Relation of Alleles of the Sodium-Potassium Adenosine Triphosphatase α2 Gene with Blood Pressure and Lead Exposure

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Lead is associated with elevated blood pressure, although the mechanism of action is unknown. Genetic differences in sodium-potassium adenosine triphosphatase (Na\(^+\)-K\(^+\)ATPase) could explain some of the variation in the strength of the blood pressure-lead relation that has been observed in previous studies. In 1996–1997, the authors studied the association of blood pressure, hypertension prevalence, and polymorphisms in the gene for the α2 subunit of Na\(^+\)-K\(^+\) ATPase (ATP1A2) among 220 former organolead manufacturing workers from New Jersey. Subjects were genotyped for a restriction fragment length polymorphism (RFLP) on the ATP1A2 gene. The association between blood lead and blood pressure was stronger among persons who were homozygous for the variant allele. Genotype was also associated with hypertension (adjusted odds ratio = 7.7; 95% confidence interval: 1.9, 31.4). Finally, the variant allele was 1.8 times more common among African Americans than among Caucasians. The RFLP may indicate susceptibility to the effect of lead on blood pressure. Moreover, the α2 gene (or a closely linked gene) may contribute to the pathophysiology of hypertension. However, because the number of subjects (especially African Americans) with the susceptible genotype in this study was small, these observations should be considered preliminary. Am J Epidemiol 2001;153:537–45.

High blood pressure is a multifactorial condition involving both genetic and environmental factors (1). Family and twin studies indicate that as much as 30 percent of hypertension, a disease defined by high blood pressure, is due to genetic causes (2). Rare forms of genetic hypertension have different molecular etiologies but a common pathophysiology mediated by abnormal sodium metabolism (3). Environmental risks for high blood pressure include dietary factors, cigarette smoking, and high alcohol intake, and possibly exposure to toxic metals such as lead (1, 4).

Several epidemiologic studies have suggested a relation between exposure to lead, particularly at blood concentrations less than 40 μg/dl, and small increases in blood pressure, but other studies have failed to find a relation (4, 5). The conflicting results among studies may be attributed to demographic differences between the populations studied, variation in genetic susceptibility, and limitations of the measures used to define lead exposure.

The mechanism of action for the effect of lead on blood pressure has not been established. However, inorganic lead is a potent inhibitor of sodium-potassium adenosine triphosphatase (Na\(^+\)-K\(^+\) ATPase) in vitro. Lead inhibits Na\(^+\)-K\(^+\) ATPase in mammalian tissues (6–10). Moreover, early epidemiologic studies observed lower enzyme activity in erythrocyte membranes of workers with elevated blood lead levels (11–13). Although data are not conclusive, lower levels of Na\(^+\)-K\(^+\) ATPase activity in erythrocytes and leukocytes have been reported in hypertensive individuals (14–17), and an inverse association between Na\(^+\)-K\(^+\) ATPase activity and blood pressure has been reported (18).

Na\(^+\)-K\(^+\) ATPase is an integral membrane protein that catalyzes the active transport of Na\(^+\) and K\(^+\) across the cell membrane. The enzyme is composed of a catalytic α subunit and an associated β subunit that is necessary for proper enzyme function. There are three distinct isozymes for the α subunit that have variable distributions in different tissues (19). The α1 subunit is ubiquitous and predominates in the kidney; the α2 subunit is most prevalent in the brain, heart, and muscle cells (19). Restriction fragment length polymorphisms (RFLPs) in the Na\(^+\)-K\(^+\) ATPase genes exist (20–25).

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Abbreviations: ATP1A2, gene for the α2 subunit of sodium-potassium adenosine triphosphatase; Na\(^+\)-K\(^+\) ATPase, sodium-potassium adenosine triphosphatase; RFLP, restriction fragment length polymorphism; SD, standard deviation.

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The RFLPs are of interest as markers of susceptibility to hypertension and as modifiers of the influence of lead on blood pressure. Indeed, studies of the normotensive offspring of hypertensive parents indicate a possible role for \( \text{Na}^+\text{-K}^+ \) ATPase (26–29).

Differences in the prevalence of genetic polymorphisms for Na\(^{+}\)-K\(^{+}\) ATPase genes may explain variation in results of studies examining the effect of lead on blood pressure. We investigated the association of two RFLPs in the gene for the \( \alpha_2 \) subunit of Na\(^{+}\)-K\(^{+}\) ATPase (\( \text{ATP}1\text{A}2 \)) with blood pressure and hypertension, as well as possible effect modification by genotype on the lead-blood pressure relation.

**MATERIALS AND METHODS**

**Study design and overview**

Data for this study were derived from a 4-year prospective study of current and former employees of a chemical manufacturing facility in New Jersey that formerly produced tetraethyl and tetramethyl lead. Subjects were enrolled over a 3-year period and followed for 2–4 years. This report is based on 226 individuals from whom measurements of blood pressure and Na\(^{+}\)-K\(^{+}\) ATPase genotype were obtained during the third year of the study.

**Selection and recruitment of study subjects**

The study was approved by the Committee on Human Research of the Johns Hopkins School of Hygiene and Public Health, and subjects provided written informed consent. The prospective study focused on individuals employed in the organolead manufacturing area (termed the tetraethyl lead facility), which was involved in the manufacture of tetraethyl lead from 1923 to 1991 and the manufacture of tetramethyl lead from 1960 to 1983 (30). Workers were eligible for recruitment if they had been employed at the facility on or after January 1, 1950, were male, were between 40 and 70 years of age in 1995, were not too ill to continue participation, resided in the state of New Jersey, and had a tibia lead measurement. For the current study, a sample of 272 subjects was selected from 543 eligible workers. To ensure sufficient numbers of individuals with high exposure levels, half of the sample consisted of participants with the longest duration of exposure to lead (10.1–44.3 years), and the remainder were randomly selected from the balance of enrolled participants.

Data collection was completed for 226 (83 percent) of 272 former lead workers who were selected for this study. Individuals were asked to participate in the blood pressure study at a supplemental visit during which tibia bone lead was measured. Twenty-five individuals refused to participate or our attempts to obtain a blood specimen were unsuccessful, and 21 individuals were not invited to participate because blood specimens could not be processed. The final study sample was comparable to the enrolled, eligible cohort with respect to data collected at the initial visit, including age, systolic and diastolic blood pressure, and blood lead concentration (data not shown).

**Data collection**

Data on demographic and personal characteristics and lead exposure were obtained during the initial visit of the prospective study. A blood sample for determination of blood lead level was also drawn. At the supplemental visit during the third year of the study, tibia lead, blood pressure, and weight were measured, and a 10-ml venous blood sample was obtained. Buffy coat was isolated, frozen in liquid nitrogen vapor on-site, and transported to the Johns Hopkins School of Hygiene and Public Health for storage at –70°C.

**Exposure assessment and dose measurements**

The methods used for assessing exposure to organic and inorganic lead (30) and validating exposure estimates (31) are described in detail elsewhere. Interview and personal industrial hygiene data were combined to derive individual measures of cumulative lead exposure (\( \mu \text{g/m}^3 \times \text{years} \)), duration of lead exposure (years), and lifetime average exposure (i.e., cumulative lead exposure/exposure duration, \( \mu \text{g/m}^3 \)).

Methods for assessment of lead dose are described in detail elsewhere (32). Blood lead levels were measured at a commercial laboratory that was certified by the Occupational Safety and Health Administration, using the National Institute for Occupational Safety and Health’s standard addition method (33), by graphite furnace atomic absorption spectrophotometry. Tibia lead was estimated by a 30-minute measurement using \(^{109}\text{Cd} \) K x-ray fluorescence at the mid-tibial shaft (34–37).

**Blood pressure measurement**

Blood pressure (systolic and fifth Korotkoff phase diastolic) was measured using a Hawksley random-zero sphygmomanometer and a protocol compatible with the recommendations of the American Society of Hypertension (38). Three measurements using an appropriately sized cuff were taken 5 minutes apart with the subject sitting. The mean of the three readings was used in the analysis. Blood pressure measurements for all subjects were taken by one trained technician.

**Na\(^{+}\)-K\(^{+}\) ATPase RFLP genotyping**

RFLP genotypes for the Na\(^{+}\)-K\(^{+}\) ATPase \( \alpha_2 \) subunit were determined using Southern blot techniques adapted from Deriaz et al. (39). DNA (10 \( \mu \text{g} \)), extracted from thawed buffy coat specimens, was digested at 37°C with Bgl II restriction enzyme, precipitated, separated electrophoretically (0.8 percent agarose), and transferred overnight to nylon membranes. The blots were prehybridized for 4 hours at 68°C in a buffer containing 250 mM Na\(_2\)HPO\(_4\), 1 mM ethylenediaminetetraacetic acid, 7 percent sodium dodecyl sulfate, and 0.1 mg/ml salmon sperm DNA. Membranes were hybridized sequentially with genomic DNA probes specific for the \( \alpha_2 \) gene provided by Dr. J. B. Lingrel (University of Cincinnati, Cincinnati, Ohio). One probe, a 2.5-kilobase \textit{EcoRI} fragment of the 5′
Statistical analysis

Linear regression was used to evaluate the relations of systolic and diastolic blood pressure with lead dose. Covariates were age (years), race (African-American, non-African-American), body mass index (weight (kg)/height (m)²), use of antihypertensive medications (any, none), a diagnosis of diabetes, alcohol consumption (lifetime reported number of drinks, in quartiles), smoking (non-smoker, current smoker, or past smoker), and season of blood pressure measurement (January/February/March, April/May/June, July/August/September). Dummy variables for genotype at each RFLP site (ATP1A2 (3′) and ATP1A2 (5′)) and an interaction term for genotype × lead dose were included. Each RFLP (ATP1A2 (3′) and ATP1A2 (5′)) was examined separately.

The range of lead dose was smaller among subjects who were homozygous for the 10.5-kilobase allele at the 3′ RFLP site. Therefore, the analysis of effect modification by genotype was repeated after we limited the analysis to subjects with lead levels present in all genotypes. This resulted in the exclusion of 12 individuals. Since antihypertensive medications alter blood pressure, the analysis was repeated after the exclusion of 56 subjects who reported taking antihypertensive medication.

Relations between hypertensive status, RFLP genotype, and lead dose were examined by means of logistic regression. The analysis used two definitions for hypertension. First, we identified 66 hypertensive subjects by defining hypertension as systolic blood pressure >160 mmHg, diastolic blood pressure >95 mmHg, or an affirmative response at the first visit regarding current use of antihypertensive medications. Second, we identified 81 individuals by defining hypertension as systolic blood pressure >150 mmHg, diastolic blood pressure >89 mmHg, or the use of medications to control blood pressure. The logistic regression analysis modeled α2 genotype using dummy variables for each α2 genotype, and as an ordinal predictor variable (zero, one, or two copies of the less common allele).

RESULTS

Characteristics of the study subjects

At the time their blood pressure was measured, participants had a mean age of 58.0 years (standard deviation (SD) 7.4) and mean systolic and diastolic blood pressures of 129.6 mmHg (SD 16.2) and 77.4 mmHg (SD 10.6), respectively; 92.3 percent were Caucasian (table 1). The mean blood lead level was 5.2 µg/dl (SD 3.1; range, 1–20 µg/dl).

Genotypes for the 3′ RFLP site on the ATP1A2 gene were obtained for 220 individuals, and the prevalence of the 10.5-kilobase allele was 24.5 percent. The 11 individuals who were homozygous for the 10.5-kilobase allele had more prevalent current tobacco use (table 1). Prevalence of the 10.5-kilobase allele was higher among the 13 African-American workers than among Caucasian workers (p = 0.02). Genotypes for the ATP1A2 (5′) RFLP site were obtained from 212 individuals. The prevalence of the 3.3-kilobase allele was 9 percent, with only one homozygous individual identified. Individuals who were homozygous for either of the less common alleles (i.e., either 10.5 or 3.3) were always homozygous for the more common allele at the other RFLP site on the gene (table 2). However, a test for the independence of the table cells was not statistically significant (χ² statistic = 5.7, p = 0.22). Cell frequencies in the 2 x 2 table representing individuals who were homozygous or heterozygous for the more common allele at either RFLP site suggested that the two loci may be linked (Fisher’s exact p value = 0.13).

Blood pressure, blood lead, and ATP1A2 genotype

Systolic blood pressure (r = 0.2; p = 0.0004) and diastolic blood pressure (r = 0.2; p = 0.004) were positively correlated with blood lead level. Mean systolic and diastolic blood pressures, exposure duration, and blood lead were slightly lower among individuals who were homozygous for the 10.5-kilobase allele (p > 0.05) (table 1). Blood and tibia lead, systolic and diastolic blood pressures, and body mass index were comparable between subjects who were homozygous and heterozygous for the common 8.0-kilobase allele at the 5′ RFLP site. The single subject who was homozygous for the uncommon 3.3-kilobase allele had relatively high values for tibia lead (31.9 g/g bone), systolic blood pressure (152 mmHg), and body mass index (39 kg/m²).

The ATP1A2 (3′) RFLP significantly modified the association between blood lead and systolic blood pressure (p = 0.01) (table 3). The slope for the relation between systolic blood pressure and blood lead was significantly greater for the 10.5/10.5 genotype compared with the other two groups. The regression coefficients for genotypes 4.3/4.3 and 4.3/10.5 did not significantly differ from each other, and results are presented for the two genotypes combined. Systolic blood pressure increased by 0.5 mmHg and 6.1 mmHg with each 1-µg/dl increase in blood lead level for genotypes 4.3/4.3 and 4.3/10.5 combined and genotype 10.5/10.5, respectively (figure 1). Exclusion of subjects with blood lead values above those in the 10.5/10.5 group or of subjects taking antihypertensive medication did not alter the
relations. Twenty-six of the 56 subjects taking antihypertensive medication were heterozygous and three were homozygous for the 10.5-kilobase uncommon allele, indicating that the lead-genotype interaction is not solely due to the association between genotype and hypertension.

The \textit{ATP1A2} (3′) RFLP also significantly modified the effect of tibia lead on systolic blood pressure (data not shown). In contrast to the findings for blood or tibia lead and systolic blood pressure, \textit{ATP1A2} (3′) genotype did not significantly modify the relation between blood or tibia lead and diastolic blood pressure. No associations with blood or tibia lead or with systolic or diastolic blood pressure were observed for the 3.3-kilobase or 8-kilobase alleles on the 5′ end of the \textit{ATP1A2} gene.

**Hypertension and \textit{ATP1A2} (3′) genotype**

Sixty-six (30 percent) of the 220 workers met the definition of hypertension. Mean age and education for persons with


<table>
<thead>
<tr>
<th>Characteristic</th>
<th>4.3/4.3</th>
<th>4.3/10.5</th>
<th>10.5/10.5</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>123</td>
<td>86</td>
<td>11</td>
<td>220</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>58.2 (7.4)†</td>
<td>57.7 (7.2)</td>
<td>57.7 (9.3)</td>
<td>58.0 (7.4)</td>
</tr>
<tr>
<td>Education‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤12 years</td>
<td>74.0</td>
<td>68.6</td>
<td>63.6</td>
<td>71.3</td>
</tr>
<tr>
<td>&gt;12 years</td>
<td>26.0</td>
<td>31.4</td>
<td>36.4</td>
<td>28.7</td>
</tr>
<tr>
<td>Race‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>96.8</td>
<td>87.2</td>
<td>81.8</td>
<td>92.3</td>
</tr>
<tr>
<td>African American§</td>
<td>2.4</td>
<td>10.5</td>
<td>9.1</td>
<td>5.9</td>
</tr>
<tr>
<td>Other</td>
<td>0.8</td>
<td>2.3</td>
<td>9.1</td>
<td>1.8</td>
</tr>
<tr>
<td>Tobacco use‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>26.0</td>
<td>27.9</td>
<td>9.1</td>
<td>25.9</td>
</tr>
<tr>
<td>Current</td>
<td>24.4</td>
<td>15.1</td>
<td>36.4</td>
<td>21.4</td>
</tr>
<tr>
<td>Past</td>
<td>49.6</td>
<td>57.0</td>
<td>54.6</td>
<td>52.7</td>
</tr>
<tr>
<td>Alcohol use‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>2.4</td>
<td>2.3</td>
<td>0.0</td>
<td>2.3</td>
</tr>
<tr>
<td>Current</td>
<td>74.8</td>
<td>70.9</td>
<td>63.6</td>
<td>72.7</td>
</tr>
<tr>
<td>Past</td>
<td>22.8</td>
<td>26.7</td>
<td>36.4</td>
<td>25.0</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>29.3 (4.8)</td>
<td>29.9 (4.5)</td>
<td>27.3 (4.1)</td>
<td>29.4 (4.7)</td>
</tr>
<tr>
<td>Antihypertensive medications‡</td>
<td>22.0</td>
<td>31.4</td>
<td>36.4</td>
<td>26.4</td>
</tr>
<tr>
<td>Mean exposure duration (years)</td>
<td>13.7 (10.9)</td>
<td>13.2 (11.9)</td>
<td>12.1 (12.0)</td>
<td>13.4 (11.3)</td>
</tr>
<tr>
<td>Mean blood lead level (g/dl)</td>
<td>5.4 (3.3)</td>
<td>5.0 (2.7)</td>
<td>4.1 (2.2)</td>
<td>5.2 (3.1)</td>
</tr>
<tr>
<td>Mean tibia lead level (g/g bone)</td>
<td>16.5 (8.8)</td>
<td>16.3 (9.9)</td>
<td>13.7 (9.9)</td>
<td>16.3 (9.3)</td>
</tr>
<tr>
<td>Mean systolic blood pressure (mmHg)</td>
<td>130.2 (16.6)</td>
<td>129.1 (13.8)</td>
<td>127.0 (27.4)</td>
<td>129.6 (16.2)</td>
</tr>
<tr>
<td>Mean diastolic blood pressure (mmHg)</td>
<td>77.4 (10.0)</td>
<td>77.6 (10.7)</td>
<td>76.0 (16.5)</td>
<td>77.4 (10.6)</td>
</tr>
<tr>
<td>Hypertension§,¶,#</td>
<td>25.2</td>
<td>32.6</td>
<td>63.4</td>
<td>30.0</td>
</tr>
</tbody>
</table>

* Genotypes were measured using the Bgl II restriction endonuclease and Southern blot techniques. Three genotypes are identified, signified by the length in kilobases of the DNA fragment (visualized by a specific radioactive probe) after endonuclease digestion, electrophoresis, and blotting.

† All numbers in parentheses are standard deviations.

‡ Percentage of column total.

§ The distribution of African Americans by genotype is different from that of other races (Fisher’s exact test: \(p = 0.04\)).

¶ The distribution of persons with hypertension differs significantly by genotype (\(p = 0.03\)).

# Hypertension was defined as systolic blood pressure >160 mmHg or diastolic blood pressure ≥96 mmHg or use of antihypertensive medications.

### TABLE 2. Numbers of subjects by genotype for two polymorphic sites on the \textit{ATP1A2} gene among former organolead manufacturing workers in New Jersey, 1996–1997

<table>
<thead>
<tr>
<th>\textit{ATP1A2} (3′) RFLP genotype†</th>
<th>\textit{ATP1A2} (5′) RFLP genotype‡</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.3/4.3 (123)‡</td>
<td>4.3/4.3 (175)†</td>
<td>116</td>
</tr>
<tr>
<td>4.3/10.5 (86)</td>
<td>4.3/10.5 (36)‡</td>
<td>82</td>
</tr>
<tr>
<td>10.5/10.5 (11)</td>
<td>10.5/10.5 (1)‡</td>
<td>11</td>
</tr>
<tr>
<td>No.</td>
<td>174</td>
<td>209</td>
</tr>
</tbody>
</table>

* RFLP, restriction fragment length polymorphism.

† Genotypes were measured using the Bgl II restriction endonuclease and Southern blot techniques. Three genotypes are identified at each locus, signified by the length in kilobases of the DNA fragment (visualized by a specific radioactive probe) after endonuclease digestion, electrophoresis, and blotting.

‡ Numbers in parentheses are the total number of individuals in the sample with the genotype. These numbers do not match the column and row totals because data are not available for both genotypes on all subjects.

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DISCUSSION

An RFLP at the 3’ end of the gene for the α2 subunit of Na+–K+ ATPase (ATP1A2 (3’)) modified the relation between blood lead and systolic blood pressure among these male former organolead manufacturing workers. These results suggest that ATP1A2 (3’) genotype may identify individuals who are susceptible to the effect of lead on blood pressure. The ATP1A2 (3’) RFLP was directly associated with hypertension in this sample, and it may confer susceptibility to hypertension or be linked to another gene on the same chromosome causing susceptibility. Because there were relatively few individuals with the at-risk allele and because susceptibility was identified primarily among homozygotes, the findings must be viewed as preliminary.

The prevalence of the 10.5-kilobase allele at the 3’ RFLP site in this sample was higher than that reported in a Canadian study (25 percent vs. 18 percent), while the prevalence of the 3.3-kilobase allele at the 5’ RFLP site was lower (9 percent vs. 12 percent) (39). However, the distribution of genotypes at either locus did not vary from the Hardy-Weinberg equilibrium. Cell frequencies in a 2 × 2 table of individuals who were homozygous or heterozygous for the more common allele at each of the two RFLP sites (upper left of table 2) suggested that the two loci on the ATP1A2 gene may be linked, albeit not tightly. Indeed, while hypertension was associated with genotype at the 3’ RFLP site, no health endpoints were related to genotype at the 5’ RFLP site. In contrast, Rankinen et al. (40) found significant linkage disequilibrium between the two sites in a sibling-pair analysis of participants in the Quebec Family Study. The 5’ genotype was not directly related to blood pressure in the Canadian study, although it was associated with other hemodynamic phenotypes. The 3’ genotype was related to resting systolic blood pressure, 12-year change in systolic blood pressure, and exercise diastolic blood pressure, and the 3’5’ haplotype was related to diastolic blood pressure. The findings of our study and of the Canadian study are in conflict with those of Shull et al. (41), who failed to observe segregation of polymorphisms in the α2
FIGURE 1. Association of blood lead level with systolic blood pressure and effect modification by the ATP1A2 (3′) genotype among 220 former organolead manufacturing workers in New Jersey, 1996–1997. The figure shows results from a linear regression model of systolic blood pressure, blood lead, and genotype for a restriction fragment length polymorphism at the 3′ end of the sodium-potassium adenosine triphosphatase α2 subunit gene, containing a cross-product term for blood lead and genotype. The model also contained age, use of antihypertensive medications, current smoking, quartile of lifetime alcohol consumption, and season (January/February/March, April/May/June, July/August/September). The lines represent the rise in systolic blood pressure with increasing blood lead level for genotypes 4.3/4.3 and 4.3/10.5 combined and genotype 10.5/10.5. The symbols represent the genotype of each individual: open circle = 4.3/4.3 or 4.3/10.5; open triangle = 10.5/10.5. The graph depicts results from the regression model that included all subjects. Systolic blood pressure increased by 0.54 mmHg and 6.12 mmHg with each 1-µg/dl increase in blood lead level for genotypes 4.3/4.3 and 4.3/10.5 combined and genotype 10.5/10.5, respectively. The relation between blood lead and systolic blood pressure in 10.5-kilobase homozygotes achieved statistical significance (p = 0.01).

and β genes with essential hypertension, a study that only involved three families and no individuals homozygous for the uncommon allele.

It is possible that the relations between genotype and hypertension and genotype and race may be due to differential survival in this lead-exposed occupational group. However, while study subjects had past occupational exposure to lead, they had not been exposed to lead occupationally for an average of 14 years. Indeed, the range of blood lead in study participants was similar to that reported for other middle-aged or elderly males residing in the eastern United States with environmental exposure to lead (42). The study sample was selected from all previously lead-exposed workers, minimizing selection bias. Therefore, genetic variation in the α2 subunit of Na⁺-K⁺ ATPase may contribute significantly to the prevalence of hypertension in the general population, but this conclusion must await studies of ATP1A2 (3′) genotype and hypertension in subjects without past occupational lead exposure. The association between genotype and race probably did not result from selective loss. African Americans with the 10.5/10.5 genotype would have been more strongly subject to selection given the association of race and genotype with hypertension, a risk factor for cardiovascular disease and mortality (43). Therefore, we do not think that differential survival explains the higher prevalence of the 10.5 allele among African Americans compared with Caucasians.

The difference in allele frequency by race raises the possibility that the α2 subunit of Na⁺-K⁺ ATPase plays a significant role in the pathogenesis of hypertension among African Americans. A higher proportion of African Americans are hypertensive compared with other racial groups (44). Most of the hypertension in African Americans is salt-sensitive and associated with low plasma renin, conditions that appear to be under genetic control (44). Moreover, mean blood lead levels of African Americans are higher than those of Caucasians (45). Consequently, African Americans may be especially affected by the interaction between...
between lead and ATP1A2 (3′) genotype and elevations in systolic blood pressure. Unfortunately, the number of African Americans or individuals of other racial groups in the sample was too small to examine the relations between genotype, lead, and blood pressure by race.

Little is known about the number, identity, and function of genes involved in blood pressure control. Two rare autosomal dominant forms of hypertension have been described, glucocorticoid-suppressible hyperaldosteronism and an abnormality of sodium reabsorption in the renal distal tubule (Liddle’s syndrome) (3). Several genes that may increase susceptibility to hypertension have been studied, including the renin, angiotensin I converting enzyme, angiotensin type II receptor, angiotensinogen, and adducin genes. While none have been definitively ruled out, strong evidence of linkage with hypertension has been documented for the angiotensinogen and adducin genes (46–49). Angiotensinogen is the first substrate in a regulatory cascade resulting in the release of aldosterone from the adrenal cortex, a hormone that regulates sodium retention and excretion (50). The polymorphism in the adducin gene was associated with a measure of salt sensitivity in 86 hypertensive patients (48). Na⁺-K⁺ ATPase is another example of a gene that regulates homeostasis and may influence blood pressure.

The effect, if any, of the nucleotide change identified by the Bgl II restriction endonuclease at the 3′ RFLP site on enzyme function is not clear. The change has not been specified, and its location (exon or intron) is not known. The polymorphic site is identified in Southern blots by a 1-kilobase probe containing exons 21 and 22. Exons 19–22 code for hydrophobic domains in the protein that may be transmembrane domains (22). Site-directed mutagenesis studies indicate that changes in the transmembrane region can alter enzyme kinetics (51–54).

The role of Na⁺-K⁺ ATPase in the mechanism of blood pressure elevation is not known. In experimental studies, inhibition of Na⁺-K⁺ ATPase results in increased peripheral vascular resistance and enhanced vascular responsiveness to vasoconstrictor hormones (55, 56). Na⁺-K⁺ ATPase inhibition in the brain also may influence blood pressure. A compound that is structurally similar to the Na⁺-K⁺ ATPase inhibitor, ouabain, has been identified in the human circulation and may originate in adrenocorticoid tissue (57). Circulating levels of “endogenous ouabain” are increased in many forms of hypertension, and its infusion into rats elevates blood pressure (57). Increased levels of endogenous ouabain in serum and in the hypothalamus could inhibit Na⁺-K⁺ ATPase, increase sympathetic activity and aldosterone levels, and elevate blood pressure. The association between hypertension and an RFLP in the gene for the α2 subunit, which is present in significant quantity in the brain, is consistent with this hypothesis. Future research is needed to identify intermediate phenotypes that vary with α2 genotype.

The results of this study provide additional evidence that lead dose is associated with elevated blood pressure. The findings suggest that Na⁺-K⁺ ATPase genotype can alter this relation. Na⁺-K⁺ ATPase may also have a direct role in the pathophysiology of hypertension. However, because of the small number of individuals with the susceptible genotype, these observations must be considered preliminary.

### Table 4. Logistic regression model for hypertension* in former organolead manufacturing workers in New Jersey with ATP1A2 (3′) genotype measurements, 1996–1997†

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Crude OR‡</th>
<th>Adjusted§ OR</th>
<th>95% CI‡</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>1.03</td>
<td>1.02</td>
<td>0.98, 1.07</td>
<td>0.3</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>1.12</td>
<td>1.15</td>
<td>1.07, 1.23</td>
<td>0.00</td>
</tr>
<tr>
<td>Lifetime number of alcoholic drinks/1,000¶</td>
<td>0–1.57%</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>1.58–5.50</td>
<td>1.23</td>
<td>1.08</td>
<td>0.41, 2.84</td>
<td>0.87</td>
</tr>
<tr>
<td>5.51–19.0</td>
<td>2.14</td>
<td>2.79</td>
<td>1.09, 7.16</td>
<td>0.03</td>
</tr>
<tr>
<td>&gt;19.0</td>
<td>1.81</td>
<td>2.11</td>
<td>0.84, 5.28</td>
<td>0.11</td>
</tr>
<tr>
<td>ATP1A2 (3′) genotype**</td>
<td>4.3/4.3#</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>4.3/10.5</td>
<td>1.33</td>
<td>1.31</td>
<td>0.68, 2.54</td>
<td>0.43</td>
</tr>
<tr>
<td>10.5/10.5</td>
<td>4.91</td>
<td>7.69</td>
<td>1.89, 31.39</td>
<td>0.004</td>
</tr>
</tbody>
</table>

* Hypertension was defined as systolic blood pressure >160 mmHg, diastolic blood pressure ≥96 mmHg, or current use of medications for high blood pressure.
† Data for all variables in the model were available for 210 of the 220 subjects.
‡ OR, odds ratio; CI, confidence interval.
§ Controlled for other variables in the model.
¶ Alcohol consumption (lifetime number of drinks, where one drink was defined as 1 ounce of distilled spirits, 5 ounces of wine, or 12 ounces of beer) was divided into quartiles for this analysis, which compares quartiles of increasing alcohol consumption with the lowest quartile reference group.
# Reference group.
** Genotypes were measured using the Bgl II restriction endonuclease and Southern blot techniques. Three genotypes are identified, signified by the length in kilobases of the DNA fragment (visualized by a specific radioactive probe) after endonuclease digestion, electrophoresis, and blotting.
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REFERENCES


