR/Jr rats that a restriction fragment length polymorphism for the renin gene cosegregated with blood pressure. A single dose of the SS/Jr rat renin allele was associated with a 10 mm Hg increase in blood pressure. Mullins et al have reported on a transgenic rat with the mouse Ren-2 gene in which fulminating hypertension developed where levels of renin and Ang II were lower than in control rats.

The present studies were conducted in children and adolescents, and the relevance of the findings to hypertension could not be examined. However, as previously reported, black children have higher blood pressures and a lower level of aldosterone production than white children. In the present study, familial aggregation for aldosterone excretion was strong for black siblings and not significant for white siblings. Although the familial aggregation observed may have stemmed from common environmental influences, adjustments were made for those known to affect aldosterone production, namely dietary sodium and potassium. The findings thus indicate that there is a strong familial component to the regulation of aldosterone excretion in black children. This may represent an unknown environmental effect or it could be genetic in origin. When a subgroup analysis of these data was performed, the familial aggregation was significant only for black girls and not black boys. Whether this lack of significant familial aggregation in black boys resulted from the smaller sample size used in the subgroup analysis or whether familial aggregation was limited to black girls remains to be determined. Also, it is unknown if this familial influence on aldosterone excretion is in any way causally related to the lower aldosterone excretion rates observed in black children. Conceivably, a familial influence on aldosterone production is related to a propensity for the development of hypertension. It may be, for example, that low aldosterone excretion serves as a familial marker for a mechanism predisposing to higher blood pressure.

Finally, results of the present study may have relevance to previous reports that urinary kallikrein excretion was genetically regulated and negatively associated with family history of hypertension. The urinary excretion of kallikrein parallels the rate of aldosterone production. For example, kallikrein excretion rates are high in patients with primary aldosteronism and in individuals in whom sodium is restricted in the diet. Kallikrein excretion increases with the administration of fludrocortisone and decreases with spironolactone, suggesting that mineralocorticoids control kallikrein production. In children, kallikrein excretion was found to be lower in blacks than in whites, similar to the observations that have been made for excretion of aldosterone. Also, hypertensive blacks were shown to have lower kallikrein excretion rates than normotensive blacks or normotensive and hypertensive whites under conditions of dietary sodium restriction. Thus, the genetic regulation for aldosterone and kallikrein production may be similar, and their relation to blood pressure control may be the same.

In summary, using MZ and DZ twins to estimate H2, a significant genetic influence on aldosterone excretion was observed in children and adolescents. In studies of siblings from both black and white families, estimates of familial effects on aldosterone excretion were found to be high for blacks. The relevance of these findings to blood pressure control or to the greater predisposition for the development of hypertension in black children remains to be determined.

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