Associations Between Human Aldosterone Synthase (CYP11B2) Gene Polymorphisms and Left Ventricular Size, Mass, and Function

Markku Kupari, MD; Aarno Hautanen, MD; Laura Lankinen, MB; Pekka Koskinen, MD; Juha Virolainen, MD; Heli Nikkila, PhD; Perrin C. White, MD

**Background**—Aldosterone has direct and indirect effects on the heart, and genetic variations in aldosterone synthesis could therefore influence cardiac structure and function. Such variations might be associated with polymorphisms in the gene encoding aldosterone synthase (CYP11B2), the enzyme catalyzing the last steps of aldosterone biosynthesis.

**Methods and Results**—A Finnish population sample of 84 persons (44 women) aged 36 to 37 years was studied by M-mode and Doppler echocardiography to assess left ventricular size, mass, and function. Subjects were genotyped through the use of the polymerase chain reaction for two diallelic polymorphisms in CYP11B2: one in the transcriptional regulatory region (promoter) and the other in the second intron. In multiple regression analyses, the CYP11B2 promoter genotype predicted statistically significant variations in left ventricular end-diastolic diameter ($\beta = .40$, $P < .0001$), end-systolic diameter ($\beta = .33$, $P = .0009$), and mass ($\beta = .17$, $P = .023$). These effects were independent of potentially confounding factors, including sex, body size, blood pressure, physical activity, smoking, and ethanol consumption. Genotype groups also differed in a measure of left ventricular diastolic function, the heart rate–adjusted atrial filling fraction ($P = .018$). Increased dietary salt, which is known to predict increased left ventricular mass, had this effect only in association with certain CYP11B2 genotypes ($P < .001$).

**Conclusions**—Genetic variations in or near the aldosterone synthase (CYP11B2) gene strongly affect left ventricular size and mass in young adults free of clinical heart disease. These polymorphisms may also influence the response of the left ventricle to increases in dietary salt. (*Circulation. 1998;97:569-575.*)

**Key Words:** ventricles ■ echocardiography ■ genes

Left ventricular size, mass, and function are important predictors of cardiovascular morbidity and mortality in cardiac patients and in the general population. A number of constitutional and environmental factors have been identified that influence left ventricular size; these include age, sex, body size, blood pressure, physical activity, salt intake, alcohol consumption, and the presence of heart disease or diabetes. Studies of monozygotic and dizygotic twins have suggested that inherited factors are also important.

Aldosterone controls sodium balance and intravascular volume and thus helps regulate blood pressure (reviewed in Ref 11). Therefore, genetic variations in the regulation of aldosterone synthesis might influence the structure and function of the left ventricle.

Aldosterone secretion is regulated primarily by the renin–angiotensin system. In response to decreased intravascular volume, renin is secreted by the renal juxtaglomerular apparatus and converts angiotensinogen to angiotensin I, which is then converted to angiotensin II by ACE. The genes encoding these components have been investigated as risk factors for hypertension and, in some cases, left ventricular hypertrophy. A deletion polymorphism in an intron (an internal region of the gene that does not encode part of the protein) has been associated with increased ACE levels, increased risk of cardiovascular mortality, and increased risk of left ventricular hypertrophy. However, we have been unable to confirm any association between ACE genotype and left ventricular size or mass in normal young adults, and a recent large population-based study also failed to confirm either association or genetic linkage between ACE and left ventricular hypertrophy.

In the adrenal cortex, aldosterone is synthesized from deoxycorticosterone by a mitochondrial cytochrome P450 enzyme, aldosterone synthase (CYP11B2). The corresponding gene is located on chromosome 8, band 8q22. It is adjacent to a closely related gene that encodes steroid 11β-hydroxylase (CYP11B1), an enzyme required for cortisol biosynthesis.

Mutations in CYP11B2 can cause aldosterone deficiency.

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From the Division of Cardiology (M.K., P.K., J.V.), Department of Medicine, Helsinki University Central Hospital, Helsinki, Finland; Institute of Biomedicine and Physiology (A.H., L.L.), University of Helsinki, Finland; and Department of Pediatrics (H.N., P.C.W.), University of Texas Southwestern Medical Center (Dallas).

Reprint requests to Perrin C. White, MD, Division of Pediatric Endocrinology, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX 75235-9063.

E-mail pwhite2@mednet.swmed.edu

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Conversely, an inherited form of hypertension, glucocorticoid-suppressible hyperaldosteronism, is caused by genetic recombinations between \textit{CYP11B1} and \textit{CYP11B2} that increase expression of \textit{CYP11B2} and lead to inappropriate secretion of aldosterone.\textsuperscript{21-23} Therefore, it is plausible that other polymorphisms in \textit{CYP11B2} might affect aldosterone biosynthesis and thus perhaps influence left ventricular mass or size.

Several frequent polymorphisms have recently been described in the transcriptional regulatory region and the second intron of \textit{CYP11B2}.\textsuperscript{24} We now report that two of these polymorphisms have significant associations with left ventricular size, mass, and function in an age-homogeneous population sample with a low prevalence of cardiovascular diseases.

\section*{Methods}

\subsection*{Study Population}

We previously examined left ventricular structure and function in a population sample with a low prevalence of cardiovascular diseases.\textsuperscript{16,25} We solicited a random sample of 120 persons living in Helsinki who were 36 to 37 years old at the time of our investigation; 42 men and 51 women (78\% of the sample) entered the study.\textsuperscript{25} We obtained both high-quality echocardiographic data and leukocyte DNA samples on 84 subjects. Several subjects had been followed for borderline blood pressure elevation, but none was on antihypertensive drug therapy. No subject had heart disease by history, clinical examination, or 12-lead ECG. At echocardiography, 1 woman had moderate tricuspid regurgitation, and 1 man had a possible incipient dilated cardiomyopathy; both were asymptomatic.

The subjects’ daily physical activity and ethanol and cigarette consumption were determined by 2-month prospective daily recording.\textsuperscript{25} The estimation of salt intake was based on a 7-day food record.\textsuperscript{26} Body height and weight were measured in light indoor clothing just before the echocardiographic examination. Resting brachial artery cuff blood pressure was averaged over three measurements made over the 2-month follow-up period. Hematocrit and serum insulin were determined on venous blood obtained after an overnight fast.\textsuperscript{25}

All subjects were studied by M-mode echocardiography to determine left ventricular size (end-diastolic and end-systolic cavity diameter), wall thickness, and mass and by pulsed Doppler velocimetry to determine the peak early and late (atrial) diastolic transmural velocities, atrial filling fraction, and deceleration of the early diastolic flow velocity. Details of the technique and measurements, including calculation of peripheral arterial resistance,\textsuperscript{25} have been presented previously.\textsuperscript{25,26} Data were analyzed without knowledge of the subjects’ other characteristics, including the \textit{CYP11B2} genotype.

\subsection*{Molecular Analysis of the Aldosterone Synthase (\textit{CYP11B2}) Gene}

DNA was extracted from whole blood. Segments of \textit{CYP11B2} (Fig 1) were amplified from \textasciitilde 20 ng of each DNA sample by the polymerase chain reaction in 20-\textmu L reactions containing 0.2 U Taq DNA polymerase, 1X concentration of the supplied buffer, 0.2 mmol/L concentration of each deoxynucleotide triphosphate, and 10 pmol of each primer. After initial denaturation at 94°C for 5 minutes, a manual hot start at 80°C was used, followed by 35 cycles of 94°C for 1 minute, 67°C or 68°C annealing for 1 minute, and 72°C extension for 2 minutes.

Subjects were genotyped for the -344 promoter polymorphism using primers CAGGAGAGACCCATGTGAC (sense) and CCTCACCCTGTTCAGCCC (antisense). Then, 5 \textmu L from each reaction (consisting mainly of a 537-bp product) was digested with 10 U of restriction endonuclease \textit{Hae} III in the supplied buffer for 2 hours at 37°C. Reactions were subjected to electrophoresis in 2.5\% agarose gels. The -344T allele lacks an \textit{Hae} III site (GGCC) present in the -344C allele, so -344T alleles are detected as \textit{Hae} III fragments of 273 bp and -344C alleles as fragments of 202 bp (plus smaller fragments in each case). Subjects were typed for a gene conversion polymorphism in the second intron of \textit{CYP11B2} by allele-specific polymerase chain reaction. Both reactions used the same antisense primer: AGGAACCTCTCTGACCGCC. Reactions to detect allele 1 with the gene conversion (1013-bp product) also contained the primer CAGAAAAATCCCTCCCCCTCTTA (67°C annealing temperature), whereas reactions to detect allele 2 lacking the gene conversion (yielding a 1017-bp product) instead used the sense primer TGGGAAAAAGCCCTACCTGTT.

\subsection*{Statistical Analysis}

Group comparisons were made by ANOVA and the Kruskal-Wallis test (asymmetrical data distribution). In comparing the groups for the Doppler transmural velocity measurements, heart rate was used as a covariate. Frequency data were analyzed by the \chi^2 test. A hypothesis of a “gene dosage effect” on left ventricular measurements was tested by linear regression analysis with the number of -344C alleles in the \textit{CYP11B2} gene promoter (0, 1, and 2, corresponding to \textit{T}, \textit{T} and \textit{C}, and \textit{C} and \textit{C} genotypes) as the explanatory factor. Stepwise multiple linear regression analysis was used to examine whether the number of -344C alleles carried by each subject had statistical influence on left ventricular diameters and mass independent of sex, body size, blood pressure, hematocrit, serum insulin, and lifestyle factors, including daily salt intake, physical activity index (square-root transformed), smoking, and ethanol consumption (square-root transformed). The \alpha level for entry and removal of terms at each forward step was .10. Asymmetrically distributed variables were square-root transformed before regression analysis. Continuous data are summarized as mean\pm SEM or as median (range). The associations found in multivariate analyses are reported as multiple regression coefficients (b) \pm SEM and as standardized regression coefficients (\beta). Squared multiple correlation coefficients (\textit{R}^2) were also calculated. Two-tailed values of \textit{P}<.05 were considered statistically significant.

\subsection*{Results}

\subsection*{Genotyping of Subjects}

We genotyped 84 individuals for two polymorphisms in the aldosterone synthase (\textit{CYP11B2}) gene.\textsuperscript{24} The first is located in the transcriptional regulatory region, or promoter, of \textit{CYP11B2}, 344 nucleotides before the start of the protein coding sequence. This position can be either a cytosine
(-344C) or thymidine (-344T). Persons homozygous for C, heterozygous for C and T, or homozygous for T will be referred to as having the genotypes -344CC, -344CT, or -344TT, respectively. The second polymorphism is in the second intron of CYP11B1; in some individuals, the usual sequence of this intron has been largely replaced by the sequence typically found in the related gene, CYP11B1. Such replacement is termed gene conversion, and the alleles at this locus will be referred to as 1 (conversion) and 2 (no conversion) (Fig 1). The genotype at the -344 position was TT in 22 persons, CT in 42, and CC in 20. The distribution is not significantly different across the genotypes in our study population. The data confirm linkage disequilibrium (ie, nonrandom associations of alleles) between these two polymorphic loci in the CYP11B2 gene; allele 1 in intron 2 was seen only in association with -344T. Intron 2 genotype did not influence any of the factors listed above (not shown).

**TABLE 1. Characteristics of Subjects in the Different Genotype Groups by Cytosine/Thymidine (C/T) Polymorphism in the Promoter of the Aldosterone Synthase Gene**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>TT (n=22)</th>
<th>CT (n=42)</th>
<th>CC (n=20)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, M/F</td>
<td>10/12</td>
<td>19/23</td>
<td>10/10</td>
<td>.935</td>
</tr>
<tr>
<td>Height, cm</td>
<td>171±9</td>
<td>171±9</td>
<td>171±10</td>
<td>.991</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>66±10</td>
<td>72±15</td>
<td>69±14</td>
<td>.207</td>
</tr>
<tr>
<td>Body surface area, m²</td>
<td>1.77±0.18</td>
<td>1.84±0.21</td>
<td>1.80±0.22</td>
<td>.439</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>22.8±2.8</td>
<td>24.7±4.6</td>
<td>23.6±2.7</td>
<td>.104</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>67±11</td>
<td>66±9</td>
<td>65±9</td>
<td>.878</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>122±13</td>
<td>126±14</td>
<td>128±17</td>
<td>.307</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>79±9</td>
<td>81±9</td>
<td>82±10</td>
<td>.533</td>
</tr>
<tr>
<td>Peripheral arterial resistance, mm Hg/L/min</td>
<td>27±6</td>
<td>26±5</td>
<td>26±6</td>
<td>.976</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>41±3</td>
<td>42±3</td>
<td>42±4</td>
<td>.517</td>
</tr>
<tr>
<td>Serum insulin, mU/L</td>
<td>4.0 (1.6–13.4)</td>
<td>5.2 (1.8–30.4)</td>
<td>4.3 (2.8–19.4)</td>
<td>.273</td>
</tr>
<tr>
<td>Physical activity, MET·h/d</td>
<td>2.6 (0–13.8)</td>
<td>1.7 (0–8.7)</td>
<td>2.2 (0–6.5)</td>
<td>.258</td>
</tr>
<tr>
<td>Smoking, yes/no</td>
<td>11/11</td>
<td>20/22</td>
<td>9/12</td>
<td>.316</td>
</tr>
<tr>
<td>Ethanol use, g/d</td>
<td>18.3 (5–75)</td>
<td>15.9 (0–84)</td>
<td>7.2 (0–71)</td>
<td>.347</td>
</tr>
<tr>
<td>Salt intake, mEq/d³</td>
<td>164±44</td>
<td>150±51</td>
<td>158±47</td>
<td>.586</td>
</tr>
</tbody>
</table>

Data are mean±SD of normally distributed continuous variables, medians (range) of asymmetrically distributed continuous variables, and frequencies of categorical variables.

*Allele 1 carries a gene conversion from the corresponding intron of CYP11B1; allele 2 does not carry the conversion.

Table 2 shows the distribution of the CYP11B2 intron 2 conversion genotypes in relation to the -344 C/T promoter genotypes in our study population. The data confirm linkage disequilibrium (ie, nonrandom associations of alleles) between these two polymorphic loci in the CYP11B2 gene; allele 1 in intron 2 was seen only in association with -344T. Intron 2 genotype did not influence any of the factors listed above (not shown).

**TABLE 2. Distribution of the Promoter -344 C/T Genotypes and Intron 2 Conversion Genotypes* of CYP11B2 in the Study Population**

<table>
<thead>
<tr>
<th>Intron 2</th>
<th>1/1</th>
<th>1/2</th>
<th>2/2</th>
</tr>
</thead>
<tbody>
<tr>
<td>-344TT</td>
<td>11</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>-344CT</td>
<td>0</td>
<td>29</td>
<td>13</td>
</tr>
<tr>
<td>-344CC</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
</tbody>
</table>

*Allele 1 carries a gene conversion from the corresponding intron of CYP11B1; allele 2 does not carry the conversion.

Table 3 summarizes the M-mode and Doppler echocardiographic measurements for the three CYP11B2 promoter genotype groups: -344CC, -344CT, and -344TT. ANOVA revealed a statistically significant main effect of the promoter genotype on left ventricular end-diastolic diameter (Fig 2), end-systolic diameter, early-to-late transmitral velocity ratio, and atrial filling fraction. The data on left ventricular diameters, mass, and atrial filling fraction were consistent with a gene dosage effect, such that these parameters increased in a linear relationship with the number of -344C alleles carried by each subject.

Stepwise multiple regression analyses also were performed. Table 4 lists the factors selected into the multivariate models. The CYP11B2 promoter polymorphism was the single most important statistical predictor of left ventricular diameters and also independently predicted left ventricular mass. The result was the same if weight, height, body mass index, or the second or third power of height were substituted for body surface area or if sex was forced into the equation (data not shown). In sex-specific analyses, the CYP11B2 promoter polymorphism was independently associated with left ventricular end-diastolic diameter (β=.43, P=.007), end-systolic diameter (β=.34,
The plots show that the regression of left ventricular mass with salt intake in relation to the CYP11B2 promoter from .65 to .70. Fig 3 illustrates the association of left ventricular mass with salt intake of CYP11B2. Short horizontal lines indicate group mean values.

In the regression model for left ventricular mass (see Table 4), added to the equation, the product term “salt intake*CYP11B2 promoter polymorphism” increased the statistical power of the model. The interaction between systolic blood pressure and salt intake on left ventricular mass was strong and statistically highly significant in the -344CT group and nonexistent in the -344TT group. We previously reported an interaction between systolic blood pressure and salt intake on left ventricular mass in the present study population.16 The interaction between salt intake and CYP11B2 promoter polymorphism remained statistically significant (P=.045) even after the term “systolic blood pressure*salt intake” was added to the model; R² rose to .73. No interaction between salt intake and CYP11B2 promoter genotype was observed in the models predicting left ventricular diameters.

Analyses of the echocardiographic data using the same covariates as with the promoter polymorphism showed that intron 2 polymorphism (expressed as the number of nonconversion alleles) was an independent predictor of left ventricular end-diastolic diameter (β=.29, P=.002) and mass (β=.18, P=.010) and was associated with differences in the peak early diastolic transmitral velocity (P=.025). The associations with other echocardiographic measurements did not reach statistical significance.

Discussion

CYP11B2 Genotype Influences Left Ventricular Size

This study shows that polymorphic variations in the CYP11B2 gene are associated with left ventricular size, mass, and, to some extent, diastolic function in persons free of clinical cardiovascular disease. The influence of CYP11B2 gene polymorphism is statistically independent of constitutional and environmental factors that also predict left ventricular measurements. We believe these observations represent genuine associations because the nature of the study population makes a selection bias unlikely, because we used validated and reproducible methods for echocardiography and CYP11B2 genotype assessment, and because the investigators responsible for the respective analyses were blinded to each other’s work. The results are extremely
unlikely to be due to unsuspected admixture of populations in our study sample because the population of Helsinki is ethnically highly homogeneous and the allele frequencies obtained in the present study are identical to those of Caucasian populations in the United States and the United Kingdom (unpublished observations). To the best of our knowledge, \textit{CYP11B2} genotype is the only genetic factor identified thus far that affects heart size in young adults.

The apparent effect of the \textit{CYP11B2} promoter polymorphism on left ventricular size and mass is of considerable magnitude. The data on left ventricular diameters (see Table 2) suggest that homozygotes for the $-344\text{CT}$ allele (i.e., $-344\text{CC}$ genotype) average, by cubic approximation, 28% larger end-diastolic volumes and 21% greater mass than homozygotes for the $-344\text{T}$ allele ($-344\text{TT}$). The Doppler indexes of left ventricular diastolic function fit logically with the size measurements in that the genotype group with the smallest left ventricular mass ($-344\text{TT}$) has the “best” preserved diastolic function as indicated by the highest early-to-late transmitral velocity ratio and the lowest atrial filling fraction.

### Possible Explanations for \textit{CYP11B2} Genotype Effects

The mechanism of the association of left ventricular size and mass with the \textit{CYP11B2} promoter polymorphism is unknown at present. The M-mode echocardiographic data on left ventricular size (Table 3) are compatible with an increased volume load on the left ventricle in persons with $-344\text{CC}$ and, to a lesser extent, with $-344\text{CT}$ compared with the $-344\text{T}$ genotype. The measurements also suggest that the increase of left ventricular mass with the number of $-344\text{C}$ alleles reflects increasing ventricular volume rather than differences in wall thickness. Previous echocardiographic studies in humans have demonstrated that increases or decreases in dietary sodium result in corresponding changes in left ventricular volumes and mass.\textsuperscript{28,29} Thus, differences in the body’s sodium balance and intravascular volume related to the \textit{CYP11B2} genotype are a plausible mechanism for our observations. This idea is supported by the interaction between the effects of salt intake and \textit{CYP11B2} promoter genotype on left ventricular mass.
In addition to indirect cardiovascular effects, aldosterone has had direct actions on the heart in animal studies, including induction of myocardial hypertrophy and fibrosis. These effects are probably mediated via mineralocorticoid receptors in the myocardium. Although the mineralocorticoid receptor itself binds both mineralocorticoids and glucocorticoids, mineralocorticoid target tissues express an enzyme, 11β-hydroxysteroid dehydrogenase, that oxidizes glucocorticoids and prevents them from occupying the mineralocorticoid receptor. Although it was originally thought that the heart did not express this enzyme, more recent studies have confirmed that the human heart does express the same isozyme as the kidney and other mineralocorticoid target tissues. This permits mineralocorticoids to have direct effects on the heart.

It is not yet known whether the polymorphisms that we studied actually affect aldosterone levels in young adults. Although dietary sodium intake is known in our subjects, renin and aldosterone levels and urinary sodium excretion values are not available. Unpublished studies in other populations have thus far yielded inconsistent results, with a trend toward higher urinary aldosterone excretion associated with the −344C allele in healthy white US schoolchildren but a statistically significant disequilibrium between loci is usually directly proportional to the polymorphism that is thought to be essential for expression of steroidcontrolled conditions of diet and activity.

24-hour urinary aldosterone excretion were measured under examine a population in whom blood aldosterone levels or their proximity, it is more likely that the −344 polymorphism is close or identical to the polymorphism that is responsible for the observed effects on heart size.

Comparisons With Previous Studies

The design of the present study differs in important respects from other studies of genetic influences on left ventricular characteristics. Our study population was relatively young and homogeneous for age. Moreover, our population was almost completely free of hypertension and other cardiovascular disease, whereas other studies examined patients from cardiology clinics or individuals with ECG evidence of left ventricular hypertrophy. Thus, studies of our population should only detect factors that affect left ventricular size or function in young, healthy adults. Conversely, although the present study demonstrates that CYP11B2 genotype has major effects on heart size in normal individuals, it cannot answer the question of whether CYP11B2 genotype is, like ACE, a possible risk factor for the development of cardiovascular pathology such as myocardial infarction. Case-control studies of patients with cardiovascular disease would be the best way to answer this question.

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