The δ–Aminolevulinic Acid Dehydratase (*ALAD*) Polymorphism and Bone and Blood Lead Levels in Community-Exposed Men: The Normative Aging Study

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Recent research has indicated that a polymorphic variant of δ-aminolevulinic acid dehydratase (*ALAD*) may influence an individual's level of lead in bone and blood and, as a result, may also influence an individual's susceptibility to lead toxicity. In this study, we investigated whether this *ALAD* polymorphism is associated with altered levels of lead in bone and blood among 726 middle-aged and elderly men who had community (nonoccupational) exposures to lead. We measured levels of blood and bone lead by graphite furnace atomic absorption spectroscopy and a K X-ray fluorescence (KXRF) instrument, respectively. We determined the *ALAD* MspI polymorphism in exon 4 by a polymerase chain reaction restriction fragment length polymorphism (RFLP). Of the 726 subjects, 7 (1%) and 111 (15%) were, respectively, homozygous and heterozygous for the variant allele. The mean (SD) of blood lead (micrograms per deciliter), cortical bone (tibia) lead (micrograms per gram), and trabecular bone (patella) lead (micrograms per gram) were 6.2 (4.1), 22.1 (13.5), and 31.9 (19.5) in subjects who did not have the variant allele (*ALAD 1-1*), and 5.7 (4.2), 21.2 (10.9), and 30.4 (17.2) in the combined subjects who were either heterozygous or homozygous for the variant allele (*ALAD 1-2* and *ALAD 2-2*). In multivariate linear regression models that controlled for age, education, smoking, alcohol ingestion, and vitamin D intake, the *ALAD 1-1* genotype was associated with cortical bone lead levels that were 2.55 μg/g [95% confidence interval (CI) 0.05–5.05] higher than those of the variant allele carriers. We found no significant differences by genotype with respect to lead levels in trabecular bone or blood. In stratified analyses and a multivariate regression model that tested for interaction, the relationship of trabecular bone lead to blood lead appeared to be significantly modified by *ALAD* genotype, with variant allele carriers having higher blood lead levels, but only when trabecular bone lead levels exceeded 60 μg/g. These results suggest that the variant *ALAD-2* allele modifies lead kinetics possibly by decreasing lead uptake into cortical bone and increasing the mobilization of lead from trabecular bone. *Key words*: blood, bone, δ-aminolevulinic acid dehydratase, lead. *Environ Health Perspect* 109:827–832 (2001). [Online 13 August 2001]
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Lead toxicity remains a critical environmental health issue, given the widespread nature of lead exposure from both community and occupational exposures as well as the demonstration of lead's deleterious effect on multiple organ systems at ever lower levels of exposure.

One important question in the study of lead toxicity that has only recently begun receiving attention involves understanding the factors that explain differences in symptoms among individuals who have similar lead exposures. In addition, significant variation has been observed in the relationship between biologic markers of lead exposure and measures of organ dysfunction. Understanding the nature of this variation could also lead to a more profound understanding of the mechanism of toxicity of lead.

A genetic polymorphism in the δ-aminolevulinic acid dehydratase (*ALAD*) gene has been suggested (*1*,*2*) to modify the kinetics and distribution of lead (and therefore its toxicity). This gene codes for the second enzyme in the biosynthetic pathway of heme (*3*,*4*). In 1981, Battistuzzi et al. (*3*) showed that human *ALAD* protein is a polymorphic enzyme. Subsequently, Wetmur et al. (*4*) characterized the molecular nature of this polymorphism, showing it to be caused by a G-to-C transversion in nucleotide 177. This produces coding for lysine rather than asparganine. Although initially Battistuzzi et al. (*3*) noted no detectable difference in the *in vitro* activities of the variant enzymes (*ALAD 1-1*, *ALAD 1-2*, or *ALAD 2-2*) in erythrocytes, Bergdahl et al (*5*) found that the *ALAD-2* subunit binds lead more tightly than does the *ALAD-1* subunit.

Epidemiologic studies have suggested that among lead-exposed workers (*6*,*7*) and environmentally exposed children (*6*), individuals with either the *ALAD 1-2* or *2-2* genotype had blood lead levels that were significantly higher than the blood lead levels of individuals with the *ALAD 1-1* genotype. These findings suggest that the *ALAD-2* polypeptide binds lead more tightly and effectively than *ALAD-1*. However, Smith et al. (*8*) did not find any meaningful difference in mean blood lead concentration between *ALAD-2* carriers and those who were homozygous for the *ALAD-1* allele among 688 members of a construction trade union. The relatively low blood lead levels of these union members [mean ± standard deviation (SD): 7.8 ± 3.6 μg/dL] led the authors to suggest that the *ALAD* polymorphism may influence lead kinetics, but only at relatively high levels of exposure. In studies of lead smelter workers whose blood lead levels ranged from 20–30 μg/dL, Bergdahl et al. (*9*) did not find any significant differences in bone and blood lead levels by genotype among 123 subjects, but Fleming et al. (*10*) found that lead workers with the *ALAD 1-2/2-2* had significantly higher blood lead levels than those with *ALAD 1-1* among 381 subjects.

In this study, we investigated the impact of the *ALAD* polymorphism on lead levels in both blood and bone among participants of the Normative Aging Study (NAS) (*11*). This is a well-studied cohort of men, now middle-aged and elderly, who have had general community (nonoccupational) exposures to lead. Among the advantages of this study are the relatively large sample size (n = 726), the availability of a host of other well-validated demographic and lifestyle data, and

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the use of a K X-ray fluorescence (KXRF) instrument for making *in vivo* measurements of bone lead. These measurements are well-validated (*12*) and have produced data for a number of previous successful studies on low-level lead toxicity and biomarker determinants (*13–16*). We examined the relationship of the *ALAD* polymorphism to bone and blood lead levels. In addition, we conducted exploratory analyses to see if the *ALAD* polymorphism modifies the relationship of bone or blood lead levels to any of their well-known determinants.

This study was approved by the Human Subjects Committees of the Brigham and Women's Hospital and the Harvard School of Public Health.

Materials and Methods

Study subjects. The NAS is a longitudinal study of aging established by the U.S. Veterans Administration in 1963 (*11*). 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 who presented with either systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg at entry were disqualified. Between 1963 and 1968, 2,280 men who met the entry criteria were enrolled, ranging in age from 21 to 81 years, with 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 permission was sought to take KXRF bone lead measurements. Consenting individuals reported to the outpatient Clinical Research Center of the Brigham and Women's Hospital in Boston.

Blood and bone lead measurements. We obtained blood samples and analyzed by graphite furnace atomic absorption spectroscopy (GF-AAS; ESA Laboratories, Chelmsford, MA). Values below the minimum detection limit of 1 μg/dL were coded as 0.5 μg/dL. We calibrated the instrument after every 21 samples with National Institute of Standards and Technology (Gaithersburg, MD) materials. We ran 10% of samples in duplicate; at least 10% of the analyses were controls and 10% blanks. In tests on reference samples from the Centers for Disease Control and Prevention (Atlanta, GA), precision (the coefficient of variation) ranged from 8% for concentrations from 10 to 30 μg/dL to 1% for higher concentrations. In comparison with a National Bureau of Standards target of 5.7 μg/dL, 24 measurements by this method gave a mean of 5.3 μg/dL (SD 1.23 μg/dL).

We took bone lead measurements at two bone sites, the mid-tibial shaft and the patella, with an ABIOMED KXRF instrument (ABIOMED, Inc., Danvers, MA). The tibia and patella have been targeted for bone lead research because these two bones consist mainly of pure cortical and trabecular bone, respectively, which represent the two main bone compartments. A technical description and validity specifications of this instrument have been published elsewhere (*12,17*). This instrument provides an unbiased estimate of bone lead levels (normalized to bone mineral content as micrograms of lead per gram of bone mineral) and an estimate of the uncertainty associated with each measurement (equivalent to a single SD if multiple measurements were taken). Negative estimates of bone lead concentration may occur for lead values close to zero. The technicians who measured the bone lead were blinded to the participant's health status.

ALAD *exon 4 genotypes analysis.* We determined the *ALAD* polymorphism in exon 4 by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP), according to the method of Schwartz et al. (*18*). We performed PCRs in duplicate with blank controls included in each set.

Statistical analyses. We first compared characteristics of subjects who had all data of interest, including genotypes and blood and bone lead levels, with subjects who were not included in this analysis because of missing data. Gene frequencies and tests for Hardy-Weinberg equilibrium were calculated. Only 7 (1%) subjects in this population were classified as *ALAD 2-2*, so we combined *ALAD 1-2* and *ALAD 2-2* into one category (*ALAD 1-2/2-2*) for most of the subsequent analyses.

We compared the distributions of demographic and lifestyle characteristics (as categories) and bone and blood lead levels (as continuous variables) by genotype (*ALAD 1-1* versus *ALAD 1-2/2-2*), and tested the differences using chi-square and Student's *t*-test statistics. We adjusted dietary vitamin D for total caloric intake and divided it into quintiles, as we had done before (*15*). We used multivariate linear regression to model determinants of tibia lead, patella lead, and blood lead; we began with core models including determinants we had identified in previously published research (*13,15*). For the tibia and patella lead, these core-model determinants included age, educational levels, cumulative smoking, alcohol consumption, and dietary intake of vitamin D; for blood lead, these core-model determinants included age, current smoking status, current alcohol consumption, dietary intake of vitamin C, and patella lead [the major internal bone source of circulating lead (*13,15*)]. We repeated each of these regressions after adding a term for genotype (*ALAD 1-1* vs. *ALAD 1-2/2-2*).

To assess whether genotype may serve as an effect modifier (versus an independent determinant) of bone lead, we also compared the β-estimates of core-model determinants in regressions of tibia and patella bone lead that were stratified by genotype (*ALAD 1-1* versus *ALAD 1-2/2-2*). If a core-model β-estimate was markedly different between genotypes, we ran an additional exploratory regression of the entire sample; the regression included a cross-product term that tested for interaction between genotype and the core-model determinant of interest. This allowed us to test for the significance of the interaction term.

In each of the above regressions, we ran generalized additive models for continuous covariates to examine the shape of associations with bone lead and blood lead. These analyses allowed us to assess for potential nonlinearities and the need to transform these covariates.

All data were analyzed using the SAS and S-plus statistical packages (*19*). All *p*-values reported are two-sided.

Results

The 776 NAS subjects who participated in the KXRF bone lead study were comparable to the 1,532 NAS subjects who were seen for their regularly scheduled visits between 1991 and 1995 with respect to distributions of age, race, education, smoking status, consumption of alcohol, retirement status, and blood lead level, as noted in an earlier report (*15*). Of these 776 NAS subjects, 726 had information on genotype status. We found no significant differences in distributions of age, education, alcohol consumption, and blood and bone lead levels among subjects with and without genotype (data not shown). Nine of 726 (1%) and 8 of 50 subjects (16%) with and without genotype, respectively, did not provide information on smoking status. However, among the subjects with information on smoking status, the distribution of never, former, and current smokers was not significantly different between those with and those without genotype information.

The prevalence (number) of *ALAD 1-1*, *ALAD 1-2*, and *ALAD 2-2* was 83.5% (580), 15.5% (108), and 1.0% (7), respectively. The distribution of the different *ALAD* genotypes among the 726 NAS study

subjects conformed to Hardy-Weinberg expected frequencies (chi-square = 0.28; df = 2; $p = 0.87$).

Table 1 shows the demographic characteristics and blood and bone lead levels categorized by genotype. The *ALAD 1-1* genotype was associated with a higher percentage of current smokers and drinkers 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. The mean (SD) blood lead of individuals with the *ALAD 1-1* genotype [6.3 (4.1) µg/dL], was significantly higher than that of the variant allele carriers [5.7 (4.2) µg/dL (*t*-statistic = 2.11, $p = 0.04$)]. We found no differences in mean bone lead levels, including tibia and patella lead as well as tibia and patella lead adjusted by age. We also did not find any association between the variant allele and the difference between tibia and patella bone lead concentrations (the cortical-trabecular bone lead differential, Table 1), a parameter that had previously been found to differ by genotype among lead-exposed construction workers (*8*).

As in our previous investigation (*15*), older age, lower education level, higher pack-years of cigarette smoking, and low intake of vitamin D were important predictors of bone lead levels in the core regression models (Table 2 Model A, Table 3 Model A). We found that the *ALAD* genotype significantly influenced tibia lead, but not patella lead. Subjects with the *ALAD 1-2/2-2* genotype had tibia lead levels that were 2.55 µg/g (95% CI, 0.05–5.05) lower than those with the *ALAD 1-1* genotype after adjusting for other covariates (Table 2 Model B). In the regression models stratified by presence or absence of variant allele, having a less-than-high school education was associated with a mean tibia lead level that was substantially higher among the *ALAD 1-1* genotype subjects (8.15 µg/g) than among the *ALAD 1-2/2-2* genotype subjects (4.88 µg/g; Table 4 Models C and D). In addition, dietary intake of Vitamin D in the highest versus the lowest quintiles was associated with a mean difference in tibia lead level that was substantially greater among the *ALAD 1-1* genotype subjects (–7.31 µg/g) than among the *ALAD 1-2/2-2* genotype subjects (–3.14 µg/g; Table 4 Models C and D). However, no such differences were seen in the models of patella lead stratified by genotype (Table 5 Models C and D). In subsequent models of tibia lead that re-combined the *ALAD 1-1* and *ALAD 1-2/2-2* groups, interaction terms for genotype with less-than-high school education and interaction terms for genotype with dietary intake of vitamin D in the highest quintile failed to reach statistical significance (data not shown).

In the core model of blood lead, high intakes of Vitamin C and iron were associated with low blood lead, as seen before (*15*). In addition, higher patella bone lead was associated with higher blood lead and was the dominant statistical determinant of blood lead (partial R^2 of 0.14; total adjusted R^2 of 0.19). When added to this core model, a term for presence or absence of variant allele was not associated with any meaningful change in blood lead. However, in the models of blood lead that were stratified by the *ALAD* genotype (Table 6 Models D and E), the effect estimate that was associated with each microgram per gram increase in patella bone lead was substantially higher among the *ALAD 1-2/2-2* genotype subjects (0.15 µg/dL) than among the *ALAD 1-1* genotype subjects (0.07 µg/dL). In a model that re-combined the *ALAD 1-1* and *ALAD 1-2/2-2* genotype subjects (Table 7 Model C), an interaction term for *ALAD 1-2/2-2* genotype and patella bone lead was associated with a significantly higher blood lead level. In the same model, the term for *ALAD 1-2/2-2* genotype alone was associated with a significantly lower blood lead level. In a smoothed plot of blood lead in relation to patella lead that was stratified by genotype and adjusted for other covariates in the model (Figure 1), this interaction can be appreciated as, among the *ALAD 1-2/2-2* genotype individuals, a lower blood lead when bone lead levels are below 40 µg/g, and a higher blood lead level when bone lead levels are above 60 µg/g.

Table 1. Demographic characteristics and blood and bone lead concentrations by genotypes among 726 study subjects, 1991–1995.

Variable	Genotype		*p*-Value
	ALAD 1-1 (*n* = 608)	*ALAD 1-2/2-2* (*n* = 118)	
Age (years)[a]			
47-59	100 (16)	14 (12)	0.46
60-69	297 (49)	61 (52)	
≥70	211 (35)	43 (36)	
Education[a]			
≤ High school	326 (54)	68 (58)	0.75
Some college/technical school	86 (14)	15 (13)	
> College	168 (28)	31 (26)	
Missing	28 (4)	4 (3)	
Current smoking status[a]			
Never smoker	175 (29)	42 (36)	0.04
Former smoker	366 (60)	72 (61)	
Current smoker	59 (10)	3 (3)	
No information	8 (1)	1 (1)	
Cumulative smoking (pack years)[a]			
0	175 (29)	42 (36)	0.49
1–20	153 (25)	28 (24)	
> 20	248 (41)	44 (37)	
No information	32 (5)	4 (3)	
Currently consuming ≥ 2 alcoholic drinks/day[a]			
Yes	136 (22)	15 (13)	0.02
No	472 (78)	103 (87)	
Blood lead (µg/dL)[b]	6.3 ± 4.1 (0, 5, 35)	5.7 ± 4.2 (0, 5, 27)	0.04
Bone lead (µg/g)[b]			
Tibia	22.2 ± 13.9 (–3, 19, 126)	21.2 ± 10.9 (3, 19.5, 67)	0.62
Patella	32.2 ± 19.9 (1, 28, 165)	30.4 ± 17.2 (–10, 27, 85)	0.36
Adjusted tibia[b]	22.3 ± 13.1 (–8.5, 20, 121.3)	20.5 ± 10.6 (0.4, 19.4, 64.2)	0.17
Adjusted patella[b]	32.3 ± 19.1 (–6.9, 28.5, 158.7)	29.5 ± 16.5 (–14.4, 25.7, 79.8)	0.14
Patella–tibia difference[b]	10 ± 12.5 (–0.37, 9, 63)	9.2 ± 11.6 (–30, 8, 41)	0.83

[a]No. (%). [b]Mean ± SD (minimum, median, maximum).

Discussion

In this study, we found no significant differences among participants according to *ALAD* genotype with respect to levels of lead in blood or patella (trabecular) bone. Although a mean blood lead level that was slightly higher among *ALAD 1-1* than among *ALAD 1-2/2-2* genotype individuals was suggested in the bivariate analyses, this difference disappeared in the multivariate regression analysis and may have been caused by confounding by patterns of alcohol ingestion and smoking.

On the other hand, we found in the multivariate analyses that tibia (cortical) bone lead levels were significantly lower among the *ALAD 1-2/2-2* than in the *ALAD 1-1* genotype individuals, with a mean difference that

was modest (2.55 µg/g). If one considers membership in the lowest educational class a proxy for significant environmental/occupational lead exposure, the differences in the β-coefficients associated with this exposure with respect to tibia bone lead of the two *ALAD* genotypes (as seen in the stratified regressions) suggest that at high levels of lead exposure, *ALAD 1-2/2-2* individuals absorb less lead into cortical bone than *ALAD 1-1* individuals. An alternative explanation is that *ALAD 1-2/2-2* individuals experience greater loss of lead from cortical bone than *ALAD 1-1* individuals.

Furthermore, we found an interaction between patella (trabecular) bone lead and *ALAD* genotype with respect to blood lead levels. *ALAD 1-2/2-2* genotype individuals had a steeper trabecular bone lead–blood lead relationship, with higher blood lead levels at a given trabecular bone lead level, but only when trabecular bone lead levels exceeded around 60 µg/g. Interestingly, there appears to be a crossover phenomenon, with the *ALAD 1-2/2-2* genotype individuals showing significantly lower blood lead levels at a given trabecular bone lead level when blood lead levels were < 60 µg/dL. To our knowledge, the latter effect has not been published previously, but there has been little other research that has examined both bone and blood lead levels in relation to *ALAD* genotype, and even less in populations with relatively low levels of exposure.

These particular results are only suggestive, because they are based upon a few *ALAD 1-2/2-2* individuals who had very high bone lead levels. Nevertheless, this observation suggests that the *ALAD* polymorphism modifies movement of lead from bone into blood, with *ALAD 1-2/2-2* genotype individuals possibly being less prone to mobilizing bone lead when bone lead levels are low, and more prone to mobilizing bone lead when bone lead levels are high. An alternative explanation could be that *ALAD 1-2/2-2* genotype individuals are initially more likely to store lead in trabecular bone, but at a certain low saturation point, they are less likely to store lead in trabecular bone. Under this hypothesis, the interaction at higher levels of lead burden, for example, could be interpreted as reflecting lower bone lead levels at a given blood lead level (with blood lead deriving mostly from exogenous sources) rather than higher blood lead levels at a given bone lead level (with blood lead deriving mostly from endogenous bone). It is not possible to distinguish these possibilities given the cross-sectional nature of this study.

The gene frequency of the *ALAD* variant allele in our study was 8%, which is similar to the 6% among 1,278 children reported in New York (*6*) and the 8% among 691 members of a construction trade union reported in the United States (*8*). However, it was relatively lower than the 11% reported in Korean lead-exposed workers (n = 308) (*18*) and the 13% reported in Germany lead-exposed workers (n = 202) (*6*). This is likely attributable to ethnic variation in allele frequency.

Two studies have suggested that the *ALAD* genotype may be a selection factor for working in lead industries. Should this be true, it may have implications for the generalizability of findings from occupationally exposed groups (*9,18*). Our research, on the other hand, was conducted in the community, and the distribution of the *ALAD* polymorphism conformed closely to the Hardy-Weinberg equilibrium. Selection similar to a healthy worker effect was unlikely in our study (*9,18*), and our findings can probably be generalized to low lead-exposed populations of men.

In *in vitro* studies, Battistuzzi et al. (*3*) examined enzyme activities among different *ALAD MspI* genotypes and found that mean *ALAD* enzyme activities (± SD; in η/µg Hb) are similar among *ALAD 1-1*, *1-2*, and *2-2* individuals: 52 ± 17 (n = 195), 49 ± 20 (n = 43), and 55 ± 7 (n = 5). Subsequently, Bergdahl et al. (*5*) found that the presence of the *ALAD-2* subunit was associated with more bound lead when they studied seven homozygous wild-type and seven (*ALAD*) homozygous variant individuals who were lead-exposed workers with blood lead levels

Table 2. Predictors of tibia lead (µg/g) among all subjects in the Normative Aging Study, 1991–1995 (n = 689)

	Model A		Model B	
Variable	Parameter estimate	95% CI	Parameter estimate	95% CI
Intercept	18.55		19.01	
Genotype				
(*ALAD 1-2/2-2* vs. *ALAD 1-1*)			−2.55	−5.05–−0.05
Age (years)	0.71	0.58–0.84	0.71	0.58–0.84
Education				
≤ High school	7.98	5.87–10.09	7.98	5.87–10.09
Some technical school	2.88	−0.16–5.91	2.96	−0.07–5.99
Missing	3.43	−1.51–8.36	3.28	−1.65–8.21
Cumulative smoking (pack-years)				
1–20	2.13	−0.35–4.61	2.05	−0.43–4.52
> 20	4.75	2.63–6.88	4.59	2.47–6.71
No information	−0.90	−5.39–3.58	−1.00	−5.48–3.47
Alcohol				
≥ 2 drinks/day	−1.04	−3.29–1.21	−1.11	−3.36–1.14
Vitamin D (IU/day)				
179–262	−4.71	−7.37–−2.05	−4.79	−7.45–−2.13
262–375	−5.00	−7.89–−2.11	−4.90	−7.78–−2.01
375–589	−3.98	−6.87–−1.10	−3.88	−6.75–−1.00
> 589	−3.44	−5.99–−0.90	−3.55	−6.09–−1.01
Total model R^2	0.27		0.27	

Table 3. Predictors of patella lead (µg/g) among all subjects in the Normative Aging Study, 1991–1995 (n = 689)

	Model A		Model B	
Variable	Parameter estimate	95% CI	Parameter estimate	95% CI
Intercept	25.76		25.94	
Genotype				
(*ALAD 1–2/2–2* vs. *ALAD 1–1*)			−1.01	−4.55–2.54
Age (years)	0.89	0.71–1.08	0.90	0.71–1.08
Education				
≤ High school	10.23	7.17–13.29	10.24	7.18–13.30
Some technical school	3.51	−0.79–7.80	3.50	−0.80–7.80
Missing	11.72	4.91–18.53	11.69	4.87–18.50
Cumulative smoking (pack-years)				
1–20	2.13	−1.44–5.69	2.10	−1.46–5.67
> 20	6.63	3.40–9.85	6.59	3.37–9.82
No information	2.83	−3.51–9.16	2.75	−3.60–9.09
Alcohol				
≥ 2 drinks/day	1.93	−1.37–5.23	1.88	−1.43–5.19
Vitamin D (IU/day)				
179–262	−7.10	−10.83–−3.27	−7.08	−10.86–−3.30
262–375	−5.49	−9.59–−1.39	−5.46	−9.56–−1.35
375–589	−4.80	−8.90–−0.70	−4.75	−8.86–−0.64
> 589	−5.27	−9.34–−1.19	−5.26	−9.33–−1.18
Total model R^2	0.19		0.19	

around 30 µg/dL. No significant difference was found in *ALAD* lead-binding among 20 unexposed controls whose blood lead levels were around 4 µg/dL. It is possible that the difference in *ALAD*-bound lead in erythrocytes may be detectable only at higher lead levels. In this case, the *ALAD-2* protein may either bind lead more tightly than the *ALAD-1* protein or have more binding sites. Alternatively, the lack of difference may be explained simply by the difficulty inherent in quantitating binding at low lead levels (*5*).

Ziemsen et al. (*7*) examined blood lead levels in different *ALAD* genotypes among 202 lead-exposed workers whose mean (± SD) (µg/dL) blood lead levels were 40 (± 17) (*7*). They found that blood lead levels (mean ± SD, µg/dL) among the *ALAD 1-1* (n = 160), *ALAD 1-2* (n = 32), *ALAD 2-2* (n = 10) groups were 38 ± 17, 44 ± 17, and 56 ± 18, respectively, although these were not significantly different. Wetmur et al. (*6*) examined the association of *ALAD* genotype and blood lead levels among 202 lead workers in Germany and 1,278 children in New York. They found that the blood lead levels among *ALAD-2* carriers had median values that were about 9 to 11 µg/dL greater than similarly exposed individuals who were homozygous for the *ALAD-1* allele. These findings suggested that the *ALAD-2* polypeptide binds lead more tightly and effectively than *ALAD-1*. These studies had mean blood levels that were higher than 20 and 40 µg/dL among environmentally exposed children and lead-exposed workers, respectively. Smith et al. (*8*) investigated the association between the presence of *ALAD-2* allele and lead concentrations in blood and bone among 688 members of a construction trade union. They did not find a significant difference in mean blood lead concentrations between *ALAD-2* carriers (n = 96; mean ± SD, 7.7 ± 3.5 µg/dL) and those homozygous for the *ALAD-1* allele (n = 592; mean ± SD, 7.8 ± 3.6 µg/dL). However, they found a borderline statistically significant difference (p = 0.06) by subtracting the tibia lead concentrations from the patella lead concentrations for each subject between these two groups (*ALAD-2* carriers: n = 21; mean ± SD, 8.6 ± 9.5 µg/g; homozygous *ALAD-1* allele: n = 101; mean ± SD, 3.4 ± 12.0 µg/g) among a subset of 122 study subjects. Blood lead levels in the union members were relatively low (mean ± SD, 7.8 ± 3.6 µg/dL), and the authors suggested that *ALAD* polymorphism may modify lead kinetics but only at higher blood lead levels, which would be consistent with the *in vitro* study by Bergdahl et al. (*5*).

Recently, two studies investigated the modulating effect of *ALAD* polymorphism on both blood and bone lead in lead smelter

Table 4. Predictors of tibia lead (µg/g) among participants in the Normative Aging Study, 1991–1995, stratified by genotype.

	Model C		Model D	
	ALAD 1-1 (n = 580)		*ALAD 1-2/2-2* (n = 109)	
Variable	Parameter estimate	95% CI	Parameter estimate	95% CI
Intercept	18.64		19.51	
Age (years)	0.75	0.60–0.89	0.44	0.17–0.72
Education				
≤ High school	8.15	5.79–10.50	4.88	0.17–9.58
Some technical school	2.64	−0.78–6.07	3.69	−2.58–9.95
Missing	3.19	−2.10–8.49	3.61	−11.37–18.59
Cumulative smoking (pack-years)				
1–20	1.90	−0.90–4.69	3.79	−1.42–9.00
> 20	4.70	2.31–7.08	3.92	0.58–8.42
No information	−0.12	−5.12–4.88	−4.14	−14.27–5.99
Alcohol				
≥ 2 drinks/day	−0.97	−3.44–1.50	−1.63	−7.08–3.82
Vitamin D (IU/day)				
179–262	−4.58	−7.49–−1.66	−4.91	−11.49–1.67
262–375	−4.55	−7.81–−1.28	−6.50	−12.33–−0.68
375–589	−3.92	−7.16–−0.68	−3.26	−9.23–2.71
> 589	−3.14	−5.97–−0.30	−7.31	−13.26–−1.36
Total model R^2	0.28		0.22	

Table 5. Predictors of patella lead (µg/g) among participants in the Normative Aging Study, 1991–1995, stratified by genotype.

	Model C		Model D	
	ALAD 1-2 (n = 580)		*ALAD 1-2/2-2* (n = 109)	
Variable	Parameter estimate	95% CI	Parameter estimate	95% CI
Intercept	26.01		23.22	
Age (years)	0.91	0.71–1.12	0.83	0.42–1.23
Education				
≤ High school	9.69	5.79–10.50	12.44	5.58–19.30
Some technical school	2.98	−1.81–7.77	5.86	−3.79–15.50
Missing	11.12	−2.10–8.49	14.88	−3.50–33.27
Cumulative smoking (pack-years)				
1–20	2.42	−1.62–6.46	2.67	−4.96–10.30
> 20	7.36	3.71–11.02	4.34	−2.53–11.21
No information	4.72	−2.24–11.67	−8.07	−24.85–8.70
Alcohol				
≥ 2 drinks/day	1.84	−1.79–5.47	2.35	−6.05–10.75
Vitamin D (IU/day)				
179–262	−7.26	−11.40–−3.12	−5.76	−15.47–3.95
262–375	−5.11	−9.74–−0.47	−7.02	−15.75–1.71
375–589	−6.40	−11.30–−1.76	1.94	−6.93–10.82
> 589	−4.65	−9.20–−0.11	−6.37	−15.71–2.98
Total model R^2	0.19		0.27	

Table 6. Predictors of blood lead (µg/dL) among participants in the Normative Aging Study, 1991–1995, stratified by genotype.

	Model D		Model E	
	ALAD 1-1 (n = 580)		*ALAD 1-2/2-2* (n = 109)	
Variable	Parameter estimate	95% CI	Parameter estimate	95% CI
Intercept	5.12		3.48	
Patella lead (µg/g)	0.07	0.05–0.09	0.15	0.10–0.19
Genotype (*ALAD 1-1* vs. *ALAD 1-2/2-2*)				
Interaction				

Also in model: age, smoking, alcohol ingestion, vitamin C intake, iron intake.

Table 7. Predictors of blood lead (µg/dL) among all subjects in the Normative Aging Study, 1991–1995 (n = 689).

	Model A		Model B		Model C	
Variable	Parameter estimate	95% CI	Parameter estimate	95% CI	Parameter estimate	95% CI
Intercept	4.90		4.95		5.19	
Patella lead (µg/g)	0.08	0.07–0.10	0.08	0.07–0.10	0.07	0.06–0.09
Genotype (*ALAD 1-1* vs. *ALAD 1-2/2-2*)			−0.27	−1.05–0.50	−2.24	−3.83–−0.65
Interaction					0.06	0.02–0.11

Also in model: age, smoking, alcohol ingestion, vitamin C intake, iron intake.

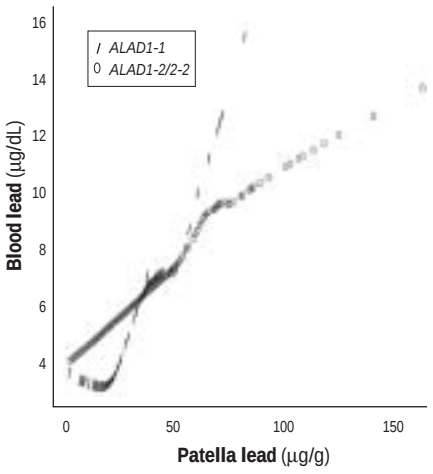

Figure 1. Smoothed line of blood lead versus patella lead by genotypes in 689 community-exposed men, with adjustment for age, alcohol consumption, current smoking, vitamin C intake, and iron intake: the Normative Aging Study, 1991–1995.

workers (*9*,*10*). The average blood lead levels in both groups of lead smelter workers were approximately 20–30 µg/dL, which is higher than in our study (mean, 6.2 µg/dL) (Tables 2 and 4). Bergdahl et al. (*9*) did not find any significant association between *ALAD* genotype and blood or bone lead levels among 89 lead-exposed workers. They also examined 34 unexposed workers whose median blood lead level was 4 µg/dL and found no difference in blood lead levels between 24 *ALAD 1-1* and 10 *ALAD 1-2* individuals. Because the sample size was small in this study, it is difficult to interpret these findings. However, the authors did find that the concentrations of urinary calcium and the ratio of urinary creatinine/serum creatinine were significantly lower in 7 *ALAD 1-2/2-2* than those of 82 *ALAD 1-1* lead workers [medians: urinary calcium (milligrams per liter) 76 vs. 188; the ratio urinary creatinine/serum creatinine 84 vs. 180]. This study suggested the presence of *ALAD* allele-specific differences in kidney function.

In another study, Fleming et al. (*10*) found the average blood lead levels were 22.9 ± 0.4 and 25.2 ± 1.0 µg/dL in 303 *ALAD 1-1* and 65 *ALAD 1-2/2-2* active lead workers ($p < 0.05$). But they did not find any significant difference in tibia bone lead by genotype (mean ± SD, 41.2 ± 1.8 in *ALAD 1-1* and 42.7 ± 3.4 in *ALAD 1-2/2-2*). In addition, they did not find any apparent difference in the contribution of bone lead (either tibia or calcaneus lead) to blood lead levels by genotype. However, using a cumulative blood lead index (CBLI) for each worker, on the basis of individual blood lead histories and bone lead measurements to estimate total lead body burden, they found in linear regressions that the slopes of bone lead to CBLI were steeper among *ALAD 1-1* workers. The more efficient uptake of lead from blood into the bone of workers with the *ALAD 1-1* genotype is consistent with our findings and suggests a decreased transfer of blood lead into bone in *ALAD 1-2/2-2* lead workers (*10*).

In our study, we found that patella lead is the major predictor of blood lead in this aging community-exposed population and that the *ALAD* polymorphism significantly modifies this association. When patella lead was > 60 µg/g, blood lead levels in *ALAD 2* carriers were higher than those in *ALAD 1-1* individuals. However, when patella lead was < 40 µg/g, blood lead levels in *ALAD 1-1* individuals were higher than that in *ALAD 2* carriers. Our results imply that when blood lead levels are relatively low (< about 8 µg/dL), *ALAD 1-1* individuals will have higher blood lead levels than *ALAD 2* carriers. This finding suggests that the modulating effect of the *ALAD* polymorphism on blood lead depends on the bone lead burden. Again, the finding is tentative and must be verified in other community-exposed population studies.

We conclude that the *ALAD* polymorphism may modify the exchange of lead between blood and bone. This, in turn, may modify an individual's ultimate risk for toxicity. Indeed, several studies have found that this *ALAD* polymorphism modifies indicators of possible lead toxicity, such as kidney function (*8*,*9*) and reproductive (*20*) and neuropsychologic function (*21*). The net effect on clinical function of the *ALAD-2* allele may be detrimental [e.g., on renal function, as seen by Bergdahl et al. (*9*) and Smith et al. (*8*)] or protective [e.g., on neuropsychologic function, as seen by Bellinger et al. (*21*) and on sperm count as seen by Alexander et al. (*20*)], possibly depending on the impact of this polymorphism on the tissue distribution of lead. Further research is needed to define precisely the mechanism of function and the potential impact of the *ALAD* polymorphism on lead kinetics and toxicity.

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