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Interaction of the δ -aminolevulinic acid dehydratase (ALAD) polymorphism and lead burden on cognitive function: the VA Normative Aging Study

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Abstract

Objective—We evaluated the modifying influence of a δ -aminolevulinic acid dehydratase (ALAD) polymorphism on the relation between lead burden and cognition among older men.

Methods—Information on ALAD genotype, lead measurements, potential confounders, and cognitive testing was collected from 982 participants. For each cognitive test and lead biomarker, we fit separate multiple linear regression models which included an interaction term for ALAD genotype and the lead biomarker, and adjusted for potential confounders.

Results—With higher levels of tibia lead, ALAD 1-2/2-2 carriers exhibited worse performance on a spatial copying test in comparison with ALAD 1-1 carriers ($p_{\text{interaction}}=0.03$). However, there was no consistent pattern of an ALAD genotype-lead interaction for the other tests.

Conclusions—The results provide some evidence that ALAD genotype modifies the relation between lead burden and cognition among older men with low lead burden, particularly for cognitive domains sensitive to the effects of cumulative lead burden.

Keywords

blood; bone and blood; lead; environmental; polymorphism; genetic; cognition; neuropsychological tests

INTRODUCTION

Occupational exposures to lead are associated with impaired cognitive function.^{1–5} Recent research also suggests that, among older adults, decline in cognitive function at the sub-clinical level can be accelerated by low-level cumulative lead burden.⁶ These effects are potentially influenced by current exogenous lead exposures, existing body burden, and genetic factors. Among environmental lead-exposed populations (<10µg lead/dL blood), Shih et al. observed cross-sectionally that tibia lead (representative of cumulative lead burden) was consistently associated with worse performance for tests in several cognitive domains.⁷ In a longitudinal study of elderly men, we observed a greater decline over time on multiple cognitive tests—particularly for tests assessing visuospatial abilities—in relation to both higher patella (potentially representative of bioavailable lead in bone) and tibia lead levels.⁸

As part of understanding the association between lead burden and cognition among adults it would be of particular interest to identify people who may have heightened vulnerability to lead-related neurotoxicity, for example, because of their genotype. One gene that has been of interest, due to its product's biologic interplay with lead, is the gene encoding δ-aminolevulinic acid dehydratase (ALAD), the second enzyme on the heme synthesis pathway, and principal lead binding protein in erythrocytes. The ALAD enzyme is polymorphic (ALAD 1-1, ALAD 1-2, and ALAD 2-2) because of a G-to-C transversion of nucleotide 177 in *exon 4* of the *ALAD* gene⁹ (rs1800435) that renders the ALAD 1-2 and ALAD 2-2 isoenzymes more electronegative than the ALAD 1-1 isoenzymes. This charge differential may provide ALAD 1-2 and ALAD 2-2 isoenzymes with an enhanced affinity for lead, binding lead more tightly than ALAD 1-1 isoenzymes and altering lead bioavailability.¹⁰ A limited understanding of the *ALAD* polymorphism's influence on endogenous lead distribution resulting from this postulated difference in affinity for lead may contribute to the variable findings reported for the *ALAD* polymorphism interacting with lead in association with renal and neurobehavioral function.^{11–18}

The purpose of this study was to evaluate the effect of the *ALAD* polymorphism on the relation between current and cumulative lead levels and cognition among NAS participants. Building upon our prior analysis of the *ALAD* polymorphism interaction with lead in relation to psychiatric symptoms¹⁸, we anticipated that the inverse dose-response between bone and blood lead and cognitive test performance would be steeper among men with the wild-type genotype (*ALAD 1-1*) than among men carrying the variant allele (*ALAD 1-2/2-2*), reflecting the increased retention of lead bound to ALAD 1-2/2-2 isoenzymes in blood.

MATERIALS AND METHODS

Study Population and Design

The Normative Aging Study, (NAS) begun in 1961, is an on-going longitudinal study of aging established by the U.S. Veterans Administration.¹⁹ NAS participants are likely to have had only modest exposures to lead, similar to current overall population exposures in the US. The NAS cohort has been described in detail elsewhere.^{20,21} Briefly, the original cohort, recruited between 1961 and 1970, consisted of 2,280 community-dwelling men from

the Greater Boston, Massachusetts, area, who were 21 to 80 years old at the time of enrollment in the study. Prior to study onset, candidates for participation with known chronic medical conditions (i.e., history of hypertension, heart disease, diabetes, cancer, peptic ulcer, gout, recurrent asthma, bronchitis, or sinusitis) were excluded. At 3-year intervals, participants have undergone reevaluations including routine physical examination and laboratory tests, and self-reported information on medical history, smoking history, dietary intake, and other factors influencing health has been collected. Over the life of the study, the rate of attrition from all causes has been less than 1% per year. 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.

The analyses reported here focused on a subgroup of the NAS cohort for whom bone and blood lead, *ALAD* genotype, and cognitive function assessments were complete. A participant was included in the study if he had at least one cognitive test score, one lead biomarker measurement, *ALAD* genotype status, and information on age and education. In 1991, we invited the 1171 men still being monitored by the NAS to take part in a study of lead exposure. Between 1993 and 2001, 1102 NAS participants completed at least one cognitive test, and of these men 988 were genotyped for the *ALAD* polymorphism. Three men missing data on educational status, an important covariate, were excluded from analysis. A total of 982 men were used in our main analysis of the interaction between *ALAD* genotype and lead in relation to cognitive function. Blood lead measurements and bone lead measurements within acceptable uncertainty values were available for 978 and 741 men, respectively. Of the 1178 men without cognitive test scores, 433 were deceased and 407 were no longer visiting the VA for examinations in 1993. Between 1993 and 2001, 250 additional men died and 67 men visited the VA without completing a cognitive test.

Blood Lead Measurements

Measurement of blood lead levels in the NAS cohort began in 1988, and was repeated at the participant's subsequent examinations. For the present study we identified the blood lead measurement that was closest to each man's baseline cognitive assessment; 94% of fresh blood samples were collected on the same date as cognitive testing. Samples were sent for analysis to ESA Laboratories, Inc. (Chelmsford, MA) and analyzed by Zeeman background-corrected graphite furnace atomic absorption (GF-AAS). After every 20 samples, the GF-AAS instrument was calibrated with National Institute of Standards and Technology Standard Reference Material (NIST SRM 955a, lead in blood). Ten percent of samples were run in duplicate; at least 10% of the analyses were controls and 10% were blanks. In tests on reference samples from the Centers of Disease Control and Prevention (CDC), the coefficient of variation (i.e. precision) ranged from 8% for concentrations below 30 µg/dL to 1% for concentrations higher than 30 µg/dL. The detection limit for this method is 1 µg/dL, and blood lead levels below the detection limit were set to zero.

Bone Lead Measurements

Beginning in 1991, and at the subject's follow-up examinations, we used a K-x-ray fluorescence (KXRF) instrument (ABIOMED, Inc., Danvers, MA) to assess lead levels in participant's cortical (tibia) and trabecular (patella) bone measures of time-integrated lead dose. For each man, we identified the bone lead measurement closest to his baseline cognitive assessment; on average, tibia and patella lead levels were measured 3.6 months prior to the baseline cognitive assessment. A 30-minute measurement was taken at the midshaft of the left tibia and at the left patella after each region was washed with a 50% solution of isopropyl alcohol. The KXRF beam collimator was sited perpendicular to the bone surface, providing an unbiased point estimate of bone lead levels that is normalized for bone mineral content as µg lead/g bone mineral.²² In addition, an estimate of uncertainty

(equivalent to a single standard deviation if multiple measures were taken) was provided for each measurement that is derived from a goodness-of-fit calculation and counting statistics of the spectrum curves. We excluded from analysis ten bone lead readings had high uncertainty estimates (greater than 10 $\mu\text{g/g}$ tibia bone and 15 $\mu\text{g/g}$ patella bone, respectively), a standard protocol in the analysis of bone lead. Technical details and validity specifications of the KXRF instrument have been previously described.²³

ALAD Genotyping

The *ALAD* polymorphism in *exon 4* (reference single-nucleotide polymorphism identification number 1800435) was determined by polymerase chain reaction (PCR) with restriction fragment length polymorphism, according to previously described methods.²⁴ In brief, modified PCRs were performed sequentially, and in duplicate with blank controls included in each set, on 0.5 μl whole blood by using nested primers. Reactions were completed using 1 unit of *Taq* polymerase in a buffer containing 300ng of each primer. The initial amplification, using 3' and 5' oligonucleotide primers, generated a 916-base-pair fragment; a second round of amplification using a pair of nested primers, generated an 887-base-pair fragment. This fragment was cleaved at the diagnostic *MSP*I endonuclease restriction site, was electrophoresed, and was visualized by fluorography.

Assessment of Cognitive Function

The cognitive test battery assessed the following cognitive domains: visuospatial abilities, attention, general intelligence, executive function, visual memory, verbal memory, working memory, language, and perceptual speed. The cognitive test battery included tests from the Consortium to Establish a Registry for Alzheimer's disease (CERAD), the Neurobehavioral Evaluation System (NES2), and the Wechsler Adult Intelligence Scale-Revised (WAIS-R).^{25–27} We administered the CERAD tests for constructional praxis (visuospatial), verbal fluency (executive function), Boston naming (language), and the word list memory (immediate and delayed verbal memory); the NES2 battery tests for pattern comparison (visuospatial and perceptual speed), continuous performance (perceptual speed), and pattern memory (visual memory and perceptual speed); and the WAIS-R digit span backward (working memory) and vocabulary (general intelligence) tests. Details of each test have been previously described.²⁰

Data Analysis

Since less than 1% of the study population were homozygous for *ALAD* 2-2, we combined men of *ALAD* 1-2 and 2-2 genotype for all analyses. The distributions of cognitive test scores were examined for departures from normality. We evaluated the association between each lead biomarker and cognitive test score for deviation from linearity, using R software²⁸ to create generalized additive models (GAMs) that included smoothing parameters for lead biomarker variables. To avoid biased standard errors previously reported for earlier versions of GAMs, we used penalized splines to estimate nonlinear associations, with the penalty estimated using generalized cross validation criteria.²⁹ If smoothing parameters for lead biomarkers in relation to cognitive test scores were significant at a 95% level of confidence, their difference from a model with a linear lead biomarker term was assessed with a likelihood ratio test.

For associations between lead and cognitive test score that did not significantly deviate from linearity, we used multiple linear regression models to estimate the adjusted mean differences in cognitive scores per increment in lead biomarker concentration, and to further determine whether these mean differences varied by *ALAD* genotype. We fit separate multiple linear regression models for each cognitive test and lead biomarker (blood, tibia, and patella). For ease in comparison, we converted all cognitive test scores into z-scores.

(An individual's *z* score for a given cognitive test is the number of standard deviations his raw score is from the study population mean score.) Some test scores measured time to response (continuous performance, pattern memory, pattern comparison) such that longer time represented worse performance. We inverted these scores (subtracting them from zero) so that a positive score indicates better test performance.

All of our regression models included a term for the main effect of the *ALAD* genotype (*1-2/2-2* vs. *1-1*), and adjusted for variables identified in previous studies^{8,19,20,30} as potential confounders of the lead-cognition association, including age at cognitive test, education (less than high school, high school, some college, college and/or post-graduate studies), alcohol consumption (0 g/day, 0.1 to 11.2 g/day, >11.2 g/day; note that 1 standard drink = 10 g of alcohol), cumulative smoking (pack-years), English as a first language. In addition, we adjusted analyses of performance on computer-based cognitive tests (continuous performance, pattern memory, and pattern comparison) for computer experience. A few men lacked data on alcohol, cumulative smoking, English as a first language, or computer experience, consequently we included missing indicator terms for these variables in models to maximize the number of subjects who could be included in the analyses. We also conducted analyses excluding these participants with missing data to verify that the missing indicators did not bias our parameter estimates. Additional adjustment for potential confounders, including current/former smoking status, income, physical activity, and diagnosis of diabetes and/or coronary heart disease did not substantially affect the magnitude of lead coefficients.

To assess whether the *ALAD* genotype modified the relation of blood or bone lead to cognitive test performance we introduced a cross-product term for the interaction between *ALAD* genotype status (*1-2/2-2* vs. *1-1*) and lead biomarker into each model. We estimated the covariate-adjusted difference between the two genotype groups' cognitive scores per interquartile range (IQR) of the lead biomarker by multiplying the IQR by the cross-product regression parameter estimate.

Due to the large number of cognitive outcomes, in tandem with the three lead biomarkers, we were concerned about chance findings. In an alternative approach to analyzing our data, we created summary outcome scores based on an exploratory factor analysis of the 13 cognitive test scores. This procedure, which accounted for the correlations among some of the test scores, identified a likely set of cognitive domains – or factors – represented in the test battery scores.³¹ The principal factor method was used to extract the factors, followed by a promax (i.e. oblique) rotation. We computed factor scores for each participant by multiplying each of his test scores by its corresponding factor loading (for the given factor), and then summing the products for all 13 tests. We then evaluated the relation of lead and *ALAD* genotype to these derived factor scores using multiple linear regression as described previously for the individual cognitive tests.

RESULTS

The prevalences of *ALAD 1-1*, *ALAD 1-2*, and *ALAD 2-2* genotypes were 83% (818), 16% (155), and 1% (9), respectively. Age, education, computer experience, English as a first language, cumulative smoking, and lead biomarker levels, were similar among *ALAD 1-2/2-2* and *ALAD 1-1* carrier groups (Table 1). *ALAD 1-1* carriers were more likely than *ALAD 1-2/2-2* carriers to be current smokers (7% vs. 2.4%) and, on average, consumed more alcohol (13.4g/day vs. 9.6 g/day). As expected of a community-exposed population, participants' blood lead levels were low, with a mean (SD) blood lead level of 5.2 (2.9)µg/dL. Blood and bone lead levels among *ALAD 1-1* carriers were slightly higher than those of *ALAD 1-2/2-2* carriers.

We observed significant non-linearity in the associations between blood lead and vocabulary test performance, blood lead and pattern memory (response latency), and tibia lead and pattern comparison (total number correct). Log-likelihood ratio tests of the difference between smoothing parameters and linear lead biomarker terms indicated that linear regressions for these four associations adequately fit the data for these models. Therefore, bone and blood lead were retained as linear terms in subsequent regression analyses.

Higher tibia lead levels among participants with the *ALAD* 1-2/2-2 genotype were associated with significantly worse performance on the constructional praxis (spatial copying) test, while this analogous association was comparably muted among participants with the *ALAD* 1-1 genotype ($p_{\text{interaction}}=0.03$; Table 2). Among men who were *ALAD* 1-2/2-2 carriers, an inter-quartile increment (comparing the 75th percentile with the 25th percentile of lead biomarker concentration) in tibia lead (15 $\mu\text{g/g}$ bone mineral) was associated with a 0.28 SD (95% CI: -0.51, -0.04) decrement in constructional praxis z score. In contrast, among *ALAD* 1-1 carriers, a one-IQR increment in tibia lead corresponded to a 0.03 SD (95% CI: -0.12, 0.06.) decrement in constructional praxis z score. No consistent evidence of an interaction between *ALAD* genotype and tibia lead was observed for the remaining 12 cognitive tests.

Higher patella lead levels among participants with the *ALAD* 1-2/2-2 genotype were associated with better performance on the pattern comparison response test (perceptual speed), while this association was much smaller in participants with the *ALAD* 1-1 genotype ($p_{\text{interaction}}=0.05$; Table 2). Among men who were *ALAD* 1-1 carriers, an inter-quartile increment in patella lead (20 $\mu\text{g/g}$ bone mineral) was associated with a 0.02 SD (95% CI: -0.06, 0.10) increase in pattern comparison response z score. Among men who were *ALAD* 1-2/2-2 carriers, a similar increase in patella lead was associated with a 0.23 SD (95% CI: 0.02, 0.44) increase in pattern comparison response z score. We did not observe a consistent *ALAD* genotype interaction with patella lead in association with performance on the remaining 12 tests.

We found evidence suggesting that *ALAD* genotype might modify the association between blood lead and vocabulary test score ($p_{\text{interaction}}=0.06$; Table 2). Among men who were *ALAD* 1-2/2-2 carriers, an inter-quartile increment in blood lead was associated with a 0.33 SD (95% CI: -0.55, -0.10) decrease in vocabulary z score. Among men who were *ALAD* 1-1 carriers, the same increment in blood lead was associated with a 0.12 SD (95% CI: -0.22, -0.02) decrease in vocabulary z score. We did not observe a consistent *ALAD* genotype interaction with blood lead in association with performance on the remaining 12 tests.

A covariate term for *ALAD* genotype status (1-2/2-2 vs. 1-1) included in all models without an interaction term did not independently predict cognitive test performance after adjustment for potential confounders (data not shown).

Three factors were extracted and retained for oblique rotation in the exploratory factor analysis of the 13 test scores. Cognitive tests exhibiting a factor loading 0.35 or greater were considered to load on the particular factor. Three tests (verbal fluency, word list total, vocabulary) assessing verbal abilities loaded on the first factor. One test (digit span backward) assessing working memory loaded on the second factor. Two tests (pattern comparison and pattern memory response latencies, respectively) assessing perceptual speed abilities loaded on the third factor. The inter-factor correlations between the first and second factor, the first and third factor, and the second the third factor were 0.37, -0.37, and -0.24, respectively. In analyses adjusted for potential confounders, none of the three lead biomarkers was significantly associated with any of the three factor scores (data not shown)

nor were statistically significant interactions observed between the lead biomarkers and ALAD genotype for the three factor scores.

DISCUSSION

In this study of 982 older men, we evaluated the modifying influence of *ALAD* genotype on lead-induced neurotoxicity across multiple cognitive domains in community exposed individuals. We found no clear pattern of *ALAD* genotype modifying the relation between blood and bone lead and cognition. However, the cognitive tests where we observed a *ALAD*-bone lead interaction (construction praxis and pattern comparison) were the same tests that were significantly associated with cumulative lead burden in our longitudinal analysis in these same men.⁸ Similarly, among community exposed adults Shih et al. also observed that bone lead (measured in tibia) remained significantly associated with the visuoconstruction domain score after adjustment for race/ethnicity and wealth in addition to other covariates.⁷

There has been limited investigation how *ALAD* polymorphism might modify lead-induced neurotoxicity, particularly among environmentally exposed individuals. In a study of attentional correlates of dentin and bone lead levels among adolescents, Bellinger et al. observed that 5 participants with the *ALAD 1-2/2-2* genotype performed consistently better on all measures of neurobehavioral function.¹⁴ Similarly, in our recent study among NAS participants of the longitudinal association of recent and cumulative lead exposure with psychiatric symptoms, we found that at higher tibia and patella lead levels men with the *ALAD 1-1* genotype exhibited significantly higher reporting of phobic anxiety symptoms in comparison to men with the *ALAD 1-2/2-2* genotype.¹⁸ However, in another study of NAS participants we did not observe that the *ALAD* polymorphism modified the association between current and cumulative lead burden and performance on the Mini-Mental Status Examination (MMSE), an assessment of global cognition.¹⁷ These results suggest that the influence of the *ALAD* polymorphism on the relation between lead and cognition is likely weak at low levels of lead burden.

An occupational study with comparable blood lead levels between *ALAD 1-1* and *1-2/2-2* carrier groups found that *ALAD 1-1* carriers performed worse in comparison to *ALAD 1-2/2-2* carriers on cognitive tests assessing motor and perceptual speed abilities.¹⁵ However, the results of a study on the *ALAD* polymorphism-lead interaction and essential tremor suggested that *ALAD 1-2/2-2* carriers may be more susceptible to the toxic effects of lead.¹⁶ With higher blood lead levels, the risk of essential tremor was greater among *ALAD 1-2/2-2* carriers, in comparison with *ALAD 1-1* carriers. A higher risk of essential tremor among *ALAD 1-2/2-2* carriers indicates the potential for greater cerebellar damage at higher levels of current lead exposure.

Interpreting these contradictory results is further complicated by a poor understanding of the etiological mechanism underlying potential differences in lead-induced neurotoxicity between *ALAD* genotypes. More generally, the complex relationship between *ALAD* isoenzymes and lead requires further elucidation. It has been suggested that that *ALAD 1-2/2-2* carriers form a tighter bond between lead and the zinc binding site of the *ALAD* isoenzyme, resulting from the alteration in protein subunit charge when asparagine is present instead of lysine.¹⁰ In an occupational study evaluating the lead binding affinity of *ALAD* isoenzymes by carrier status (blood lead $x = 29.1 \mu\text{g/dL}$), Bergdhal et al. found that *ALAD 1-2/2-2* carriers exhibited a modest but significantly higher percentage (84% vs. 81%) of lead bound to the *ALAD* protein.³² *ALAD 1-2/2-2* carriers with occupational lead exposures have exhibited significantly higher *ALAD* enzymatic activity compared to *ALAD 1-1* carriers, but only at blood lead levels above $50 \mu\text{g/dL}$.³³ Similarly, Jaffe et al. did not

observe strong variations in lead induced displacement of zinc or differences in lead inhibition of *ALAD* enzymatic activity between *ALAD 1-1* and *ALAD 1-2/2-2* carriers.³⁴ These results indicate that the *ALAD* polymorphism may alter the relationship between *ALAD* isoenzymes and lead through both the enzymatic charge differential and lead concentration in the circulating compartment. However, the influence of the *ALAD* polymorphism on lead kinetics may only be clinically relevant at blood lead levels that are much higher than those related to environmental exposures.

Lead present in the circulating compartment, which is mostly bound to erythrocytes, may represent recent exogenous and past endogenous exposures that can be partitioned into soft tissue compartments (e.g. brain, kidney). Since the amount of lead found in plasma is relatively small (less than 3% of whole blood lead), an *ALAD* genotype-related variation in the lead binding capacity of *ALAD* isoenzymes can in turn increase levels of bioavailable plasma lead. In addition, the *ALAD* genotype may also modify lead kinetics through promoting the retention of lead in blood and migration of lead from bone into blood in *ALAD 1-2/2-2* carriers. Our prior investigation of *ALAD* genotype modulation of bone and blood lead levels supports this assertion.³⁵ In that study, *ALAD 1-2/2-2* carriers exhibited a steeper trabecular bone lead to blood lead relationship at patella lead levels above 60µg/g, below which *ALAD 1-1* carriers had significantly higher blood lead levels for a given trabecular bone lead level.

Variations in the exposure profile between occupational and community-exposed individuals in conjunction with *ALAD* carrier status may influence the release or uptake of *ALAD* isoenzyme bound lead, and as a consequence the internal distribution of lead. Presumably, occupationally exposed individuals would have experienced relatively high exposures for a short duration followed by minimal exposures, whereas in contrast, community-exposed individuals, such as our study participants, would have experienced comparatively lower and constant levels of lead exposures over their lifetime. Consequently, *ALAD* genotype modification of the association between lead and cognition may vary over an individual's lifetime, depending on the time period and intensity in which exogenous exposures occurred, past uptake of lead into bone and subsequent release into the circulating compartment, as well as concurrent transfers of bioavailable lead across the blood-brain-barrier.

Potential limitations of this study deserve consideration. We have adjusted our analyses for numerous individual characteristics potentially correlated with lead exposure and test performance, although confounding by unmeasured risk factors may have influenced our study findings. The loss of NAS participants who had died (19%) or no longer visited the VA (18%) prior to the initiation of cognitive assessments may have biased our results. In particular, our estimate of the adverse association between lead exposure and cognition may have been underestimated if men who were excluded from our study had elevated lead levels and poor test performance. This may have further obscured a potential *ALAD* genotype interaction with lead in relation to cognition. We did observe that men still visiting the VA at the time of cognitive assessment who did not participate in cognitive testing tended to be less educated (a correlate of lead exposure and cognition) than study participants. Inadequate power to detect a gene-environment interaction is a potential limitation, particularly involving measurements of bone lead, which have larger error in comparison with blood lead measurements and may have attenuated estimates of the *ALAD* genotype interaction on the association between bone lead and cognition.

The cross-sectional analysis of lead burden in association with neurobehavioral outcomes presents inherent limitations. Namely, prospective analyses on neurobehavioral outcomes can separate effects arising from past exposure and recent exposures. Since bioaccumulation

of lead occurs predominantly in the human skeleton, bone lead reflects past lead exposure and a source for chronic lead toxicity arising from the endogenous release of lead.³⁶ The half-life of lead found in cortical bone is on the order of decades, potentially reflecting a reservoir for long-term storage and endogenous release. Therefore, the measurement of bone lead and blood levels in this study provides information on the relation between recent and past exposures to lead to cognition.

Since this study fit several statistical models, the possibility of chance findings is of concern. Although we based our analysis on previous research that has observed associations between blood and bone lead and distinct cognitive domains, our inconsistent results on the *ALAD* genotype interaction with lead in association with test performance suggests a potential role for chance. We also performed an exploratory factor analysis to elucidate the underlying cognitive domain structure assessed by the cognitive tests, and evaluated the appropriateness of using summary measures (factor scores), rather than individual test scores, for evaluating the *ALAD* genotype effect on the association between lead and cognition. Although the absence of significant associations between all lead biomarker covariates and the 3 factors scores does not entirely eliminate the potential for chance findings in this study, these results suggest that summary measures of cognitive function were inadequate for determining the relation between concurrent lead exposure and cumulative lead burden and distinct cognitive domains.

Overall, these results suggest that the association between cumulative lead burden and neurobehavioral test performance among community-exposed men is potentially modulated by the *ALAD* polymorphism for domains that are particularly sensitive to the neurotoxic effects of cumulative lead burden. However, it remains unclear whether *ALAD* genotype groups exhibit a differential susceptibility to lead associated changes in cognition. Further study is required to understand the biological mechanism potentially responsible for any differential lead-induced neurotoxicity between *ALAD* genotypes, the nature of the role the *ALAD* polymorphism plays in the internal distribution of lead over an individual's lifetime, as well as determining the etiologically relevant time period for cognitive function assessment.

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Table 1

Characteristics and blood and bone lead concentrations by *ALAD* genotype, VA Normative Aging Study, 1993–2001

Characteristic [†]	<i>ALAD</i> 1-1 (n=818)	<i>ALAD</i> 1-2/2-2 (n=164)
Age at cognitive interview (years)		
49–61	133 (16.3)	18 (11.0)
62–71	398 (48.7)	76 (46.3)
72	287 (35.1)	70 (42.7)
Education (years)		
12	63 (7.7)	10 (6.1)
12	237 (29.0)	52 (31.7)
13–15	214 (26.2)	41 (25.0)
16	304 (37.2)	61 (37.2)
Computer Experience	295 (37.5)	60 (37.7)
First Language English	734 (89.8)	140 (85.4)
Smoking Status		
Never Smoker	221 (27.0)	52 (31.7)
Former Smoker	537 (65.6)	107 (65.3)
Current Smoker	57 (7.0)	4 (2.4)
Missing	3 (0.4)	1 (0.6)
Cumulative smoking (pack-years)		
0	221 (27.6)	52 (31.7)
1–20	237 (29.6)	45 (27.3)
>20 – 146	344 (42.9)	64 (39.0)
Alcohol Consumption (g/day)		
0	195 (23.8)	52 (31.7)
1 – 10	248 (30.3)	51 (31.1)
> 10	319 (39.0)	46 (28.1)
Missing	56 (6.9)	15 (9.1)
Lead Biomarker Concentration [‡]		
Blood Lead (µg/dL)	5.3±2.9	4.8±2.7
Tibia bone lead (µg/g)	21.9±13.8	21.2±11.6
Patella bone lead (µg/g)	29.3 ±19.1	27.9±17.3

[†]No.(%), unless otherwise specified.

[‡]Mean±SD

Table 2

Adjusted[†] difference in cognitive test z score per inter-quartile range higher lead concentration, comparing *ALAD 1-2-2* vs. *ALAD 1-1* genotype, VA Normative Aging Study, 1993–2001.

Domain/Cognitive test	N	Blood [*] <i>ALAD</i> Parameter Estimate [†]	95% CI	N	Tibia [*] <i>ALAD</i> Parameter Estimate [†]	95% CI	N	Patella [*] <i>ALAD</i> Parameter Estimate [†]	95% CI
<i>Visuospatial</i>									
Constructional Praxis (number correct)	959	-0.05	-0.23, 0.13	728	-0.25**	-0.49, -0.02	728	0.02	-0.19, 0.23
Pattern Memory (total number correct)	490	0.19	-0.05, 0.42	397	0.12	-0.20, 0.44	395	0.12	-0.16, 0.39
Pattern Comparison (total number correct)	907	-0.01	-0.20, 0.18	705	-0.04	-0.28, 0.20	705	-0.09	-0.31, 0.13
<i>Executive Function/Language/General Intelligence</i>									
Verbal Fluency (total number of animals named)	916	-0.03	-0.22, 0.16	709	-0.11	-0.34, 0.13	709	-0.025	-0.24, 0.19
Boston Naming Test (total number of objects named)	500	0.04	-0.20, 0.29	403	-0.16	-0.50, 0.18	401	-0.15	-0.44, 0.13
Vocabulary (total #)	501	-0.21*	-0.43, 0.01	401	-0.27	-0.60, 0.05	399	-0.15	-0.42, 0.12
<i>Verbal Memory/Working Memory</i>									
Word List Memory (total number of words recalled)	913	0.003	-0.18, 0.19	708	0.08	-0.15, 0.31	708	0.14	-0.07, 0.34
Word List Delayed Recall (total number of words recalled)	912	-0.05	-0.23, 0.13	707	0.13	-0.10, 0.36	707	0.09	-0.12, 0.30
Digit Span Backward (total number of spans recalled)	863	-0.17	-0.36, 0.03	678	-0.12	-0.36, 0.12	677	-0.005	-0.22, 0.22
Digit Span Backward (longest span recalled)	864	-0.14	-0.33, 0.06	679	-0.06	-0.30, 0.18	678	0.02	-0.20, 0.24
<i>Perceptual Speed</i>									
Continuous Performance (mean response latency, 2 best trials, ms)	486	-0.18	-0.42, 0.06	392	-0.25	-0.59, 0.08	390	-0.16	-0.44, 0.12
Pattern Memory (response latency, ms)	490	-0.04	-0.28, 0.19	397	-0.08	-0.41, 0.26	395	0.06	-0.22, 0.35

Domain/Cognitive test	N	Blood*ALAD Parameter Estimate [‡]	95% CI	N	Tibia*ALAD Parameter Estimate [‡]	95% CI	N	Patella*ALAD Parameter Estimate [‡]	95% CI
Pattern Comparison (response latency, ms)	907	0.02	-0.16, 0.20	705	0.04	-0.19, 0.27	705	0.21*	0.001, 0.41

[‡] All analyses were adjusted for main effect of lead biomarker, ALAD genotype, age, alcohol intake, education, pack-years smoking, and English as a 1st language. Computer-based cognitive tests (continuous performance, pattern memory, and pattern comparison) were also adjusted for computer experience.

[‡] Reported are parameter estimates and 95% confidence intervals (CIs) corresponding to an interquartile increment in blood lead (3 µg/dL), tibia lead (15µg/g), and patella lead (20 µg/g), respectively. All cognitive tests were z-transformed and standardized so that negative values always indicate worse performance.

** p<0.05;

* p<0.10.