Aldosterone Excretion Rate and Blood Pressure in Essential Hypertension Are Related to Polymorphic Differences in the Aldosterone Synthase Gene CYP11B2

Eleanor Davies, Christine D. Holloway, Mary C. Ingram, Gordon C. Inglis, Elaine C. Friel, Caroline Morrison, Niall H. Anderson, Robert Fraser, John M.C. Connell

Abstract—Significant correlation of body sodium and potassium with blood pressure (BP) may suggest a role for aldosterone in essential hypertension. In patients with this disease, the ratio of plasma renin to plasma aldosterone may be lower than in control subjects and plasma aldosterone levels may be more sensitive to angiotensin II (Ang II) infusion. Because essential hypertension is partly genetic, it is possible that altered control of aldosterone synthase gene expression or translation may be responsible. We compared the frequency of 2 linked polymorphisms, one in the steroidogenic factor-1 (SF-1) binding site and the other an intronic conversion (IC), in groups of hypertensive and normotensive subjects. In a larger population, the relationship of aldosterone excretion rate to these polymorphisms was also evaluated. In 138 hypertensive subjects, there was a highly significant excess of TT homozygosity (SF-1) over CC homozygosity compared with a group of individually matched normotensive control subjects. The T allele was significantly more frequent than the C allele in the hypertensive group compared with the control group. Similarly, there was a highly significant relative excess of the conversion allele over the “wild-type” allele and of conversion homozygosity over wild-type homozygosity in the hypertensive group compared with the control group. In 486 subjects sampled from the North Glasgow Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) population, SF-1 and IC genotypes were compared with tetrahydroaldosterone excretion rate. Subjects with the SF-1 genotypes TT or TC had significantly higher excretion rates than those with the CC genotype. The T allele was associated with higher excretion rates than the C allele. However, no significant differences were found in excretion rate between subjects of different IC genotype. Urinary aldosterone excretion rate may be a useful intermediate phenotype linking these genotypes to raised BP. However, no causal relationship has yet been established, and it is possible that the polymorphisms may be in linkage with other causative mutations. (Hypertension. 1999;33:703-707.)

Key words: polymorphism ▪ binding sites, SF-1 ▪ intronic conversion ▪ blood pressure ▪ aldosterone

Essential hypertension is a complex disorder. Although environmental factors are major determinants of the rise in blood pressure (BP), a significant genetic contribution is now generally accepted.1,2 For example, young offspring of hypertensive parents have a significant predisposition to hypertension.3 Recent studies in humans have drawn attention to genes that influence renal sodium handling. Components of the renin-angiotensin system (RAS) and factors that affect regulation of secretion and action of aldosterone are strong contenders.4 Excess production of aldosterone such as occurs in primary or idiopathic hyperaldosteronism or in the inherited form, glucocorticoid-suppressible hyperaldosteronism (GSH), results in sodium retention, hypokalemia, suppression of the RAS, a metabolic alkalosis, and hypertension.5,6 Although this constellation of changes does not occur in essential hypertension, BP is reported to correlate positively with body sodium and negatively with body potassium, a situation not found in normotensive subjects.7 Moreover, whereas plasma aldosterone concentration is by definition within the normal range, it is on average abnormally high for the concurrent plasma renin level.8 That is, aldosterone secretion may be slightly more sensitive than normal to its principal agonist, angiotensin II (Ang II); there are experimental data that strengthen this conclusion.9-11

Expression of the gene-encoding aldosterone synthase (CYP11B2) is regulated by Ang II and potassium:12; a chimeric rearrangement of this gene with the adjacent gene-encoding 11β-hydroxylase (CYP11B1) is known to result in GSH.13 This locus is, therefore, an important candidate region in other forms of hypertension.

Steroidogenic enzyme gene promoter regions contain sites for interaction with a variety of control factors, one of which is steroidogenic factor-1 (SF-1).14,15 Differences in the structure of this site may also alter sensitivity to Ang II. A
polymorphism at this site has been described that is reported to be associated with altered left ventricular mass in a Finnish population.16 Further, White and Slutsker17 have recently described another polymorphism in which intron 2 of CYP11B1 has been transferred to CYP11B2. It is not yet known whether this affects expression of the gene or stability of mRNA, and there are no reports of the influence of this polymorphism in cardiovascular disease. In the present study, the distribution of the SF-1 binding site and intron conversion (IC) polymorphisms and their relationship to aldosterone excretion rate have been compared in large groups of hypertensive and normotensive subjects.

Methods

Patients

Approval for the present study was obtained from the appropriate hospital and community medicine ethical committees. White patients with essential hypertension (n=138) were recruited from the Blood Pressure Clinic of the Western Infirmary, Glasgow. All were aged <64 years. Secondary hypertension was excluded by physical examination and biochemical and radiological investigations where appropriate. All subjects had a positive family history of hypertension; high BP was diagnosed before the age of 60. Subjects with a history of alcohol excess (>21 U per week) and obesity (body mass index [BMI] >33) were excluded. BP was measured in the clinic by a trained observer using a mercury sphygmomanometer. The diagnosis of hypertension was based on a minimum of 3 BP readings of >160/90 before initiation of treatment although most patients were on treatment at the time of the study. GSH was excluded by molecular biology tests (either Southern blotting or a long polymerase chain reaction [PCR] assay) as part of a separate investigation.

Control subjects were drawn from the North Glasgow population in each 10-year age/sex band from 25 to 64 years. They were normotensive (<140/90), and none were receiving antihypertensive therapy, treatment for heart disease, or hormone replacement therapy. They were individually age- and sex-matched with the cases by random selection from all controls that matched the criteria of the cases. BP was measured on 2 occasions by use of the Hawksley random zero sphygmomanometer. The results were averaged.

Population Survey of Urinary Tetrahydroaldosterone Metabolite Excretion

A larger sample (n=486) of the MONICA IV survey community subjects agreed to perform a 24-hour urine collection for assay of tetrahydroaldosterone (THaldo).

Laboratory Methods

Genotyping

Blood for genotyping was taken into EDTA-containing receptacles; DNA was extracted by use of a standard phenol-chloroform method and stored at −20°C until required for batch genotyping.

The region of DNA containing the HaeIII/SF-1 polymorphism was amplified by PCR by use of conditions similar to those previously described. The primers used are shown in Table 1. A PCR product of 228 bp was amplified. This was digested with HaeIII and subjected to electrophoresis in 3% MetaPhor agarose.

The 228 bp amplicon contains 2 HaeIII restriction enzyme sites (GG CC). The presence of a C to T transition at position 344 (GG CT) removes one of these sites. After digestion, individuals homozygous for the transition (TT) produce 2 bands of 175 and 53 bp, individuals homozygous for the wild-type (CC) produce 3 bands of 104, 71, and 53 bp, and heterozygous individuals (TC) produce 4 bands.

The IC was genotyped by use of 2 separate PCR reactions, one that amplifies the normal gene (WT) and one that amplifies the conversion.18 The 2 pairs of primers and the conditions used are shown in Table 1. The size of the amplicon in each reaction is approximately 418 bp.

Urinary Corticosteroid Metabolite Measurements

Urine samples were collected for a 24-hour period in plain plastic containers without preservative. Aliquots of urine were stored at −22°C until required for THaldo assay by gas chromatography/mass spectroscopy by use of the method of Shackleton18 with minor modifications.

Statistical Methods

Comparisons between cases and controls of demographic variables and genotype or allele frequencies were carried out by paired t tests and McNemar’s test, respectively. In particular, a variation of McNemar’s test appropriate for case-control comparisons involving either 2×2 or 3×3 contingency tables was used with analyses by allele or genotype, respectively. Hardy-Weinberg equilibrium was checked by a χ2 test, and the strength of allelic association between SF-1 and IC polymorphisms was estimated by Jurg Ott’s EH program. Median THaldo levels were compared between genotype groups by use of a Kruskal-Wallis test.

Table 1. Primers and PCR Conditions

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>PCR Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF-1 sense</td>
<td>5’ GTGTCAGGGCAGGGGTGTA 3’</td>
<td>3 minute(s), 94°C, (1 minute(s) 93°C, 1 minute(s) 68°C, 1 minute(s) 72°C, 30 cycles) 72°C, 7 minute(s) Digestion with HaeIII, 3% MetaPhor gel electrophoresis</td>
</tr>
<tr>
<td>SF-1 antisense</td>
<td>5’ AGGCGTGGGGTCTGGACT 3’</td>
<td></td>
</tr>
<tr>
<td>WT intron 2 sense</td>
<td>5’ TGAGAAGCCCTACCCCTGT 3’</td>
<td>2 minute(s), 94°C, (1 minute(s) 94°C, 30 s 66°C, 30 s 72°C, 30 cycles) 72°C, 7 minute(s)</td>
</tr>
<tr>
<td>WT intron 2 antisense</td>
<td>5’ AGGAACCTCTGCAGGGCC 3’</td>
<td></td>
</tr>
<tr>
<td>Conversion sense</td>
<td>5’ CAGAAAAATCCTCCCTCCCCTTA 3’</td>
<td>2 minute(s), 94°C, (1 minute(s) 94°C, 30 s 66°C, 30 s 72°C, 30 cycles) 72°C, 7 minute(s) 1% agarose gel electrophoresis</td>
</tr>
<tr>
<td>Conversion antisense</td>
<td>5’ AGGAACCTCTGCAGGGCC 3’</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 2. Clinical Details of Subjects Studied

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>49.6±10.9</td>
<td>49.0±10.9</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>69.9±12.2</td>
<td>78.8±15.6</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.4±3.2</td>
<td>28.1±4.8</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>123.8±15.0</td>
<td>154.3±22.0</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>76.8±8.7</td>
<td>95.8±10.9</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>5.9±1.1</td>
<td>6.0±1.1</td>
</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>NA</td>
<td>86.0±14.7</td>
</tr>
<tr>
<td>Potassium, mmol/L</td>
<td>NA</td>
<td>4.2±0.4</td>
</tr>
</tbody>
</table>

Study included 138 controls and 138 cases (n=65 men and 73 women per group).

Results

Demographic Data

The details of patients and controls are given in Table 2; it shows that the age and sex matching of the groups was accurate. The patient BPs were those at the time of the study when many were receiving treatment. Despite this, BP in the patient group is significantly higher than in the control group.

Genetic Analysis

The general population frequency of the 2 polymorphisms was first studied in a random selection of the normal population from the MONICA IV community survey (n=256). The SF-1 binding-site polymorphism showed population frequencies of 0.49 (C allele) and 0.51 (T allele). For the intron 2 polymorphism, the wild-type allele had a frequency of 0.48 and the conversion allele a frequency of 0.52. There was significant linkage between the 2 polymorphisms so that the 3 common haplotypes were observed: C/wild-type (0.45), T/wild-type (0.13), and T/conversion (0.38).

The distribution of genotypes and alleles for the 2 polymorphisms in the case-control populations is shown in Table 3. The control group was at Hardy-Weinberg equilibrium for both polymorphisms, whereas the case group was in Hardy-Weinberg equilibrium for the IC polymorphism but not for the SF-1 binding site (P=0.0007).

There was a relative excess of TT homozygotes for the SF-1 binding site in the cases compared with controls (P=0.042), and a similar excess of T alleles (or deficiency of C alleles) was noted when analysis was done by allele counting (P=0.009). Similarly, for the IC, there was an excess of the conversion allele (P=0.016) and a corresponding excess of the conversion homozygote in the cases compared with the control group (P=0.020).

In 486 subjects in whom urinary THaldol excretion rate was measured, both polymorphisms were noted to be in Hardy-Weinberg equilibrium. THaldol levels were higher in those subjects possessing the T allele of the SF-1 binding site and the conversion allele of the IC compared with those lacking these alleles (Table 4) (P=0.024). Subjects homozygous for T or heterozygotes (TC) had higher THaldol levels than those homozygous for C (P=0.05). However, the 3 genotypes for the IC were not significantly different.

Discussion

An explanation of the genetic component of essential hypertension has been sought in associations between BP and polymorphic differences in many genes, among which are the gene coding for elements of the renin-angiotensin-aldosterone system. Positive associations in humans, from linkage and association studies, have been reported for angiotensinogen, but similar studies with renin and with angiotensin-converting enzyme (ACE) have proved negative. In the current study, differences in the distribution of polymorphisms at 2 sites in the aldosterone synthase gene CYP11B2 within hypertensive and normotensive groups have been revealed. Case/control studies of this type may be open to criticism because of potential problems with population mixture. Linkage studies (eg, affected sibling pairs) or family association studies may be less prone to false-positive results but are intrinsically lacking in power. For this reason, a careful case/control approach is still appropriate, but confirmation of positive results in separate populations is clearly important. In the present study, great care was taken to ensure that both groups were samples of the same population and that hypertensive subjects were closely and individually matched with normotensive controls. The control group was in Hardy-Weinberg equilibrium for both the SF-1 site and the conversion site. This was also the case for the conversion site in the hypertensive group, but there was a small but significant deviation from Hardy-Weinberg statistics for the SF-1 site in this group. The reason for this is not clear since the 2
sites are in close linkage disequilibrium. It may reflect a chance finding; the alternative explanation, that there is a systematic loss of the C allele homozygotes in hypertension, seems biologically implausible. We are confident that the genotyping data are accurate, as there is internal consistency in our findings in relation to both the SF-1 polymorphism and the IC polymorphism.

Our hypertensive group had a relative excess of the SF-1 site T allele and of the conversion allele of the IC. This is in agreement with the results of Soubrier (unpublished data, 1997). However, these findings contrast with those of Kupari et al16 who detected a significant association of the C allele and increased left ventricular mass. This was a cross-sectional study of normal subjects, and its relevance to raised BP is unclear.

Examination of the key intermediate phenotype, aldosterone levels, revealed that these alleles were also associated with higher excretion rates of the metabolite THaldo. This contrasts with the findings of Benetos et al27 who found higher supine plasma aldosterone concentrations in SF-1 site CC individuals than in TT individuals. Since the genes are in close linkage disequilibrium, it is not possible to conclude which, if either, of the polymorphisms is responsible for differences in excretion between normotensive and hypertensive subjects.

If high aldosterone levels are associated with SF-1 (TT) and IC (CC) and if these alleles occur significantly more frequently in patients with essential hypertension (although no causal relationship can be assumed), it is perhaps permissible to speculate on mechanisms. For example, it is possible that these polymorphisms are associated with an altered relationship between Ang II and aldosterone and hypertension, but more detailed physiological studies will be necessary to ascertain this. These studies were not possible in the current investigation because we were unable to discontinue therapy or to control sodium intake in our ambulant outpa-
tient population. It is also possible that the polymorphisms are in linkage with other causal mutations in neighboring genes. For example, the close proximity of CYP11B1 has already been mentioned, and there may be linkage to mutations in this gene that affect 11β-hydroxylase activity. To ascertain whether this relationship is indeed more sensitive in SF-1 (TT) and/or IC (CC) individuals, Ang II infusion studies or, at least, concurrent plasma renin and aldosterone levels will be necessary. It is relevant that Litchfield et al28 report relatively higher free cortisol excretion rates in essential hypertension; no mechanism to account for this has as yet been proposed.

In summary, we have observed an association between a polymorphism and the gene-encoding aldosterone synthase and hypertension in a careful case/control study. A possible intermediate phenotype has been identified on the basis of higher urinary aldosterone excretion in subjects bearing the allele that is overrepresented in patients with hypertension. Further studies of this locus to define the precise nature of the intermediate phenotype and the underlying physiological and clinical implications of this apparent association are now warranted.

**Acknowledgments**

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**References**


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**TABLE 4. Urinary THaldo vs Genotype in Normal Subjects (n=486)**

<table>
<thead>
<tr>
<th>SF-1 Site</th>
<th>TT</th>
<th>CC</th>
<th>TC</th>
<th>T allele</th>
<th>C allele</th>
<th>THaldo, nmol/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>T allele</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8.8±0.9</td>
</tr>
<tr>
<td>C allele</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td>14.6±1.1</td>
</tr>
<tr>
<td>THaldo, nmol/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12.4±1.2</td>
</tr>
<tr>
<td>IC (WT)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11 (WT)</td>
</tr>
<tr>
<td>T allele</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>22 (Conversion)</td>
</tr>
<tr>
<td>C allele</td>
<td></td>
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<td></td>
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<td></td>
<td>WT</td>
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<tr>
<td>THaldo, nmol/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11.6±1.2</td>
</tr>
<tr>
<td>IC (WT)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14.1±1.1</td>
</tr>
<tr>
<td>T allele</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>11.9±1.0</td>
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<tr>
<td>C allele</td>
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<td></td>
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<td>11.5±1.2</td>
</tr>
<tr>
<td>THaldo, nmol/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>12.7±1.1</td>
</tr>
</tbody>
</table>

Values are mean±SE.


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