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Citation

Wu, Ming-Tsang, Karl Kelsey, Joel Schwartz, David Sparrow, Scott Weiss, and Howard Hu. 2003. A delta-aminolevulinic acid dehydratase (ALAD) polymorphism may modify the relationship of low-level lead exposure to uricemia and renal function: the normative aging study. Environmental Health Perspectives 111(3): 335-341.

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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]

Chronic community and occupational exposures of adults to lead have been associated with deleterious effects on multiple organ systems, with consequent impacts on health, such as renal function impairment, hyperuricemia, and hypertension (*1,2*). Recently, attention has focused on understanding genetic factors that explain differences in symptoms between individuals who have had similar lead exposures. Identification of such genes would presumably help explain variation in the relationship between biologic markers of lead exposure and measures of organ dysfunction.

One genetic polymorphism that has been suggested (*3*) as a modifier of the pharmacokinetic distribution of lead (and therefore its toxicity) concerns the alleles that code for the production of δ-aminolevulinic acid dehydratase (ALAD), which is the second enzyme in the biosynthetic pathway of heme (*4,5*). In 1981, Battistuzzi et al. (*5*) showed that human ALAD protein is a polymorphic enzyme. Subsequently, Wetmur et al. (*6*) found that this enzyme is polymorphic because of a G-to-C transversion of nucleotide 177 in a coding region that results in replacement of the amino acid lysine with asparginine. Although no significant difference was noted in the activities of the erythrocyte ALAD 1-1, ALAD 1-2, or ALAD 2-2 (*5*), Bergdahl et al. (*3*) reported that the *ALAD-2* subunit binds lead more tightly than does *ALAD-1* subunit *in vitro.*

Epidemiologic studies have suggested that among lead-exposed workers and environmentally exposed children (*7–9*), individuals with either the *ALAD 1-2* or *2-2* genotype had blood lead levels that were significantly higher than those of individuals with the *ALAD 1-1* genotype. These findings suggest that the *ALAD-2* polypeptide binds lead more tightly and effectively than does *ALAD-1.*

Recently, two occupational epidemiologic studies in 691 members of a construction union and 89 lead-smelter workers have indicated that *ALAD* genotype may modify the effect of lead on uricemia and kidney dysfunction (*10,11*). In this study, we attempted to investigate the impact of *ALAD* polymorphism on the relationship of lead levels in both blood and bone to uricemia and kidney dysfunction among environmentally exposed men. Our *a priori* hypothesis was that our lead biomarkers would be independently correlated with hyperuricemia and renal dysfunction and that the *ALAD* variant type would modify this effect, particularly when blood lead or bone lead levels are high (*12,13*).

Methods

Participants. The Normative Aging Study (NAS) is a longitudinal study of aging established by the Veterans Administration in

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We gratefully acknowledge the research assistance of S. Harcourt, R. Heldman, G. Barbella, S. Oliveira, T. Luu, G. Fleischaker, M. Barr, L. Hennessey, and S. Datta. We thank D. Burger and F. Milder for technical assistance in the initial phase of our KXRF measurements and J. McCoy for editorial assistance. We are indebted to the continued enthusiastic cooperation of the participants in the Normative Aging Study.

Support for this research was provided by National Institute of Environmental Health Sciences (NIEHS) grants ES 05257-06A1 and P42-ES05947 (with funding from the U.S. Environmental Protection Agency) and NIEHS Occupational and Environmental Health Center grant 2 P30 ES00002. M.-T.W. received an award from the American Bureau for Medical Advancement in China, Inc. The Normative Aging Study is supported by the cooperative studies program/ERIC, Department of Veterans Affairs, and is a component of the Massachusetts Veterans Epidemiology Research and Information Center. Subjects were evaluated in the outpatient Clinical Research Center of the Brigham and Women's Hospital with support from National Institutes of Health (NIH) grant NCRR GCRC M01RR02635. The KXRF instrument used in this work was developed by ABIOMED, Inc., Danvers, MA, USA, with support from NIH grant SBIR 2R44 ES03918-02.

Received 25 January 2002; accepted 2 August 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:

$$\text{Creatinine clearance} = \frac{(140 - \text{age}) \times \text{weight (kg)}}{72 \times \text{serum creatinine } (\mu\text{g / dL})}$$

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 KXRF instrument (ABIOMED, 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 KXRF 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 make 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*) using chi-squared and Student's *t*-test statistics. Because only 7 (1%) subjects in this population were *ALAD 2-2*, our ability to distinguish between effects associated with the *ALAD 1-2* versus the *ALAD 2-2* genotypes was limited. Nevertheless, initial analyses were conducted comparing wild-type (*ALAD 1-1*) with *ALAD 2-2* individuals alone to see if the homozygous condition was associated with effects that were obscured by combining the *ALAD 2-2* and *ALAD 1-2* individuals. No such effects were seen; thus, all later analyses were conducted and reported combining *ALAD 1-2* and *ALAD 2-2* into one category (*ALAD 1-2*/*2-2*). Multivariate linear regression was used to model determinants of uric acid and renal function that started with core models including determinants we had identified in previously published studies (*15,19*). For uric acid (milligrams per deciliter), these core-model determinants included age (years), body mass index [weight (kilograms)/height (meters)^2], log-transformed alcohol consumption (grams per day), diastolic blood pressure (milligrams mercury), and serum creatinine (milligrams per deciliter); for serum creatinine and estimated creatinine clearance, these core-model determinants included age, body mass index, log-transformed alcohol consumption, hypertension (yes/no), current smoking (yes/no), former smoking (yes/no), and use of analgesic medications (yes/no). Then, blood lead, tibia lead, or patella lead levels were separately forced into the models.

To assess whether genotype may serve as an effect modifier (vs. an independent determinant) of the relationships of blood or bone lead to uric acid and renal function, each of these regressions was then repeated after adding an interaction term for blood or bone lead and 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 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 exists between blood or bone lead levels and uric acid or renal function. Akaike's Information Criterion was computed to determine the break point (*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 KXRF 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 blood and bone lead levels among subjects with and without all information (n = 709 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 1-2/2-2* 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 1-2/2-2* 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 level (for patella lead, p = 0.078; for tibia lead, p = 0.190; Table 2). When the interaction of patella lead and genotype was added to the regression model, the predictive slope coefficients of serum uric acid by patella or tibia lead level in *ALAD 1-2/2-2* individuals was about 50–100% higher than that of *ALAD 1-1* subjects (β = 0.006 vs. 0.004 for patella lead and β = 0.009 vs. 0.004 for tibia lead), but the significance levels of the terms were marginal. When we reset the negative values of tibia and patella lead (of which there were only four) to zero, the results were almost identical. We did not find any significant relation of blood lead or its interaction with genotype to serum uric acid (data not shown).

Table 3 shows the relationship of serum creatinine or estimated creatinine clearance with bone lead level with and without an interaction term for bone lead and genotype and before (n = 709) and after (n = 670, because of 39 missing data in body mass index and alcohol consumption) adjusting for other potential confounders identified in our previous investigation (*19*). Although bone lead level significantly predicted serum creatinine in the crude analysis, we found no significant independent effect of either blood lead or bone lead (tibia lead or patella lead) to serum creatinine after adjusting for age, body mass index, alcohol consumption, hypertension, current smoking status, and current analgesic medications. However, we found a significant interaction of tibia lead (but not blood lead or patella lead) and genotype in relation to serum creatinine (p = 0.025), even though tibia lead level was not a significant independent predictor (Table 3). For estimated creatinine clearance, patella lead was a significant predictor (p = 0.024) after adjusting for age, body mass index, alcohol consumption, hypertension, current smoking status, and current analgesic medications. Neither tibia lead nor blood lead significantly predicted estimated creatinine clearance after adjusting for other covariates. In addition, no interaction was found of blood lead or bone lead with genotype in relation to estimated creatinine clearance (Table 3).

In a smoothed plot of serum uric acid in relation to bone lead that is stratified by genotype and adjusted for other covariates in the model (Figure 1), there is a suggestion of a threshold effect, especially with respect to patella lead, with break points among *ALAD 1-1* and *1-2/2-2* genotype individuals at patella lead levels of 101 μg/g and 15 μg/g, respectively, based on the Akaike's Information Criterion (*24*). Using "hockey stick" regression analyses (*25,26*), we found a significant linear relationship of serum uric acid with patella lead (p = 0.040; Table 4) above the apparent

Table 1. Demographic characteristics and blood and bone lead concentrations by genotypes among 709 study subjects, 1991–1995.

Variable	Total (n = 709)	Genotype [mean ± SD (range)] *ALAD 1-1* (n = 595)	*ALAD 1-2/2-2* (n = 114)	p-Value[a]
Age (years)	67.0 ± 7.4 (48.0–93.0)	66.8 ± 7.3 (48.0–93.0)	68.1 ± 7.5 (52.0–93.0)	0.075
Body mass index (kg/m^2)[b]	27.8 ± 3.9 (16.7–44.9)	27.7 ± 3.8 (16.7–44.9)	28.3 ± 4.3 (18.8–41.5)	0.214
Diastolic blood pressure (mmHg)[c]	81.2 ± 9.8 (51.0–119.0)	80.8 ± 9.7 (51.0–119.0)	83.2 ± 9.9 (64.0–106.0)	0.021
Alcohol consumption (g/day)[c]	12.9 ± 17.5 (0–103.9)	13.5 ± 17.9 (0–103.9)	9.3 ± 14.7 (0–77.9)	0.011
Blood lead (μg/dl)	6.2 ± 4.1 (0–35)	6.3 ± 4.1 (0–35)	5.8 ± 4.2 (0–27)	0.044[d]
Bone lead (μg/g)				
Tibia	22.0 ± 13.4 (−3–126)	22.1 ± 13.9 (−3–126)	21.5 ± 11.0 (3–67)	0.816[e]
Patella	32.1 ± 19.5 (−10–165)	32.2 ± 20.0 (1–165)	31.1 ± 17.0 (−10–85)	0.612[e]
Uric acid (mg/dL)	6.5 ± 1.3 (2.9–10.6)	6.4 ± 1.3 (2.9–10.6)	6.6 ± 1.3 (3.6–9.7)	0.211
Serum creatinine (mg/dL)	1.2 ± 0.2 (0.6–2.5)	1.2 ± 0.2 (0.6–2.5)	1.3 ± 0.3 (0.6–2.4)	0.222
Estimated creatinine clearance (mL/min)[b]	71.3 ± 21.2 (16.9–215.7)	71.6 ± 20.8 (24.1–215.7)	70.0 ± 23.3 (16.9–130.4)	0.472
Hypertension [n (%)]				
Yes	528 (74.5)	445 (74.8)	83 (72.8)	0.656[f]
No	181 (25.5)	150 (25.2)	31 (27.2)	
Current smoking status [n (%)]				
Never smoker	215 (30.3)	174 (29.2)	41 (36.0)	0.016[g]
Former smoker	432 (60.9)	362 (60.8)	70 (61.4)	
Current smoker	62 (8.7)	59 (9.9)	3 (2.6)	
Current analgesic medications				
Yes	538 (75.9)	450 (75.6)	88 (77.2)	0.721[f]
No	171 (24.1)	145 (24.4)	26 (22.8)	

[a]Determined by Student's t-test except where noted. [b]Three missing in *ALAD 1-1* and one missing in *ALAD 1-2/2-2*. [c]Twenty-seven missing in *ALAD 1-1* and nine missing in *ALAD 1-2/2-2*. [d]Determined by Student's t-test after taking the natural logarithm of blood lead. [e]Determined by Student's t-test after adding the value of 35 and then taking the natural logarithm of all values. [f]Chi-square test. [g]Fisher's exact test.

patella lead threshold level of 15 μg/g among the *ALAD 1-2/2-2* genotype individuals, but not among the *ALAD 1-1* genotype individuals above the apparent patella lead level of 101 μg/g ($p = 0.141$).

Discussion

We have previously demonstrated that the patella lead level is independently associated with serum uric acid (*15*). In the present study, our sample was slightly different and the independent effect of patellar lead on serum uric acid was only of marginal significance ($p = 0.078$); however, we found a significant linear relationship of serum uric acid with patella lead among *ALAD 1-2/2-2* genotype individuals who had patella lead levels greater than an apparent threshold of 15 μg/g. There was also a suggestion of a linear relationship of serum uric acid with patella lead among *ALAD 1-1* genotype individuals, but with a much higher apparent threshold (~101 μg/g); moreover, the regression coefficient for patella was not significant.

This difference between the *ALAD 1-2/2-2* and *ALAD 1-1* individuals is fairly striking, and suggests that ALAD status modifies the relationship between lead and hyperuricemia with *ALAD 1-2/2-2* individuals having a higher risk for lead-induced hyperuricemia. On the other hand, there were relatively few subjects with patella lead levels > 101 μg/g in this community-exposed population (Figure 1). Similar results were found with respect to tibia and blood lead, but they were not as impressive as those for patella lead. The *ALAD-2* variant individuals had about a 0.5–2-fold higher serum uric acid than did those with the wild-type *ALAD 1-1* (Table 2).

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

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.

	Crude analyses ($n = 709$)				Adjusted analyses ($n = 638$)[a]			
Variable	Regression coefficient	*p*-Value	Regression coefficient[b]	*p*-Value	Regression coefficient	*p*-Value	Regression coefficient[b]	*p*-Value
Patella								
Lead	0.005	0.048	0.004	0.093	0.005	0.078	0.004	0.129
Lead–*ALAD*[c]	—	—	0.007	0.074	—	—	0.006	0.089
Tibia								
Lead	0.006	0.089	0.005	0.169	0.005	0.190	0.004	0.274
Lead–*ALAD*[c]	—	—	0.012	0.028	—	—	0.009	0.080

[a]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. [b]Lead–ALAD interaction in the regression model. [c]Interaction between bone lead and genotype (*ALAD 1-2/2-2* vs. *ALAD 1-1*).

Table 3. Relationship of serum creatinine or estimated creatinine clearance with bone lead (μg/g) without and with *ALAD* genotype interaction in the Normative Aging Study, 1991–1995.

	Crude analyses ($n = 709$)				Adjusted analyses ($n = 670$)[a]			
Variable	Regression coefficient	*p*-Value	Regression coefficient[b]	*p*-Value	Regression coefficient	*p*-Value	Regression coefficient[b]	*p*-Value
Serum creatinine (mg/dL)								
Patella								
Lead	0.001	0.021	0.0008	0.053	0.0004	0.408	0.0003	0.518
Lead–*ALAD*[c]	—	—	0.002	0.024	—	—	0.0009	0.172
Tibia								
Lead	0.002	0.019	0.001	0.055	0.0006	0.354	0.0005	0.495
Lead–*ALAD*[c]	—	—	0.003	0.003	—	—	0.002	0.025
Estimated creatinine clearance (mL/min)								
Patella								
Lead	–0.190	< 0.001	–0.186	< 0.001	–0.069	0.024	–0.069	0.026
Patella Lead–*ALAD*[c]	—	—	–0.042	0.485	—	—	–0.006	0.884
Tibia								
Lead	–0.271	< 0.001	–0.263	< 0.001	–0.078	0.082	–0.075	0.098
Lead–*ALAD*[c]	—	—	–0.090	0.310	—	—	–0.044	0.499

[a]Adjusted for age (years), body mass index, hypertension (yes/no), current smoking (yes/no), former smoking (yes/no), log-transformed alcohol intake (g/day), and current analgesic medications (yes/no); some variables have missing data. [b]Lead–ALAD interaction in the regression model. [c]Interaction between genotype (*ALAD 1-2/2-2* vs. *ALAD 1-1*).

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 Shaikh (*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 level does 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 rises 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 *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 (*12,32*). 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*). Sithisarankul 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.

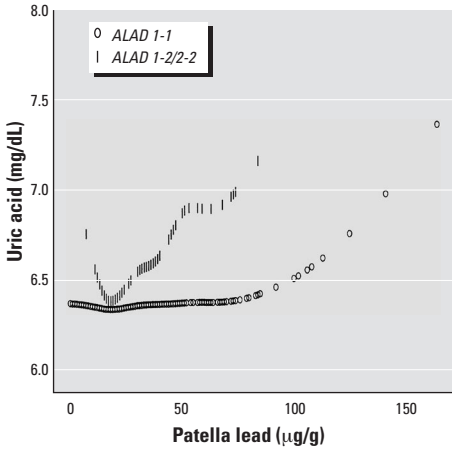

Figure 1. Smoothed line of uric acid versus patella lead by genotypes in 638 community-exposed men, adjusted for age, body mass index, log-transformed alcohol consumption, diastolic blood pressure, and serum creatinine in the Normative Aging Study, 1991–1995.

Table 4. Relationship of uric acid (mg/dL) with patella lead (μg/g) by genotype in the Normative Aging Study, 1991–1995.[a]

	ALAD 1-1 ($n = 540$)			*ALAD 1-2/2-2* ($n = 98$)		
Variable[b]	Parameter estimate	*t*-Statistic	*p*-Value	Parameter estimate	*t*-Statistic	*p*-Value
Patella lead > 101 μg/g	0.021	1.473	0.141	—	—	—
Patella lead > 15 μg/g	—	—	—	0.016	2.084	0.040

[a]Some variables with missing data. [b]Adjusted for age (years), body mass index, log-transformed alcohol consumption (g/day), diastolic blood pressure (mm Hg), and serum creatinine (mg/dL).

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