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Cumulative lead exposure is associated with reduced olfactory recognition performance in elderly men: the Normative Aging Study

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Abstract

Introduction—Olfactory dysfunction has been identified as an early warning sign for Alzheimer's disease, Parkinson's disease, dementia and more. A few occupational and environmental exposures have also been associated with reduced olfactory function, although the effects of long term environmental exposure to lead on olfactory dysfunction have not been explored. Here we performed olfactory recognition testing in elderly men in a community-dwelling cohort and examined the association with cumulative lead exposure, as assessed by lead in tibial and patellar bone.

Methods—Olfactory recognition was measured in 165 men from the Normative Aging Study (NAS) who had previously taken part in bone lead measurements using K-X-Ray fluorescence (KXRF). Olfactory recognition was measured using the University of Pennsylvania Smell Identification Test (UPSIT). Associations between olfactory recognition, global cognition and cumulative lead exposure were estimated using linear regression, with additional adjustment for age, smoking, and functional polymorphism status for hemochromatosis (*HFE*), transferrin (*TfC2*), glutathione-s-transferase Pi1 (*GSTP1*) and apolipoprotein E (*APOE*) genotypes. Sensitivity analyses explored olfactory recognition in men with high global cognitive function as measured using the Mini-Mental Status Exam (MMSE).

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Conflict of interest statement:
The authors declare that there are no conflicts of interest.

Results—The average age of the NAS participants at the time of olfactory recognition testing was 80.3 (standard deviation or SD = 5.7) years. Mean tibia lead was 16.3 (SD = 12.0) μg/g bone, mean patella lead was 22.4 (SD = 14.4) μg/g bone, and mean UPSIT score was 26.9 out of 40 (SD = 7.0). Consistent with previous findings, age at olfaction testing was negatively associated with UPSIT score. Tibia (but not patella) bone lead was negatively associated with olfaction recognition (per 15 μg/g tibia lead: β = −1.57; 95% CI: −2.93, −0.22; p = 0.02) in models adjusted for smoking and age. Additional adjustment for education did not significantly change results. Of all the genes explored, only the presence of one or more HFE variant alleles was significantly associated with olfaction recognition (HFE β = 2.26; 95% CI: 0.09, 4.43; p = 0.04). In a model containing the HFE term and a lead term, the tibia lead parameter estimate dropped by 21% (per 15 μg/g tibia lead: β = −1.25; 95% CI: −2.64, 0.14; p = 0.08) while the HFE term dropped 15% (β = 1.91; 95% CI: −0.28, 4.10; p=0.09). None of the other gene terms were associated with olfactory recognition in this cohort, nor were any gene-lead interaction terms significant. Additional sensitivity analysis in men with MMSE scores of 25 or higher (n = 149) showed a similar but slightly attenuated association between lead and olfactory recognition (per 15 μg/g tibia lead β = −1.39; 95% CI: −3.00, 0.22; p = 0.09)

Conclusion—Cumulative exposure to lead is associated with reduced olfactory recognition in a cohort of elderly men. The association was similar but not significant in men with better cognitive function as measured by the MMSE. Iron metabolism gene status may also affect olfactory function.

Keywords

Olfaction; aging; bone lead; HFE; ApoE; GSTP1

1. INTRODUCTION

Olfactory dysfunction is regarded as an early warning sign of Parkinson's disease (Louis et al., 2008, Mollenhauer et al., 2013, Ross et al., 2012), Alzheimer's Disease (Murphy et al., 1990, Thompson et al., 1998, Wang et al., 2010), and cognitive decline (Royall et al., 2002, Seo et al., 2009, Sohrabi et al., 2012, Swan and Carmelli, 2002). Given that lead, pesticides like DDT and other environmental exposures also show associations with these diseases (Richardson et al., 2014, Richardson et al., 2009, Weisskopf et al., 2010), and are known to be rhinotoxic in occupationally exposed populations and animal models (Sunderman, 2001), it is plausible that environmental exposures such as lead could also be related to olfactory dysfunction.

Occupational exposures including cadmium (Rose et al., 1992, Sulkowski et al., 2000), solvents (Schwartz et al., 1990), pesticides (Dick et al., 2001), industrial chemicals (Schwartz et al., 1989) and manganese (Antunes et al., 2007) have been associated with decrements in olfactory function. An Italian occupational study found significant associations between lead measured via air sampling and performance on an olfactory threshold task (Caruso et al., 2007), while two other studies on occupational lead exposure found detrimental but not significant associations with performance on an odor identification task (Bolla et al., 1995, Schwartz et al., 1993). Environmental manganese emitted from a ferroalloy plant was associated with reduced performance on an olfactory task in Italian

adolescents (Lucchini et al., 2012), and reduced olfactory scores were associated with air pollution exposure in children and young adults in Mexico (Calderon-Garciduenas et al., 2010).

While environmental lead exposure's effect on olfactory function has yet to be investigated, cumulative lead exposure has been previously shown to be associated with multiple types of cognitive dysfunction in adults (Bandeen-Roche et al., 2009, Schwartz, Bolla, 1993, Shih et al., 2006, van Wijngaarden et al., 2009). Specifically among men in the Normative Aging Study (NAS), bone lead measured in either the tibia or patella has been shown to be associated with impaired visuomotor skills (Payton et al., 1998), increased rate of cognitive decline (Weisskopf et al., 2007, Weisskopf et al., 2004), lower scores on the Mini-Mental Status Exam (Wright et al., 2003), reduced associative learning (Grashow et al., 2013a), and poorer hand-eye coordination (Grashow et al., 2013b).

A number of genes related to metal ion transport (TfC2) and absorption (HFE) have been shown to affect how lead is processed and stored in the body. Lead exposure may interfere with iron metabolism (Eaton and Qian, 2002, Samson and Nelson, 2000) and be associated with toxic levels of non-transferrin bound iron in plasma leading to neurodegenerative disease (Huang et al., 2004, Todorich and Connor, 2004). The hemochromatosis gene encodes a protein that is in part responsible for iron sensing and regulation and HFE variant homozygosity results in clinical hemochromatosis, which is characterized by iron overload. The presence of one or more polymorphism of the HFE gene (either H63D or C282Y) has been shown to exacerbate the detrimental effects of lead on cognitive function in elderly men (Wang et al., 2007), and has been associated with increased susceptibility to neurodegenerative disease (Eum et al., 2014, Mariani et al., 2013, Nandar and Connor, 2011). Interestingly, variant HFE gene expression has also been associated with lower blood and bone lead in the NAS cohort (Wright et al., 2004). Based on this finding, we hypothesized that carriers of any HFE variant would have reduced circulating blood and bone lead and therefore reduced effects on olfactory recognition performance.

Other genes, such as the ApoE gene, may also play a role in age-related cognitive function and olfaction. ApoE is a polymorphic gene that encodes a protein regulating transport of cholesterol, lipids, and fat-soluble vitamins. Certain ApoE polymorphisms such as ApoE-ε4 have been found to predispose individuals to neurodegenerative diseases like Alzheimer's (Poirier et al., 2014, Teter et al., 2002). Recently, it was shown that metals may be involved in the regulation of Alzheimer's related proteins, and that aberrant metal ions concentrations are more likely to occur in AD-diagnosed patients (Xu et al., 2014). ApoE is expressed in olfactory brain structures, and those with the ApoE-ε4 variant allele show reduced odor identification (Olofsson et al., 2010) and altered brain responses to olfactory stimuli (Green et al., 2013).

Glutathione-s-transferase Pi1(GSTP1) has been shown to reduce the effects of oxidative stress through free radical clearance (Hayes and Strange, 2000), and may modify the relationship between lead and cognitive function in the elderly (Eum et al., 2013) and inflammatory markers in adult males (Sirivarasai et al., 2013). Finally, the gene TfC2 encodes the transferrin protein that is responsible for iron transport, and interacts with HFE

(Namekata et al., 1997). We therefore examined the association between cumulative lead exposure as measured in bone and olfactory recognition in a population of elderly men in the Boston, MA area, with additional analysis of associations with HFE, GSTP1, TfC2 and ApoE polymorphism status. Additional analyses explored associations between olfactory identification and lead in men with higher MMSE scores.

2. METHODS

2.1 Study population

The Normative Aging Study (NAS) began recruiting men from the Boston area community beginning in the 1960s. Since that time, NAS subjects have been encouraged to return every three to five years for medical examinations (Bell et al., 1966, Hu et al., 1996, Weisskopf, Proctor, 2007). Current NAS participants are elderly and are mostly Caucasian. Starting in 1991, subjects were invited to participate in bone lead testing using K-shell X-ray fluorescence (KXRF). 68% (876 participants) agreed to bone lead testing. Two hundred and forty-three NAS subjects participated in olfactory recognition testing between January of 2009 and March of 2012, with 231 completing the entire olfactory recognition test (12 subjects did not complete all questions). Of those with completed tests, 165 had participated in bone lead testing. None of the men participating in the olfactory recognition testing reported diagnosis of Alzheimer's Disease or Parkinson's Disease. Approval from the Institutional Review Boards at the VA Boston Healthcare System, Brigham and Women's Hospital and the Harvard School of Public Health was obtained prior to study commencement. All subjects provided written informed consent before participating.

2.2 KXRF measurement of bone lead

Lead concentrations in tibial and patellar bone are considered to be markers of cumulative lead exposure: patella lead reflects exposure over the previous 8–10 years, while tibial shaft bone represents exposure occurring over decades (Wilker et al., 2011). For this study, KXRF was used to measure bone lead concentrations at the patella and midtibial shaft using either an ABIOMED KXRF prototype or upgraded system (ABIOMED, Danvers, MA). As previously explained (Weuve et al., 2009), a linear relationship was established between the two instrument types, and data used in this study adjust for the linear difference between the two machines. Additional detail on the testing protocol has been previously described (Aro et al., 1994, Chettle et al., 2003, Hu et al., 1998). Bone lead concentration is measured in μg of lead per gram of bone.

2.3 Olfactory recognition testing

The University of Pennsylvania Smell Identification Test (UPSIT, Sensonics, Inc., Haddon Heights, NJ) is considered a highly reliable test of olfactory recognition or identification (Doty et al., 1989). Detail on this test can be found elsewhere (Doty et al., 1984). Briefly, 40 microfragrances (e.g. licorice, rose and pine) were embedded in capsules, and fragrances are released when scratched by the subject. A trained tester instructs the subject to smell each sample and then choose the best answer from a multiple choice list of four items. The score ranges from 0 to 40, with 40 representing 40 correct identifications.

2.4 Cognitive function assessment

The Mini-Mental Status Exam (MMSE) is used as a screening instrument to identify dementia and global cognitive function. NAS subjects who participated after 1993 were invited to take a series of cognitive tests, including the MMSE. The MMSE has a maximum score of 30. However, in this population the question related to “county of residence” was ignored because it is not generally used for geographical identification in the greater Boston area. Therefore, the maximum score for this population was 29. For these analysis, we treated the MMSE score as continuous and also dichotomously (between 25 and 29, and 24 or below). All models including MMSE score were additionally adjusted for education.

2.5 Genotyping

Archived blood samples taken from participants were genotyped for two single nucleotide polymorphisms (SNPs) of the HFE gene, H63D and C282Y; for two SNPs of the APOE gene (T392C and T530C), the GSTP1 gene Ile105Val SNP, and for the transferrin gene pro570ser SNP (TfC2). Genotypes were ascertained using multiplex polymerase chain reaction (PCR) in conjunction with restriction fragment length polymorphism (RFLP) analysis assays (Sequenom, Inc., San Diego, CA). 10% of samples were run in duplicate for quality control. Further information on laboratory methods can be found elsewhere (Park et al., 2006, Wright, Silverman, 2004). Due to the small sample size, subjects were categorized as being homozygous wild type, or having at least one variant allele (either H63D or C282Y). Subjects were dichotomized into having no copies of the APOE E4 allele, or having at least one copy of the APOE -ε4 allele. Similarly, TfC2 and GSTP1 were also dichotomized into two categories of wild-type or having at least one variant allele. Genotyping for the GSTP1 Ile105Val polymorphism was done using Multiplex PCR assays (Sequenom, San Diego, CA). A 384 well spectroCHIP was used to spot the extension product prior to MALDI-TOF mass spectrometry (Eum, Wang, 2013).

2.6 Covariates

Covariates considered were based on biology, previous literature on olfactory function and prior studies on cumulative lead exposure. Age at time of olfactory recognition test (continuously in years) and smoking (dichotomized as current smoker or not) were included in our base models. Other variables considered in other models were the interval between XRF and olfactory recognition testing (continuously in years) and years of education (continuously and also categorized into high school or less [12 years or fewer], some college [between 12 and 16 years] and some graduate school [greater than 16 years]).

2.7 Statistical analysis

Olfactory identification test score was quantified as the raw number of correctly identified odors out of a total of 40 total odors. Initial spline regression analyses were performed (using R version 2.14.2) and did not suggest any non-linear associations with bone lead, therefore this was treated linearly using ordinary least squares regression using PROC GENMOD in SAS software (version 9.4; SAS Inc., Cary, NC). Models including tibia and patella lead concentrations were run separately. One subject was missing patella lead, and was therefore excluded from all models of patella lead and olfactory function. Six subjects

who completed the XRF measurement and olfactory testing did not have genotype data, and were excluded from models that included variant allele status terms. All genotypes analyzed in this study were in Hardy-Weinberg equilibrium as assessed in the entire NAS population.

To assess effect modification between bone lead and genotype, cross product interaction terms were created and included in models with the lead term, gene term and other covariates. We explored the lead-olfaction relationship in a subset of men who received an MMSE score of 25 or higher. Additional sensitivity analyses was conducted using non-smokers only, and including terms for years of education as both a continuous and categorical variable.

3. RESULTS

Among the 165 NAS subjects who completed both bone lead and olfactory recognition testing, the average age at the time of XRF testing was 68.4 years (standard deviation or SD = 6.6) and the average age at the time of the olfactory recognition testing was 80.3 (SD = 5.7). There was no significant difference in smell scores between the men that did (26.9. SD = 7.0) and did not (28.5, SD = 6.3) participate in bone lead testing (p=0.11). Men with bone lead measures were slightly older (average age 80.0 years, SD = 5.7) when compared to men without bone lead (average age 77.4 years, SD = 6.6). Only subjects with completed olfactory test results were included in analyses.

An average of 12.0 years (SD = 2.6) elapsed between the KXRF bone lead measure and the UPSIT. Mean tibia lead in this population was 16.3 (SD = 12.0) μg/g bone, and the mean patella lead was 22.4 (SD = 14.4) μg/g bone. Subjects had on average 14.7 (SD = 2.6) years of education. Only 3.0% currently smoked, while 66.1% were former smokers.

The mean UPSIT olfactory score was 26.9 (SD=7.0) out of 40. Olfactory recognition test performance in NAS subjects who participated in K-XRF bone lead testing by genotype is shown in Table 1. 70.6% of subjects had the wild type allele for the H63D single nucleotide polymorphism (SNP), while 86.9% of subjects were identified as wild type carriers for the C282Y SNP. Men with all wild type versions of the HFE SNPs studied here had slightly higher bone lead levels (Table 1). Among men with wildtype HFE the mean tibia lead was 18.0 (SD = 13.5) μg/g bone and mean patella lead was 24.0 (SD= 15.8) μg/g bone, while men with at least one variant allele had a mean tibia lead of 13.7 (SD = 8.6) μg/g bone and a mean patella lead of 20.0 (SD = 12.0) μg/g bone. There was little difference in bone lead levels by other genotypes (Table 1).

As expected, age at the time of olfactory recognition testing was negatively associated with UPSIT score (Doty, 1989). In smoking-adjusted models the olfactory score was .25 points lower (95% CI: −0.44, −0.07; p=0.008) per year of age. This was reduced when tibia lead was added to the model (−0.19 per year of age; 95% CI: −0.38, 0.004; p=0.05), while higher tibia lead was significantly associated with worse olfactory recognition score (Table 2). Patella bone lead was not significantly associated with olfactory recognition in unadjusted or adjusted models (adjusted model per IQR of parent population [20 μg/gbone]: β= −0.80; 95% CI: −2.32, 0.72; p = 0.30). When patella bone lead at the time of olfactory identification testing was estimated using an exponential decay model, results were stronger,

but still not significant (adjusted model per IQR of parent population [20 μg/gbone]: β= −2.61; 95% CI: −6.31, 1.08; p = 0.17). When a term for the interval in years between KXRF lead measures and the UPSIT was added to the models, results for both patella and tibia lead were effectively unchanged. Results were also effectively unchanged if we included a term for years of education, or modeled smoking as current, former and never smokers or excluded the five current smokers (data not shown).

The presence of an HFE variant allele was associated with a higher UPSIT score (Table 2; β = 2.26; 95% CI: 0.09, 4.43; p = 0.04). When tibia lead and HFE variant status were in the model together, the association with each term was reduced somewhat relative to estimates from separate models (Table 2). There was no interaction between tibia lead and HFE status (p=0.55). None of the other genotypes were associated with olfaction recognition score whether or not tibia lead was in the model, and their inclusion did not change the tibia lead estimate (Table 2). There were also no interactions between tibia and patella lead and any of the other genotypes (all p>0.50).

We wanted to determine whether the relationship between lead and olfactory recognition would still be seen in men with higher global cognitive function as measured by the the Mini-Mental Status Exam (MMSE). For all participants, the mean (SD) for the MMSE was 26.6 (2.3), and the mean (SD) interval between the MMSE and the olfactory recognition task was 1.1 (SD=2.0) years. Of the 165 participants, 4 (2.4%) took the MMSE after the olfactory test, 111 (67.2%) took both tests on the same day, and 50 (30.3%) took the smell test before the MMSE. In this population tibia lead was significantly negatively associated with MMSE performance (adjusted model per IQR of tibia lead in parent population [15 μg/gbone]: β= −0.73; 95% CI: −1.19, −0.27; p = 0.002). To eliminate overall cognitive impairment as the cause of olfactory recognition dysfynction, we explored the lead-UPSIT relationship in men who had an MMSE score of 25 or higher (n = 149). In this group, the parameter estimate for lead remained similar, although this term was no longer significant (per 15 μg/g tibia lead: β = −1.39; 95% CI: −3.00, 0.22; p = 0.09). When the lead-MMSE association was modeled in this group, the tibia lead term dropped by 40%, and remained significantly associated with MMSE (per 15 μg/g tibia lead: β = −0.44; 95% CI: −0.76, −0.12; p = 0.007).

4. DISCUSSION

Studies showing associations between environmental risk factors and neurodegenerative diseases imply that exposures are incurring physiological, chemical and anatomical changes in the brain. In fact, olfactory dysfunction has been shown to precede motor symptoms in Parkinson’s disease and cognitive deficits in Alzheimer’s patients (Hawkes, 2006, Kranick and Duda, 2008, Takeda et al., 2014) and even distinguish between disease subtypes (Katzenschlager et al., 2004). Given that many environmental exposures occur via inhalation such as air pollution, olfactory dysfunction may be an important link between neurotoxicant exposures and neurological disease. In a cohort of elderly men residing in the greater Boston area, we found that cumulative lead exposure as measured in tibial bone was associated with reduced performance on the UPSIT, a well-established olfactory recognition task.

Additionally, we saw that the presence of at least one variant HFE allele was associated with increased olfactory recognition.

Nasal passages serve to filter and protect the lungs and upper airways from airborne exposures. However, airborne metals inhaled into nasal passages will bypass the circulatory system and blood brain barrier to accumulate in the olfactory bulb and other types of neural tissue. In addition to passing through the olfactory pathway, metal-binding molecules such as carnosine and metallothionein are abundant in the olfactory bulb, thus increasing ion uptake into the brain (Sunderman, 2001). Interestingly, a study of untreated rats found similar lead concentrations in the olfactory bulb and other areas of the brain (Scheuhammer and Cherian, 1982), however it is difficult to know if this pattern would also be seen in studies in animals and humans exposed to higher levels of lead.

Lead's effects on the brain have been in part attributed to its ability to substitute for calcium (Sanders et al., 2009, White et al., 2007), which is central to functions such as neurotransmission, mitochondrial function, apoptosis and more. The mechanism behind the effects of lead on olfactory function is unknown, but animal models indicate that lead exposure could alter neurotransmission in olfactory-related areas. Exposure to lead via intraperitoneal injection altered gene and enzyme expression patterns in the nitric oxide signaling pathway in the olfactory bulbs of adult mice (Kim et al., 2011). Additionally, prenatal lead exposure has been shown to alter olfactory discrimination in rats (Lim et al., 2005) as well as acetylcholine function in the olfactory bulb (Gietzen and Woolley, 1984, Widmer et al., 1992).

Olfactory recognition testing involves the olfactory system as well as brain processing areas that include cognitive and language abilities. There is a risk that a decline in olfactory recognition performance could be incorrectly attributed to dysfunction in olfactory areas, when in fact it results from dysfunction in cognitive and language abilities. We therefore investigated whether subjects with higher global cognitive function as measured by the MMSE showed a similar association between lead and olfactory recognition scores. In this smaller subset of the population, the term for tibia lead was essentially unchanged, although the p-value got larger clearly in part because the sample size was smaller. Interestingly, in this subset with better MMSE scores, the lead-MMSE relationship was reduced by almost half, while remaining significant. These results suggest that the effect of lead exposure seen in this study is not primarily due to cognitive effects, but instead lead may be directly influencing olfactory function. Further study is necessary to determine the impact of cumulative lead exposure on olfaction is affected by lead-related dysfunction in other brain areas.

Associations between tibia lead and olfactory identification ability were found, while no significant associations were found for patella. This could be due to the different time courses of lead deposition into bone type: the half-life of lead in tibial (cortical) bone is on the order of decades, while the half-life in patellar (trabecular) bone is only a few years (Wilker, Korrick, 2011).

Subjects with one or more copy of a variant HFE allele showed significantly increased UPSIT olfactory scores over those with wild type genes. Why this is the case is not clear, but one possibility is that it is related to differential effects on metal transport between the olfactory bulb and other regions. Although variant HFE allele carriers have general iron excess, which can have neurotoxic effects, it has been found that HFE knockout mice do not accumulate iron in the olfactory bulb (Kim et al., 2013). Furthermore, while H67D mutant mice show higher whole brain iron levels, they have similar olfactory bulb iron levels when compared to wildtype (personal communication, JK Kim). As this may relate to HFE mutant effects on metal transport, a similar phenomenon could occur with other metals like lead. It has been shown previously that HFE variant allele carriers have lower bone and blood lead concentrations than those with wildtype HFE (Wang, Hu, 2007). This is not likely the result of different external lead exposures and so suggests differences in lead distribution within the body. If variant HFE carriers tend to distribute lead to locations other than the olfactory bulb, similar to what is seen for iron, this could afford protection from adverse effects of lead. In this regard, having a variant HFE allele could be an indicator of reduced lifetime lead exposure to the olfactory bulb. This is consistent with the effect estimate for tibia lead being slightly reduced in the model with HFE allele status. In addition, HFE variant carriers show reduced blood levels of manganese (Mn), a known neurotoxin (Claus Henn et al., 2011). It is therefore possible that improved olfactory identification may be due to reduced circulating Mn in HFE variant carriers.

APOE-ε4 allele was not related to UPSIT score, nor did it modify the association with lead in this cohort. However this may be due to a small sample size. Studies with much larger sample sizes have shown odor identification impairment in APOE-E4 carriers (Finkel et al., 2011, Olofsson, Nordin, 2010). To our knowledge, this is the first investigation into the effects of TfC2 and GSTP1 on olfactory function. We found no olfaction differences between genotypes.

A limitation of our study is that for many of the subjects, a number of years elapsed between the bone lead XRF testing and the olfactory task. This may partially explain why no associations were seen with patellar bone, which has a half-life that is half as long as the average XRF-olfaction testing interval. The half-life of tibia bone is believed to be on the order of decades (Wilker et al, 2011). Our overall numbers were also somewhat small, particularly for analyses of interactions. It should also be noted that most of the subjects in this study were Caucasian men. It cannot be determined from these data whether older women or minority populations would show similar effects.

None of the subjects in this study reported diagnoses of Alzheimer's Disease (AD) or Parkinson's Disease (PD). Given that olfactory dysfunction may be an early sign of both of these conditions, we cannot rule out the possibility that some of the participants with poorer olfactory recognition scores were displaying subclinical symptoms of AD or PD.

Taken together, these results indicate that in addition to acting as a premorbid signal for serious neurological disease, olfactory recognition deficits may also occur in response to cumulative exposure to lead. Given other findings that cumulative lead exposure is associated with Parkinson's disease (Coon et al., 2006, Gorell et al., 1999, Weisskopf,

Weuve, 2010), our results raise the possibility that reduced olfactory recognition dysfunction is an early indicator of neurological effects of lead exposure that ultimately result in Parkinson's disease.

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Highlights

- We explored olfactory recognition in elderly men with environmental exposure to lead.
- Higher tibia lead was associated with reduced olfactory recognition performance
- Variant HFE allele status was associated with improved olfaction recognition
- APOE-ε4, GSTP1 and TfC2 were not associated with olfactory recognition.
- Lead exposure and iron metabolism gene status may affect olfactory recognition

Table 1

Study population characteristics shown by HFE, ApoE-ε4, TfC2, or GSTP1 genotype.

Characteristic	HFE wild-type (n = 99)	HFE variant allele (n= 61)	ApoE-ε2 or ε3 (n = 130)	ApoE-ε4 (n = 33)	GSTP1 wild-type (n = 75)	GSTP1 variant allele (n= 78)	TfC2 wild-type (n= 110)	TfC2 Variant allele (n=50)
Age at olfactory testing [mean (SD)]	80.3 (5.8)	80.4 (5.6)	80.5 (5.7)	80.0 (5.5)	80.6 (5.4)	80.4 (6.0)	80.2 (6.1)	80.8 (4.7)
UPSIT olfaction score [mean (SD)]	26.0 (7.2)	28.2 (6.6)	27.0 (7.2)	26.5 (6.2)	27.1 (7.7)	26.9 (6.5)	26.6 (7.5)	27.4 (6.1)
XRF-olfactory testing interval (years; [mean (SD)])	11.6 (2.5)	12.6 (2.5)	11.9 (2.7)	12.1 (2.2)	12.1 (2.4)	11.8 (2.7)	12.0 (2.5)	12.0 (2.7)
Tibia lead (μg/g bone; [mean (SD)])	18.0 (13.5)	13.7 (8.6)	16.5 (12.2)	16.0 (11.3)	16.5 (9.3)	16.0 (14.5)	15.3 (13.0)	18.6 (9.6)
Patella lead (μg/g bone; [mean (SD)])*	24.0 (15.8)	20.0 (12.0)	20.1 (14.7)	20.0 (13.2)	23.0 (12.8)	22.2 (16.5)	22.9 (15.5)	21.6 (12.4)

* patella data missing for one participant

Table 2

Adjusted association between tibia lead (per 15 μg/g tibia[a]), genotypes and olfaction score in individual and combined models.

		Models with tibia or gene term alone[b]		Models with tibia and gene terms together[b,c]			
				Tibia lead		Gene term	
Variable	**n**	**β (95% CI)**	**p-value**	**β (95% CI)**	**p-value**	**β (95% CI)**	**p-value**
Tibia lead	165	−1.57 (−2.93,−0.22)	0.02	--	--	--	--
HFE	160	2.26 (0.09. 4.43)	0.04	−1.25 (−2.64,0.14)	0.08	1.91 (−0.28, 4.10)	0.09
APOE-e4	163	−0.66 (−3.29, 1.96)	0.62	−1.56 (−2.93, −0.12)	0.02	−0.69 (−3.27, 1.90)	0.60
GSTP1	153	0.24 (−2.44, 1.96)	0.83	−1.58 (−2.98, −0.17)	0.03	−0.29 (−2.45, 1.88)	0.79
TfC2	160	0.97 (−1.33,3.27)	0.41	−1.56 (−2.95, −0.18)	0.03	1.29 (−0.99, 3.57)	0.27

[a] The interquartile range among the parent population (Weisskopf, Proctor, 2007)

[b] Adjusted for age and smoking.

[c] Models with both tibia lead and the gene term (no interaction term).