ORIGINAL ARTICLE

Delta-aminolevulinic acid dehydratase polymorphism and the relation between low level lead exposure and the Mini-Mental Status Examination in older men: the Normative Aging Study

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Occup Environ Med 2006;63:746–753. doi: 10.1136/oem.2006.027417

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Accepted 26 May 2006
Published Online First
6 June 2006

Objective: To determine whether a polymorphism in the δ -aminolevulinic acid dehydratase (ALAD) gene modifies the neurotoxicity of lead in older adults.

Methods: The authors studied men participating in the Department of Veterans Affairs' Normative Aging Study, assessing their recent exposure to lead by measuring blood lead ($n=915$) at each triennial clinic visit, and, beginning in 1991, assessing their cumulative exposure by measuring lead levels in tibia ($n=722$) and patella ($n=720$), using K-shell x ray fluorescence. Starting in 1993 and again at each triennial visit, the authors administered the Mini-Mental State Examination (MMSE) to assess their cognitive functioning. The relation of the lead biomarkers to MMSE score was evaluated and this association was compared among men who carried the variant allele, ALAD-2, versus men without the allele.

Results: Sixteen per cent of men carried the ALAD-2 allele. Median tibia and patella lead levels (first-third quartile) were 19 (13–28) and 27 (18–39) $\mu\text{g/g}$. Blood lead levels were consistent with non-occupational exposure: only 6% of men had levels $\geq 10 \mu\text{g/dl}$. In multivariable adjusted analyses, higher levels of blood lead were associated with poorer performance on the MMSE. This association was most pronounced among ALAD-2 carriers, among whom a 3 $\mu\text{g/dl}$ increment in blood lead (the interquartile range) was associated with a 0.26 point lower mean MMSE score (95% CI –0.54 to 0.01), compared with a 0.04 point lower score (95% CI –0.16 to 0.07) among non-carriers. The modest 0.22 point difference in these associations did not attain statistical significance, however ($p_{\text{interaction}}=0.13$). The associations between bone lead levels and MMSE score did not vary by ALAD-2 status.

Conclusions: Although not statistically significant, these findings suggest that ALAD genotype may modify blood lead's adverse association with cognition among older men who had community exposures to lead. However, despite a relatively large sample size and the use of sensitive methods for measuring lead burden, the evidence overall was fairly weak.

Lead is a neurotoxicant whose adverse effects on childhood neurological development appear even at low levels of exposure.¹ Lead is also neurotoxic to adults as documented by studies of high lead exposure in occupational settings,^{2–5} but the potential adverse effects of lower lead exposures experienced in community settings have not been extensively described. Of particular concern is lead's effect on decline in cognitive functions in older age. With the coming surge in the population of older adults in the United States, the number of people with Alzheimer's disease (AD) is expected to triple in 50 years,⁶ likely complemented by a rise in the number of individuals with other age related dementias. The causes of age related dementia are not well understood, but many factors probably contribute to its development.

Recent research suggests that relatively small increases in lead exposure may be associated with decrements in cognition in older age,^{7,8} but further research is required to confirm and elaborate upon these results. Specifically, genetic variation may render some people especially susceptible to lead's neurotoxicity, and identifying such heightened susceptibility is important both for better understanding of the aetiological relation between lead and cognition in older

adulthood and for more accurately defining the threshold of acceptable lead exposure. One potential source of genetic susceptibility lies in the gene that codes for δ -aminolevulinic acid dehydratase (ALAD), the enzyme that catalyses the second step in the production of heme. A polymorphism in exon 4 of the ALAD gene produces the ALAD-2 allele, which is codominant with the more common ALAD-1 allele. The ALAD-2 allele, present in 15%–20% of white populations,⁹ arises from a guanine-to-cytosine transversion in nucleotide 177.¹⁰ In the resulting enzyme subunit, the neutral asparagine replaces the positively charged lysine and, consequently, the resulting isozymes vary in their electronegativity, from the least electronegative 1-1 isozyme to the most electronegative 2-2 isozyme.

This gradient in electronegativity may underlie differences in susceptibility to lead's neurotoxicity, as lead may bind to the 2-2 isozyme with greater affinity than to the 1-2 isozyme and with still greater affinity than to the 1-1 isozyme.^{10,11} The neurotoxic consequences of such differential binding are

Abbreviations: ALAD, δ -aminolevulinic acid dehydratase; GAM, general additive model; GAMM, general additive mixed model; MMSE, Mini-Mental State Examination; NAS, Normative Aging Study

unclear, however. One hypothesis proposes that the electro-negativity of the 1-2 and 2-2 isozymes renders them “lead distributors”, differentially withholding lead from long term repositories, such as bone, and making it available to vulnerable soft tissue, such as brain. This distributor hypothesis implies that lead is more neurotoxic to *ALAD*-2 carriers (1-2 and 2-2 genotypes) than to people who are homozygous for *ALAD*-1 (1-1 genotype). A second hypothesis proposes the 1-2 and 2-2 isozymes act as “lead misers”, preventing the distribution of lead to soft tissue. This hypothesis would suggest that lead is less neurotoxic to *ALAD*-2 carriers.¹²

In addition, lead inhibits ALAD, causing an increase in ALAD's substrate, 5-aminolevulinic acid (ALA), which is itself neurotoxic.¹³ When exposed to lead, *ALAD*-1 homozygotes have larger elevations of plasma ALA than do *ALAD*-2 carriers.^{14–16} Hence, by this mechanism, *ALAD*-1 homozygotes would be expected to be more vulnerable than *ALAD*-2 carriers to the neurotoxicity of lead.

We previously reported a deleterious association between lead exposure and cognition in a large cohort of older men.^{7, 17} Building on this work, we conducted a study of *ALAD* genotype, non-occupational lead exposure, and cognitive function in the same cohort of older men. With long term cumulative exposure, lead may damage (or kill) the neuron by inducing oxidative damage and impairing cellular responses to oxidative damage. With acute exposure, lead likely interrupts calcium dependent neuronal functions, which may impair cognition in the short term and might possibly be reversible.⁷ Therefore, we investigated the interaction of *ALAD* genotype with both cumulative exposure to lead, as indicated by levels of lead in bone, and acute exposure to lead, as indicated by levels of lead in blood.

METHODS

This research was approved by the Human Subjects Committees of the Boston VA Medical Center, the Brigham and Women's Hospital, and the Harvard School of Public Health.

Study population

We studied a subgroup of participants from the Normative Aging Study (NAS), a longitudinal study of aging established in 1961 by the US Veterans Administration (VA) and based at the Boston VA Medical Center.¹⁸ The study enrolled 2280 men from the Greater Boston area who were 21–80 years old and who were free of known chronic medical conditions, including cancer, asthma, and elevated blood pressure. The men have undergone detailed evaluations every five years since their enrollment—and every three years since 1984—including collection of medical history information, routine physical examinations, laboratory tests, and completion of questionnaires on smoking history, education level, food intake, and other factors that may influence health. To date, the annual attrition due to all causes has been less than 1%, and more than 80% have responded to mailed questionnaires supplementing on-site examinations.

Study of cumulative exposure to lead (bone lead)

In 1991, we invited the 1171 men still being monitored by the NAS to take part in a study of lead exposure, as assessed by lead content in cortical and trabecular bone; 878 (75%) have now completed these assessments, but six of these men had cortical readings with uncertainty values greater than 10 µg/g bone and were excluded as unreliable, a standard protocol in the analysis of bone lead.¹⁹ Of the 872 remaining men, we had *ALAD* genotype data for 808 (93%). Cognitive assessments began in 1993, and these included the Mini-Mental State Examination (MMSE),²⁰ which is the focus of our

study. Of the men with reliable cortical bone lead readings and *ALAD* genotype data, 730 (90%) took the MMSE at least once; we had data pertaining to all covariates of interest for 722 of these men, who were included in our investigation of how *ALAD* genotype modifies the effect of cumulative lead exposure on cognition in older age. About three years later (SD 1 year), the MMSE was re-administered to 471 men who constituted the sample for our parallel exploratory analyses of cognitive change. The analysis of trabecular bone lead and baseline MMSE score comprised 720 men (two fewer than for the analysis of cortical bone lead); the corresponding analyses of change in MMSE score comprised 471 men.

Study of acute exposure to lead (blood lead)

We measured lead levels in blood specimens provided at the time of the cognitive testing. Because blood lead, with a half-life of 30 days, largely reflects recent exposure to lead, and because the neurocognitive effects associated with modestly elevated blood lead appear to diminish when the blood lead level is lowered,²¹ we surmised that blood lead levels would be most directly related to proximal cognitive ability. Therefore, of the NAS participants with *ALAD* genotype data, we identified 924 men who had blood lead measurements and baseline cognitive assessments within 30 days of each other. Of these, 915 had data pertaining to all covariates of interest; 564 had blood lead measurements at the time of their second cognitive examination, giving a total of 1479 observations in our investigation into how *ALAD* genotype modifies the effect of acute lead exposure on cognition in older age.

Assessment of lead exposure

Assessment of lead in bone

Beginning in 1991, those who gave their informed consent visited the Ambulatory Clinical Research Center of the Brigham and Women's Hospital for a K x ray fluorescence (KXRF; ABIOMED, Inc, Danvers, MA, USA) measurement of lead content in their mid-tibial shaft and patella. These sites are targets for bone lead research, because the tibia consists mainly of cortical bone, and the patella of trabecular bone. A technical description and the validity specifications of the KXRF instrument have been published elsewhere.^{22, 23}

The residence time of lead in trabecular bone lead varies by age and previous exposure, but in the NAS cohort it has been estimated to be eight years, while the residence time of lead in cortical bone is much longer.²⁴ Therefore, trabecular lead levels are likely to represent lead exposure over the past decade, while tibia lead levels likely represent lead exposure over the past two decades or longer. Due to increased bone resorption with age and decreased levels of lead in the environment, it is likely that older measures of tibia lead in these aging men are better indicators of lifetime lead exposure than more recent measures. Therefore, for participants who had more than one bone lead measurement, we analysed the earliest available measurement, a mean of 1.6 (SD 2.3) years before the MMSE.

Assessment of lead in blood

We analysed samples of whole blood for lead by graphite furnace atomic absorption with Zeeman background correction (ESA Laboratories, Chelmsford, MA, USA). Values below the minimum detection limit of 1 µg/dl were coded as 1 µg/dl divided by the square root of 2, that is, 0.71 µg/dl. The instrument was calibrated with National Institute of Standards and Technology Standard Reference Material (NIST SRM 955a, lead in blood) after every 20 samples. Ten per cent of samples were run in duplicate; at least 10% of the samples were controls, and 10% were blanks. In tests on reference samples from the Centers for Disease Control and

Prevention (Atlanta, GA, USA), precision [coefficient of variation] ranged from 8% for lead concentrations of 10–30 µg/dl to 1% for higher concentrations. Compared with an NIST target of 5.7 µg/dl, 24 measurements by this method gave a mean (SD) of 5.3 (1.23) µg/dl.

Determination of *ALAD* genotype

The *ALAD* gene is located on chromosome 9q32, and the reference SNP identification number for the polymorphism is rs1800435. We determined participants' *ALAD* genotype according to the methods described by Schwartz *et al.*²⁵ Specifically, we performed amplifications on 0.5 µl of whole blood using two sets of primers specific for a portion of the *ALAD* gene. Primer sequences for the initial round of amplification were (5'-AGACAGACATTAGCTCAGTA-3') and (5'-GGCAAAGAACACGTCCATTC-3'), which generate a 916 base pair fragment. To increase the amplification yield, which was inconsistent from this first amplification step when using whole blood, we performed a second round of amplification, using a nested pair of primers (5'-CAGAGCTGTTCCACAGTGGGA-3') and (5'-CCAGCACAATGTGGGAGTGA-3') which generate an 887 base pair fragment. Amplified DNA was then restricted using *MSPI* and electrophoresed through 2.0% agarose. *ALAD* alleles are differentiated based on the existence of a *MSPI* endonuclease restriction site specific to the *ALAD*-2-derived PCR fragment, which yields a diagnostic restriction band. Heterozygotes exhibit both the *ALAD*-1 and *ALAD*-2 fragments and can thus be differentiated from homozygotes of either type. We performed PCR reactions in duplicate, with blank controls included in each set.

Assessment of cognition

In 1993, cognitive assessments administered by trained interviewers became part of the NAS participants' triennial evaluations. One of the tests in the battery was the Mini-Mental Status Examination (MMSE),²⁰ a global examination of cognition that assesses orientation to place and time, memory, attention, language, and spatial abilities. In compiling the total score, we excluded the question on county ("What county are we in?") as counties in Massachusetts have little political significance and are generally unknown to residents, and thus not of diagnostic utility.²⁶ Thus, for this study, the maximum MMSE score was 29.

Statistical analyses

For all analyses, we classified the men as *ALAD*-2 carriers (1-2 and 2-2) or non-carriers (1-1). For each lead biomarker, we estimated the covariate adjusted mean difference in MMSE score associated with an increment in lead biomarker, and then compared this mean difference among *ALAD*-2 carriers versus non-carriers. A core requirement of using conventional linear regression models to analyse these data is that any association between lead biomarker and MMSE score is linear for both genotype groups. To determine whether the association among either *ALAD*-2 carriers or non-carriers between bone lead biomarker and MMSE score was linear, we modeled the lead biomarker as a penalised spline in a general additive model (GAM).²⁷ A penalised spline is a technique for flexibly modeling dose-response by dividing the range of exposure into intervals, and fitting a separate cubic polynomial within each interval. A penalty term is added to the log likelihood that is proportional to how "wiggly" the resulting dose-response curve is, which prevents excessive non-linearity. Further details have been published.²⁸ Then, using an F test, we contrasted the GAM results with those from a multiple linear regression model, where the bone lead biomarker was modeled as a single continuous term. (Small p

values from this test indicate non-linearity.) Our analyses of blood lead included repeated measures of MMSE for some men, and we applied an analogous procedure for the analysis of this biomarker, modeling blood lead as a penalised spline in a general additive mixed model (GAMM)²⁸ and contrasting it, using a likelihood ratio test, with a linear mixed effect model, where blood lead was modeled as a single continuous term. (In essence, penalised splines in GAMMs are an adaptation of penalised splines in GAMS to a setting that has repeated measures of an outcome.)

Interaction of *ALAD* genotype and lead in bone

Among non-carriers, we detected a trend toward non-linearity in the association between both bone lead measures and baseline MMSE score (p values from the F tests = 0.08 and 0.10 for tibia and patella, respectively). We proceeded by fitting conventional multiple linear regression models that included bone lead level as a continuous variable and a term for the cross product of bone lead and *ALAD*-2 carriership. In alternate analyses, however, we fitted GAMs where we modeled bone lead level as a penalised spline and included a cross product term between the spline and *ALAD* genotype.

For analyses of bone lead in association with change in MMSE score over about three years, we regressed the difference between the baseline and second scores on each bone lead level. In addition to the covariates listed, these models included a term for the amount of time between MMSE tests and, to improve the precision of the fitted estimates, a term for the residuals from a model of baseline MMSE as a function of bone lead and all covariates.⁷

Interaction of *ALAD* genotype and lead in blood

We did not detect significant deviations from linearity in the association between blood lead and MMSE score (all p values from the likelihood ratio tests >0.15). Therefore, we fitted mixed linear regression models that included blood lead level as a continuous variable and a term for the cross product of blood lead and *ALAD*-2 carriership.

Covariates

We adjusted our models for variables that were likely to confound the association between bone lead and MMSE score. To gauge the level of confounding in our data, we performed these adjustments in two stages, first adjusting for variables related to basic demographic characteristics and timing of the lead and cognitive assessments (Set 1), and then additionally adjusting for a group of variables related to health status and behaviour (Set 2). The variables in Set 1 were age at cognitive assessment and age-squared, years of education (≤ 8 , 9–11, 12, 13–15, 16, ≥ 17 years), computer experience (an additional measure of socioeconomic status), and length of time between the lead and cognitive assessments. For the analysis of blood lead, we also accounted for timing of the cognitive assessments (0 for the baseline MMSE, and years since the baseline MMSE for subsequent assessments). The variables in Set 2 were smoking status (current v past or never), alcohol consumption (none, 0.1–4.9 g/day, 5.0–9.9 g/day, ≥ 10 g/day, or missing), calorie adjusted calcium intake (in tertiles), regular energy expenditure on leisure time physical activity (in tertiles), and diabetes (physician diagnosed or fasting blood glucose >126 mg/dl). With the exception of age, which we computed directly, covariate values were determined from participants' self reports occurring at the time of lead assessment (smoking, diabetes, dietary variables) or cognitive assessment (education, computer experience). To reduce measurement error and instability in the measurement of alcohol consumption, calorie adjusted calcium intake, and physical activity, computation of these quantities was based on

Table 1 Mean lead biomarker levels by characteristics of the study participants

	Blood lead* (n = 915)		Tibia lead (n = 722)	
	n (%)	Mean (SD) blood lead, µg/dl	n (%)	Mean (SD) tibia lead, µg/g
ALAD genotype				
ALAD-2 carrier	149 (16.3)	4.8 (2.7)	122 (16.9)	21.0 (11.1)
Wild type	766 (83.7)	5.3 (2.9)	600 (83.1)	21.7 (13.5)
Age at lead measurement (years)				
45–59	91 (9.9)	4.5 (2.3)	115 (15.9)	15.2 (9.3)
60–64	208 (22.7)	4.9 (2.7)	170 (23.5)	18.4 (9.0)
65–69	233 (25.5)	5.3 (2.9)	205 (28.4)	22.2 (12.3)
70–74	215 (23.5)	5.4 (2.9)	131 (18.1)	25.7 (16.3)
75+	168 (18.4)	5.5 (3.1)	101 (14.0)	27.3 (15.1)
Education (years)				
<12	69 (7.5)	6.3 (2.9)	49 (6.8)	30.9 (18.0)
12–15	509 (55.6)	5.3 (3.0)	400 (55.4)	22.3 (13.4)
16+	337 (36.8)	4.7 (2.5)	273 (37.8)	18.7 (10.5)
Computer experience				
Yes	337 (36.8)	4.7 (2.5)	266 (36.8)	18.2 (9.9)
No	558 (61.0)	5.5 (3.0)	423 (58.6)	23.9 (14.5)
Missing	20 (2.2)	4.6 (2.6)	33 (4.6)	18.6 (10.3)
Smoking status				
Current	58 (6.3)	6.2 (3.4)	55 (7.6)	21.3 (11.5)
Past	600 (65.6)	5.2 (2.9)	450 (62.3)	22.2 (13.2)
Never	257 (28.1)	5.0 (2.7)	217 (30.1)	20.4 (13.2)
Alcohol consumption, g/day				
0	172 (18.8)	5.0 (2.7)	138 (19.1)	22.9 (13.0)
0.1–4.9	219 (23.9)	4.5 (2.6)	183 (25.3)	21.6 (12.9)
5–9.9	134 (14.6)	5.0 (2.6)	101 (14.0)	19.7 (11.6)
10+	390 (42.6)	5.7 (3.1)	300 (41.6)	21.5 (13.8)
Tertile of calcium intake (calorie adjusted)†				
Lowest (99–484 mg/day)	305 (33.3)	5.7 (2.8)	240 (33.2)	21.4 (12.9)
2nd (485–657 mg/day)	305 (33.3)	5.3 (3.1)	241 (33.4)	22.7 (14.3)
Highest (>656 mg/day)	305 (33.3)	4.6 (2.5)	241 (33.4)	20.5 (12.0)
Tertile of physical activity				
Lowest	303 (33.1)	5.5 (3.0)	239 (33.1)	22.9 (13.0)
2nd	305 (33.3)	5.0 (2.6)	242 (33.5)	21.7 (14.6)
Highest	307 (33.6)	5.1 (2.9)	241 (33.4)	20.1 (11.5)
Diabetes				
Yes	153 (16.7)	4.9 (2.8)	115 (15.9)	23.8 (13.1)
No	762 (83.3)	5.2 (2.9)	607 (84.1)	21.1 (13.1)

*Pertains to blood lead measured in first wave of measures.

†Ranges of intake in tertiles pertain to men in analysis of blood lead. Ranges are similar for men in the bone lead analyses.

responses to the participant's last three completed questionnaires.

In all models that included cross products of lead biomarker level and *ALAD*-2 carriership, the lead biomarker level was centered at the sample median to improve model stability. (Centering does not alter the overall interpretation of the results.) We conducted GAM and GAMM analyses using R version 2.01 (R Foundation for Statistical Computing, Vienna, Austria) and all other analyses using SAS version 9 (SAS Institute, Cary, NC, USA). We used $p < 0.05$ as the level of significance.

RESULTS

Levels of lead in bone varied widely: the median (and first and third quartiles) of tibia and patella were 19 (13, 28) and 27 (18, 39) µg/g. Still, blood lead levels were consistent with exposures in the community: only 6% of men had levels ≥ 10 µg/dl (mean (range) of the first wave of blood lead measurements: 5.2 (≤ 1 –28) µg/dl). The *ALAD* genotype distribution did not deviate from Hardy-Weinberg equilibrium ($p > 0.9$). In both the bone lead and the blood lead study populations, about 16% of men were *ALAD*-2 carriers. For example, in our blood lead cohort, 84% (766) were homozygous for *ALAD*-1, 15% (141) were heterozygous, and 1% (8) were homozygous for *ALAD*-2.

Bone lead levels tended to be higher among men who were older and had less formal education (table 1; patella results not shown). Blood lead levels varied similarly along these gradients, and in addition, tended to be higher among

current smokers and those who reported drinking at least 10 g/day of alcohol (about one drink). Both bone and blood levels were only slightly lower among *ALAD*-2 carriers than among *ALAD*-1 homozygotes (table 2).

Interaction of *ALAD* genotype and lead in bone

Generally, we found little evidence of an interaction between *ALAD* genotype and bone lead in association with MMSE score. In linear regression models adjusted for all covariates, baseline MMSE scores tended to be lower with higher levels of tibia and patella lead. Although this association was more pronounced among *ALAD*-2 carriers, it did not differ substantially by *ALAD* genotype ($p_{\text{interaction}} > 0.3$; table 3). Results from the GAM (non-linear) analyses were similar ($p_{\text{interaction}} > 0.3$). In exploratory adjusted linear regression analyses of change in MMSE score over three years, we found no evidence of an interaction between *ALAD* genotype and bone lead in relation to this outcome ($p_{\text{interaction}} = 0.8$ and 1.0 for tibia and patella, respectively; data not shown).

Interaction of *ALAD* genotype and lead in blood

In analyses adjusted only for Set 1 covariates, higher levels of blood lead were associated with significantly poorer performance on the MMSE (at baseline and at second assessment). While this adverse association was modest among *ALAD*-1 homozygotes, it was particularly pronounced among *ALAD*-2 carriers, among whom a 3 µg/dl increment in blood lead (equivalent to the interquartile range of blood lead among the men in our study) was associated with an average 0.29

Table 2 Characteristics of the participants in the blood lead analyses*, by *ALAD* genotype

	<i>ALAD</i> genotype	
	<i>ALAD 1-1</i> , n = 766	<i>ALAD-2</i> carriers, n = 149
Mean blood lead, $\mu\text{g}/\text{dl}$ (SD)	5.3 (2.9)	4.8 (2.7)
Mean patella lead, $\mu\text{g}/\text{g}$ (SD)†	30.8 (19.7)	29.7 (17.3)
Mean tibia lead, $\mu\text{g}/\text{g}$ (SD)†	21.7 (13.5)	21.0 (11.1)
Mean age, years (SD)	68.5 (7.3)	70.0 (7.4)
Education, n (%)		
<12 years	60 (7.8)	9 (6.0)
12–15 years	424 (55.4)	85 (57.0)
16+ years	282 (36.8)	55 (36.9)
Computer experience, n (%)		
Yes	281 (36.7)	56 (37.6)
No	466 (60.8)	92 (61.7)
Missing	19 (2.5)	1 (0.7)
Smoking status, n (%)		
Current	54 (7.0)	4 (2.7)
Past	501 (65.4)	99 (66.4)
Never	211 (27.5)	46 (30.9)
Mean alcohol consumption, g/day (SD)	14.0 (17.0)	10.2 (14.1)
Tertile of calcium consumption, n (%)‡		
Lowest (99–484 mg/day)	262 (34.2)	43 (28.9)
2nd (485–657 mg/day)	262 (34.2)	43 (28.9)
Highest (>656 mg/day)	242 (31.6)	63 (42.3)
Tertile of physical activity, n (%)		
Lowest	246 (32.1)	57 (38.3)
2nd	256 (33.4)	49 (32.9)
Highest	264 (34.5)	43 (28.9)
Diabetes, n (%)		
Yes	126 (16.4)	27 (18.1)
No	640 (83.6)	122 (81.9)

*Pertains to blood lead measured in first wave of measures.

†Pertains to men in the bone lead analyses.

‡Ranges of calcium intake in tertiles pertain to men in analysis of blood lead. Ranges are similar for men in the bone lead analyses.

point decrement in MMSE score (95% CI -0.56 to -0.02 ; table 3). This association was 0.24 unit greater (worse) than that among *ALAD-1* homozygotes, a difference of borderline significance (95% CI -0.53 to 0.05 ; $p_{\text{interaction}} = 0.10$). Additional adjustment for remaining covariates (Set 2) slightly attenuated the interaction between blood lead and the *ALAD* polymorphism. Among *ALAD-1* homozygotes, a 3 $\mu\text{g}/\text{dl}$ increment in blood lead was associated with an average 0.04 point decrement in MMSE score (95% CI -0.16 to 0.07). In our data, this decrement in MMSE corresponded to the mean difference in baseline MMSE scores in men who

were half a year apart in age. Among *ALAD-2* carriers, the corresponding average decrement in MMSE was 0.26 (95% CI -0.54 to 0.01), a decrement which, in our data, corresponded to that associated with men who were over three years apart in age. Although this blood lead-MMSE association was 0.22 point larger than that among *ALAD-1* homozygotes, the difference was not statistically significant (95% CI -0.51 to 0.07 ; $p_{\text{interaction}} = 0.13$; table 3). We performed additional analyses in which we fitted separate models for *ALAD-2* carriers and *ALAD-1* homozygotes and assessed the overlap of the confidence intervals for the lead term, using previously

Table 3 Lead biomarker levels in association with MMSE score, by *ALAD* genotype

	Adjusted for Set 1 covariates*		Adjusted for Set 2 covariates*	
	Mean difference in MMSE score per IQR in lead biomarker (95% CI)	$p_{\text{interaction}}$	Mean difference in MMSE score per IQR in lead biomarker (95% CI)	$p_{\text{interaction}}$
Tibia lead (n = 722; IQR = 15 $\mu\text{g}/\text{g}$)				
Among <i>ALAD-2</i> carriers	-0.20 (-0.62 to 0.22)		-0.16 (-0.58 to 0.27)	
Among <i>ALAD</i> wildtypes	-0.07 (-0.23 to 0.10)		-0.05 (-0.21 to 0.12)	
Difference in estimates†	-0.13 (-0.58 to 0.31)	0.55	-0.11 (-0.55 to 0.33)	0.63
Patella lead (n = 720; IQR = 21 $\mu\text{g}/\text{g}$)				
Among <i>ALAD-2</i> carriers	-0.28 (-0.66 to 0.11)		-0.26 (-0.64 to 0.12)	
Among <i>ALAD</i> wildtypes	-0.08 (-0.24 to 0.07)		-0.07 (-0.23 to 0.09)	
Difference in estimates†	-0.19 (-0.60 to 0.21)	0.35	-0.19 (-0.59 to 0.21)	0.35
Blood lead (n = 915; IQR of 3 $\mu\text{g}/\text{dl}$)				
Among <i>ALAD-2</i> carriers	-0.29 (-0.56 to -0.02)		-0.26 (-0.54 to 0.01)	
Among <i>ALAD</i> wildtypes	-0.05 (-0.16 to 0.06)		-0.04 (-0.16 to 0.07)	
Difference in estimates†	-0.24 (-0.53 to 0.05)	0.10	-0.22 (-0.51 to 0.07)	0.13

*Set 1: age, age², education, experience with computers, time between lead assessment and cognitive assessment, and, for the blood analyses, time between cognitive measures. Set 2: all variables in Set 1 and physical activity, calorie adjusted calcium intake, alcohol intake, diabetes, and smoking status.†The 95% CI for the difference in estimates and the $p_{\text{interaction}}$ are complementary statistics in that they both test for equivalence in the lead-MMSE associations across *ALAD* groups.
IQR, interquartile range.

developed statistical methods;²⁹ the blood lead-MMSE associations and statistical significance of the difference between them were identical.

DISCUSSION

In this study of 915 older, community dwelling men, we investigated how *ALAD* genotype modifies the relation between low level lead exposure and cognition. Although our results did not reach statistical significance, they did suggest a modestly more deleterious association between blood lead and general cognition among *ALAD*-2 carriers than among *ALAD*-1 homozygotes. By studying a population of non-occupationally exposed individuals, not only have we examined the lower end of the lead exposure spectrum, but our study is less likely to be affected by selection biases present in some occupational studies.^{25–30}

Several limitations of our study nevertheless merit consideration. First, our study population is small for detecting gene/environment interactions of modest magnitude. Its size also renders it vulnerable to the influence of extreme MMSE scores, extreme lead biomarker values, or both. Nonetheless, our study is among the largest to date to examine the interaction of *ALAD* genotype and lead exposure in relation to cognition. Although the interaction between *ALAD* genotype and blood lead was not statistically significant after adjusting for potential confounders, additional research among larger study populations, including women, might be able to shed further light on whether this constitutes a true gene/environment interaction and, if so, identify its underlying mechanism and determine its public health implications. Larger studies may also further elaborate any interaction between bone lead and *ALAD* in relation to cognition, if such an interrelation exists.

A second limitation to our study is the use of the MMSE as a global test of cognition. Imperfect reproducibility and validity in the MMSE diminishes the statistical precision of our analyses. Although it cannot stand alone as a diagnostic tool, the MMSE is among the most extensively characterised and most widely used tests of cognitive status for older adults. Moreover, given the practical constraints of a large population study, the MMSE performs with a reasonable degree of reproducibility and validity. Past studies have found high correlations between scores from administrations of the MMSE up to 64 days apart ($r = 0.38$ to 0.98 , with most between 0.70 and 0.95);^{26–31} high correlations between MMSE scores and scores on other well described cognitive tests, such as the Blessed Information-Memory-Concentration test;²⁶ and reasonable sensitivity and specificity in delineating individuals with and without dementia.^{26–31}

Measurement error in our lead biomarkers is, in all likelihood, non-differential with respect to mental status and *ALAD* genotype, and thus, we would expect it to attenuate the estimates of lead's association with MMSE score and, possibly, estimates of the lead-*ALAD* genotype interaction as well.

Two small studies have examined *ALAD* genotype in relation to cognition. A study of 72 young adults, aged 19–20 years, reported generally better performance on tests of cognition and intelligence among *ALAD*-2 carriers than among non-carriers, after adjustment for levels of lead in dentin (teeth). The investigators qualified these results as preliminary, however, as only five participants were *ALAD*-2 carriers, and the study was not powered to detect differences between carriers and non-carriers.³²

In a cross sectional study of 120 men, aged 21–63 years, who worked at two lead stabiliser factories in South Korea, *ALAD*-2 carriers performed significantly better than *ALAD*-1 homozygotes on three out of eight tests of psychomotor function ($p < 0.04$), after adjustments for age, duration of

occupational exposure to lead, and blood lead level.¹⁴ Participants in this study generally had blood lead levels reflecting occupational exposure: the mean blood lead level was $22.1 \mu\text{g/dl}$, with levels ranging from 3.9 to $51.3 \mu\text{g/dl}$. Thus, although the analyses did not explicitly describe the joint effect of *ALAD* genotype and lead exposure on cognition, the results generally describe the variant allele's association with cognition among men with moderate to high levels of lead exposure. Nonetheless, only 11% of participants ($n = 13$) in this study were *ALAD*-2 carriers, suggesting that these results should be interpreted with caution.

In a small case control study of essential tremor, another neurological disorder, the deleterious association between blood lead levels and essential tremor was markedly more pronounced among *ALAD*-2 carriers.³³ A one tertile increase in blood lead (about $0.25 \mu\text{g/dl}$) corresponded to an 80-fold increase in the odds of essential tremor among *ALAD*-2 carriers (95% CI 3.1 to 2096.4) versus a tripling of odds among non-carriers (95% CI 0.6 to 11.8).

The interaction of *ALAD* genotype and lead has been investigated in relation to renal, reproductive, and cardiovascular outcomes. In a study of renal outcomes in the NAS, the positive (deleterious) association between bone lead and serum creatinine was significantly steeper among *ALAD*-2 carriers, significantly so with respect to tibia lead ($p_{\text{interaction}} = 0.025$). *ALAD*-2 carriers also had a steeper positive association between bone lead and serum uric acid ($p_{\text{interaction}} = 0.09$ and 0.08 for patella and tibia lead, respectively). These results suggest that the *ALAD* genotype may modulate internal lead distribution, in particular, between bone lead compartments and vulnerable soft tissue compartments such as the kidney. No interactions were found between *ALAD* genotype and blood lead.³⁴ Studies among lead workers found no interactions between *ALAD* genotype and lead biomarkers in association with blood pressure,³⁵ as well as sperm count and concentration.³⁶

Findings from several in vitro and in vivo studies suggest that lead is differentially neurotoxic to *ALAD*-2 carriers. Although the inhibition of ALAD by lead is well described,³⁷ data from an animal model suggest that ALAD is also an effective distributor of lead to soft tissues. Claudio and colleagues³⁸ studied two laboratory strains of mice that differ in their expression of the *ALAD* gene; mice from the DBA/2J strain produce twice the amount of ALAD as do mice from the C57BL/6J strain. When exposed to set amounts of lead, mice from the high ALAD production strain exhibited levels of lead in their blood, kidney, liver, and brain that were over twice those found in the mice from the low production strain.^{12–38} Blood levels of zinc protoporphyrin (ZPP) were also associated with blood lead levels among mice in the low ALAD production strain, but not among mice in the high production strain. In addition to inhibiting ALAD, lead also inhibits ferrochelatase, the last enzyme in the production of heme, and ZPP is a byproduct of this inhibition. The investigators interpreted the difference in the lead-ZPP association as evidence that if more ALAD is available, it will bind to lead, leaving little lead available to inhibit ferrochelatase. Although this mouse model is not directly analogous to the *ALAD* genotype framework found in humans, it offers insights on ALAD's role as lead distributor. If the *ALAD*-2 isozyme is more effective in this role, whether due to its differential affinity for lead or upregulation in the face of inhibition, then one would expect greater accumulation of lead in the soft tissues of *ALAD*-2 carriers.

In parallel to this mouse study, human studies have generally found higher blood lead levels among *ALAD*-2 carriers than among non-carriers.^{9–39–40} Moreover, several studies have reported higher levels of ZPP among *ALAD*-1 homozygotes than among *ALAD*-2 carriers,^{15–25–41} and a study

of 124 male workers observed a steeper blood lead-ZPP gradient among *ALAD-1* homozygotes than among *ALAD-2* carriers ($p_{\text{interaction}} < 0.06$).³⁶

Nonetheless, several lines of evidence contradict an influence of *ALAD* genotype on neuropsychological susceptibility to lead. In the only study to examine protein bound lead in erythrocytes by *ALAD* genotype, 84% of protein bound lead was bound to ALAD in the *ALAD-2* carriers, and 81% of protein bound lead was bound to ALAD in *ALAD-1* homozygotes.⁴² Although this difference is consistent with the isozymes' gradient in electronegativity, it seems unlikely that a difference of this magnitude has marked implications for differences in the distribution of and susceptibility to lead. In addition, significant differences in ALAD activity for a given blood lead level have not been observed by *ALAD* genotype,^{14 15 43} but such a relation would be difficult to establish in humans if *ALAD* genotype affects blood lead level. Indeed, a preponderance of data suggests that *ALAD-2* carriers have significantly higher levels of blood lead, although some investigations, the present study included, have not observed such a difference.^{14 15 43 44}

Moreover, the available data do not support enhanced susceptibility of *ALAD-2* carriers to lead due to greater build up of ALA, a neurotoxin. Levels of ALA in plasma and urine have generally been found to be lower in *ALAD-2* carriers than in non-carriers,^{14 15 43 45} and one small study of male lead workers observed no difference between *ALAD-2* carriers and non-carriers in the blood lead-ALA association.¹⁶ The molecular and cellular basis for differential susceptibility continues to be researched and debated and is likely more complex than mere differences in the isozymes' electronegativity (see, for example, Jaffe *et al*⁴⁶).

Still, the divergent evidence described may not indicate the absence of differential neurological susceptibility as much as it indicates a need for research that addresses the methodological limitations of previous research—such as small sample sizes, selection bias, and cross sectional designs—and that more sharply focuses on the hypothesised causal mechanisms. For example, a study of 142 adults found plasma lead levels among *ALAD-2* carriers that were over twice those among non-carriers, even though lead levels in whole blood were similar across the two groups,⁴⁷ suggesting that plasma lead, rather than whole blood lead, is critical to the interplay between lead exposure and *ALAD* genotype. Further, if the *ALAD-2* isozyme more effectively distributes lead to the brain partially by partitioning lead to plasma, then plasma lead level is a potential causal intermediate between *ALAD* genotype and cognition, and as such, the interaction between *ALAD* genotype and lead should be evaluated with respect to a marker of external rather than internal exposure to lead.

In conclusion, in our study of 915 older, community dwelling men, we observed a suggestion that the association between blood lead and cognition is more deleterious among *ALAD-2* carriers than among *ALAD-1* homozygotes, although this distinction did not attain statistical significance. Our results did not support an interaction between bone lead and *ALAD* genotype in relation to MMSE score and change in MMSE score.

Main message

- Older adults with the *ALAD-2* allele may be more susceptible to the adverse effects of blood lead on cognitive function, even at low levels of exposure.

ACKNOWLEDGEMENTS

The views expressed in this paper are those of the authors and do not necessarily represent the views of the US Department of Veterans Affairs. This research was primarily supported by NIH ES R01-ES05257, P42 ES05947, and NIEHS Center Grant 00002. Participants were evaluated for bone lead levels in the outpatient Clinical Research Center of the Brigham and Women's Hospital with support from NIH grant no NCRR GCRC M01RR02635. The KXRF instrument used in this work was developed by ABIOMED, Inc, of Danvers, Massachusetts, with support from NIH grant no SBIR 2R44 ES03918-02. The core and cognitive data were collected under the auspices of the VA Normative Aging Study as well as the Cognition and Health in Aging Men Project (CHAMP) with support from the Research Services and the Cooperative Studies Program/ERIC of the US Department of Veterans Affairs, the Massachusetts Veterans Epidemiology Research and Information Center (MAVERIC), the National Institutes of Health (grants R01-AA08941, R01-AG13006, R01-AG14345, R01-AG18436), and the US Department of Agriculture, Agricultural Research Service (contract 53-K06-510).

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Competing interests: none.

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