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Lead Burden and Psychiatric Symptoms and the Modifying Influence of the δ-*Aminolevulinic Acid Dehydratase* (*ALAD*) Polymorphism:

The VA Normative Aging Study

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Abstract

The authors evaluated the association between lead burden and psychiatric symptoms and its potential modification by a δ-*aminolevulinic acid dehydratase* (*ALAD*) polymorphism. Lead measurements in blood or bone and self-reported ratings on the Brief Symptom Inventory from 1991 to 2002 were available for 1,075 US men participating in the Department of Veterans Affairs (VA) Normative Aging Study. The authors estimated the prevalence odds ratio for the association between interquartile-range lead and abnormal symptom score, adjusting for potential confounders. An interquartile increment in tibia lead (14 μg/g) was associated with 21% higher odds of somatization (95% confidence interval of the odds ratio: 1.01, 1.46). An interquartile increment in patella lead (20 μg/g) corresponded to a 23% increase in the odds of global distress (95% confidence interval of the odds ratio: 1.02, 1.47). An interquartile increment in blood lead (2.8 μg/dl) was associated with 14%

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higher odds of hostility (95% confidence interval of the odds ratio: 1.02, 1.27). In all other analyses, lead was nonsignificantly associated with psychiatric symptoms. The adverse association of lead with abnormal mood scores was generally stronger among *ALAD 1-1* carriers than *1-2/2-2* carriers, particularly regarding phobic anxiety symptoms ($p_{interaction}$= 0.004). These results augment evidence of a deleterious association between lead and psychiatric symptoms.

Keywords

blood; bone and bones; lead; neuropsychological tests; polymorphism, genetic

Alterations in mood among the elderly not only disrupt their regular physical, mental, and social functioning but also may place them at a higher risk of developing clinically relevant mental disorders (1,2) and cardiovascular disease (3–5). In particular, depressive symptoms are associated with a higher risk of functional decline (6,7) and self-neglect (8). Lead exposure is associated with increased psychiatric symptomatology in some occupationally exposed adults (9–11). However, the results of other occupational studies are inconsistent with these findings and report that cumulative and concurrent lead burden is not associated with psychiatric symptoms (12,13). Few studies have addressed the impact of low-level cumulative and concurrent lead burden on psychiatric symptoms among the general population. In our evaluation of lead and psychiatric symptoms in environmentally exposed older men, higher chronic and cumulative lead burden (measured by lead levels in the patella and tibia) was significantly associated with phobic anxiety symptoms and a composite index of depression, anxiety, and phobic anxiety symptoms (14). In the same study, higher levels of concurrent lead exposure (measured by lead levels in blood) were also significantly associated with the composite symptom index, suggesting that even modest levels of lead exposure may increase the risk of developing psychiatric symptoms.

For this study, we expanded upon our earlier work by incorporating newly available data from up to two additional assessments of both psychiatric symptoms and lead burden. In addition, we evaluated the influence of a polymorphism in the gene encoding δ-*aminolevulinic acid dehydratase* (*ALAD*) on the relation of lead to mood. This *ALAD* polymorphism (rs1800435) encodes three distinctively charged erythrocyte isoenzymes in the heme synthesis pathway: ALAD 1-1 (52.1 mIU/g hemoglobin), ALAD 1-2 (49 mIU/g hemoglobin), and ALAD 2-2 (54.6 mIU/g hemoglobin) (15). In comparison with *ALAD 1-1* carriers, *ALAD 1-2/2-2* carriers have been reported to have a higher percentage of lead bound to the ALAD protein (16), potentially altering the kinetic distribution of lead to target organs (17,18). However, *ALAD* genotype modulation of lead toxicokinetics is poorly understood, and prior studies on the relation between *ALAD* genotype, lead, and neurologic outcomes have reported inconsistent findings (19–21). We anticipated that the dose-response association between biomarkers of lead burden and psychiatric symptoms would be steeper among men with the wild-type genotype (*ALAD 1-1*) in comparison with men with a variant allele (*ALAD 1-2/2-2*), reflecting a potential increase in the retention of lead in blood among this latter group and therefore decreased bioavailability for crossing the blood-brain barrier.

MATERIALS AND METHODS

Study population

The VA Normative Aging Study is an ongoing longitudinal study of aging established by the US Veterans Administration (now the Department of Veterans Affairs) (22). The study cohort has been described in detail elsewhere (23,24). Briefly, the original cohort, recruited between 1961 and 1970, consisted of 2,280 community-dwelling men from the greater Boston, Massachusetts, area, who were aged 21–80 years at the time of enrollment. 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. Attrition for all causes has been less than 1 percent per year over the life of the study. 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.

This study focused on a subgroup of the VA Normative Aging Study cohort for whom blood lead measurements, *ALAD* genotype status, and psychiatric symptom assessments were complete. Bone lead levels were also assessed for a subset of participants with blood lead measurements. Beginning in 1991, we collected blood samples to evaluate blood lead levels and administered a self-report psychiatric symptom assessment to 1,075 of the 1,171 still-active VA Normative Aging Study participants. *ALAD* genotype status and information on all covariates of interest were available for 939 of these participants. On participants' two potential follow-up visits, blood lead levels and psychiatric symptoms were again assessed for 768 and 478 participants, respectively. Thus, our analysis of blood lead, *ALAD* genotype, and psychiatric symptoms included 2,185 total observations among 939 men.

From 1991 to 2002, 774 VA Normative Aging Study participants completed a bone lead assessment and at least one psychiatric symptom assessment. However, seven bone lead readings that produced high uncertainty values (>10 μg/g for tibia bone and >15 μg/g for patella bone) were excluded as unreliable, a standard protocol in the analysis of bone lead (25). (High uncertainties usually reflect excessive patient movement during the bone scan.) *ALAD* genotype status and information on all covariates of interest were available for 690 of these participants. On participants' two potential triennial follow-up visits, psychiatric symptoms were again assessed in 646 and 469 participants with a baseline bone lead measurement, respectively. In total, our analysis of bone lead, *ALAD* genotype, and psychiatric symptoms included 1,805 total observations among 690 men.

Exposure assessment

Blood lead measurements—Blood lead levels were measured beginning in 1991, and subsequently at 3-year intervals on up to two additional occasions, to examine the relation between concurrent lead exposures occurring in a 6-year period and abnormal psychiatric symptoms. For our analyses, we identified the blood lead measurement closest to the date, up to 90 days before or after, on which psychiatric symptoms were assessed. Fresh blood samples were sent for lead analysis to ESA Laboratories, Inc. (Chelmsford, Massachusetts) and were analyzed by Zeeman background-corrected graphite furnace atomic absorption spectrophotometry. After every 20 samples, the spectrophotometer 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 percent of the analyses were controls and 10 percent were blanks. In tests on reference samples from the Centers for Disease Control and Prevention (Atlanta, Georgia), the coefficient of variation ranged from 8 percent for concentrations of 10–30 μg/dl to 1 percent for concentrations of more 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, a K-x-ray fluorescence instrument (ABIOMED, Inc., Danvers, Massachusetts) was used to determine lead levels in cortical (mid-shaft of the tibia) and trabecular (patella) bone as measures of cumulative lead burden. For each measurement, an estimate of uncertainty (equivalent to a single standard deviation if

multiple measures were taken) was derived from a goodness-of-fit calculation and the counting statistics of the spectrum curves. Technical details and validity specifications of the K-x-ray fluorescence instrument have been previously described (25,26). For our analyses, we selected bone lead measurements obtained closest to a participant's first psychiatric symptom assessment. On average, tibia and patella lead levels were measured within 1.3 years of each participant's baseline psychiatric symptom assessment.

ALAD genotyping

An *ALAD* polymorphism in exon 4 (reference single-nucleotide polymorphism identification number 1800435) was determined by polymerase chain reaction with restriction fragment length polymorphism, according to previous methods (27). In brief, modified polymerase chain reactions were performed sequentially, and in duplicate with blank controls included in each set, on 0.5 μl of whole blood by using nested primers. Reactions were completed by using one unit of Taq polymerase in a buffer containing 300 ng of each primer. The initial amplification, using 3' and 5' oligonucle-otide 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 *MSP1* endonuclease restriction site, was electrophoresed, and was visualized by fluorography.

Psychiatric symptom assessment

Psychiatric symptoms were evaluated by using the Brief Symptom Inventory, a self-administered 53-item questionnaire that assesses nine primary symptom dimensions (anxiety, depression, hostility, interpersonal sensitivity, obsessive-compulsive, paranoid ideation, phobic anxiety, psychoticism, somatization) experienced by the respondent in the last 30 days (28). Three composite indices of distress (Global Severity Index, Positive Symptom Total, and Positive Symptom Distress Index) gauge overall psychopathologic status. Brief Symptom Inventory symptom dimensions and composite indices of distress have been previously described (14). Prior research suggests that lead is associated with increased reporting of anger, confusion, aggression, hyperactivity, depression, and anxiety (9,10,29); therefore, this study evaluated five related Brief Symptom Inventory dimensions (anxiety, depression, phobic anxiety, hostility, somatization) as well as the three composite indices.

Statistical analysis

An abnormal response for a Brief Symptom Inventory dimension or composite score was defined as a score one standard deviation above the mean for our study population. We explored the possibility that associations between continuous predictor variables and the abnormal Brief Symptom Inventory responses on each of the eight Brief Symptom Inventory scores were nonlinear (on the log scale) by plotting the fitted curves from generalized additive mixed models using R software (30) that included smoothing parameters for continuous variables. To avoid biased standard errors previously reported for earlier generalized additive models, generalized additive mixed models in R use penalized splines to estimate nonlinear associations, with the penalty estimated by using generalized cross-validation (31).

If we could not detect substantial nonlinearity between continuous predictor variables and abnormal Brief Symptom Inventory responses, we used repeated-measures logistic regression models with generalized estimating equations to estimate the prevalence odds ratio of an abnormal response on each Brief Symptom Inventory score per unit increment in each lead biomarker, fitting a separate model for each Brief Symptom Inventory score–biomarker pair, and a continuous term for lead biomarker (blood, tibia, and patella). Psychiatric assessments occurred for each man on up to three occasions, and, to account for within-person correlations between abnormal responses on a given score over time, we incorporated in our generalized estimating equations models the assumption that the within-person pair-wise correlations

among the repeated responses had an unstructured covariance matrix. For the regression parameter variances (i.e., var($\hat{\beta}$)) and 95 percent confidence intervals, we used empirical "sandwich" estimators, which are robust to misspecification of model covariance (32).

We adjusted all regression models for age at lead biomarker measurement, education (less than high school, high school, some college, college, and/or postgraduate studies), alcohol consumption (0 g/day, 0.88–11.2 g/day, >11.2 g/day), and cumulative smoking (pack-years). In addition to these covariates, analyses of tibia and patella lead models were also adjusted for the time between assessments of psychiatric symptoms. For models of bone lead, we used the measurement of bone lead closest to the individual's first psychiatric assessment; only time since last psychiatric assessment varied by assessment occasion. In models of blood lead, blood lead level and age at blood lead measurement corresponded to assessment occasion. We modeled each lead biomarker as a continuous term and then used the resulting regression parameter to compute the odds ratio of each Brief Symptom Inventory outcome per interquartile range (IQR) of the lead biomarker (i.e., $\exp(\beta_{\text{Pb biomarker}} \times \text{IQR}_{\text{Pb biomarker}})$).

To evaluate whether *ALAD* genotype modified the relation of lead exposure to psychiatric symptoms, we introduced an interaction term for *ALAD* genotype (*1-2/2-2* vs. *1-1*) and lead biomarker into each model and estimated the covariate-adjusted difference (on the log scale) between the two genotype groups' lead–psychiatric symptom odds ratios. We then used these interaction models to calculate, separately for *ALAD 1-1* and *ALAD 1-2/2-2* carriers, the adjusted odds ratio and 95 percent confidence interval corresponding to the association between lead biomarker and abnormal psychiatric symptom response. All statistical tests were two sided. We performed generalized estimating equations analysis by using SAS software (33).

RESULTS

Table 1 shows the participants' characteristics across three visits. At their first Brief Symptom Inventory assessment, the men ranged in age from 48 years to 94 years, and most had at least a high school education. Mean lead levels in blood (6.2 (standard deviation, 4.1) μg/dl), tibia (22.1 (standard deviation, 13.8) μg/g), and patella (31.4 (standard deviation, 19.6) μg/g) reflect the incidental community exposures of the population. The prevalence of *ALAD 1-1* and *ALAD 1-2/2-2* carriers was 83 percent ($n = 779$) and 17 percent ($n = 160$), respectively. The distribution of the different *ALAD* genotypes was in Hardy-Weinberg equilibrium. The percentage of participants exceeding cutoff levels for psychiatric symptom outcomes at baseline varied between 8.2 percent and 14.2 percent. The prevalence of abnormal responses remained fairly stable over follow-up.

Smoothing plots and analysis of variance tests of generalized additive mixed models indicated that the associations of the lead biomarkers with psychiatric symptoms did not noticeably deviate from linearity, with the exception of the associations between blood lead and abnormal responses for depression, phobic anxiety, and Positive Symptom Total scores. Log-likelihood ratio test results indicated that marginal logistic regressions for these three outcomes with a linear blood lead term adequately fit the data for these models. Therefore, bone and blood lead were retained as linear terms in subsequent regression analyses.

A higher burden of cumulative lead was associated with an elevated prevalence of psychiatric symptoms (table 2). An interquartile increment in tibia lead (14 μg/g of bone mineral) was associated with 21 percent higher odds of abnormal somatization symptoms (odds ratio = 1.21, 95 percent confidence interval (CI): 1.01, 1.46), reflecting distress arising from perceptions of physical dysfunction. Higher tibia lead levels were also associated with prevalent abnormal responses on the four other Brief Symptom Inventory dimensions and the three composite scores, although none of these associations were statistically significant.

For patella lead, the strongest association pertained to abnormal responses on the Global Severity Index, where an interquartile increment in patella lead (20 μg/g of bone mineral) corresponded to an adjusted odds ratio of 1.23 (95 percent CI: 1.02, 1.47). Higher patella lead levels were also nonsignificantly associated with increased prevalence of abnormal responses on the five other Brief Symptom Inventory dimensions and the two other composite scores.

We observed a significant association between higher concurrent lead burden and hostility symptoms. An inter-quartile increment in blood lead (2.83 μg/dl) corresponded to a 14 percent increase in the odds of abnormal hostility symptoms (odds ratio = 1.14, 95 percent CI: 1.02, 1.27). Higher blood lead levels were also associated with greater reporting of anxiety and phobic anxiety symptoms, global distress, and a high Positive Symptom Total, but none of these associations were statistically significant (data not shown).

Higher cumulative lead burden was associated with increased odds of abnormal psychiatric symptoms among *ALAD 1-1* carriers but not among *ALAD 1-2/2-2* carriers. This distinction was statistically significantconcerning the association between tibia lead and phobic anxiety ($p_{\text{interaction}} = 0.004$) (table 3). Among men who were *ALAD 1-1* carriers, the adjusted odds ratio for an interquartile increment in tibia lead corresponding to phobic anxiety was 1.23 (95 percent CI: 0.97, 1.55) in comparison with 0.47 (95 percent CI: 0.24, 0.92) among *ALAD 1-2/2-2* carriers. This pattern of associations was similar with respect to patella lead (table 4), with an interquartile increment in patella lead corresponding to an odds ratio of 1.31 (95 percent CI: 1.04, 1.65) for abnormal phobic anxiety symptoms among *ALAD 1-1* carriers, in comparison with an odds ratio of 0.79 (95 percent CI: 0.48, 1.29) among *ALAD 1-2/2-2* carriers ($p_{\text{interaction}} = 0.01$). The association between blood lead and psychiatric symptoms did not significantly differ with respect to *ALAD* genotype status (data not shown).

DISCUSSION

In this expanded study of the association between lead burden and psychiatric symptoms, we found that biomarkers of both recent (blood lead) exposure and cumulative (bone lead) exposure were associated with increased prevalence of some types of abnormal psychiatric symptoms. We also found that some of these associations appeared to be modified by the *ALAD* polymorphism, with *ALAD 1-1* carriers exhibiting a greater likelihood than *ALAD 1-2/2-2* carriers of reporting psychiatric symptoms at a higher burden of cumulative lead.

Our present results build upon a previous cross-sectional study of a smaller subset ($n = 526$) of the participants included in the present study (14). In concordance with our prior study's results, we observed that bone and blood lead were nonsignificantly associated with higher reporting of anxiety, depression, and phobic anxiety. We report here the new finding that patella lead was significantly associated with a higher Global Severity Index score. The prior study's smaller sample size may have limited our ability to detect this significant association. In addition, blood and tibia lead were significantly associated with hostility and somatization symptoms, respectively. These two symptoms were not evaluated in association with lead burden in the prior study.

The few studies that have evaluated the relation of lead exposure to central nervous system symptoms have been conducted primarily within occupational cohorts. Several studies have found associations between elevated blood lead levels (>40 μg/dl) and reports of depression, irritability, anxiety, and fatigue (9,10,29). In a study of 467 lead-exposed workers, investigators found that cumulative lead dose (integrated blood lead over working lifetime) was associated with general distress (11). In contrast, a longitudinal study of past lead exposures and psychiatric symptoms among 535 former organolead workers found that peak tibia lead was not significantly associated with depression and a global measure of distress (12). Similarly,

in a study of 124 occupationally exposed adults, tibia and blood lead levels did not significantly predict reporting of psychiatric symptoms (13).

Animal studies suggest that chronic lead exposure during both early development (pregnancy and lactation) and adulthood can induce aggression, hyperactivity, and anxiety (34,35). Among children 11 years of age, tibia lead was associated with teachers' or parents' ratings of somatic complaints, aggressiveness, hyperactivity, and anxiety (36). In children at younger ages, subclinical elevations in blood lead levels were associated with hyperactivity, inattention, and withdrawal (37,38). However, the etiologic mechanisms underpinning current lead exposure and cumulative lead burden in relation to emotional processing are unclear. Further investigation is required to elucidate interactions between lead and relevant biochemical processes or cellular targets, and their potential modification by genetic polymorphisms.

This study supports our a priori hypothesis that *ALAD 1-1* carriers would be more likely to report psychiatric symptoms at a higher lead burden in comparison with *ALAD 1-2/2-2* carriers. At higher lead levels, *ALAD 1-1* and *ALAD 1-2/2-2* carriers differ in their levels of circulating aminolevulinc acid, a porphyrin precursor and inhibitor of the γ-amino-butyric acid neurotransmitter, and this difference may underlie *ALAD*-related variation in susceptibility to lead-induced neurotoxicity. At similar blood levels, higher aminolevulinc acid levels in plasma have been observed among *ALAD 1-1* carriers compared with *ALAD 1-2/2-2* carriers (39). However, the influence of the *ALAD* genotype on the lead–aminolevulinc acid relation is not consistent (40). A meta-analysis of published data on the *ALAD* polymorphism found a statistically significant association between *ALAD 1-2/2-2* carriers and higher blood lead levels among occupationally exposed adults, but this association was not observed among environmentally exposed adults with blood lead levels of less than 10 μg/dl (41). Similar lead levels in tibia and patella bone between *ALAD 1-1 and 1-2/2-2* carriers were reported among occupationally and community-exposed adults (17,18,42,43).

The few studies evaluating the modifying influence of the *ALAD* polymorphism on the association between lead and neurobehavioral outcomes have reported inconsistent results. A study among adolescents found that five participants with the *ALAD 1-2/2-2* genotype performed consistently better on all measures of neurobehavioral function in comparison with those with the *ALAD 1-1* genotype (19). Similarly, an occupational study found that at higher blood lead levels, *ALAD 1-1* carriers performed worse on neurobehavioral tests assessing motor and perceptual speed abilities in comparison with *ALAD 1-2/2-2* carriers (20). In contrast, a recent study observed a greater risk of essential tremor among *ALAD 1-2/2-2* carriers, in comparison with *ALAD 1-1* carriers, for a given blood lead concentration, indicating the potential for greater cerebellar damage at higher levels of current lead exposure (21).

These inconsistent results are potentially attributable to complicated interactions between *ALAD* isoenzymes and lead, which remain poorly understood. It is commonly presumed that *ALAD 1-2/2-2* carriers form a tighter bond between lead and the zinc binding site on the *ALAD* isoenzyme because of the alteration in protein subunit charge when asparagine is present instead of lysine (44). This difference in lead binding would likely result in a lower plasma lead level at a given blood lead level concentration among *ALAD 1-2/2-2* carriers compared with *ALAD 1-1* carriers. However, an investigation of allelic differences in ALAD protein structure found no differences in the lead-induced displacement of zinc and inhibition of enzymatic activity between *ALAD 1-1* and *ALAD 1-2/2-2* carriers (45). These results suggest that any *ALAD*-genotype-mediated variation in the binding mechanism between the ALAD enzyme and lead is likely more complex than differences in protein subunit electronegativity.

We used measures of cumulative and concurrent lead burden to distinguish associations arising from recent exposures from exposures accumulated over past years. Since our study

participants had low levels of lead in blood and bone that arose from environmental rather than occupational exposures, these results are not likely to be affected by selection biases noted in occupational cohorts (27). Our repeated-measures study design offers greater efficiency in estimating the association between lead burden and psychiatric symptoms than a study entailing only a single wave of psychiatric assessments.

Several limitations of our study merit consideration. The cohort size, restricted range of bone lead levels, and low blood lead levels limited our power to detect *ALAD* genotype modification of the association between lead burden and psychiatric symptoms. Evaluating the modifying influence of the *ALAD* polymorphism on the associations observed in this study should be replicated in larger study populations with greater variability in blood and bone lead levels to provide a more powerful test of the hypothesis that such an interaction exists.

Only 523 men had three Brief Symptom Inventory assessments; thus, bias from loss to follow-up is a concern. However, participants who did not complete all follow-up visits were generally similar to those who remained in the study, having similar educational attainment and alcohol consumption levels, although they were slightly more likely to be current smokers and had higher blood lead levels than those participants who completed all follow-up visits. If men lost to follow-up were more likely to have had higher lead exposure and more psychiatric symptoms, the ensuing bias in our results would be conservative, and we would have underestimated the adverse association between lead exposure and abnormal psychiatric symptoms.

In addition, there remains the potential for underreporting of psychiatric symptoms by our elderly study participants because of social stigma. It is unlikely, however, that underreporting would be differentially related to exposure status, and we would thus expect attenuation in our parameter estimates, lessening our ability to detect lead-symptom associations and differences in those associations by *ALAD* genotype. Because this study fit several statistical models, the possibility of chance findings is of concern. We report five statistically significant associations among the 48 total lead-symptom relations assessed (eight symptom models for tibia, eight symptom models for patella, and eight symptom models for blood lead; eight symptom models for interactions with each of these lead biomarkers and *ALAD* genotype), approximately three more associations than would have been expected by chance. In addition, overall, our results (table 2 and table 3) indicated that higher lead burden was consistently associated with increased reporting of psychiatric symptoms.

In conclusion, we observed a deleterious association between current and cumulative lead burden and risk of psychiatric symptoms. At a given cumulative lead burden, participants with the *ALAD 1-1* genotype appeared to be at greater risk than participants who were carriers of at least one variant *ALAD* allele. These results are specific to men and may not be generalizable to women, who experience comparatively different rates of bone metabolism at older ages. Early detection and treatment of lead-related psychiatric disorders among susceptible subgroups may reduce subsequent risk of severe mental disorders in older adults. These findings are consistent with evidence suggesting that lead is a potential risk factor for the development of psychiatric symptoms. Minimizing sources of lead exposure, particularly among susceptible subgroups, may reduce subsequent risk of severe mental disorders in older adults.

Abbreviations

ALAD, δ-aminolevulinic acid dehydratase; CI, confidence interval..

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TABLE 1

Characteristics of men by wave of Brief Symptom Inventory assessment, VA Normative Aging Study, United States, 1991–2002*

Characteristic	Wave 1 (*n* = 1,075)		Wave 2 (*n* = 849)		Wave 3 (*n* = 523)	
	No.	%	No.	%	No.	%
Age (years) at blood draw	67.3 (7.5)†		69.9 (7.1)		72.9 (6.7)	
Age (years) at bone scan	67.5 (7.3)					
Education (no. of years)						
<12	119	11.1	83	9.8	51	9.7
12	376	35.0	298	35.1	183	35.0
13–15	282	26.2	230	27.1	140	26.8
≥16	298	27.7	238	28.0	149	28.5
Alcohol consumption (g/day)						
0	275	25.6	213	25.1	119	22.8
0.88–11.2	375	34.9	298	35.1	210	40.1
>11.2	380	35.3	311	36.6	175	33.5
Missing	45	4.2	27	3.2	19	3.6
Current smoking status						
Never	301	28	234	27.8	162	31.1
Former	684	63.7	559	63.4	339	65.1
Current	89	8.3	49	5.8	20	3.8
Missing	1	0.09				
Lead biomarker concentration						
Blood (μg/dl)	6.2 (4.1)		4.9 (2.6)		4.3 (2.5)	
Tibia (μg/g)‡	22.1 (13.8)					
Patella (μg/g)‡	31.4 (19.6)					
Abnormal response on Brief Symptom Inventory symptom score						
Anxiety (>0.55)	113	10.6	94	11.4	55	10.9
Depression (>0.63)	128	12.0	100	12.2	65	12.9
Hostility (>0.60)	87	8.2	64	7.8	47	9.3
Phobic anxiety (>0.33)	89	8.4	68	8.3	48	9.5
Somatization (>0.61)	108	10.2	113	13.8	59	11.7
Global Severity Index (>0.57)	112	10.5	100	12.1	63	12.4
Positive Symptom Total (>20.22)	151	14.2	134	16.2	76	14.9
Positive Symptom Distress Index (>1.53)	84	9.3	94	12.8	53	11.5

*Participants at each assessment had at least one symptom score and a lead biomarker measurement.

†These centered values are expressed as mean (standard deviation).

‡*n* = 767.

TABLE 2

Association between bone lead measured in tibia and patella* and prevalent abnormal responses on mood dimension scores (n = 744), VA Normative Aging Study, United States, 1991–2002

Mood dimension score†	Tibia OR	95% CI	Patella OR	95% CI
Anxiety	1.18	0.98, 1.42	1.18	0.98, 1.41
Depression	1.16	0.97, 1.38	1.11	0.92, 1.33
Hostility	1.12	0.91, 1.38	1.13	0.93, 1.39
Phobic anxiety	1.13	0.90, 1.41	1.24	0.99, 1.55
Somatization	1.21	1.01, 1.46	1.09	0.90, 1.32
Global Severity Index	1.15	0.96, 1.38	1.23	1.02, 1.47
Positive Symptom Total	1.09	0.91, 1.31	1.11	0.92, 1.34
Positive Symptom Distress Index	1.08	0.90, 1.29	1.06	0.89, 1.26

*Reported are odds ratios (ORs) and 95% confidence intervals (CIs) corresponding to an interquartile increment in both tibia lead (14 μg/g) and patella lead (20 μg/g).

†All analyses were adjusted for age at bone scan, alcohol consumption (categorical), education (categorical), time between Brief Symptom Inventory assessments, and cumulative smoking (pack-years).

TABLE 3

Interaction between *ALAD*[*] genotype and tibia lead[†] in association with prevalent abnormal responses on mood dimension scores, VA Normative Aging Study, United States, 1991–2002

Mood dimension score[‡]	*ALAD 1-1* (*n* = 587)		*ALAD 1-2/2-2* (*n* = 121)		$p_{interaction}$
	OR	95% CI	OR	95% CI	
Anxiety	1.27	1.04, 1.56	0.76	0.47, 1.24	0.08
Depression	1.17	0.96, 1.43	0.93	0.55, 1.59	0.39
Hostility	1.19	0.97, 1.48	0.95	0.58, 1.55	0.35
Phobic anxiety	1.23	0.97, 1.55	0.47	0.24, 0.92	0.004
Somatization	1.25	1.00, 1.56	1.25	0.87, 1.78	0.98
Global Severity Index	1.23	1.01, 1.50	0.84	0.47, 1.50	0.19
Positive Symptom Total	1.16	0.95, 1.42	0.66	0.37, 1.17	0.05
Positive Symptom Distress Index	1.08	0.89, 1.31	1.21	0.81, 1.83	0.56

[*] ALAD, δ-aminolevulinic acid dehydratase.

[†] Reported are odds ratios (ORs) and 95% confidence intervals (CIs) corresponding to an interquartile increment in tibia lead (14 μg/g).

[‡] All analyses were adjusted for age at bone scan, alcohol consumption (categorical), education (categorical), time between Brief Symptom Inventory assessments, and cumulative smoking (pack-years).

TABLE 4

Interaction between *ALAD*[*] genotype and patella lead[†] in association with prevalent abnormal responses on mood dimension scores, VA Normative Aging Study, United States, 1991–2002

Mood dimension score[‡]	*ALAD 1-1* (*n* = 589)		*ALAD 1-2/2-2* (*n* = 119)		$p_{interaction}$
	OR	95% CI	OR	95% CI	
Anxiety	1.23	1.02, 1.48	0.97	0.60, 1.56	0.30
Depression	1.09	0.89, 1.34	1.14	0.69, 1.87	0.68
Hostility	1.19	0.98, 1.46	1.26	0.71, 2.24	0.61
Phobic anxiety	1.31	1.04, 1.65	0.79	0.48, 1.29	0.01
Somatization	1.09	0.88, 1.36	1.20	0.76, 1.90	0.46
Global Severity Index	1.25	1.03, 1.52	1.21	0.71, 2.08	0.84
Positive Symptom Total	1.13	0.92, 1.39	1.00	0.63, 1.59	0.59
Positive Symptom Distress Index	1.05	0.87, 1.27	1.27	0.76, 2.14	0.26

[*] ALAD, δ-aminolevulinic acid dehydratase.

[†] Reported are odds ratios (ORs) and 95% confidence intervals (CIs) corresponding to an interquartile increment in patella lead (20 μg/g).

[‡] All analyses were adjusted for age at bone scan, alcohol consumption (categorical), education (categorical), time between Brief Symptom Inventory assessments, and cumulative smoking (pack-years).