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Modification of the Association between Lead Exposure and Amyotrophic Lateral Sclerosis by Iron and Oxidative Stress Related Gene Polymorphisms

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Abstract

Objective—To examine whether functional polymorphisms in hemochromatosis (*HFE*; H63D and C282Y), transferrin (*TfC2*), and glutathione-s-transferase Pi1 (*GSTP1;* Ile105Val) genes modify any lead-ALS association.

Methods—We measured blood lead using atomic absorption spectroscopy and bone lead—a biomarker of cumulative lead exposure—using K-shell-X-ray fluorescence in 100 neurologist-confirmed ALS cases and 194 controls, the latter recruited as part of two separate studies; all subjects lived in New England. Participants were considered variant carriers or wild-type for each polymorphism. To assess effect modification, we included cross-product terms between lead biomarkers and each polymorphism in separate adjusted polytomous logistic regression models.

Results—Compared with wild-type, the odds ratio (OR) per 15.6μg/g patella lead (interquartile range; IQR) was 8.24 (95% CI: 0.94–72.19) times greater among C282Y variant carriers, and 0.34 (95% CI: 0.15–0.78) times smaller among H63D variant carriers. Results were weaker for tibia lead. Compared with wild-type the OR per 2μg/dL blood lead (IQR) was 0.36 (95% CI: 0.19–0.68) times smaller among H63D variant carriers, and 1.96 (95% CI: 0.98–3.92) times greater among *GSTP1* variant carriers.

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Disclosure of Interests

None.

Conclusions—We found that *HFE* and *GSTP1* genotypes modified the association between lead biomarkers and ALS. Opposite modification by the *HFE* polymorphisms H63D and C282Y may suggest that the modification is not simply the result of increased iron.

Keywords

Amyotrophic lateral sclerosis; Epidemiology; Hemochromatosis; Iron metabolism; Oxidative stress

INTRODUCTION

Excessive oxidative stress may contribute to ALS onset through increased death of motor neurons (1). Lead and other heavy metals can increase oxidative stress, and several studies have explored the possible association between heavy metals, particularly lead, and ALS risk (2–4). Although the results of these studies are somewhat inconsistent, two more recent papers, both of them our own, did see increased risk of ALS with higher lead exposure (5, 6). Increased iron loading also contributes to cellular oxidative damage, and elevated iron levels have been observed in ALS cases (7). Aberrant expression of genes linked to regulation of iron metabolism or reactive oxygen species detoxification may modify the neurological effects of heavy metal exposure via oxidative stress. However, no studies have explored the role of genetic polymorphisms associated with iron metabolism or oxidative stress in the association between lead exposure and ALS.

The hemochromatosis (HFE) protein is a major histocompatibility class 1-like molecule that is involved in iron regulation (8). Both the H63D and C282Y *HFE* gene variants are associated with the iron overload disorder known as hemochromatosis, and both are associated with a higher labile iron pool and increased oxidative stress as well as other changes (9, 10). Transferrin is a transmembrane iron-transport protein that interacts with HFE (11). Glutathione-s-transferases (*GSTs*) are involved with detoxification processes and free radical clearance (12). GSTs play an essential role in the defense against oxidative stress—which is thought to contribute to ALS (1)—as they catalyze the conjugation of glutathione with electrophilic compounds and also display glutathione peroxidase activities (12).

Therefore, we expanded on our previous study that found an association between lead exposure and ALS (5) to examine in a case-control study whether any association between lead exposure and risk of ALS is modified by known functional polymorphisms in *HFE*, transferrin, and *GST* genes.

METHODS

Study Population

The original ALS case-control study upon which the current study is based has been described in detail elsewhere (5). Briefly, ALS cases were recruited in 1993–1996 from two locations in New England: the Neuromuscular Research Unit at New England Medical Center and the Neurophysiology Laboratory at Brigham and Women’s Hospital. Potential cases were evaluated by board-certified neurologists, and diagnoses were confirmed using

standard criteria (13). Confirmed cases were eligible to participate in the study if they had been diagnosed within the prior 2 years, lived in New England more than half the year, spoke English, and were mentally competent. Controls were identified by random telephone screening and matched to cases by age, sex, and region within New England. Eligibility criteria for controls were the same as those for cases; in addition individuals with Alzheimer's disease, dementia, Parkinson's disease or Parkinsonism, ALS or other motor neuron disease, neuropathy, or post-polio syndrome were excluded. 71% and 76% of eligible cases and controls, respectively, enrolled in the study. Among enrolled subjects who were invited for bone lead measurements and a blood sample, 95% of cases and 41% of controls agreed. Controls who were invited but declined the laboratory visit were similar in age, gender, education, physical activity, smoking, and alcohol use to those who did participate (5). Because >95% of subjects were white and not Hispanic, we excluded 8 participants of other races and ethnicities from the present analysis. We also excluded two controls for whom we did not have lead biomarker measurements. This left 100 cases and 36 controls from the original ALS study who contributed genetic data and blood, tibia, and patella lead measurements. The mean age was 59 (sd=12.5) years for cases and 61 (sd=12.4) years for controls.

Because of the small number of controls from the original study, we included in the present analysis additional New England area participants who had been recruited between 2003–2007 from several sources in the Boston area as controls for a separate study on Parkinson's disease (PD) (14). Of 231 controls who provided a blood sample, 205 were successfully genotyped for our single nucleotide polymorphisms (SNPs) of interest. Of these, we excluded non-whites (n=43), and those without lead biomarker measurements (n=4). The mean age of the remaining 158 controls was 70 (sd=9.4) years. Thus, the final study sample for the current analyses was 100 cases and 194 controls. The mean age of all controls was 68 (sd=10.7) years.

Genotyping

The *HFE* his63asp (H63D), *HFE* cys282tyr (C282Y), transferrin pro570ser (*TfC2*), and *GSTP1* Ile105Val single nucleotide polymorphisms were genotyped using the Sequenom MassARRAY System. Genotyping assays were designed for each SNP using automated assay design software (SpectroDESIGNER 3.0, Sequenom). DNA samples were subjected to multiplex polymerase chain reaction (PCR) to amplify genomic DNA flanking the target polymorphisms. Amplified PCR product was used as a template in a second, modified single-primer minisequencing reaction. After amplification, the PCR product was purified and analyzed by MALDI-TOF spectrometry (Sequenom), with the resulting spectra being translated into a nominal genotype by SpectroTYPER-RT software (Sequenom).

Measurement of Blood and Bone Lead

Lead concentrations in bone were measured by K-x-ray fluorescence (KXRF). When we began measuring bone lead, we used an instrument developed by ABIOMED (Danvers, MA) (15). In 1999, we replaced our prototype ABIOMED instrument with an upgraded instrument (16). Intercalibration data using lead-doped phantoms and persons who were measured on both instruments demonstrated a linear relationship with intercept 0 and slope

of 0.87 for predicting the upgraded instrument's measurements from the prototype's measurements (17). Using this correction, we are able to combine data from our prototype and upgraded KXRF machines. Thirty-minute measurements were taken of the left tibia and patella, and measurements with estimated uncertainties >10 and 15μg/g bone, respectively, were excluded as reflecting excessive subject movement during the measurement. Tibia and patella are measured because these consist primarily of cortical and trabecular bone, respectively. Lead in tibia is mobilized very slowly and appears to average over much of adult life, while lead in patella turns over more rapidly and averages over roughly the past decade (18, 19). Blood lead concentrations were analyzed using graphite furnace atomic absorption spectroscopy.

Statistical Analysis

Differences between groups were calculated using a chi-square test or, if there were 5 or fewer subjects in a cell, Fisher's exact test. Since variant alleles were rare, we used a dominant allele model and dichotomized genotypes into "reference" (wild-type homozygotes) and "non-reference" (variant allele carrier) groups and conducted analyses for each SNP separately. Potential confounders considered in different models were age (years at time of recruitment/lead measurement), sex, education (≤high school, some college or more), and pack-years of smoking. We also conducted sensitivity analyses excluding 5 subjects with both H63D and C282Y variants, and analyses excluding 2 subjects with potential extreme outliers of patella or tibia lead (>3 times the inter-quartile range [IQR] away from the 3rd quartile). We additionally conducted analyses restricted to subjects from the original ALS case-control study.

Because we had two control groups, we used polytomous logistic regression Polytomous logistic regression extends conventional logistic regression from two outcome categories (*e.g.*, case vs. control) to more than two outcome categories (in our application, three – the case group and two distinct control groups) (ref: Kleinbaum and Klein). The polytomous model allows us to simultaneously and efficiently estimate pairs of log-odds-ratio parameters that compare each control group separately to the case group. The simultaneous estimation also allows us to test whether pairs of underlying parameters are equal while accounting for correlations due to the common case group. If parameters are regarded as equal, a combined estimate can be formed as a weighted average of the two individual estimates using the inverse of their respective variances as weights. We used this approach in models that included only a specific polymorphism, as well as in models that included main effect terms for blood or bone lead (continuous) and a specific polymorphism, and a cross-product term between the lead biomarker and the polymorphism. A polytomous model with cross-product terms, therefore, estimates two parameters for each term in the model: namely, two main effects for lead[dmu1], two main effects for polymorphism, and two interaction parameters. If the test for heterogeneity between the two interaction terms was non-significant these were pooled, as described above, to yield a single estimate. Sensitivity analyses considered all polymorphisms in the same model for each lead biomarker.

Because of declining levels of lead in the environment over time, and the fact that a large number of controls for this study had their lead measurements an average of 12 years after

the cases and first set of controls, the main effect of lead biomarkers on ALS should be biased. However, it has been shown that interaction effects can remain unbiased in such a situation under specified conditions (20–22). Therefore, although we need to include the main effect term for lead in our models of lead-gene interactions, we do not report the estimate for this main effect. Instead, we report only the multiplicative interactions of the effect of lead on odds of ALS with the genetic polymorphisms.

The conditions needed so that estimates of the multiplicative interaction terms from this polytomous logistic model are unbiased are the same conditions that apply to each group separately (21). One condition that will guarantee an unbiased interaction term, even when the main effects are biased, is the plausible but difficult to verify requirement that, conditional on exposure and disease status, the probability of selection into (participation in) the study does not depend on genotype (20). If we knew *a priori* that one control group was properly constituted (no selection bias) and would provide unbiased estimates for all parameters, a sufficient condition for the other control group to also provide an unbiased estimate of interaction, even when main effects estimated using it are biased, is that genotype and exposure do not interact on the probability of selection into the control groups (21). We indirectly checked this latter condition (under the assumption that our original control group was properly constituted) by examining the genotype-lead product term in a logistic regression model with control set as the dependent variable and genotype, lead concentration, genotype times lead, and covariates as independent variables.

We performed all analyses using SAS version 9.3 (SAS Institute, Cary, NC, USA). We consider a p-value of 0.05 as statistically significant and do not apply any further multiple comparisons adjustment (23, 24). We recognize the limited sample size of the study and consider this work as hypothesis generating. This study was approved by the Institutional Review Boards of the National Institute of Environmental Health Sciences, New England Medical Center, Brigham and Women's Hospital, Survey Research Associates-Batelle (Durham, NC) and CODA (Durham, NC). All participants gave informed consent.

RESULTS

The median concentrations of patella, tibia, and blood lead among cases were 15.0μg/g (25%–75%: 9.5–25.5), 13.0μg/g (25%–75%: 9.5–22.0), and 4μg/dL (25%–75%: 3–6), respectively. The median concentrations among controls were 9.6μg/g (25%–75%: 3.5–19.1), 8.7μg/g (25%–75%: 2.6–14.8), and 2μg/dL (25%–75%: 1–3), respectively. Cases were more often male, had lower education, were more likely to be smokers, and tended to be younger than the controls (Table 1). Some of the differences between cases and controls, or between control groups, were significant in bivariate analyses, so adjustment for these factors was considered in the analyses.

Lead levels differed by sex, age, education and smoking (Table 2). The distributions of *HFE* H63D and C282Y, *TfC2* and *GSTP1* ile105val polymorphisms among controls were in Hardy-Weinberg equilibrium (H63D: $\chi 2=3.16$, p=0.21; *C282Y*: $\chi 2=0.57$, p=0.86; *TfC2*: $\chi 2=0.40$, p=0.82; *GSTP1*: $\chi 2=1.12$, p=0.55). Among controls, patella, tibia, and blood lead concentrations did not differ significantly by H63D (all p>0.52), C282Y (all p>0.50), *TfC2*

(all p>0.24), and *GSTP1* (all p>0.11) genotype (Table 2; the distribution by genotype among cases is shown in supplemental table 1). Although not significant, mean bone lead concentrations were somewhat higher among *HFE* H63D variant carriers and C282Y wildtypes.

In analyses adjusted for age and sex that considered only genotype and not lead, we found elevated odds for ALS among H63D variant allele carriers compared with non-carriers but odds of ALS were not changed among C282Y, *TfC2* or *GSTP1* variant carriers (Table 3). Results were similar with additional adjustment for education and smoking. The distribution of genotype among the two control groups separately are shown in supplemental table 2.

As described above, because most controls were recruited later, the effect estimate for the association with lead is likely to be biased; for example, in the age and sex adjusted model for patella lead, the OR per IQR increase [15.6μg/g] using the later controls was 4.84; 95% CI: 2.99–7.84), which was highly significantly different from the OR using the original controls (p<0.0001). Our analyses to probe genotype-exposure interactions in comparisons of the two control groups supported the assumption needed for unbiased lead-gene interaction terms for all polymorphisms except *TfC2*. For that polymorphism, we saw some suggestion of gene by lead interactions on the selection of controls in our study that reached statistical significance only for blood lead (p-values with tibia, patella, and blood were 0.14, 0.11, and 0.02, respectively). No other polymorphisms showed any evidence of interaction on selection of controls: the p-value for the blood lead by C282Y polymorphism was 0.19 and for tibia lead by *GSTP1* polymorphism was 0.20, while all other p-values were >0.54. In polytomous logistic regression analyses, the p-values for heterogeneity between separate gene-lead interaction terms were statistically significant for the interaction between each lead biomarker and *TfC2*, as well as for the interaction between tibia lead and *GSTP1*.

After adjusting for age and sex, the OR per IQR increase in patella lead was 8 times higher among C282Y variant carriers compared with wild-type, and two-thirds lower among H63D variant carriers compared with wild-type (Table 4). The pattern of results was similar for tibia lead, but weaker. The OR per IQR increase in blood lead was two-thirds lower among H63D variant carriers compared with wild-type, but the association with blood lead was not modified by C282Y. The associations between patella lead and ALS was not affected by *GSTP1*, but the association of blood lead with ALS was almost two times stronger among *GSTP1* variant carriers. We do not report interactions between tibia lead and *GSTP1*, and all interactions with *TfC2* because our efforts to check assumptions suggested that combining the control groups was not justified. The polytomous logistic regression results for interactions by each control group separately and the p-values for heterogeneity are shown in Supplemental table 3—estimates based only on subjects from the original control group were similar, although with much wider confidence intervals.

We found similar effect modification after further adjusting for education and pack-years of smoking. Results were also similar when we included all SNPs and their interaction terms in the model for a given lead biomarker simultaneously. Although all additional controls recruited later had to come to Boston for bone lead analysis, 8 of them did not provide their current residence information and 13 reported a residence outside the New England area.

When we excluded these 21 controls, the results were similar. Similar results were found after excluding 5 subjects with both H63D and C282Y variants, and after excluding 2 subjects with extremely high patella or tibia lead concentrations.

DISCUSSION

We found an increased risk of ALS among *HFE* H63D variant carriers, but not C282Y carriers nor *TfC2* or *GSTP1* ile105val carriers. Both H63D and C282Y genotypes modified the association between patella lead and ALS, but in opposite directions: it was decreased among H63D carriers and increased among C282Y carriers. Results for blood and tibia lead were similar. The association between blood lead and ALS was increased among *GSTP1* variant carriers. While these results are intriguing, they are a first examination of this issue in a small sample and so must be interpreted with caution. In addition several tests were done and significant ones could be the result of chance alone. Larger studies to follow up these findings are warranted.

Given that lead in trabecular bone (patella) has a half-life on the order of years while that in cortical bone (tibia) has a half-life on the order of decades (18, 19), and that the half-life of lead in blood is much shorter than in bone, the generally stronger findings with patella and blood lead suggest that lead exposure in the recent past may have the most relevance for development of ALS. One possibility is that lead-related effects only contribute to ALS risk if some other necessary event has occurred, at which point the lead-related effects—for example neuro-inflammation as a result of lead-induced oxidative stress—come into play with more immediate consequences. Although we could not directly examine the main effect of lead on ALS in this study, it should be noted that our finding of a gene-by-lead interaction in the development of ALS strengthens the evidence for some effect of lead. Interaction effects may in some regards be less easily explained by confounding bias than main effects, because they require more complicated conditions to explain observed effects. For example, to confound the lead-gene interactions we report here, a variable would have to be related to both lead and ALS differently across levels of our gene polymorphisms.

No prior studies have examined *TfC2* or *GSTP1* ile105val and ALS. The C282Y polymorphism has not been found to be associated with ALS risk (25–28), which agrees with our findings. Four of the first five studies of H63D and ALS found an increased risk of ALS among H63D carriers (25–29), although the results of one of the four were not significant (25). The other study did not find an association among H63D heterozygotes but did observe an increased risk among homozygotes (26). More recently, a French study and a large European meta-analysis did not find an overall association between H63D and ALS (30, 31). However, our results raise concerns for comparisons across studies that look only at the association with H63D and do not consider lead exposure. If an interaction exists between lead exposure and H63D, then the association with H63D estimated ignoring the interaction would be different across different studies, possibly even appearing null in some studies, if the lead exposures differed across populations.

Ours is the first study to evaluate effect modification of the association between lead exposure and the risk of ALS by *HFE* H63D and C282Y, and *GSTP1* ile105val. Lead is

capable of promoting oxidative damage, and differences in phenotypic expression of *GSTP1* in polymorphic variants may alter the clearance rate of lead-induced oxidative stressors and thereby influence a lead-ALS association.

The stronger association between lead and ALS among C282Y variant carriers is consistent with the hypothesis of increased lead-induced oxidative stress in the presence of more iron. The C282Y variant is more strongly associated with hemochromatosis than the H63D variant, which might explain why we did not see a similar interaction with H63D. The opposite interaction with H63D could relate to effects more specific to the H63D *HFE* variant. Examples include increased glutamate release and reduced uptake (32), or altered innate immunity (33). Different mechanisms underlying the interactions of lead with H63D and C282Y could also explain why the pattern of interactions with the three lead biomarkers differed for these two polymorphisms.

Both *HFE* variants affect iron levels via interference with normal iron transport, and this interference extends to other metals as well (8, 34, 35). If *HFE* variants alter the distribution of lead in blood and bone, or across the blood brain barrier, this action could account for different associations between lead and ALS because the same blood or bone lead measurement in a variant carrier compared with wild-type may mean different lead concentrations that actually reach the central nervous system. The slightly higher bone lead concentrations among H63D variant carriers and C282Y wildtype could reflect this type of process, However, it would remain unclear why the two *HFE* polymorphisms would alter the bone lead concentrations in opposite directions.

A limitation of our study is that bone lead measurements were made after the onset of ALS. However, the time between diagnosis and bone lead measurement was at most two years, and bone lead reflects long-term exposure, limiting the potential for reverse causation. The possibility that the association of ALS with blood lead is partly explained by mobilization of lead from bone resorbed due to inactivity cannot be excluded, although a more recent report found blood lead to be associated with ALS even after controlling for markers of bone turnover (6). Nonetheless, increased mobilization of lead from bone among ALS cases would not, in and of itself, result in an interaction with gene variants.

An unusual characteristic of this study is the inclusion of additional controls from a later study on PD because of low participation in the laboratory portion of the study among controls in the original ALS study. The inclusion of additional controls not contemporaneous with cases is expected to induce bias in the main effect of lead related to secular changes in lead levels; the additional controls will tend to have lower lead exposures simply because they were recruited later than cases. The literature has, however, identified conditions that, if met, guarantee unbiased estimation of interactions even when main-effect estimates are biased (20, 21). Analogous but slightly simpler conditions will guarantee unbiasedness of main effects in studies of one risk factor (20, 21). In particular, for our polytomous logistic analysis with two control groups, conditions for unbiasedness of interaction or of genetic effects must be met in each group separately (21). A key condition is that the probability of selection into (participation in) the study does not depend on genotype, conditional on exposure level and disease status for interactions or conditional on

disease status for genetic main effects (20). Because the SNPs studied seem unlikely to affect complex behaviors like participating in a study, this kind of assumption is often viewed as plausible. Because we do not have genotypes and exposures for non-participants, these assumptions are impossible to verify directly. For the original study, where controls are contemporaneous with cases, the possibility of selection bias arises because of low participation in the bone and blood measurements. We did find the controls with bone/blood measurements similar demographically to other original controls who did not participate in blood and bone measurements (5). This evidence together with the plausibility argument leads us to regard the original controls providing a proper comparison group.

Using results in Wacholder *et al.* (21) under the assumption that our original controls were proper, we checked whether the second control group would likely provide unbiased estimation of interaction by seeing whether the genotype and exposure interacted in a comparison of the two control groups. We found no evidence of interaction ($p>0.54$) for any of the lead-gene combinations that were significantly associated with odds of ALS (Table 4). In addition, the polytomous logistic approach allows us to directly compare interaction terms based on the different control groups for heterogeneity. We only report interactions for lead-gene combinations with no evidence of such heterogeneity – though we recognize that tests for heterogeneity are underpowered with samples as small as ours. Finally, we found that the combined estimates were similar to the estimates based only on subjects from the original ALS study, although with much less precision because of reduced numbers (Supplemental table 3). Consequently, we feel that the overall evidence argues that the second control group is not inducing bias in estimates of interaction, despite the likely bias in the lead main effect.

Overall, our results suggest that different polymorphisms in the *HFE* gene differentially influence the association between lead exposure and ALS, regardless of the lead biomarker evaluated, and that the *GSTP1* polymorphism increases the association of ALS with blood lead. Although the current study was limited by its small sample size, and thus should be interpreted with caution, the results suggest important avenues for further investigation—in particular a potential role of the *HFE* and *GSTP1* genes in modifying the effect of lead on the development of ALS. If such interaction is present, associations with the genetic polymorphisms estimated ignoring lead levels will lead to different findings in populations with different lead levels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Distribution of participant characteristics by case status.

Characteristic	Case N (%)	All Controls N (%)	Original Controls N (%)	Later Controls N (%)
Sex				
Male	62 (62.0)	83 (42.8)	28 (77.8)	55 (34.8)
Female	38 (38.0)	111(57.2)	8 (22.2)	103 (65.2)
p-value		*<0.01*		*<0.001*
Age (yrs)				
<60	49 (49.0)	34 (17.5)	12 (33.3)	22 (13.9)
60–69	27 (27.0)	61 (31.4)	15 (41.7)	46 (29.1)
70+	24 (24.0)	99 (51.0)	9 (25.0)	90 (57.0)
p-value		*<0.001*		*<0.01*
Education				
High school or below	35 (35.0)	25 (12.9)	3 (8.3)	22 (13.9)
Some college or above	65 (65.0)	169 (87.1)	33 (91.7)	136 (86.1)
p-value		*<0.001*		*0.58*
Pack-years of smoking				
Never	29 (29.0)	92 (47.4)	15 (41.7)	77 (48.7)
<10	19 (19.0)	40 (20.6)	3 (8.3)	37 (23.4)
10–30	25 (25.0)	39 (20.1)	9 (25.0)	30 (19.0)
>30	27 (27.0)	23 (11.9)	9 (25.0)	14 (8.9)
p-value		*<0.01*		*0.02*
Control groups				
Original ALS Case-Control Study	100 (100)	36 (18.6)	36 (100)	-
Controls recruited later	-	158 (81.4)	-	158 (100)

p-values in the All Controls column refer to differences between cases and all controls, and those in the Later Controls column refer to differences between the two control groups.

Table 2

Mean (sd) concentrations* of lead exposure biomarkers among controls by general characteristics and genotype (N=194).

Characteristic	N	Patella lead (μg/g)	Tibia lead (μg/g)	Blood lead (μg/dL)
Sex*				
Male	83	14.3 (12.6)	10.4 (9.6)	2.8 (1.8)
Female	111	10.2 (12.9)	7.9 (11.4)	1.7 (1.8)
Age (yrs)*				
<60	34	7.0 (9.9)	5.3 (9.6)	1.9 (1.2)
60–69	61	9.5 (12.2)	8.1 (9.9)	2.3 (2.0)
70+	99	15.2 (13.4)	10.7 (11.3)	2.2 (2.0)
Education				
High school or below	25	16.2 (11.3)	13.3 (11.1)	2.1 (1.3)
Some college or above	169	11.3 (11.6)	8.3 (10.1)	2.2 (1.9)
Pack-years of smoking				
Never	92	8.7 (9.3)	6.6 (9.7)	1.9 (1.2)
<10	40	12.8 (11.9)	9.8 (10.3)	2.5 (2.8)
10–30	39	16.2 (15.1)	12.3 (11.9)	2.3 (1.5)
>30	23	16.3 (12.9)	11.3 (8.6)	2.7 (2.0)
C282Y				
Wildtype (GG)	174	12.0 (12.3)	9.1 (10.6)	2.2 (1.8)
Variant (AG)	20	11.5 (8.7)	8.1 (8.8)	2.5 (1.9)
H63D				
Wildtype (CC)	150	11.7 (11.5)	8.8 (9.9)	2.2 (1.5)
Variant (CG + GG)	44	13.0 (13.8)	9.6 (11.9)	2.2 (2.6)
TfC2				
Wildtype (CC)	129	11.5 (11.9)	9.5 (10.1)	2.1 (1.6)
Variant (CT + TT)	65	12.8 (12.3)	8.0 (11.0)	2.4 (2.2)
GSTP1				
Wildtype (AA)	90	12.5 (12.7)	9.4 (10.5)	2.4 (2.2)
Variant (AG + GG)	104	11.5 (11.4)	8.6 (10.3)	2.0 (1.3)
Control groups				
Original ALS Case-Control Study	36	20.5 (10.9)	14.6 (9.9)	3.1 (1.9)
Controls recruited later	158	10.0 (11.6)	7.7 (10.2)	2.0 (1.7)

*Lead concentrations by all variables except age and sex are adjusted for age (year) and sex.

Table 3

Age and sex-adjusted odds ratios (OR) and 95% confidence intervals (CI) for ALS by genotype.

Genotype	Cases	Controls	OR (95% CI)
C282Y			
Wildtype (GG)	86	174	Ref
Variant (AG)	14	20	1.41 (0.64–3.10)
H63D			
Wildtype (CC)	69	150	Ref
Variant (CG + GG)	31	44	1.81 (0.98–3.32)
TfC2			
Wildtype (CC)	73	129	Ref
Variant (CT + TT)	27	65	0.63 (0.35–1.13)
GSTP1			
Wildtype (AA)	42	90	Ref
Variant (AG + GG)	58	104	1.21 (0.71–2.07)

Table 4

Multiplicative effect* of gene polymorphisms# on the odds ratio per interquartile range increase of patella lead (15.6 μg/g), tibia lead (12.2 μg/g), or blood lead (2 μg/dL) and ALS.

Genotype	Patella lead	Tibia lead	Blood lead
C282Y variant	8.24 (0.94–72.19)	2.92 (0.66–12.83)	1.17 (0.44–3.12)
H63D variant	0.34 (0.15–0.78)	0.60 (0.28–1.30)	0.36 (0.19–0.68)
GSTP1 variant	1.10 (0.56–2.18)	#	1.96 (0.98–3.92)

*Adjusted for age (year) and sex. Polytomous regression OR by each control group were combined by inverse variance weighting as described in the methods.

#The p-value for heterogeneity from the polytomous logistic regression for all interactions with *TfC2* and between tibia lead and GSTP1 were <0.05 and so combined ORs for these are not shown.