Original Contribution

Association Between Blood Lead and the Risk of Amyotrophic Lateral Sclerosis

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The authors conducted a 2003–2007 case-control study including 184 cases and 194 controls to examine the association between blood lead and the risk of amyotrophic lateral sclerosis (ALS) among US veterans and to explore the influence on this association of bone turnover and genetic factors related to lead toxicokinetics. Blood lead, plasma biomarkers of bone formation (procollagen type 1 amino-terminal peptide (PINP)) and resorption (C-terminal telopeptides of type 1 collagen (CTX)), and the K59N polymorphism in the δ-aminolevulinic acid dehydratase gene, *ALAD*, were measured. Odds ratios and 95% confidence intervals for the association of blood lead with ALS were estimated with unconditional logistic regression after adjustment for age and bone turnover. Blood lead levels were higher among cases compared with controls ($P < 0.0001$, age adjusted). A doubling of blood lead was associated with a 1.9-fold increased risk of ALS (95% confidence interval: 1.3, 2.7) after adjustment for age and CTX. Additional adjustment for PINP did not alter the results. Significant lead-ALS associations were observed in substrata of PINP and CTX levels. The K59N polymorphism in the *ALAD* gene did not modify the lead-ALS association ($P = 0.32$). These results extend earlier findings by accounting for bone turnover in confirming the association between elevated blood lead level and higher risk of ALS.

amyotrophic lateral sclerosis; bone and bones; bone resorption; lead; odds ratio; osteogenesis

Abbreviations: *ALAD*, δ-aminolevulinic acid dehydratase gene; ALS, amyotrophic lateral sclerosis; CTX, C-terminal telopeptides of type 1 collagen; GENEVA, Genes and Environmental Exposures in Veterans with Amyotrophic Lateral Sclerosis; ICD-9, *International Classification of Diseases,* Ninth Revision; PINP, procollagen type 1 amino-terminal peptide; VALE, Veterans with ALS and Lead Exposure.

An association between lead exposure and amyotrophic lateral sclerosis (ALS) is a long-standing hypothesis. Most previous studies have supported this relation but in general have relied on indirect measures of lead exposure (1–12). Previously, we reported that increases in blood and bone lead levels were associated with a higher risk of ALS (1, 12). Another recent study reported a similarly strong association for blood, albeit not bone, lead level (13).

Blood lead levels may reflect both current environmental lead exposure and mobilization of lead from bone (14). The distribution of lead between blood and bone may change during ALS progression as a patient's level of physical activity declines, but no known study has taken bone turnover into account by using direct measurements. Lead toxicokinetics

may also modify the lead-ALS association. For example, the K59N polymorphism of the δ-aminolevulinic acid dehydratase gene, *ALAD*, influences lead toxicokinetics, leading to lower bone lead levels and sometimes to higher blood lead levels in carriers of the variant allele (*ALAD2*) (15), and should thus also be accounted for. In a previous study, we found no evidence for such an effect modification by *ALAD* genotype, but we had limited power to evaluate this issue (1).

In the present study, Veterans with ALS and Lead Exposure (VALE), we sought to corroborate our earlier findings on the relation of lead to ALS risk in a different and larger population. In addition, we evaluated the role of bone turnover and *ALAD* genotype in the lead-ALS relation.

MATERIALS AND METHODS

Cases: the National Registry of Veterans with ALS

Cases for the VALE study were derived from the National Registry of US Veterans with ALS. Details of this study have been provided elsewhere (16). Briefly, between April 2003 and September 2007, nationwide publicity efforts and a search of Veterans Administration databases were used to identify living US veterans with motor neuron diseases. Veterans or their proxies completed a screening questionnaire to verify eligibility, and veterans were invited to participate in the registry DNA bank by donating a blood sample. Subsequently, neurologists with expertise in ALS reviewed medical records to determine motor neuron disease diagnosis in accordance with the original El Escorial Criteria (17), including ALS (*International Classification of Diseases*, Ninth Revision (ICD-9) code 335.20), progressive muscular atrophy (ICD-9 code 335.21), progressive bulbar palsy (ICD-9 code 335.22), pseudobulbar palsy (ICD-9 code 335.23), primary lateral sclerosis (ICD-9 code 335.24), and other motor neuron diseases (ICD-9 code 335.29). Ultimately, 1,998 veterans with motor neuron diseases enrolled in the registry, of whom 1,167 donated a blood sample to the DNA bank (16, 18).

VALE included a subset of registry cases comprising motor neuron disease cases who donated a blood sample between January 23, 2007, and September 30, 2007; 208 cases were eligible, of whom 200 were enrolled, including 163 ALS cases, 30 progressive muscular atrophy cases, and 7 primary lateral sclerosis cases (Table 1). The remaining 8 cases were excluded because their final diagnosis was not motor neuron disease. Cases included in VALE were similar to those in the registry as a whole with respect to age, gender, race, and distribution of diagnoses.

Controls: Genes and Environmental Exposures in Veterans with Amyotrophic Lateral Sclerosis Study

Controls for the VALE study were obtained from the Genes and Environmental Exposures in Veterans with Amyotrophic Lateral Sclerosis (GENEVA) study; enrollment procedures for GENEVA have been described previously (18). Briefly, in June 2005, a random sample of 10,000 US veterans were identified; control recruitment was initiated in January 2006. Eligible controls, free of ALS or other neurologic disorders per the telephone screener, were frequency matched to the cases by age (within 5 years), gender, race (white/nonwhite), and past use of the Veterans Administration system for health care.

Between May 2007 and May 2008, VALE contacted 359 controls already enrolled in GENEVA for additional informed consent and blood sample collection. A total of 252 controls consented to participate in VALE, of whom 229 ultimately donated a blood sample. There were no differences in age, gender, race, smoking, or education between those who enrolled and provided a blood sample and those who did not ($P > 0.41$ for all comparisons).

Sample collection

The National Registry of Veterans with ALS conducted home visits with case enrollees and collected as many as 4 tubes of blood to provide DNA and plasma. For cases enrolled in VALE, blood collection procedures remained the same except that the first whole-blood sample was collected in a 6-mL BD Vacutainer blue-top Trace Element metal-free tube for lead measurement (Becton, Dickinson and Company, Franklin Lakes, New Jersey).

For GENEVA controls, saliva samples, collected by mail using Oragene kits (DNA Genotek Inc., Kanata, Ontario, Canada), were used as a source of DNA. VALE conducted a home visit for controls, during which 2 blood samples were collected: a 6-mL whole-blood sample in a metal-free tube for lead measurement and a 9-mL plasma sample for bone turnover biomarkers.

For both cases and controls, blood samples were chilled immediately, shipped with cold packs, and processed and frozen within approximately 48 hours after blood draw. All samples were stored at −80°C until assay. Samples were collected and processed in the same way for cases and controls.

Lead measurement

Lead concentration in blood samples was determined by inductively coupled plasma mass spectrometry. The testing laboratory was blinded to case-control status and made extensive efforts to prevent metal contamination, including use of a class 100 plastic hood for sample preparation and ultrex-grade acids and oxidants as well as approximately 18-MΩ-quality deionized water to eliminate contamination. Before analysis, samples were digested in a digitally controlled digestion block with high-purity acids and oxidants. Quality control samples processed with each batch of study samples to continuously monitor assay performance indicated good precision: the relative standard deviation percentage was less than 10% for all and less than 5% for 96% of the batches. Method blanks and aliquots of digestion reagents were carried through the analytical procedure to monitor the analyte background contribution from the reagents and the procedure. Aliquots of a Standard Reference Material (National Institute of Standards and Technology (NIST) SRM 966 Toxic Elements in Bovine Blood) were also processed as an accuracy check. In addition, approximately 5% of the study samples were prepared and analyzed in duplicate to monitor precision.

Bone turnover measurement

Because bone formation and bone resorption are coupled processes, we measured plasma biomarkers for both. We conducted a pilot study to determine whether collecting samples under field conditions affected biomarker stability. We subjected blood samples collected from nonveteran volunteers to 1 of 3 conditions: processed immediately, held at 4°C for 24 hours before processing, and stored at room temperature for 24 hours before processing. Plasma was then stored at −80°C until assay. On the basis of stability

Table 1. Characteristics of Participants in the Veterans With ALS and Lead Exposure Study, United States, 2003–2007

	All Participants				White Men Only			
	Cases (*n* = 200)		Controls (*n* = 229)		Cases (*n* = 184)		Controls (*n* = 194)	
	No.	%	No.	%	No.	%	No.	%
Age, years[a]	63.3 (34–83)		63.4 (34–84)		63.4 (34–83)		64.3 (34–84)	
Gender								
Men	196	98	216	94	184		194	
Women	4	2	13	6	0		0	
Race								
White	187	94	205	90	184		194	
Nonwhite	13	6	24	10	0		0	
Cigarette smoking[b]								
Ever	133	66	154	67	123	67	136	70
Never	62	31	75	33	56	30	58	30
Missing	5	2			5	3		
Reviewed diagnosis[b]								
Clinically definite ALS	31	16			29	16		
Clinically probable ALS, laboratory supported	35	18			33	18		
Clinically probable ALS	73	36			67	36		
Clinically possible ALS	24	12			22	12		
Progressive muscular atrophy	30	15			28	15		
Primary lateral sclerosis	7	4			5	3		
Site of onset								
Bulbar	39	20			35	19		
Spinal	161	80			149	81		
Symptom onset to diagnosis								
≤1 year	102	51			96	52		
>1 year	98	49			88	48		
Diagnosis to sample collection								
≤2 years	159	80			144	78		
>2 years	41	20			40	22		

Abbreviation: ALS, amyotrophic lateral sclerosis.
[a] Values are expressed as mean (range).
[b] Percentages may not add to 100% because of rounding.

observed under these conditions, we assessed bone formation by measuring plasma procollagen type 1 amino-terminal peptide (PINP) using the UniQ PINP RIA radioimmunoassay (Orion Diagnostica Oy, Espoo, Finland; intraassay coefficient of variation: 8.8%; interassay coefficient of variation: 5.1%) and assessed bone resorption by measuring plasma C-terminal telopeptides of type 1 collagen (CTX) using the Serum CrossLaps ELISA assay (Nordic Bioscience Diagnostics, Herlev, Denmark; intraassay coefficient of variation: 5.1%; interassay coefficient of variation: 6.7%). Both assays were run with negative and positive controls and met specified assay requirements for all kit-calibrated standards. These biomarkers are both specific to bone relative to other connective tissues.

ALAD genotyping

DNA was extracted from whole blood for cases and Oragene (DNA Genotek Inc.) saliva collection kits by using Puregene reagents (Gentra Systems, Inc., Minneapolis, Minnesota) for controls. Previous studies have documented that DNA extracted from Oragene kits generates both high yields and high-quality DNA, as judged by polymerase chain reaction and genotyping success rates (19, 20). Results

for our study were similar. The coding change (K59N, rs1800435) in the *ALAD* gene was genotyped with a TaqMan assay (Applied Biosystems Inc., Foster City, California) at the Duke Center for Human Genetics Molecular Genetics Core. We required 95% genotyping efficiency and that genotypes of quality control samples match within and across all plates before including samples in the statistical analysis.

Statistical analysis

The aims of VALE included examining whether 1) elevated blood lead level is associated with a higher risk of ALS among the veterans, 2) the lead-ALS association is influenced by bone turnover, and 3) the lead-ALS association is modified by *ALAD* genotype. We based our power calculations on 200 cases and 200 controls and treated lead as a continuous variable. For aim 1, on the basis of an expected mean lead level of 3.4 μg/dL (standard deviation, 2.5) (12) but conservatively assuming a standard deviation of 1.0, we had greater than 80% power to detect a 1.4-fold increase in ALS risk for each 1-μg/dL increase in blood lead. For aim 2, on the basis of expected increases in bone turnover biomarkers in ALS patients, we had greater than 80% power to detect an odds ratio of 1.9–2.0 for interaction of blood lead with these biomarkers. For aim 3, on the basis of an expected prevalence of 0.2 for the *ALAD2* allele, we had greater than 80% power to detect an odds ratio of 1.8 for the main effect and an odds ratio of 2.2 for an interaction with blood lead.

Most study participants were white men. To reduce sample heterogeneity, we excluded women and nonwhites from the main analyses, leaving 184 cases and 194 controls (Table 1). We compared the means of lead, PINP, and CTX levels between cases and controls using analysis of covariance (PROC GLM; SAS version 9.1.3 software, SAS Institute, Inc., Cary, North Carolina); *P* values were calculated after adjustment for age (as a continuous variable; age at diagnosis for cases and age at interview for controls). Since case samples with various lag times after diagnosis were collected, changes in physical activity associated with different disease stages might have influenced blood lead and bone turnover levels. Consequently, we examined whether the means of lead, PINP, and CTX levels varied with the time interval between diagnosis and sample collection (<1 year, 1–2 years, and >2 years) among the cases.

We used unconditional logistic regression models to estimate odds ratios for ALS and their 95% confidence intervals. We used log_2-transformed blood lead level as a continuous variable; this transformation was used to ensure linearity in model fitting and better interpretability. All models included adjustment for age as a continuous variable. In some models, we further adjusted for smoking, which may be associated with ALS (21–23).

In additional models, we adjusted for PINP and CTX separately and jointly, as log_2-transformed continuous variables. Analyses were also conducted after stratification by untransformed PINP (≤34.45 μg/L and >34.45 μg/L, the median for controls) or CTX (≤0.32 ng/mL and >0.32 ng/mL, the median for controls) levels; log_2-transformed

PINP and CTX were still included in these models as continuous variables to mitigate residual confounding.

To evaluate robustness of results, we conducted several additional analyses. First, we repeated analyses after excluding progressive muscular atrophy and primary lateral sclerosis cases (*n* = 33) to ensure that results were pertinent to ALS. Second, the time interval between diagnosis and sample collection was 2 years or less for 144 cases (78.3%, Table 1), and we repeated analyses after excluding cases for whom this interval exceeded 2 years to allay potential concern that survival bias would influence our results. Third, diagnostic delay was 1 year or less for 96 cases (52.2%, Table 1), and we repeated the analyses after stratification by this factor (≤1 year or >1 year) to assess whether diagnostic delay affected the lead-ALS relation. Finally, we repeated the analyses including women and nonwhites and adjusting for gender and race to evaluate the generalizability of results from the main analyses.

Since the *ALAD2* allele is rare, few individuals are homozygous for that allele. Accordingly, we dichotomized *ALAD* genotype into those homozygous for *ALAD1* versus those with at least one copy of *ALAD2*. To evaluate potential effect modification of the lead-ALS relation, we conducted an analysis stratified by *ALAD* genotype. In a separate analysis, a potential interaction between blood lead and *ALAD* genotype was tested by adding an interaction (product) term of these 2 variables in the logistic regression model.

The VALE study was approved by the institutional review boards at the National Institute of Environmental Health Sciences, the Durham VA Medical Center, Duke University, and the Copernicus Group.

RESULTS

The unadjusted mean level of untransformed blood lead was 1.76 μg/dL (range, 0.32–6.90) among the controls and 2.41 μg/dL (range, 0.72–7.58) among the cases; the difference was statistically significant after adjustment for age (Figure 1). The mean levels of blood lead were 2.38 μg/dL (standard deviation, 1.31) among ALS cases, 2.67 μg/dL (standard deviation, 1.28) among progressive muscular atrophy cases, and 1.93 μg/dL (standard deviation, 0.79) among primary lateral sclerosis cases; these values did not differ (*P* = 0.25). The interval between diagnosis and sample collection did not influence the mean lead level of cases (Figure 2), although lead was weakly correlated with time since diagnosis among cases when time was used as a continuous variable (*r* = 0.18; *P* = 0.01). After adjustment for age, a 1-unit increment of log_2-transformed lead (equivalent to a doubling of blood lead) was associated with a 2.6-fold higher odds of ALS (95% confidence interval: 1.9, 3.7), indicating a dose response (Table 2). Adjustment for smoking (ever/never) in addition to age did not change the results (data not shown).

Compared with controls, cases had higher CTX but not PINP levels (Figure 1). The interval between diagnosis and sample collection did not influence mean levels of PINP or CTX among cases (Figure 2). Lead was correlated with CTX among cases (*r* = 0.20; *P* = 0.008) and controls
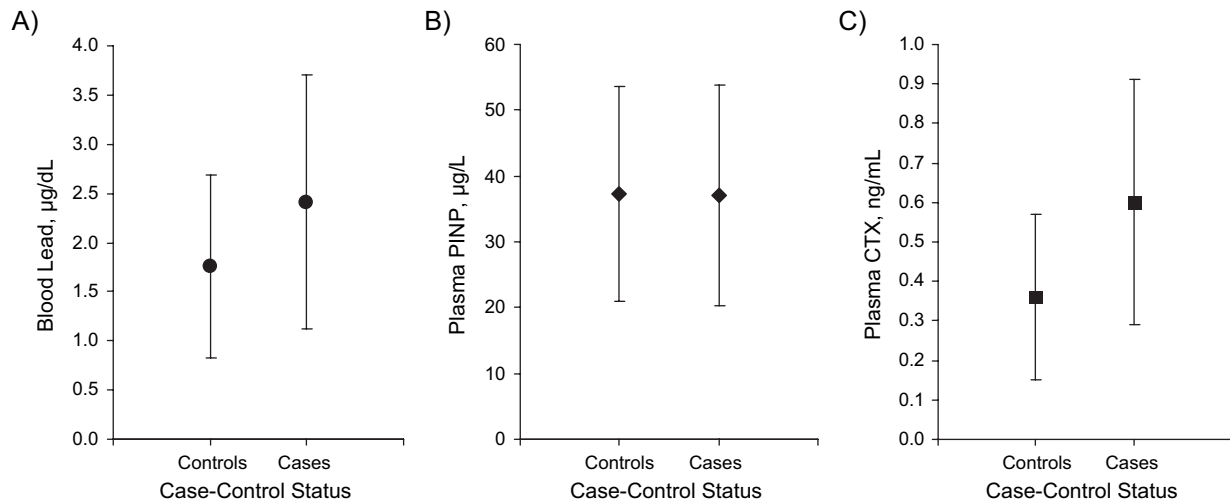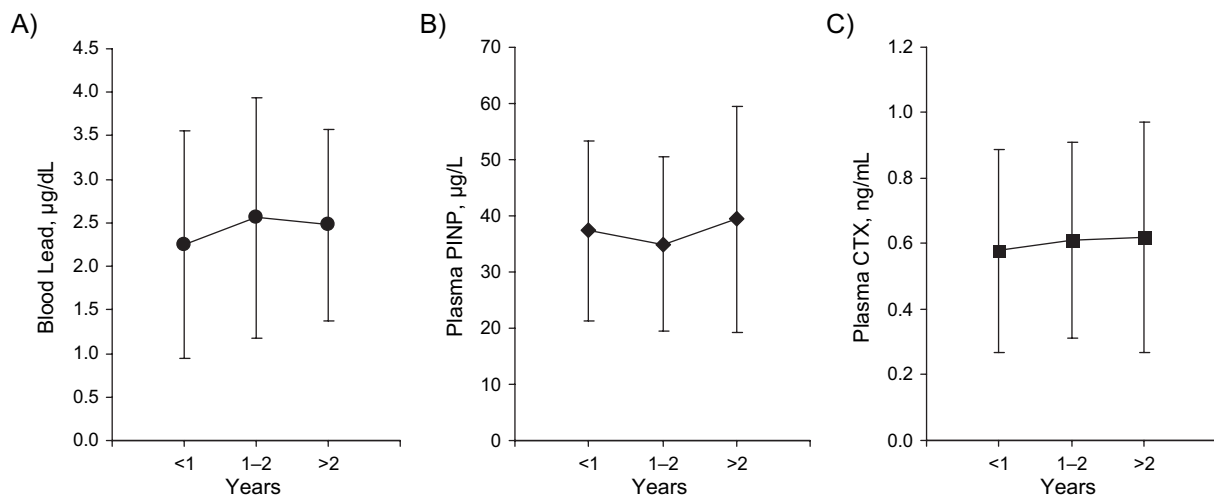
Figure 1. Unadjusted, untransformed means and standard deviations of A) blood lead, B) plasma procollagen type 1 amino-terminal peptide (PINP), and C) plasma C-terminal telopeptides of type 1 collagen (CTX) levels among amyotrophic lateral sclerosis (ALS) cases and controls, the Veterans with ALS and Lead Exposure study, United States, 2003–2007. *P* values were calculated after adjustment for age (as a continuous variable; age at diagnosis for cases and age at interview for controls)—A): *P* < 0.0001, B): *P* = 0.77, C): *P* < 0.0001.

($r = 0.23$; $P = 0.002$) but not with PINP ($P > 0.20$ for both groups). Adjustment for CTX diminished the magnitude but did not eliminate the association of lead with ALS (odds ratio = 1.9, 95% confidence interval: 1.3, 2.7). Further adjustment for PINP alone or jointly with CTX did not alter results; thus, we present results adjusted for age and CTX only. A dose response for the lead-ALS association was also seen when blood lead was categorized in tertiles; after adjustment for age and CTX, the odds ratio for the highest compared with the lowest tertile was 2.1 (95% confidence interval: 1.1, 3.8; $P_{trend} = 0.008$).

Models stratified by either PINP or CTX showed a significant association of blood lead with ALS in all strata (Table 2). Slightly stronger associations of lead with ALS were suggested among individuals with lower CTX or higher PINP levels (i.e., less demineralization), but a statistically significant interaction was not noted ($P > 0.20$).

Excluding cases with progressive muscular atrophy or primary lateral sclerosis did not change results substantially (Table 2). After we excluded cases with an interval of more than 2 years between diagnosis and sample collection, the odds ratio for the association of lead with ALS was 1.7 (95% confidence interval: 1.2, 2.5). Odds ratios were 1.9 (95% confidence interval: 1.3, 2.9) for cases with more than a 1-year diagnostic delay and 1.7 (95% confidence interval: 1.1, 2.7) for cases with a diagnostic delay of 1 year or less.

Figure 2. Unadjusted, untransformed means and standard deviations of A) blood lead, B) plasma procollagen type 1 amino-terminal peptide (PINP), and C) plasma C-terminal telopeptides of type 1 collagen (CTX) levels among amyotrophic lateral sclerosis (ALS) cases by interval between diagnosis and sample collection (years), the Veterans with ALS and Lead Exposure study, United States, 2003–2007. *P* values were calculated after adjustment for age at diagnosis (as a continuous variable), A): *P* = 0.38, B): *P* = 0.45, C): *P* = 0.76.

Table 2.   Associations Between Log$_2$-Transformed Blood Lead Level and ALS Risk, Veterans With ALS and Lead Exposure Study, United States, 2003–2007

	No. of Controls	%	All Cases (n = 184)						ALS Cases (n = 151)					
			No. of Cases	%	OR[a]	95% CI	OR[b]	95% CI	No. of Cases	%	OR[a]	95% CI	OR[b]	95% CI
Overall	194	100	184	100	2.6	1.9, 3.7	1.9	1.3, 2.7	151	82	2.5	1.8, 3.4	1.8	1.2, 2.5
Stratified by PINP[c]														
PINP ≤34.45 μg/L	97	50	90	49	2.4	1.5, 3.7	1.5	0.9, 2.6	76	41	2.2	1.4, 3.5	1.4	0.8, 2.3
PINP >34.45 μg/L	97	50	90	49	2.9	1.8, 4.8	2.3	1.4, 3.9	72	39	2.7	1.6, 4.5	2.2	1.3, 3.8
Stratified by CTX[c]														
CTX ≤0.32 ng/mL	95	49	31	17	3.0	1.5, 5.8	2.8	1.4, 5.5	28	15	2.8	1.4, 5.5	2.6	1.3, 5.3
CTX >0.32 ng/mL	99	51	149	81	2.0	1.3, 2.9	1.6	1.1, 2.4	120	65	1.9	1.3, 2.8	1.5	1.0, 2.3
Stratified by ALAD genotype[d]														
ALAD1	156	80	157	85	2.7	1.9, 3.8	2.0	1.3, 2.9	127	69	2.5	1.7, 3.6	1.8	1.2, 2.7
ALAD2	32	16	25	14	1.9	0.8, 4.5	1.2	0.4, 3.1	23	12	1.9	0.8, 4.6	1.1	0.4, 3.1

Abbreviations: ALAD, δ-aminolevulinic acid dehydratase gene; ALS, amyotrophic lateral sclerosis; CI, confidence interval; CTX, C-terminal telopeptides of type 1 collagen; OR, odds ratio; PINP, procollagen type 1 amino-terminal peptide.

[a] Adjusted for age (continuous; age at diagnosis for cases and age at interview for controls); in analyses stratified by PINP and CTX, 4 cases (3 with ALS) were excluded because of missing PINP/CTX level.

[b] Adjusted for age and log$_2$-transformed CTX level; 4 cases (3 with ALS) were excluded because of missing CTX level.

[c] Categorizations were made at the medians of PINP/CTX among the controls; 4 cases (3 with ALS) were excluded because of missing PINP/CTX level.

[d] Two cases and 6 controls were excluded because of missing ALAD data.

When women and nonwhites were included, the odds ratio was 1.6 (95% confidence interval: 1.2, 2.2) after adjustment for gender and race in addition to age and CTX.

Two cases and 6 controls had no ALAD genotype data and were excluded from corresponding analyses (Table 2). ALAD2 carriers did not have different odds of ALS compared with ALAD1-1 homozygotes (age-adjusted odds ratio = 0.8, 95% confidence interval: 0.4, 1.4). Among ALAD1-1 homozygotes, mean lead levels were 2.43 μg/dL (standard deviation, 1.31) for cases and 1.77 μg/dL (standard deviation, 0.94) for controls; among ALAD2 carriers, they were 2.26 μg/dL (standard deviation, 1.13) for cases and 1.88 μg/dL (standard deviation, 0.89) for controls. Using log$_2$-transformed lead as a continuous variable, we noted a significant lead-ALS association among ALAD1-1 carriers after adjustment for age and CTX, whereas the association was weaker and not significant among ALAD2 carriers (Table 2). The interaction between lead and ALAD genotype was not statistically significant ($P = 0.32$), however.

DISCUSSION

In the VALE study, we extended our previous findings to show that elevated blood lead levels were associated with a higher ALS risk for a new and larger population and after taking bone turnover into account. ALAD genotype did not seem to modify the lead-ALS association.

Association of lead exposure with ALS is a long-standing hypothesis (1–12), although many previous studies used indirect measures of lead exposure. In a previous study, we showed that measured blood and bone lead levels were both associated with a higher risk of ALS; the association was particularly marked for blood lead (odds ratio = 1.9 for each

1-μg/dL increment) (1, 12). Recently, a similar association between blood lead and ALS was also shown in a Northern California population (13). Findings from our previous and present studies are similar both qualitatively and quantitatively. In the previous study, the possible contribution of bone demineralization was taken into account by controlling for physical activity. The present study extended these findings by measuring bone turnover directly.

Although blood lead is often considered an indicator of current lead exposure, it may also reflect bone lead levels. In older individuals with no obvious sources of external exposure, bone lead is the largest source of blood lead (14), suggesting that the latter may serve as an indirect indicator of cumulative lifetime exposure. Alternatively, increased blood lead level may be a consequence of the disease process among ALS patients: decreased physical activity could increase bone turnover, leading to increased release of lead from bone. VALE addressed the latter possibility directly by taking measured bone turnover into account and showed that adjustment for or stratification by bone turnover biomarkers did not substantively alter our results. Interestingly, we found hints of a stronger lead-ALS association among individuals with lower bone resorption or higher bone formation, that is, individuals likely to have less release of lead from bone to blood. These findings suggest that reverse causality does not fully account for the association between blood lead and ALS.

Despite the strong lead-ALS association observed, both cases and controls had low levels of blood lead. However, a small difference in blood lead levels may be biologically significant given the low absolute lead level observed in the controls. Furthermore, a small difference in current blood lead may reflect large differences in past environmental lead

exposure or a long period of increased bone lead release after cessation of environmental lead exposure (14). It is possible that a long-term increase in release of lead from bone to blood, slightly elevating blood lead level, might result in greater exposure to neural target tissues. The mechanisms relating lead neurotoxicity to ALS are still unclear. However, several mechanisms proposed to play a role in ALS pathogenesis, including oxidative stress, excitotoxicity, and mitochondrial dysfunction (24), are also involved in lead neurotoxicity (25–27).

Veterans may be exposed to lead from firing practice (28) and other military-related sources, so the observed lead-ALS association may partly explain the higher risk of ALS noted for military service personnel compared with the general population (29–32). However, lead exposure can result from many different sources, including residential history, nonmilitary occupational history, and hobbies, and more research is necessary to determine which of these sources (if any) best explains the observed increased lead levels in the VALE cases.

The K59N polymorphic variant of the *ALAD* gene may affect an individual's blood and bone lead levels and thus influence susceptibility to lead exposure (33). In VALE, lead levels did not differ substantially by genotype in either cases or controls; however, this result is not surprising given that an effect of *ALAD* genotype on blood lead is primarily observed at much higher blood lead levels (33). We found a significant lead-ALS association among *ALAD1-1* homozygotes but not *ALAD2* carriers, but we did not find a significant interaction between *ALAD* genotype and blood lead, consistent with our previous finding (11). In our previous study, we observed an association of *ALAD* genotype with ALS, independent of lead level—a finding we did not replicate in VALE (11). Reasons for this difference between the 2 studies are not apparent, although the present study had a larger sample size.

VALE has several strengths, including its sample size and the available information on both bone turnover and *ALAD* genotype. In addition, we utilized a highly sensitive assay for lead measurement, which enabled us to detect small differences in blood lead levels in a population with a low absolute level of blood lead.

Limitations should also be appreciated. First, about 22% of the cases were diagnosed more than 2 years before sample collection, and they may represent a selected group of cases with better survival and with blood lead or bone turnover levels different from those of other ALS cases. However, the interval between diagnosis and sample collection did not substantially affect lead levels, nor did excluding individuals diagnosed more than 2 years before sample collection alter the results. Second, the exposure data were collected cross-sectionally, and thus we cannot entirely rule out reverse causality. However, the fact that the lead-ALS relation persisted after adjustment for or stratification by a biomarker of bone resorption suggests that disease-related lead mobilization from bone, the most likely reason to suspect reverse causality, does not fully explain the association. Third, we did not evaluate other genes that may modify the relation between lead exposure and neurologic outcomes, for example, the hemochromatosis gene, *HFE* (34). Finally, residual confounding from other factors could not be completely ruled out. For example, socioeconomic status might be associated with both blood lead level and the risk of ALS. We did not detect a significant association between blood lead levels and years of education, however, a commonly used proxy of socioeconomic status, among either the cases (data available for 100 cases, $P = 0.33$) or controls (data available for all controls, $P = 0.68$).

In summary, we found that elevated blood lead level was associated with higher odds of ALS among US veterans, regardless of bone turnover or *ALAD* genotype. More studies are needed to support a causal relation between blood lead and ALS risk. Although it would be difficult to collect blood lead data prospectively, before ALS symptom onset, some insight might be achieved by linking ALS to conditions involving abnormal bone resorption.

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The views expressed in this manuscript are those of the authors and do not necessarily represent the views of the Department of Veterans Affairs.

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