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A δ-Aminolevulinic Acid Dehydratase (ALAD) Polymorphism May Modify the Relationship of Low-Level Lead Exposure to Uricemia and Renal Function: The Normative Aging Study

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In this study we investigated whether a known δ-aminolevulinic acid dehydratase (ALAD) exon 4 polymorphism has a modifying effect on the association of blood or bone lead level with uricemia and indices of renal function among middle-aged and elderly men. We performed a cross-sectional study of subjects who participated between 1991 and 1995 in the Department of Veterans Affairs Normative Aging Study. Information on blood lead levels, bone lead levels (measured by K-shell X-ray fluorescence), serum uric acid, serum creatinine, estimated creatinine clearance, and ALAD polymorphism status was available in 709 subjects. Regression models were constructed to examine the relationships of serum uric acid, serum creatinine, and estimated creatinine clearance to blood or bone lead level, stratified by genotype. We also adjusted for age, body mass index, blood pressure, smoking, alcohol consumption, and ingestion of analgesic medications (n = 638). Of the 709 subjects, 7 (1%) and 107 (15%) were homozygous and heterozygous for the variant (ALAD-2) allele, respectively. The mean (range) serum uric acid and creatinine levels were 6.5 (2.9–10.6) and 1.2 (0.6–2.5) mg/dL. No significant differences were found in serum uric acid, serum creatinine, or estimated creatinine clearance by ALAD genotype. However, after adjusting for other potential confounders, we found a significant linear relationship between serum uric acid and patella bone lead (p = 0.040) among the ALAD 1-2/2-2 genotype individuals above a threshold patellar lead level of 15 µg/g. In contrast, among the wild-type (ALAD 1-1) individuals, there was a suggestion of a significant linear relationship of serum uric acid with patella bone lead (p = 0.141), but only after a threshold of 101 µg/g. There was evidence of a significant (p = 0.025) interaction of tibia lead with genotype (ALAD 1-1 vs. ALAD 1-2/2-2) regarding serum creatinine as an outcome, but in the same linear regression model tibia lead alone was not a significant predictor of serum creatinine. Conversely, for estimated creatinine clearance, patella lead, but not the interaction of patella lead with genotype, was a significantly independent predictor (p = 0.026). Our findings suggest that ALAD genotype may modify the effect of lead on the renal excretion of uric acid as well as overall renal function among middle-aged and elderly men who had community (nonoccupational) exposures to lead. Additional research is needed to ascertain whether this constitutes a true gene–environment interaction and, if so, its clinical impact. Key words: δ-aminolevulinic acid dehydratase, bone lead, serum creatinine, serum uric acid. Environ Health Perspect 111:335–340 (2003). doi:10.1289/ehp.5504 available via http://dx.doi.org/ [Online 31 October 2002]
1963 (14). Healthy male volunteers from the Greater Boston, Massachusetts, area were screened at entry and accepted into the study if they had no history of heart disease, hypertension, diabetes mellitus, cancer, peptic ulcer, gout, recurrent asthma, bronchitis, or sinusitis. Men with either systolic blood pressure > 140 mm Hg or diastolic blood pressure > 90 mm Hg at entry were disqualified. Between 1963 and 1968, a total of 2,280 men who met the entry criteria were enrolled, ranging in age from 21 to 80 years, with a mean age of 42 years at entry. Study participants were asked to return for examinations every 3-5 years. At each visit, extensive physical examination, laboratory, anthropometric, and questionnaire data were collected. Beginning in 1991, during the course of each continuing participant’s regularly scheduled evaluation at the Department of Veterans Affairs outpatient clinic in Boston, a fresh blood specimen was obtained for measurement of lead and other biochemical indicators such as uric acid and serum creatinine after an overnight fast and abstinence from smoking, and permission was sought to take bone lead measurements by K-shell X-ray fluorescence (KXRF). Consenting individuals reported to the outpatient Clinical Research Center of the Brigham and Women’s Hospital in Boston. This study was approved by the Human Subjects Committees of the Brigham and Women’s Hospital and the Harvard School of Public Health.

Uric acid and renal function. Serum uric acid levels were measured by the colorimetric phosphotungstic method (N-30) with the Technicon Autoanalyzer (Technicon Instruments, Tarrytown, NY, USA) (15). The assay included standards from the College of American Pathologists. In addition, the correlation between the NAS colorimetric phosphotungstic method and the more specific urease method at the clinical laboratory of the Massachusetts General Hospital in 23 split serum samples was 0.93, indicating that these two methods are comparable (15,16).

Serum creatinine was measured by an Astra 8 analyzer (Beckman Instruments, Inc., Fullerton, CA, USA) (17). Creatinine clearance was estimated on the basis of the serum creatinine concentration according to the method of Cockcroft and Gault (18). The following formula predicts creatinine clearance from serum creatinine:

\[
\text{Creatinine clearance} = \frac{(140 - \text{age}) \times \text{weight (kg)}}{72 \times \text{serum creatinine (mg/dL)}}
\]

Derivation included the relation between age and 24-hr creatinine excretion per kilogram of body weight (19).

Blood and bone lead measurements. Blood lead was measured by ESA Laboratories, Inc. (Chelmsford, MA, USA) (20). In tests on reference samples from the U.S. Centers for Disease Control and Prevention, precision (coefficient of variation) ranged from 8% for concentrations below 30 μg/dL to 1% for higher concentrations. Measurements below a concentration of lowest detection limit (1.0 μg/dL) were coded at 0.5 μg/dL (seven study subjects).

Bone lead measurements were taken of each subject’s mid-tibia shaft and patella using an ABIOMED KXR instrument (ABIO-MED, Inc., Danvers, MA, USA). The physical principles, technical specifications, validation, and quality control procedures of this instrument have been described in detail elsewhere (20,21). Briefly, the fluorescent photons from both bone lead and calcium were counted simultaneously to provide the measurement of lead concentration per unit of bone mineral (microgram per gram). Although the KXR instrument may generate negative point estimates of bone lead when the true values are close to zero, we have found that retention of all point estimates makes better use of the data in epidemiologic studies (21). In addition, only three and one study subjects were found to have the negative values for tibia and patella lead levels, respectively. The technicians who measured the bone lead were blind to the participant’s health status.

ALAD exon 4 genotype analysis. The ALAD polymorphism in exon 4 was determined by polymerase chain reaction (PCR) with restriction fragment length polymorphism, according to the methods described by Schwartz et al. (22). We performed PCR reactions in duplicate, with blank controls included in each set.

Statistical analyses. We first compared the distribution of demographic and lifestyle characteristics, bone and blood lead levels, and uric acid and renal function (serum creatinine and estimated creatinine clearance) by genotype (ALAD 1-2/2-2 vs. ALAD 1-1). In addition, we also compared the coefficients of core-model and lead biomarker determinants in regressions of uric acid and renal function that were stratified by genotype (ALAD 1-2/2-2 vs. ALAD 1-1). In each of the above regressions, generalized additive models (23) were used to examine the shape of associations between continuous variables (e.g., blood or bone lead) and uric acid and renal function. These analyses allowed us to assess for potential nonlinearities and the need for transforming these covariates. Finally, we applied statistical tests to determine whether a threshold existed between blood or bone lead levels and uric acid or renal function. Akaike’s Information Criterion was used to determine the threshold (24). Failure to identify a threshold (break point), if one exists, would erroneously attribute a relationship at low blood or bone lead levels where it is not present (25,26). In addition, such failure would underestimate both the size and significance of the effect at blood or bone levels where it is truly present, by averaging levels where the relationship is nonexistent with those where it is significant. All data were analyzed using the SAS (SAS Institute, Cary, NC, USA) and S-PLUS (Insightful Corp, Seattle, WA, USA) statistical packages. All p-values reported are two sided.

Results

Between 1991 and 1995, there were 1,194 active NAS subjects, of whom 776 participated in the KXR bone lead study. Of these, 710 subjects had all information on serum uric acid and creatinine levels, genotype status, and blood and bone lead levels. One subject with a serum creatinine of 9.0 mg/dL (severe renal disease) was excluded (19). No significant differences were found with respect to the distributions of age, body mass
index, alcohol consumption, diastolic blood pressure, current smoking status, and bone and blood lead levels among subjects with and without all information \((n = 709 \text { vs. } 66)\) on serum uric acid and creatinine, genotype status, and blood and bone lead (data not shown). Therefore, the subsequent analyses were focused on the 709 study subjects.

The prevalence (number) of \(ALAD\ 1-1\), \(ALAD\ 1-2\), and \(ALAD\ 2-2\) was 83.9\% (595), 15.1\% (107), and 1.0\% (7), respectively. Table 1 shows the demographic characteristics, blood and bone lead levels, and serum uric acid and creatinine and estimated creatinine clearance categorized by genotype. Except for age, alcohol consumption, current smoking status, diastolic blood pressure, and blood lead levels, there were no meaningful differences of characteristics by genotype. Subjects with \(ALAD\ 1-2\)/2 genotype had a slightly older mean age (68.1 years) and a slightly higher diastolic blood pressure (83.2 mm Hg) than those of the \(ALAD\ 1-1\) genotype subjects (66.8 years and 80.8 mm Hg). Conversely, the \(ALAD\ 1-1\) genotype was associated with a higher percentage of current smokers than the \(ALAD\ 1-2\)/2 genotype. Blood lead levels in this population were relatively low, as expected, with a mean ± SD of 6.2 ± 4.1 μg/dL. We found that mean blood lead level in \(ALAD\ 1-1\) subjects was slightly and significantly higher than that in \(ALAD\ 1-2\)/2-2 individuals \((p = 0.044)\). The same results were not noted with respect to mean bone lead levels by genotype. The mean ± SD serum uric acid and creatinine were 6.5 ± 1.3 and 1.2 ± 0.2 mg/dL, respectively, for all subjects. We did not find any meaningful difference in mean serum uric acid, serum creatinine, or estimated creatinine clearance between the allele groups.

Our hypotheses were that a) blood lead or bone lead level was a risk factor for hyperuricemia and renal dysfunction and b) \(ALAD\) variant type modified this effect especially when blood lead or bone lead level was relatively high (12,27). Table 2 shows the relationship of serum uric acid with bone lead level (patella or tibia lead) with and without an additional term for the interaction of bone lead with genotype, and before \((n = 709)\) and after \((n = 638)\), because of missing data in body mass index, alcohol consumption, and diastolic blood pressure) adjusting for other potential confounders identified in our previous investigation (15). Bone lead level marginally predicted uric acid level after adjusting for age, body mass index, alcohol consumption, diastolic blood pressure, and serum creatinine after adjusting for other covariates. In addition, no interaction was found of bone lead or bone lead with genotype in relation to estimated creatinine clearance (Table 3).
Table 2. Relationship of uric acid (mg/dL) with bone lead (µg/g) in multivariate linear regression without and with ALAD genotype interaction in the Normative Aging Study, 1991–1995.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Crude analyses (n = 709)</th>
<th>Adjusted analyses (n = 638)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Regression coefficient p-Value</td>
<td>Regression coefficient p-Value</td>
</tr>
<tr>
<td>Patella</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lead</td>
<td>0.005</td>
<td>0.048</td>
</tr>
<tr>
<td>Lead–ALADc</td>
<td></td>
<td>0.007</td>
</tr>
<tr>
<td>Tibia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lead</td>
<td>0.006</td>
<td>0.089</td>
</tr>
<tr>
<td>Lead–ALADc</td>
<td></td>
<td>0.012</td>
</tr>
</tbody>
</table>

*Adjusted for age (years), body mass index index, log-transformed alcohol consumption (g/day), diastolic blood pressure (mm Hg), and serum creatinine (mg/dL); some variables have missing data. *Lead–ALAD interaction in the regression model. *Interaction between bone lead and genotype (ALAD 1-2 vs. ALAD 1-1).

Hyperuricemia is the strongest risk factor for gout. Campion et al. (16) found an increased incidence of gout with serum uric acid levels > 9.0 mg/dL. Among patients with lead nephropathy, there is no evidence of overproduction of uric acid (28,29). Therefore, chronic lead toxicity causes hyperuricemia mainly due to a defect in the tubular secretion of uric acid. In this study, we found an association between hyperuricemia and bone lead, especially patella lead. Toxic levels of lead (e.g., blood lead levels > 60 µg/dL) are clearly associated with gouty arthritis (15,28). However, to what extent low-level lead exposure contributes to the development of hyperuricemia and clinical risk of gout is unclear. The middle-aged and elderly men in this cohort currently have low levels of lead in blood (mean, 6.2 µg/dL), which may help to explain why our results are not stronger.

Studies in humans have indicated that renal function may be impaired by high levels of lead exposure or prolonged low-level lead exposure (2,19). Renal biopsies in humans with long-term high lead exposure have revealed abnormal renal peritubular and interstitial fibrous tissue. Lead-induced interstitial nephritis could lead to a true reduction in the glomerular filtration rate or could alter factors that influence the glomerular filtration rate (e.g., surface area, permeability, and oncotic and hydrostatic pressure gradients across the capillary walls) (30). In this cross-sectional study, bone lead level (tibia or patella lead) was positively associated with serum creatinine, but these results did not reach significance after adjusting for other covariates. On the other hand, we detected a positive and significant interaction of tibia lead (but not patella lead) with ALAD genotype in relation to serum creatinine (Table 3), showing ALAD-2 subjects had about 3–4-fold higher serum creatinine than did individuals with ALAD-1. This finding suggests that ALAD may modify the chronic renal toxicity of lead. In a similar vein, Bergdahl et al. (11) did not find any modifying effect of ALAD genotype on the relationship of lead to clinical kidney disease indicators among 89 lead-exposed workers, but they did find that the concentrations of urinary calcium and
the ratio of urinary creatinine/serum creatinine were significantly lower in the seven ALAD 1-2/2-2 subjects compared with those of the 82 ALAD 1-1 lead workers [median urinary calcium (mg/L), 76 vs. 188; urinary creatinine/serum creatinine ratio, 84 vs. 180]. Thus, their study did suggest the presence of ALAD allele-specific differences in kidney function. In another study, Smith et al. (10) investigated the association between the presence of ALAD-2 allele, renal function, and lead concentrations in blood and bone among 688 members of a construction trade union. They found marginally higher levels of blood urea nitrogen \((p = 0.06)\) and uric acid \((p = 0.09)\) among the ALAD-2 genotype individuals after adjusting for blood lead level, age, and alcohol consumption. Smith et al. (10) suggested that ALAD-2 genotype may influence chronic renal toxicity by differential binding of lead to the variant ALAD-2 protein. Our results are consistent with these previous reports in demonstrating that ALAD genotype status may modify the relationship between lead and kidney function. In addition to our findings with serum creatinine, we observed that creatinine clearance, estimated by age, body weight, and serum creatinine, is significantly and negatively associated with bone lead, especially patella lead. One possible explanation is that serum creatinine is not as sensitive a marker for subclinical renal damage as is estimated creatinine clearance. Notably, Nolan and Shaihik (30) found that N-acetyl-D-glucosaminidase, a lysosomal marker of tubular cell toxicity, is a sensitive marker for detecting subclinical kidney damage and is associated with blood lead levels.

We did not find any significant association between blood lead and impaired renal function as measured by serum creatinine or estimated creatinine clearance. Current blood lead levels do not represent long-term lead exposure, which may explain why it is not an adequate dosimeter with respect to kidney toxicity. Regarding our measures of bone lead, it is not clear why we found that patella bone lead was the best predictor of elevated serum uric acid whereas tibia bone lead was the best predictor of elevated serum creatinine.

Compared with the patella, which is composed mostly of trabecular bone, the tibia is mostly cortical bone with a slow rate of bone turnover and a longer half-life with respect to lead levels. As a consequence, the tibia is a better reflection of long-term cumulative lead exposure, whereas the high resorption rates of trabecular bone make it the most important skeletal source of circulating lead (31). It is possible that lead-induced rises in serum uric acid are mostly dependent on active resorption of bone lead stores, whereas lead-induced increases in serum creatinine are mostly dependent on a cumulative effect of lead on the kidney taking place over many years.

How might this ALAD polymorphism modify the impact of lead on uric acid? The effect of lead on uric acid metabolism has been thought to be mediated by a toxic effect on the proximal renal tubule, which is the site of uric acid excretion as well as active secretion by renal tubular cells. It is possible that the lead–ALAD-2 subunit complex is more toxic to proximal tubular cells than is the lead–ALAD-1 subunit (11). This same mechanism might be more broadly explanatory of a modifying effect of the ALAD polymorphism on general kidney function, as reflected by our findings with respect to serum creatinine.

Although in \textit{in vitro} studies and epidemiologic studies suggest that the ALAD-2 polypeptide binds lead more tightly and effectively than does ALAD-1 (3,7,8), the theoretical differential binding of lead by the ALAD isoenzymes does not indicate greater susceptibility to lead for persons with the ALAD-2 genotype. Susceptibility is dependent on different partitioning of lead and how bioavailable different complexed forms of lead are in different tissues. In the present study, ALAD-2 modified lead’s relation to uricemia and indices of renal function. However, the modifying influence of ALAD polymorphism on the neurologic effect of lead may be different (12,33). An elevated concentration of plasma δ-aminolevulinic acid, an early biologic effect of lead poisoning, was higher in 44 battery workers with homozygous ALAD-1, compared with 21 ALAD-2 workers (12). Sithisanankul et al. (12) suggested that if the neurologic effects of lead poisoning are partly due to elevated δ-aminolevulinic acid, then the ALAD-2 genotype may be protective for this end point. This is consistent with the results of a study of neuropsychologic effects of lead in adolescents, showing that the ALAD-2 subjects performed better on tests of attention than did the ALAD-1 subjects after adjusting for lead exposure (32). These potential contrasting roles of ALAD genotype demand further study to clarify the effect of susceptibility to lead toxicity.

Our study has a number of limitations including its cross-sectional epidemiologic design and, perhaps most important, its focus on community-exposed men with relatively modest lead burdens, as reflected by bone lead levels less than a third of those seen in workers in a primary lead industry, such as lead smelting (11,33). It is quite possible that the modifying effect of ALAD polymorphism on lead toxicity would be more apparent among populations with higher levels of lead exposure. Clearly, our findings must be viewed only as suggestive, and other studies are needed to see if these relationships can be replicated.

In conclusion, we found evidence that a polymorphism of ALAD that has been the recent focus of research may modify the impact of lead on uric acid excretion and general kidney function, with ALAD-2 carriers manifesting effects at levels of lead burden with an apparently lower threshold than that of ALAD 1-1 individuals. Additional research is required to determine if this represents a true gene–environment interaction with clinical significance.

**References and Notes**

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