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Blood Levels of Trace Metals and Amyotrophic Lateral Sclerosis

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Abstract

Some trace metals may increase risk of amyotrophic lateral sclerosis (ALS), whereas others may be beneficial. Our goal was to examine associations of ALS with blood levels of selenium (Se), zinc (Zn), copper (Cu), and manganese (Mn). We conducted a case-control study of 163 neurologist confirmed patients from the National Registry of Veterans with ALS and 229 frequency-matched veteran controls. We measured metal levels in blood using inductively coupled plasma mass spectrometry and estimated odds ratios (ORs) and 95% confidence intervals (CIs) for associations between ALS and a doubling of metal levels using unconditional logistic regression, adjusting for age, gender, and race/ethnicity. ALS was inversely associated with both Se (OR=0.4, 95% CI: 0.2–0.8) and Zn (OR=0.4, 95% CI: 0.2–0.8). Inverse associations with Se were stronger in patients with bulbar compared to spinal onset, worse function, longer diagnostic delay, and longer collection delay; inverse associations with Zn were stronger for those with worse function and longer collection delay. In contrast, ALS was positively associated with Cu (OR=3.4, 95% CI: 1.5–7.9). For Mn, no linear trend was evident (OR=0.9, 95% CI: 0.6–1.3, P_{trend}=0.51). Associations of Se, Zn, Cu, and Mn with ALS were independent of one another. Adjustment for lead levels attenuated the positive association of ALS with Cu but did not change associations with Se, Zn, or Mn. In conclusion, Se and Zn were inversely associated with ALS, particularly among

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those with worse function, suggesting that supplementation with these metals may benefit such patients, while Cu was positively associated with ALS. Deficiencies of Se and Zn and excess Cu may have a role in ALS etiology.

Keywords

Amyotrophic Lateral Sclerosis; Motor Neuron Disease; Risk Factors; Trace Metals; Case-Control Study

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease affecting motor neurons in the brain and spinal cord (Wijesekera and Leigh, 2009). Most patients are in their fifties and sixties, and ALS is more common in men than women (Wijesekera and Leigh, 2009). ALS has a complex etiology: about 10% of patients have a family history of ALS, and their disease may have a genetic origin; the remaining 90% are likely due to a combination of genetic and environmental factors, with the latter playing an important role (Al-Chalabi and Hardiman, 2013).

Metal exposure is a potentially relevant environmental factor, although previous studies have produced inconsistent results, perhaps partly attributable to limited exposure assessment (Sutedja et al., 2009). Few studies have evaluated metal exposure using levels measured in blood or other tissues. Lead (Pb), the metal most often studied using measured levels, is generally positively associated with ALS risk (Fang et al., 2010; Kamel et al., 2005). Studies of other metals, including selenium (Se), zinc (Zn), copper (Cu), and manganese (Mn), have primarily evaluated the presumed toxic effects of high exposures (Bergomi et al., 2002; Garzillo et al., 2014; Kapaki et al., 1997; Nagata et al., 1985; Pamphlett et al., 2001; Roos et al., 2013; see Supplemental Table 1). Metal deficiencies could also plausibly increase ALS risk because metals are essential trace elements and play a role in mitigating oxidative stress as well as other critical cell processes (Jellinger, 2013). Previous studies have yielded inconsistent results, possibly because of small sample sizes, choice of comparison groups, or the different types of samples used for measurement (Supplemental Table 1). No previous study has evaluated modification of the ALS-metal associations by clinical features, such as site of onset.

For the present analysis, we used data from the Veterans with ALS and Lead Exposure (VALE) study, a case-control study of US military veterans (Fang et al., 2010). We focused on four trace metals, Se, Zn, Cu, and Mn: Se because it has been reported to increase ALS risk (Vinceti et al., 2010); Zn and Cu because they are co-factors for superoxide dismutase (SOD1), an enzyme implicated in familial ALS (Trumbull and Beckman, 2009); and Mn because it has been implicated in neurodegenerative disease (Jellinger, 2013). In addition, Se, Zn, and Cu all affect oxidative stress (Jellinger, 2013; Navarro-Alarcon and Cabrera-Vique, 2008), a mechanism potentially involved in ALS pathogenesis (Wijesekera and Leigh, 2009). We report associations of ALS with these metals, variation of these associations by clinical features and effects of Pb on the relationships between ALS and each of the four metals.

2. Materials and Methods

2.1 Study Population

VALE patients came from the US Department of Veterans Affairs (VA) National Registry of Veterans with ALS (VA Registry) (Allen et al., 2008). Veterans or their caretakers who passed a telephone screening questionnaire were asked to provide medical records, and approximately half donated blood samples for a DNA bank (DiMartino et al., 2007). Neurologists specializing in motor neuron disease (MND) used information from the medical records to assign a diagnosis using an algorithm based on the revised El Escorial Criteria (Brooks et al., 2000). The VALE study included a subset of VA Registry patients who donated blood samples between January and September 2007. Our main analyses focused on 163 patients with clinically definite, probable, or possible ALS. We also considered a broader group of MND patients consisting of these ALS patients plus 30 persons diagnosed with progressive muscular atrophy; patients with primary lateral sclerosis were excluded from all analyses.

VALE controls came from the Genes and Environmental Exposures in Veterans with ALS (GENEVA) study (Schmidt et al., 2008). GENEVA identified controls from an age-stratified random sample of 10,000 US veterans obtained from the Veterans Benefit Administration in June 2005. Veterans free of ALS and other neurological disorders were frequency matched to GENEVA patients on age within five years (age at diagnosis for patients and age at interview for controls), and use of the VA health care system before diagnosis/interview (as a proxy for socioeconomic status). For VALE, controls already enrolled in GENEVA were contacted between May 2007 and May 2008 and invited to donate a blood sample during a home visit. Of 359 controls contacted, 252 consented to participate, and 229 donated a blood sample.

Institutional Review Boards of the National Institute of Environmental Health Science, the Durham VA Medical Center, Duke University, and Copernicus Group approved VALE. All study participants provided written informed consent.

2.2 Blood Collection and Metal Measurements

For VALE patients, blood collection procedures were the same as those for other VA Registry patients (Allen et al., 2008) except for the addition of a whole blood sample in a 6-mL BD Vacutainer blue-top Trace Element metal-free tube (Becton, Dickinson and Company, Franklin Lakes, New Jersey) for metal measurement; this tube was collected first. Like patients, VALE controls provided two samples collected during home visits: a 6-mL whole blood sample in a metal-free tube, collected first, and a 9-mL plasma sample. Samples for cases and controls were processed similarly. For both, blood samples were chilled and shipped cold. Upon arrival at the lab, plasma samples were separated and whole blood and plasma samples were frozen within 48 hours of blood draw and stored at −80°C until assay.

The present analysis focused on Se, Zn, Cu, and Mn; Pb was considered as a covariate because it has commonly been associated with ALS. We determined metal concentrations in 1.0 ml whole blood by inductively coupled plasma mass spectrometry (ICPMS) as

previously described (Fang et al., 2010). All batches included both cases and controls. Sample contamination was minimized by using a class 100 plastic hood and trace metal-free reagents (oxidants, 18-MΩ-quality deionized water, and ultrex-grade acids). Detection limits were 0.113 μg/dl for Se, 6.24 μg/dl for Zn, 0.234 μg/dl for Cu, and 0.124 μg/dl for Mn. Assay precision was good, with percent relative standard deviation ~2% for all four metals. To additionally monitor precision, 5% of cases and controls underwent preparation and analysis twice; agreement was ≥95% for all duplicates except for Zn, where agreement was ≥80%. Because the assay was optimized for Pb measurement, no external standards for other metals were evaluated. However,values for reagent blanks, included in all batches, were below the detection limit for all four metals. Accuracy was evaluated as percent relative error using spiked blood samples; values were 68% for Se, 5% for Zn, −17% for Cu, and −8% for Zn. The high recovery for Se, contributing to the increased % relative error, is due to spectral interference that can impart a consistent positive bias to biological samples unless a tuning gas is used. This gas can interfere with sensitivity for detection of Pb and hence was not used. Historical data for Se from the laboratory indicated % relative error of −18% for spiked reagent blanks and 10% for spiked blood samples.

2.3 Covariates

Information on covariates including age, gender, race (White, other), ethnicity (Hispanic, non-Hispanic), and smoking (ever, never) was collected by interview for both patients and controls. We combined race and ethnicity to create a single race/ethnicity variable (non-Hispanic White, other).

For patients, we obtained clinical information such as site of onset (spinal, bulbar) and dates of symptom onset and first diagnosis from medical records. We used the latter two variables to create the variable diagnostic delay, defined as the time from symptom onset to diagnosis and categorized as ≤ 1 vs. > 1 year. We defined collection delay as the time from diagnosis to blood collection and dichotomized it at the median (≤ 13 vs. > 13 months).

Patients enrolled in the VA Registry completed follow-up interviews at approximately six month intervals. During each interview, participants' functional status was monitored using the revised ALS Functional Rating Scale (ALSFRS-R) (Gordon et al., 2004). The ALSFRS-R score has a possible range 0–48 with a lower score representing worse function. For the present analysis, we used the score determined closest to the time of blood draw and dichotomized at the median (≤ 29 vs. > 29).

2.4 Statistical Analysis

We calculated Spearman correlation coefficients to evaluate associations between each pair of metals among controls, ALS patients, and MND patients. We used unconditional logistic regression models to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for associations between blood metals and ALS or MND. We assessed model fit using the Akaike information criterion (AIC). We used both categorical and continuous versions of the metal variables. For the former, we created categories using quintiles of metal levels among the controls as cut points, taking the group with the lowest levels as the referent. For continuous variables, we $\log_2$-transformed the metal levels to ensure linearity in model fit

and reported P_{trend} from models using these variables. We always adjusted for age (continuous), gender, and race/ethnicity and sometimes additionally adjusted for smoking, Pb, or the other metals in addition to the metal of interest, as potential confounders.

To evaluate whether associations between metals and ALS or MND varied across sub-groups of patients, we used multinomial logistic regression models with three outcome categories: controls and two disease sub-groups defined by site of onset, dichotomized ALSFRS-R score, dichotomized diagnostic delay, or dichotomized collection delay. We adjusted multinomial logistic regression models only for age (continuous) and race/ethnicity due to the small number of female cases (n = 3) in the study.

We used SAS 9.3 (SAS Institute, Cary, NC) for all statistical analyses.

3. Results

Characteristics of the study participants are presented in Table 1. The average reference age (diagnosis for patients, interview for controls) among all participants was ~63 years. Most participants were men, non-Hispanic Whites, and ever smokers. The most common diagnosis among patients was clinically probable ALS, and most patients had spinal onset. Whole blood Zn, Cu, and Mn levels among VALE controls were similar to their respective levels in other populations, whereas Se levels among VALE controls were higher than in European populations, as were Se levels in the US population as a whole reported in NHANES (Alimonti et al., 2005; Bocca et al., 2011; Heitland and Koster, 2006; Jain et al, 2014; Rosborg et al., 2007; see Supplemental Table 2).

In general, pairwise correlations among the four metals and lead were weak – the maximum absolute Spearman correlation coefficient was 0.23 among controls, 0.30 among ALS patients, and 0.35 among MND patients – although some were statistically significant. Statistically significant correlations included a positive correlation between Zn and Se among controls but not among ALS or MND patients; a positive correlation of Cu and Pb among controls and ALS patients but not MND patients; and a positive correlation of Zn with Mn and a negative correlation between Zn and Cu among all three groups (Supplemental Table 3).

As judged using the AIC, model fit was essentially the same for the categorical and continuous variables for Se, slightly better for the continuous variables for Zn and Cu, and notably better for the categorical variable for Mn (Table 2). After adjustment for age, gender, and race/ethnicity, both Se and Zn were inversely associated with ALS, whereas Cu was positively associated with ALS, regardless of whether categorical or continuous variables were used (Table 2). The relationship of Mn with ALS was non-linear (P_{trend}=0.51) with an inverted U shape, with the strongest association observed in comparing the middle to the lowest 20% (OR=2.3, 95% CI: 1.2–4.3). For all metals, results for MND were qualitatively the same as those for ALS (Table 2).

Additional adjustment for smoking did not change associations of ALS with any of the metals (Table 3). Adjusting for Pb did not change associations of ALS with Se, Zn, or Mn, but it attenuated the positive association of ALS with Cu (Table 3). Including all four metals

of interest as well as Pb in the models gave essentially the same results as adjusting for Pb alone: associations of ALS with Zn, Se, and Mn were similar to those from models with only the metal of interest, although less precise, and the positive association with Cu was attenuated (Table 3).

Comparing ALS patients with different clinical features (spinal vs. bulbar site of onset, lower vs. higher ALSFRS-R score, shorter vs. longer diagnostic delay, shorter vs. longer collection delay), we found little difference in median blood levels for any of the metals. We did, however, find differences in ORs between ALS sub-groups for some metals, particularly for Se (Table 4). Specifically, we saw strong inverse associations of ALS with Se among patients with bulbar onset, lower ALSFRS-R scores, longer diagnostic delay, and longer collection delay whereas associations in the respective opposite categories were indistinguishable from null. The association of Zn with ALS did not differ by site of onset or by category of diagnostic delay, but it did differ by category of ALSFRS-R score or of collection delay. Namely, Zn was inversely associated with ALS only among patients with ALSFRS-R scores below the median or collection delays above the median. Associations of ALS with Cu and Mn did not vary by clinical features.

4. Discussion

We found associations of ALS with four trace metals: Se, Zn, Cu and Mn. Both Se and Zn were inversely associated with ALS. Cu was positively associated with ALS, although the association was weakened after adjustment for Pb. The odds of ALS exhibited an inverted U-shape relation with Mn levels, with the strongest positive association evident at intermediate Mn levels. Associations of ALS with the four metals were largely independent of one another. We found similar associations among a broader group of MND patients that also included individuals with progressive muscular atrophy.

Whole blood levels of Zn, Cu, and Mn in VALE controls were similar to levels in other populations (Supplemental Table 2). In contrast, Se levels in VALE controls were elevated compared to levels reported for European populations (Supplemental Table 2). Our blood Se values may be typical for the United States population; for example, levels found in NHANES were also elevated compared to European populations but still slightly lower than those in VALE controls (Jain et al., 2014; Supplemental Table 2). In general, blood Se levels are higher in the US, ranging from 16–40 μg/dl, than in most other parts of the world (Combs, 2001), but the reasons for this difference are not entirely clear. Exposure to Se occurs mainly through diet and only rarely through drinking water and occupation (Navarro-Alarcon and Cabrera-Vique, 2008; Combs, 2001). Se levels in diet vary by season, geographical location, food processing methods, and protein content (Navarro-Alarcon and Cabrera-Vique, 2008; Combs, 2001), factors which may explain differences in Se levels between populations in the United States and elsewhere.

An important question is whether the higher Se levels in controls contributed to the inverse association we observed. It is unlikely that the accuracy of our assay is a critical factor. Although the method used may impart a positive bias to biological samples, assay characteristics are unlikely to vary between cases and controls and so cannot explain the

inverse association we found. It is however possible that differences in diet between ALS cases and controls may have contributed to the inverse association of Se with ALS that we observed.

Previous studies comparing trace metal levels in ALS patients and controls have reported inconsistent results, possibly because of variations in study design and small size, with 17 to 40 patients and 9 to 40 controls in each study (Bergomi et al., 2002; Garzillo et al., 2014; Kapaki et al., 1997; Nagata et al., 1985; Pamphlett et al., 2001; Roos et al., 2013; Supplemental Table 1). These studies measured metals in various biological samples, including cerebrospinal fluid (CSF), whole blood, blood cells, serum or plasma, and toenails. The type of sample chosen for measurement does not seem to be a major determinant of results, although one study found that, compared to controls, ALS patients had high levels of Zn, Cu, and Mn in CSF but not in plasma (Roos et al., 2013). On the other hand, the choice of controls may have influenced results. For example, use of spouses, friends, or co-workers as controls (Pamphlett et al., 2001; Roos et al., 2013) may lead to overmatching and compromise a study's ability to detect associations, either positive or inverse; such individuals often live with the cases and have similar lifestyles and occupations and hence similar exposures (Rothman et al., 2008). Other factors, such as the approach to data analysis, may also have influenced results; two of the six studies did not match controls to patients (Kapaki et al., 1997; Nagata et al., 1985), and only one adjusted for any covariates (Bergomi et al., 2002).

Previous studies of Se and ALS have reported inconsistent results. Some have suggested that Se exposure increases ALS risk. Veterinary observations suggested that high levels of Se are toxic to motor neurons in swine and cattle (reviewed in Vinceti et al., 2010), and a case series reported that four farmers who lived in a Se-rich environment in South Dakota developed ALS (Kilness and Hichberg, 1977). A small case-control study (41 patients) reported that drinking well water with high Se content was associated with ALS, but possible contaminants other than metals were not considered (Vinceti et al., 2010). One cross-sectional study found that ALS patients had higher blood Se levels than controls (Nagata et al., 1985), but three other cross-sectional studies found no difference between ALS patients and controls for Se levels in blood (Kapaki et al., 1997; Pamphlett et al., 2001; Roos et al., 2013) or CSF (Roos et al., 2013). There was a strong although imprecise inverse association (RR=0.3) of ALS with Se in toenails in the one study that adjusted for covariates (Bergomi et al., 2002). In our larger study, which also adjusted for covariates, we found a statistically significant inverse association of the same magnitude of ALS with Se. Further high quality studies will be necessary to resolve these discrepancies.

Most previous studies of Zn found little evidence for an association with ALS. Two studies found no difference between cases' and controls' Zn levels in several blood compartments (Nagata et al., 1985; Pamphlett et al., 2001), and another found no association with toenail levels (Bergomi et al., 2002). One study reported that ALS patients had higher levels of Zn than controls in CSF although not in plasma (Roos et al., 2013), whereas another found no difference in either CSF or serum levels (Kapaki et al., 1997). Our finding of an inverse association is inconsistent with these reports.

Results for Cu are similarly mixed. One study found that ALS patients had lower levels of Cu than controls in both CSF and serum (Kapaki et al., 1997) while another reported higher levels in CSF and no difference in plasma (Roos et al., 2013). Other studies found no association of Cu with ALS in several blood compartments (Pamphlett et al., 2001) or in toenails (Bergomi et al., 2002). We found that Cu and Pb levels were correlated and that adjusting for Pb attenuated the positive association of ALS with Cu, suggesting that the association of ALS with Cu in the present study may reflect the association with Pb we previously observed (Fang et al., 2010).

Elevated Mn may contribute to the high-incidence foci of ALS in the Western Pacific (Bowman et al., 2011). Some studies in other locations found increased Mn levels in brain, spinal cord, and muscle from ALS patients (Kurlander and Patten, 1979; Mitchell et al., 1991; Miyata et al., 1983) while others did not (Kihira et al., 1990; Pierce-Ruhland and Patten, 1980). Most of these studies were small, however, and the observed changes in Mn levels may reflect tissue degeneration. Degenerating brain tissue may undergo changes in number, type, or spatial distribution of neurons or glia, changes in the volume of specific brain regions, accumulation of aggregated proteins or other structures, or changes in the permeability of the blood brain barrier, all of which may change metal concentrations, making it difficult to infer etiological relationships (Dickson and Weller, 2011). In addition, methods of sample collection, storage, and processing all affect brain metal concentrations (Hare et al., 2012; Schrag et al., 2011), and these may differ between cases and controls. Among studies that measured Mn levels in blood or CSF, results were mixed. One study found that, compared to controls, Mn levels were elevated in serum but not CSF of ALS patients (Kapaki et al., 1997), while another found the reverse – an elevation among patients compared to controls in CSF but not plasma (Roos et al., 2013). Another study found no difference in Mn levels in serum of ALS patients and controls (Garzillo et al., 2014). One study reported an inverse association of ALS with Mn levels in blood cells (Nagata et al., 1985), while others found null associations in several blood compartments (Pamphlett et al., 2001) and in toenails (Bergomi et al., 2002). The explanation for our finding of an inverted U-shaped relationship of ALS with Mn is unclear.

Associations of metal levels with ALS progression, assessed with the ALSFRS, have also been evaluated in one study: ALS progression was inversely associated with Se and Zn, positively associated with Cu, and not related to Mn (Bergomi et al., 2002). Metals may have different relationships with ALS risk and progression, but it is noteworthy that our findings on ALS risk are consistent with these results for progression.

For the most part, associations we observed between ALS and metals did not vary by clinical features of the disease. One notable exception was Se, where associations with ALS were stronger for individuals with bulbar onset, longer diagnostic or collection delay, or a lower ALSFRS-R score, but not for those with the opposite characteristics. Zn showed a similar pattern. Thus associations with deficiencies of Se or Zn were more pronounced in individuals with more advanced disease, possibly suggesting that supplementation might be beneficial for these individuals. This could be particularly important if changes in diet contributed to the inverse association of Se with ALS.

Several mechanisms might explain an association of ALS with metal levels in blood. Se and Zn both play a role in mitigating oxidative stress (Navarro-Alarcon and Cabrera-Vique, 2008; Prasad, 2014; Steinbrenner and Sies, 2013). This might contribute to the inverse associations we observed between ALS and these metals, because oxidative stress may play a role in ALS etiology (Jellinger, 2013; Wijesekera and Leigh, 2009). More specifically, a lower risk of ALS has been found in studies where a higher intake of antioxidants in diet occurred (Ingre et al., 2015). Cu and Mn, on the other hand, may both increase oxidative stress (Martinez-Finley et al., 2013; Rivera-Manica et al., 2010), in this case potentially contributing to the positive associations we found between ALS and these metals. Cu and Zn may also play a more direct role in ALS because both are cofactors for cytosolic SOD1. Mutations in this enzyme, found in about 20% of familial ALS patients, lead to a toxic gain of function (Wijesekera and Leigh, 2009). Most polymorphisms result in misfolding of the SOD1 monomer, reducing its affinity for Zn and exposing the Cu binding site; this conformational change leads the enzyme to generate rather than detoxify reactive oxygen species (Rivera-Mancia et al., 2010; Trumbull and Beckman, 2009). Loss of Zn also contributes to dissociation of the SOD1 dimer and formation of larger aggregates, a process likely involved in ALS (Rivera-Mancia et al., 2010; Trumbull and Beckman, 2009). Decreased availability of Zn may lead the wild type SOD1 monomer to misfold, with consequences similar to those of the genetic polymorphisms (Rivera-Mancia et al., 2010; Trumbull and Beckman, 2009). Studies showing that Zn supplementation or Cu chelation can retard disease progression in rodent models underscore these findings and suggest that metal imbalance may contribute to development of sporadic ALS (Vonk and Klomp, 2008).

The primary limitation of our study is that blood was collected after diagnosis and thus differences in metal levels in cases compared to controls may be a consequence rather than a cause of ALS. It is worth noting that dietary differences between patients and controls may have contributed to the observed inverse association of Se with ALS. Concerns regarding reverse causation arise in all cross-sectional or case-control studies that measure metals in human tissues, but it may be a less serious problem for studies of metals in blood than for studies of metals in degenerating nerve or muscle. Metals in both compartments may be affected by disease-related changes in lifestyle or exposure opportunities, but concentrations in degenerating tissue will also be affected by changes related to degeneration itself (Dickson and Weller, 2011). A second limitation is that patients in our study tended to be long survivors, so that the associations we observed between metals in blood and ALS may reflect associations with survival as well as risk. Finally, the study subjects were primarily white men who had served in the United States military, potentially limiting the generalizability of our findings. However, our primary interest was the biological relationships of trace metals to ALS which would depend on the internal validity of our study and likely not be affected by use of a veteran population.

Our study also has major strengths, including the large sample size and use of a highly sensitive metal assay, which allowed us to evaluate variation at low levels. ALS diagnoses were made by neurologists specializing in motor neuron disease. We controlled for confounding by known risk factors of ALS. Because of the large study size and the availability of clinical information, we were uniquely able to evaluate the associations of ALS with metals in sub-groups of patients with different clinical features.

5. Conclusions

We found that Se and Zn were inversely associated with ALS, particularly among patients with worse function, suggesting that supplementation with these metals may benefit such patients. In contrast, Cu was positively associated with ALS, although this relationship was attenuated by adjustment for Pb. While we cannot exclude reverse causation as an explanation for our findings, our results are consistent with those of experimental studies investigating the pathogenesis of ALS. Previous literature on the relationship of trace metals to ALS has been inconsistent. Although we cannot of course resolve all discrepancies, our study was considerably larger than previous ones and had a stronger study design. We conclude that deficiencies of Se and Zn and excess Cu may have a role in ALS etiology. This hypothesis should be considered in additional populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

AIC	Akaike information criterion
ALS	amyotrophic lateral sclerosis
ALSFRS-R	revised ALS functional rating scale
CI	confidence interval
CSF	cerebral spinal fluid
Cu	copper
GENEVA	Genes and Environmental Exposures in Veterans with ALS
ICPMS	inductively coupled plasma mass spectrometry
Mn	manganese
MND	motor neuron disease
NHANES	National Health and Nutrition Examination Survey
ns	not statistically significant

OR	odds ratio
Pb	lead
RBCs	red blood cells
RR	relative risk
SD	standard deviation
Se	selenium
SOD1	superoxide dismutase 1
VA	Department of Veterans Affairs
VALE	veterans with ALS and lead exposure
Zn	zinc

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Table 1

Characteristics of study participants.

	Controls (n=229) N (%)	ALS Cases (n=163) N (%)	MND Cases (n=193) N (%)
Age (years), mean ± (SD)	63.4 (9.9)	62.9 (10.8)	63.5 (10.8)
Quartiles *, N (%)			
34–57	65 (28)	41 (25)	48 (25)
58–64	58 (25)	48 (29)	55 (28)
65–71	50 (22)	30 (18)	32 (17)
72–84	56 (24)	44 (27)	58 (30)
Gender, N (%)			
Male	216 (94)	160 (98)	190 (98)
Female	13 (6)	3 (2)	3 (2)
Race and Ethnicity, N (%)			
Non-Hispanic White	201 (88)	152 (93)	179 (93)
Other	28 (12)	11 (7)	14 (7)
Cigarette Smoking, N (%)			
Ever	154 (67)	104 (65)	122 (65)
Never	75 (33)	56 (35)	67 (35)
Missing	0	3	4
Diagnosis, N (%)			
Clinically definite ALS	-	31 (19)	31 (16)
Clinically probable ALS	-	108 (66)	108 (56)
Clinically possible ALS	-	24 (15)	24 (12)
Progressive muscular atrophy	-	-	30 (16)
Site of Onset, N (%)			

	Controls (n=229) N (%)	ALS Cases (n=163) N (%)	MND Cases (n=193) N (%)
Spinal	-	127 (78)	156 (81)
Bulbar	-	36 (22)	37 (19)
Diagnostic Delay (years), N (%)			
≤ 1	-	88 (54)	100 (52)
> 1	-	75 (46)	93 (48)
Collection Delay (months)[†], N (%)			
≤ 13	-	76 (47)	91 (47)
> 13	-	87 (53)	102 (53)
ALS Functional Rating Scale-Revised Score[†], N (%)			
≤ 29	-	82 (51)	92 (49)
> 29	-	78 (49)	97 (51)
Missing	-	3	4

Abbreviations: SD, standard deviation.

[*] Categorized at quartiles among the controls.

[†] Dichotomized at the median among ALS cases.

Table 2

Association of blood levels of trace metals with risk of ALS and MND.

	Controls N=229 N (%)	ALS Cases N=163 N (%)	ALS OR (95% CI)*	ALS AIC	MND Cases N=193 N (%)	MND OR (95% CI)*	MND AIC
Selenium (μg/dL) †				527.729			579.044
≥ 11.9 and ≤ 26.8	48 (21)	57 (35)	1.0 (Reference)		65 (34)	1.0 (Reference)	
> 26.8 and ≤ 29.3	46 (20)	31 (19)	0.5 (0.3–0.9)		37 (19)	0.6 (0.3–1.0)	
> 29.3 and ≤ 31.5	44 (19)	20 (12)	0.3 (0.2–0.7)		27 (14)	0.4 (0.2–0.8)	
> 31.5 and ≤ 34.3	41 (18)	27 (17)	0.5 (0.3–0.9)		32 (17)	0.5 (0.3–0.9)	
> 34.3 and ≤ 73.5	50 (22)	28 (17)	0.5 (0.2–0.8)		32 (17)	0.5 (0.3–0.8)	
log_2Se §‡			0.4 (0.2–0.8)	527.761		0.4 (0.2–0.8)	576.822
Zinc (μg/dL) †				533.850			582.560
≥ 268 and ≤ 530	47 (21)	49 (30)	1.0 (Reference)		56 (29)	1.0 (Reference)	
> 530 and ≤ 593	45 (20)	23 (14)	0.4 (0.2–0.9)		26 (13)	0.5 (0.2–0.9)	
> 593 and ≤ 643	46 (20)	35 (21)	0.7 (0.4–1.2)		39 (20)	0.6 (0.4–1.2)	
> 643 and ≤ 693	46 (20)	28 (17)	0.5 (0.3–1.0)		32 (17)	0.5 (0.3–1.0)	
> 693 and ≤ 974	45 (20)	28 (17)	0.6 (0.3–1.1)		40 (21)	0.7 (0.4–1.3)	
log_2Zn §‡			0.4 (0.2–0.8)	528.888		0.5 (0.3–1.1)	581.319
Copper (μg/dL) †				531.860			583.683
≥ 47.0 and ≤ 73.5	46 (20)	23 (14)	1.0 (Reference)		30 (16)	1.0 (Reference)	
> 73.5 and ≤ 81.5	46 (20)	27 (17)	1.3 (0.6–2.5)		35 (18)	1.2 (0.6–2.3)	
> 81.5 and ≤ 89.0	46 (20)	36 (22)	1.7 (0.9–3.3)		43 (22)	1.5 (0.8–2.9)	
> 89.0 and ≤ 97.5	46 (20)	30 (18)	1.4 (0.7–2.7)		34 (18)	1.2 (0.6–2.2)	
> 97.5 and ≤ 154.0	45 (20)	47 (29)	2.5 (1.3–4.8)		51 (26)	2.1 (1.1–3.9)	
log_2Cu §‡			3.4 (1.5–7.9)	526.060		2.5 (1.2–5.5)	578.528
Manganese (μg/dL) †				521.353			571.281

	Controls N=229 N (%)	ALS Cases N=163 N (%)	ALS OR (95% CI)*	ALS AIC	MND Cases N=193 N (%)	MND OR (95% CI)*	MND AIC
≥ 0.23 and ≤ 0.74	46 (20)	25 (15)	1.0 (Reference)		32 (17)	1.0 (Reference)	
> 0.74 and ≤ 0.93	46 (20)	44 (27)	1.7 (0.9–3.3)		48 (25)	1.5 (0.8–2.7)	
> 0.93 and ≤ 1.15	44 (19)	55 (34)	2.3 (1.2–4.3)		63 (33)	2.0 (1.1–3.7)	
> 1.15 and ≤ 1.39	47 (21)	24 (15)	0.9 (0.5–1.9)		34 (18)	1.0 (0.5–2.0)	
> 1.39 and ≤ 6.03	46 (20)	15 (9)	0.6 (0.3–1.3)		16 (8)	0.5 (0.2–1.1)	
$\log_2$Mn §‡			0.9 (0.6–1.3)	534.195		0.9 (0.6–1.3)	583.489

*Adjusted for age, gender and race/ethnicity.

†Categorical metal variables were categorized at quintiles among the controls.

§Continuous metal variables were log2-transformed.

‡OR for log2-transformed levels corresponds to a doubling of the metal level.

Table 3

Association of blood levels of trace metals with ALS, controlling for smoking, lead, or other metals.

	OR (95% CI)*	OR (95% CI)† Smoking	OR (95% CI)‡ Lead	OR (95% CI)§ All Metals
Selenium (log_2Se, μg/dL) ¥				
	0.4 (0.2–0.8)	0.4 (0.2–0.8)	0.5 (0.2–1.0)	0.5 (0.2–1.0)
Zinc (log_2Zn, μg/dL) ¥				
	0.4 (0.2–0.8)	0.4 (0.2–0.8)	0.4 (0.2–0.8)	0.4 (0.2–1.1)
Copper (log_2Cu, μg/dL) ¥				
	3.4 (1.5–7.9)	3.3 (1.4–7.7)	2.1 (0.9–5.0)	1.8 (0.7–4.1)
Manganese (μg/dL) ¶				
≥ 0.23 and ≤ 0.74	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
> 0.74 and ≤ 0.93	1.7 (0.9–3.3)	1.8 (0.9–3.5)	1.7 (0.9–3.4)	1.8 (0.9–3.6)
> 0.93 and ≤ 1.15	2.3 (1.2–4.3)	2.5 (1.3–4.7)	2.3 (1.2–4.4)	2.6 (1.3–5.1)
> 1.15 and ≤ 1.39	0.9 (0.5–1.9)	1.0 (0.5–2.0)	1.0 (0.5–2.0)	1.1 (0.5–2.3)
> 1.39 and ≤ 6.03	0.6 (0.3–1.3)	0.7 (0.3–1.4)	0.6 (0.3–1.4)	0.7 (0.3–1.6)

*Adjusted for age, gender and race/ethnicity; results for models in this column are the same as those in Table 2, presented here for comparison.

†Adjusted for age, gender, race/ethnicity, and smoking.

‡Adjusted for age, gender, race/ethnicity, and lead.

§Adjusted for age, gender, race/ethnicity, and lead, selenium, zinc, copper, and manganese.

¥OR for log2-transformed levels corresponds to a doubling of the metal level.

¶Categorized at quintiles among the controls.

Table 4

Association of blood levels of trace metals with ALS risk by clinical features.

	Metal Concentration (μg/dL) Median (Range)	OR (95% CI) *¥	P-value †
Selenium (μg/dL) §			
Site of Onset			
Spinal	29.5 (15.3–66.8)	0.6 (0.3–1.3)	≤ 0.01
Bulbar	26.3 (11.9–51.0)	0.1 (0.03–0.3)	
ALSFRS-R Score ‡			
≤ 29	27.4 (11.9–57.0)	0.1 (0.04–0.3)	≤ 0.01
> 29	30.7 (21.2–66.8)	1.7 (0.7–4.2)	
Diagnostic Delay (years)			
≤ 1	29.3 (15.8–66.8)	0.7 (0.3–1.7)	0.03
> 1	28.0 (11.9–46.5)	0.2 (0.1–0.5)	
Collection Delay (months)			
≤ 13	30.4 (19.4–66.8)	1.3 (0.5–3.1)	≤ 0.01
> 13	27.5 (11.9–61.0)	0.1 (0.1–0.4)	
Zinc (μg/dL) §			
Site of Onset			
Spinal	602 (268–944)	0.4 (0.2–1.0)	0.71
Bulbar	608 (353–901)	0.3 (0.1–1.2)	
ALSFRS-R Score ‡			
≤ 29	595 (268–901)	0.2 (0.1–0.6)	0.09
> 29	619 (325–944)	0.7 (0.2–1.9)	
Diagnostic Delay (years)			
≤ 1	600 (268–783)	0.3 (0.1–0.7)	0.21
> 1	612 (353–944)	0.6 (0.2–1.6)	
Collection Delay (months)			
≤ 13	617 (268–944)	0.8 (0.3–2.2)	0.05
> 13	579 (290–901)	0.2 (0.1–0.6)	
Copper (μg/dL) §			
Site of Onset			
Spinal	88.5 (60.7–136)	2.4 (1.0–5.8)	0.21
Bulbar	88.6 (67.3–136)	6.3 (1.5–26.2)	
ALSFRS-R Score ‡			
≤ 29	88.9 (60.7–136)	4.2 (1.5–11.8)	0.23
> 29	88.3 (64.5–126)	1.9 (0.7–5.5)	
Diagnostic Delay (years)			
≤ 1	88.3 (64.5–136)	2.2 (0.8–5.8)	0.27

	Metal Concentration (μg/dL) Median (Range)	OR (95% CI) *¥	P-value †
> 1	89.8 (60.7–136)	4.4 (1.5–12.7)	
Collection Delay (months)			
≤ 13	88.3 (65.6–136)	3.3 (1.1–9.4)	0.78
> 13	88.6 (60.7–136)	2.7 (1.0–7.4)	
Manganese (μg/dL) ¶			
Site of Onset			
Spinal	1.0 (0.4–3.1)	0.9 (0.6–1.3)	0.92
Bulbar	1.0 (0.5–3.3)	0.8 (0.4–1.6)	
ALSFRS-R Score ‡			
≤ 29	1.0 (0.5–3.3)	0.9 (0.6–1.4)	0.93
> 29	1.0 (0.5–2.9)	0.9 (0.6–1.5)	
Diagnostic Delay (years)			
≤ 1	0.9 (0.5–1.8)	0.7 (0.4–1.1)	0.14
> 1	1.0 (0.4–3.3)	1.1 (0.7–1.8)	
Collection Delay (months)			
≤ 13	1.0 (0.5–3.3)	1.0 (0.6–1.7)	0.21
> 13	1.0 (0.4–2.9)	0.7 (0.5–1.1)	

*Adjusted for age, race/ethnicity.

†Comparing ORs for ALS cases with the indicated features.

§Continuous metal variables were log2-transformed. ORs correspond to a doubling of the metal level.

¶For Mn, analysis with the categorical variable produced similar results.

‡Three ALS cases were omitted from the analysis because ALSFRS-R scores were missing.