Human \(\alpha\)-Adducin Gene, Blood Pressure, and Sodium Metabolism


Abstract—The adducin genes contribute significantly to population variation in rat blood pressure and cell membrane sodium transport. The 460Trp mutation of the human \(\alpha\)-adducin gene has been associated with hypertension, in particular hypertension sensitive to sodium restriction. We studied the relationship between the 460Trp mutation and population variation in blood pressure and sodium metabolism. From 603 Scottish families, we selected 151 offspring and 224 parents with blood pressures in either the upper (high) or bottom (low) 30% of the population distribution and measured the 460Trp mutation using allele-specific hybridization. In offspring, we also measured exchangeable sodium, plasma volume, and total body water. Plasma levels of components of the renin-angiotensin system, atrial natriuretic peptide, and cellular sodium and transmembrane sodium efflux were also estimated. The overall frequency of the 460Trp mutation was 27.1%. In offspring and parent groups, we found no difference in the genotype or allele frequencies of the 460Trp mutation between subjects with high or low blood pressure. There was no overall association between the \(\alpha\)-adducin genotypes and blood pressure variation. In offspring, the 460Trp mutation was not associated with any significant differences in body fluid volumes or exchangeable sodium; levels of plasma renin, angiotensin II, aldosterone, or atrial natriuretic peptide; intracellular sodium; or ouabain-sensitive transmembrane sodium efflux. These findings suggest that in our Scottish population, the \(\alpha\)-adducin 460Trp polymorphism is not related to blood pressure and does not affect whole body or cellular sodium metabolism. (Hypertension. 1998;32:138-143.)

Key Words: blood pressure ■ adducin ■ renin ■ genetics ■ family ■ sodium

\(\alpha\)-Adducin is a component of the cytoskeleton that appears to be involved in cell-to-cell contact\(^1\) and cell membrane ion transport\(^2\) and signal transduction.\(^3\) These functions make \(\alpha\)-adducin of relevance to blood pressure and sodium balance. In humans, certain genetic variants of the \(\alpha\)-adducin gene have been found more frequently in hypertensive than normotensive subjects.\(^4\) Recently, a specific mutation in the \(\alpha\)-adducin gene was described that results in the substitution of tryptophan (Trp) for glycine (Gly) at amino acid number 460. This genetic variant known as 460Trp was associated with hypertension.\(^5\) Hypertensive subjects with the 460Trp allele had lower plasma renin and showed a significantly greater fall in blood pressure with sodium restriction or diuretic treatment. These findings suggested that the 460Trp mutation might cause sodium retention and high blood pressure.

Studies in the MHS model of genetic hypertension have linked the \(\alpha\)-adducin gene with high blood pressure and sodium metabolism.\(^6\) High blood pressure in the MHS appears to be dependent on the kidney\(^7\) and associated with faster ion transport across renal tubular membranes.\(^8\) The same defect is observed in erythrocytes and can be abolished by removal of the membrane skeleton.\(^9\) Mutations in adducin \(\alpha\)- and \(\beta\)-subunits in MHS have been associated with a significantly faster transport across the Na,K-ATPase pump,\(^2\) consistent with findings in prehypertensive animals.\(^10\) The adducin subunit mutations in MHS have been shown to cosegregate with a significant increment in blood pressure F\(_2\) populations derived by crossing MHS and control strains.\(^6\) These studies revealed that the adducin genes are involved in the population-wide variation in blood pressure, not only in hypertension.

Our aim was to study the relationship between the \(\alpha\)-adducin 460Trp mutation and blood pressure and body sodium and fluid balance and the cellular transport of sodium in families selected from the general population.

Methods

Ascertainment and Sampling Design

In this study, the “four corners” approach\(^11\) was used to select young adults with contrasting genetic predisposition to high blood pressure...
from the general population. All participants were white and drawn from an area served by 2 group general practices based at the Ladywell Medical Center in the west of Edinburgh, Scotland. Parental blood pressures were recorded during the screening phase of the Medical Research Council Trial of Treatment of Mild Hypertension, during which 76% of adults aged 35 to 64 years registered at the Medical Center took part. The screened adults included 1809 pairs of husbands and wives. The study population was formed by 1473 couples still registered with the practices in 1986. Blood pressure was measured in 864 young adults aged 16 to 24 years from 603 families. Offspring comprised 74% of all children in this age group related to these parents by blood. Blood pressure was adjusted for age and gender to calculate standardized scores for both parents and offspring. Eighteen parents receiving antihypertensive treatment were given z scores equivalent to the upper 5% of the distribution. A scatter diagram was then constructed with combined parental blood pressure z scores on 1 axis and offspring z scores on the other. Offspring and parents were selected for low or high z scores according to cutoffs that corresponded approximately to the lower or upper 30% of the distributions, respectively. By combining offspring and parental z scores, we identified 4 groups of approximately 50 offspring from each corner of the scatter diagram. Among the 4 groups, the greatest contrast in genetic predisposition to high blood pressure existed between offspring with high blood pressures who came from families in which both parents had high blood pressure, and offspring with low blood pressure who came from families in which both parents had low blood pressure.

Clinical and Hormonal Measurements

A total of 201 young adults attended the medical center for measurements of blood pressure, weight, and height. Blood pressure was measured twice after subjects rested for 10 minutes in a recumbent position with the Hawksley random zero sphygmomanometer by nurses who were unaware to which offspring group individuals belonged. Subjects were allowed to rest supine for 25 minutes with a butterfly needle in situ before blood was taken for sodium efflux studies. The methods have been described in detail elsewhere. Additional blood was taken for DNA studies. Subjects were provided with a container and detailed instructions to collect a 24-hour urine sample. Informed consent was obtained from all participants, and all procedures were carried out according to the guidelines of the ethics committees of the Ladywell Medical Center and Western Infirmary.

Sodium Studies

A sample of 100 offspring were admitted to the Western Infirmary, Glasgow, for detailed studies of fluids and electrolytes. The subjects taking part in these studies were representative in all respects of the larger group of offspring. The methods have been described in detail elsewhere. Total exchanged sodium and body water were estimated from dilution of $^2$Na and $^3$H. Plasma volume was estimated from the dilution of an injected bolus of $^{125}$I-labeled albumin. Intraerythrocytic concentrations of sodium and potassium and the total and ouabain-sensitive transmembrane sodium efflux constants were measured as described previously. In brief, sodium efflux measurements were made in RBC loaded with $^{24}$Na. RBC were incubated at 37°C with or without ouabain (10 mol/L). This mixture was sampled in triplicate at 8, 16, and 24 minutes after addition of RBC, and the ERC was calculated from the natural logarithm of the radioactivity of the RBC (as a percentage of RBC plus extracellular radioactivity) plotted against time. Red cell sodium and potassium concentrations were measured in RBC after triple washing in cold (4°C) isotonic choline chloride and lysis in 15 mmol/L lithium chloride at known hematocrit. Coefficients of variation (3 estimates within 3 months in each of 12 normal subjects) were 4.8% for ERC, 5.4% for ouabain-sensitive ERC, and 4.0% for red cell sodium and potassium concentrations.

Parental Phenotypes

Parents of the selected offspring were invited to return to our clinic for repeat measurements of blood pressure, pulse rate, height, and weight. A blood sample was also taken for DNA studies.

Determination of ζ-Adducin 460Trp Mutation

The genetic variation resulting in the 460Trp mutation at amino acid 460 is the result of a G-to-T substitution at nucleotide 614 in exon 10 of the ζ-adducin gene. High-molecular-weight DNA was isolated from peripheral blood leukocytes by routine methods. The Gly460Trp polymorphism was measured using PCR amplification of genomic DNA followed by allele-specific oligonucleotide hybridization (Reference 5; and D. Cusi, personal communication, 1997). The sequences of the sense and antisense primers for PCR were 5′-GACAGAAGGCTGAATCTGG-3′ and 5′-AGTCTTCGACCTGGAACCTG-3′, respectively. The PCR was performed using 100 ng of genomic DNA in a total volume of 50 μL containing 15 pmol of each primer, 200 μmol/L of each deoxynucleotide triphosphate, 1.5 mmol/L MgCl₂, 50 mmol/L KCl, 10 mmol/L Tris-HCl, and 1 U Tag GOLD (Perkin Elmer Cetus). The PCR product of 79 bp was hybridized with allele-specific probes for the wild-type allele (460Gly) 5′-TTCTGCCCTTCTTC-3′ and the mutant allele (460Trp) 5′-TTCTGCGATTCTTC-3′. After PCR amplification of genomic DNA, products were run on a 2.5% agarose gel, and electrophoresis samples were transferred onto nylon membranes (Hybond N+, Amersham) with alkali blotting using 0.4 mol/L NaOH. Each membrane was hybridized in Rapid-hyb buffer (Amersham) at 46°C for 1 hour with 32P–end-labeled oligonucleotide probes using bacteriophage T4-polyadenylate kinase. The filters were washed in 5× SSC and 0.1% SDS at 46°C and exposed as autoradiographs for 24 hours. Positive and negative controls were used in each hybridization panel. All assays were scored by 2 independent observers who were unaware of the subject details. Where the observers disagreed, polymorphism analysis was repeated until a clearly agreed result was obtained.

ζ-Adducin genotypes were available in 151 of the 201 screened offspring and in 224 of 349 parents. The 151 parents represented 143 families, there being 62 families for whom genotypes of only 1 parent was available. Genotypes were available in 79 of the 100 offspring who underwent sodium studies. The genotyped groups did not differ significantly from the remaining individuals in terms of age, gender ratio, systolic or diastolic blood pressure, body mass index, or the prevalence of antihypertensive drug treatment. Data presented for both parents and offspring are derived from genotyped individuals only.

Statistical Analyses

Data are expressed as mean with the 95% CI for the mean. Differences in the distribution of ζ-adducin genotypes and alleles were analyzed using χ² tests. Differences between the groups were analyzed by ANOVA.

Results

Offspring

The frequencies of the ζ-adducin genotypes and alleles (Table I) were not significantly different in offspring with high (systolic, 126.0 mm Hg [95% CI, 123.6 to 128.5]); diastolic, 71.1 mm Hg [68.8 to 73.4]) or low (systolic, 116.6 mm Hg [114.4 to 118.8] mm Hg; diastolic,
63.1 mm Hg (60.9 to 65.3) blood pressure. There was also no difference in the frequency of 460Trp alleles in offspring with high personal and parental blood pressures (460Trp alleles, 25.6%) compared with those with low personal and parental blood pressures (460Trp alleles, 31.1%; NS).

Because no differences existed between groups, all offspring were combined and then subgrouped according to α-adducin genotype (Table 2). These groups did not differ significantly in age (P=0.97) or gender ratio (P=0.98) (data not shown). Systolic and diastolic pressures did not vary significantly between the genotypic groups (Table 2), and there were no significant differences in the values of circulating components of the renin-angiotensin system or atrial natriuretic peptide (Table 2).

Given the possible interaction between the α-adducin genotype, measured phenotypes, and sodium intake, the data were reanalyzed taking into account the 24-hour urinary sodium excretion. There was no significant difference in daily sodium excretion between the 3 α-adducin genotypic groups (Table 2, P=0.98). When 24-hour urinary sodium values were entered as covariates in the ANOVA before testing the effects of α-adducin genotype, no significant effect of α-adducin genotype emerged.

We found no significant differences between the 3 α-adducin genotypic groups in total body water, exchangeable sodium, or plasma volume expressed per kilogram of body weight (Table 3). The results were not different if these variables were expressed as absolute values, percentages of the expected normal value for a given body surface area, or as proportions of lean body mass (data not shown).

Intracellular sodium and potassium concentrations did not differ according to α-adducin genotype (Table 4). No relationship was observed between total sodium ERC and the 460 Trp mutation. Furthermore, the ouabain-sensitive ERC was similar in the 3 α-adducin genotypic groups.

Parents
The average pressure of high blood pressure parents was 141.3 mm Hg (137.0 to 145.6) systolic and 83.7 mm Hg (81.7 to 85.7) diastolic; the average of low blood pressure parents was 121.9 mm Hg (118.9 to 125.0) systolic and 76.0 mm Hg (74.1 to 77.9) diastolic.

In parents with high blood pressure (n=111), the distributions of alleles (460Trp, 24.8%) and genotypes (Gly/Gly, 57.7%; Gly/Trp, 35.1%; Trp/Trp, 7.2%) were not different (allele frequencies: x²=1.18, P=0.56; genotypes: x²=1.00, P=0.61) from those of parents with low blood pressure (n=104) (460Trp allele, 28.4%; Gly/Gly, 51.0%; Gly/Trp, 41.3%; Trp/Trp, 7.7%).

When parent groups were combined, the blood pressure of those carrying the Trp/Trp genotype (systolic, 120.9 mm Hg [117.0 to 124.9]; diastolic, 78.9 mm Hg [72.8 to 85.0]) was not significantly different (P=0.69 for systolic, P=0.65 for diastolic) to those with the Gly/Trp (systolic, 122.9 mm Hg [118.9 to 125.0]; diastolic, 80.6 mm Hg [78.2 to 82.9]) or

### TABLE 1. Distribution of α-Adducin Gene Polymorphisms as Genotypes and Allele Frequencies in Offspring With Contrasting Blood Pressures

<table>
<thead>
<tr>
<th></th>
<th>Low Blood Pressure</th>
<th>High Blood Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gly/Gly</td>
<td>38 (49.4%)</td>
<td>43 (58.1%)</td>
</tr>
<tr>
<td>Gly/Trp</td>
<td>31 (40.3%)</td>
<td>25 (33.8%)</td>
</tr>
<tr>
<td>Trp/Trp</td>
<td>8 (10.4%)</td>
<td>6 (8.1%)</td>
</tr>
</tbody>
</table>

χ²=1.18, P=0.56

<table>
<thead>
<tr>
<th></th>
<th>Low Blood Pressure</th>
<th>High Blood Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gly</td>
<td>107 (69.4%)</td>
<td>111 (75.0%)</td>
</tr>
<tr>
<td>Trp</td>
<td>47 (30.6%)</td>
<td>37 (25%)</td>
</tr>
</tbody>
</table>

χ²=1.15, P=0.29

Percentage of column totals for genotype and allele frequencies are given in parentheses.

### TABLE 2. Basic Characteristics of Offspring Grouped According to α-Adducin Genotypes

<table>
<thead>
<tr>
<th>Gly/Gly</th>
<th>Gly/Trp</th>
<th>Trp/Trp</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>81</td>
<td>56</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>(119.3–124.5)</td>
<td>(117.1–122.7)</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>67.8</td>
<td>65.3</td>
</tr>
<tr>
<td>Plasma renin activity, μU/mL</td>
<td>31.2</td>
<td>28.4</td>
</tr>
<tr>
<td>Angiotensin II, pg/mL</td>
<td>11.2</td>
<td>10.4</td>
</tr>
<tr>
<td>Aldosterone, μg/100 mL</td>
<td>12.3</td>
<td>11.0</td>
</tr>
<tr>
<td>ANP, pg/mL</td>
<td>26.4</td>
<td>22.9</td>
</tr>
<tr>
<td>24-h Urinary sodium, mmol/d</td>
<td>152</td>
<td>150</td>
</tr>
</tbody>
</table>

BP indicates blood pressure; ANP, atrial natriuretic peptide. Data are mean (95% CI of mean).
Gly/Gly (systolic, 130.2 mm Hg [126.6 to 133.8]; diastolic, 79.1 mm Hg [77.2 to 81.2]) genotypes.

Discussion
The present study extends knowledge regarding the α-adducin 460Trp mutation in 3 important ways. We tested the α-adducin–blood pressure hypothesis in an independent population. We examined the importance of the α-adducin mutation in explaining blood pressure contrasts across the general population. Finally, in young adults before the development of hypertension, we determined whether the 460Trp mutation was associated with abnormalities of whole body or cellular sodium metabolism.

In our Scottish families, we found no relationship between the 460Trp mutation and blood pressure in either the parental or offspring generations. There were no significant differences in the frequency of the 460Trp mutation in subjects with high versus low blood pressure. Furthermore, the average blood pressure of all subjects carrying the 460Trp mutation was not significantly different from that of those without the mutation. These findings contrast with animal studies in which specific mutations of the adducin genes are associated with significant differences in blood pressure in a genetically heterogeneous population. Genetic effects on population variation in blood pressure are potentially important because the attributable cardiovascular risk of blood pressure at average or slightly above average levels of pressure is at least as great as that associated with clinical hypertension.

There are a number of possible explanations for the disparity between our findings and those reported recently in which the 460Trp mutation was found more frequently in hypertensive than normotensive subjects. Differences in research design might explain the discrepant results. Our aim was to examine the relationship between the α-adducin gene and population blood pressure variation. As such, we studied individuals with blood pressures at the upper and lower end of the population distribution to maximize potential genetic contrast. It is possible, although unlikely, that the 460Trp mutation is relevant to only the highest levels of pressure and unrelated to the presumed polygenic nature of population blood pressure variation. Such a situation might arise if the phenotypic effect of the 460Trp mutation depended on other independent hypertension genes.

Statistical power is another important consideration when small differences in allele frequencies do not achieve statistical significance. The size of our study provided sufficient statistical power to detect a 7% excess in frequency of the 460Trp mutation reported in hypertensive compared with normotensive subjects. We expected that genetic contrast would be at least as great between high and low groups as between hypertensive subjects and the rest of the population. However, in our population, we did not observe any excess of the 460Trp allele with high blood pressure. The overall frequency of the 460Trp allele in the 185 subjects with high

| Table 3. Body Fluid and Electrolytes of Offspring Grouped According to α-Adducin Genotypes |
|----------------------|----------------------|----------------------|----------------------|----------------------|
| Gly/Gly | Gly/Trp | Trp/Trp | P |
| Exchangeable sodium, mmol/kg | 39.4 (38.4–40.5) | 39.6 (38.3–40.8) | 38.4 (36.2–40.7) | 0.71 |
| Total body water, mL/kg | 592 (573–610) | 600 (571–628) | 556 (522–590) | 0.27 |
| Plasma volume, mL/kg | 41.0 (39.0–43.1) | 40.5 (38.2–42.8) | 41.8 (36.6–47.1) | 0.85 |

Data are mean (95% CI of mean).

| Table 4. Cellular Electrolytes of Offspring Grouped According to α-Adducin Genotypes |
|----------------------|----------------------|----------------------|----------------------|----------------------|
| Gly/Gly | Gly/Trp | Trp/Trp | P |
| Intracellular sodium, mmol/L | 6.3 (6.0–6.6) | 6.1 (5.6–6.5) | 5.8 (5.2–6.5) | 0.39 |
| Intracellular potassium, mmol/L | 92.3 (90.4–94.2) | 91.7 (90.3–93.2) | 90.2 (85.9–94.5) | 0.58 |
| Erythrocytic sodium efflux | 0.41 (0.39–0.43) | 0.44 (0.41–0.46) | 0.46 (0.40–0.51) | 0.10 |
| Ouabain-sensitive, h⁻¹ | 0.26 (0.24–0.27) | 0.27 (0.25–0.29) | 0.28 (0.26–0.31) | 0.32 |

Data are mean (95% CI of mean).
pressure was 24% compared with an overall frequency of 29% in the 181 subjects with low pressure. Therefore, our findings do not even tend to support an association between 460Trp and high blood pressure.

Discrepancies in genetic analyses may reflect important differences in the genetic or environmental factors relevant to the expression of the 460Trp mutation. In our study, the frequencies of the 460Trp α-adducin allele and genotypes were more common than reported in Italian and French populations. The 460Trp allele frequency in our study was 27.1% compared with 15.1% in Italian/French control subjects and 22.0% in hypertensive subjects. Whether the result of population stratification or differences in the genetic composition, our findings indicate the importance of regional genetic variation over relatively small geographic distances. Nevertheless, one might not expect that differences in the underlying prevalence of a particular allele per se would negate important physiological effects or conceal an important relationship with blood pressure, should it exist.

Another possibility is population differences in environmental factors such as diet, behavior, and lifestyle that might obscure underlying genetic predisposition. Of particular relevance to the α-adducin locus is sodium intake. In Italian hypertensive subjects, the average basal 24-hour sodium excretion is ≈160 mmol, comparable with the average of 151 mmol in our sample.

Our study also examined the possible relationship between the α-adducin 460Trp mutation and phenotypes relevant to sodium metabolism in young adults. This is important because other studies have suggested that the 460Trp mutation is associated with sodium retention and suppression of plasma renin activity. Also, in the MHS model, adducin gene mutations are linked genetically to primary abnormalities in sodium transport from a very young age. We undertook comprehensive studies of sodium metabolism but could find no abnormality associated with the α-adducin 460Trp mutation. Direct in vivo evidence came from the absence of differences between genotype groups in body fluid volumes or exchangeable sodium. Indirect evidence came from studies of relevant hormonal control systems. Our previous studies in this population have revealed abnormalities of plasma angiotensinogen, cortisol, and adrenaline, and polymorphisms of the glucocorticoid receptor gene in association with pre-disposition to high blood pressure. However, we could find no relationship between the 460Trp mutation of the α-adducin gene and circulating levels of atrial natriuretic peptide, plasma renin activity, aldosterone, or other components of the renin-angiotensin system. Importantly, our analysis also showed that these results were not confounded by differences in sodium intake in the 3 genotype groups.

The in vitro studies of intracellular sodium and transmembrane sodium efflux constants provided the most direct test in human subjects of a hypothesis derived from the MHS model of hypertension. Despite the weight of evidence supporting the effect of mutations in the adducin genes on transmembrane sodium movement and the activity of the Na,K-ATPase pump in the MHS, we could find no relationship between the human 460Trp mutation and intracellular concentrations or transmembrane efflux of sodium. In particular, the normal ouabain-sensitive component of sodium efflux from erythrocytes reflects normal function of the Na,K-ATPase pump in the presence of the 460Trp mutation.

The α-adducin gene is not the first to show apparent variability in its relation with blood pressure in different studies. Inconsistency between populations has been noted for a number of other proposed candidate genes for hypertension, such as the angiotensin-converting enzyme, angiotensinogen, and S$_a$ genes. It is important therefore to identify within specific groups the local relevance of particular genetic markers. In our Scottish population, the 460Trp mutation of the α-adducin gene seems to exert no influence on blood pressure or sodium metabolism.

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**References**


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