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Modifying roles of glutathione S-transferase polymorphisms on the association between cumulative lead exposure and cognitive function

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Abstract

Background—Glutathione-S-transferase gene (*GST*) polymorphisms can result in variable ability of these enzymes to remove electrophilic substrates. We investigated whether the *GSTP1* Val105 and *GSTM1* deletion polymorphisms modify the lead-cognitive function association.

Methods—We used repeated measures analysis to compare the association between cumulative lead biomarkers—bone lead measured using K-shell X-Ray Fluorescence—and Mini-Mental State Exam (MMSE) score by *GST* variants, adjusted for covariates, among Normative Aging Study participants, a Boston-based prospective cohort of men. We had complete data for 698 men (providing 1292 observations) for *GSTM1* analyses and 595 men (providing 1142 observations) for *GSTP1* analyses.

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Results—A 15 μg/g higher tibia lead concentration (interquartile range of tibia lead) was associated with a 0.24 point decrement in MMSE score among *GSTP1* Val105 variant carriers, which was significantly stronger than the association among men with only wild-type alleles (p=0.01). The association among *GSTP1* Val105 carriers was comparable to that of 3 years of age in baseline MMSE scores. The association between tibia lead and MMSE score appeared progressively steeper in participants with increasingly more *GSTP1* Val105 alleles. A modest association between tibia lead and lower MMSE score was seen among participants with the *GSTM1* deletion polymorphism. Neither of the glutathione S-transferase variants was independently associated with cognitive function, nor with lead biomarker measures. The results pertaining to patella lead were similar to those observed for tibia lead.

Conclusion—Our results suggest that the *GSTP1* Val105 polymorphism confers excess susceptibility to the cognitive effects of cumulative lead exposure.

Keywords

Lead; Glutathione S-transferase; Cognitive function; Environmental exposure; Gene-environment interaction

1. Introduction

In the United States, the population of persons aged 65 years and older is projected to increase twofold to 75 million in the next 30 years and a concomitant upsurge in the number of individuals with dementia is expected (US Census Bureau 2000a, 2000b). Cognitive decline, a risk factor for dementia, may be a transition stage spanning normal cognition and onset of diseases associated with dementia, including Alzheimer's disease (Bischkopf et al. 2002; Burns and Zaudig 2002; Pratico et al. 2002; Knopman et al. 2003; Palmer et al. 2003b).

Lead exposure has long been recognized to impair cognition. Numerous studies in children have found inverse associations between blood lead levels and tests of cognitive development (Schwartz 1994; Lanphear et al. 2005). Workers in lead-related industries have been found to experience cognitive declines proportional to current blood lead and total lead burden levels (Shih et al. 2007). The few studies that have addressed cognitive function among non-occupationally exposed older adults have predominantly reported inverse associations between body lead levels and cognitive function (Muldoon et al. 1996; Nordberg et al. 2000; Wright et al. 2003; Weisskopf et al. 2004, 2007; Shih et al. 2006, 2007; Weuve et al. 2006; Bandeen-Roche et al. 2009). Bone lead measures are biomarkers of body lead burden that have been found to correlate well with measures of cumulative external lead levels and integrated blood lead levels (Bleecker et al. 1997). With half-life estimates on the order of decades for cortical bone and several years for trabecular bone (Kim et al. 1997; Wilker et al. 2011), lead levels in bone are thought to better reflect long-term cumulative lead exposure than blood lead, which has a half-life of approximately 30 days (Rabinowitz 1991).

Oxidative stress has been proposed as a mechanism by which lead affects cognition (Volicer and Crino 1990; Feldman 1999). Glutathione S-transferases (GST) are enzymes involved in the clearance of harmful electrophilic compounds, including redox radicals. Several polymorphisms in *GST* result in phenotypic variations in enzymatic activity. The GST *pi* class (GSTP1) isoenzymes are expressed in the brain and blood brain barrier (Hayes and Strange 2000). Studies have reported that the Ile105· ·Val105 substitution in *GSTP1* is a functional polymorphism that results in differential substrate clearance efficiencies by GSTP1 isoenzymes (Spiteri et al. 2000). Of the *mu* class of isoenzymes, glutathione S-

transferase M1 (GSTM1) enzymes are the most widely expressed (Hayes and Strange 1995). It has been estimated that 45% of most populations are homozygous for the *GSTM1* deletion polymorphism and thus do not express the GSTM1 isoenzyme (Stroombergen and Waring 1999); these *GSTM1* null individuals may experience greater oxidative stress and, as a consequence, greater cognitive impairment associated with lead exposure because of their inability to clear particular reactive compounds.

Several studies have found associations between cumulative lead exposure and cognitive function in adults, but no study has yet evaluated the modifying roles of *GST* polymorphisms on this relation. Therefore, we examined the modifying roles of *GSTP1* Ile105Val and *GSTM1* null polymorphisms on the association between cumulative lead exposure—as measured by lead in bone— and cognition as measured by the Mini-Mental State Exam (MMSE), a test of global cognitive function, in a cohort of older men.

2. Material and methods

Participants in the current study were drawn from the VA Normative Aging Study (NAS), a community-based, prospective cohort study initiated in 1963 at the Veterans Affairs (VA) Outpatient Clinic in Boston to examine factors related to healthy aging among men (Bell et al. 1966). Every 3-5 years, study participants have undergone extensive in-person evaluations including medical and physical examinations and laboratory tests. They also completed questionnaires on smoking history, diet and other factors potentially related to aging and health. To date, the annual attrition due to all causes has been less than 1%, and more than 80% have responded to mailed questionnaires supplementing on-site examinations (Hu et al. 1996). This study protocol has been approved by the Human Subjects Committees of the VA Boston Healthcare System, the Brigham and Women's Hospital, and the Harvard School of Public Health.

2.1 Study Population

Beginning in 1991, bone lead measurements were taken using K-x-ray fluorescence (KXRF) among active participants who gave consent. In 1993, cognitive function assessments were initiated. At the time of the present study 1079 study participants had at least one cognitive assessment. Of the participants with cognitive measures, 789 had at least one bone lead measurement. Men with and without bone lead measures had comparable baseline characteristics and MMSE scores (Wright et al. 2003). In 1995, NAS participants were genotyped for the *GSTM1* deletion polymorphism using archived blood samples. In addition, cohort members were genotyped for the *GSTP1* Ile105Val polymorphism in 2003 and 2005. In all, 706 men had at minimum one cognitive assessment, complete information on all covariates, *GSTM1* genotyping data, and a tibia bone lead measure. Of these, 8 were excluded because of high tibia uncertainty levels. Of the 698 men (1292 observations) included in our repeated measures analysis of lead and cognition with the *GSTM1* null polymorphism, 157 completed three cognitive assessments, 280 completed two cognitive assessments, and 261 completed one cognitive assessment. For evaluation of the modifying role of the *GSTP1* Ile105Val polymorphism, 602 participants had complete data, 7 of whom were excluded because of high tibia uncertainty levels. Of the 595 men (1142 observations) in our analysis, 149 completed three cognitive assessments, 249 completed two cognitive assessments and 197 completed one cognitive assessment.

2.2 Lead Biomarker Measurement

In vivo bone measurements were taken using a KXRF instrument (ABIOMED, Inc., Danvers, MA) at the mid-tibia (shin bone) and the patella (knee cap bone) (Hu et al. 1989; Aro et al. 1994, 2000). These sites were chosen to be representative of the two predominant

bone types: cortical (tibia) and trabecular (patella) bone. These measurements have units of micrograms of lead per gram bone mineral (μg/g). Bone measurement taken closest in time to the cognitive assessment served as proxy for past lead burden. We kept all point estimates of bone lead concentrations (even including negative values). Use of all estimates without a minimum detectable limit has been identified as the most appropriate method of using these data in epidemiologic studies (Kim et al. 1995). Lead estimates with high uncertainty values (over 10μg/g for tibia, 15μg/g for patella) were censored as this usually indicates excessive movement during measurement (Hu et al. 1998).

Blood specimens were collected in trace-metal-free tubes with ethylenediaminetetra-acetic acid (EDTA), and we used the Zeeman background-corrected flameless atomic absorption spectroscopy with graphite furnace to measure the blood concentration. Before analyzing the sample, the instrument was calibrated with National Bureau of Standards Blood Lead Standard Materials before sample measurement. To maintain the internal reliability, 10% of the blood samples were run in duplicate, 10% were controls, and 10% were blanks. For the external validity, we used reference samples from the Centers for Disease Control and Prevention and the coefficient of variation was 8% for samples below 30 μg/dL. We did not find any evidence of external contamination or significant problems with reliability and the limit of detection for blood lead was 1 μg/dL.

2.3 GST Genotyping

Genotyping for the *GSTM1* polymorphism was done at the Harvard School of Public Health in 1995. The assay consisted of polymerase chain reaction (PCR) amplification of exons 4 and 5 of the *GSTM1* allele. As this polymorphism is a gene deletion, PCR product indicated the presence of one or more copies of the gene. In each case concomitant amplification of the *CYPA1A* gene was done as a positive control. The PCR amplification of *CYP1A1* resulted in a 312-bp product that was easily visualized in the presence or absence of the GSTM1 273-bp PCR product. Genotyping for the *GSTP1* Ile105Val polymorphism conducted in 2003 was performed on a subset of cohort members using the 5′ to 33 exonuclease assay in TaqMan (Applied Biosystems, Foster City, CA). In 2005, cohort members with archived DNA were re-genotyped for the *GSTP1* Ile105Val polymorphism using Multiplex PCR assays (Sequenom, San Diego, CA). The extension product was then spotted onto a 384 well spectroCHIP before being flown in the MALDI-TOF mass spectrometer.

2.4 Assessment of Cognitive Function

Participants were administered the Mini-Mental State Examination (MMSE), a global examination of cognitive function that assesses orientation, immediate and short term recall, verbal and written skills, attention and ability to follow commands (Crum et al. 1993; Folstein et al. 1975). It is widely used as a screening tool for dementia, but it is also commonly used in epidemiologic studies to assess cognitive function (Farmer et al. 1995; Izaks et al. 1995; Christensen et al. 1997; Knopman et al. 2003). Scores range from 0 to 30 with higher score denoting better cognitive performance, although in our analysis the highest possible score was 29 because we deleted the question “what county are we in?” from our tally. Other studies have reported that most Massachusetts residents do not know in which county they reside as counties in Massachusetts do not have strong governmental function (Tombaugh and McIntyre 1992).

2.5 Statistical Analysis

For all analyses, we centered the bone measures at their respective mean levels to increase the precision of effect estimates when we added interaction terms. We classified the participants as *GSTP1* wildtype (having only *GSTP1* Ile105 alleles) or *GSTP1* Val105

(having at least one *GSTP1* Val105 allele). Men with discordant *GSTP1* Ile105Val genotyping data from the two genotyping cycles were classified by the 2003 genotyping data. Among study members with *GSTM1* data, we grouped participants as *GSTM1* wildtype (having at least one copy of GSTM1) or *GSTM1* null.

We analyzed lead levels in tibia and patella separately and likewise evaluated *GSTM1* and *GSTP1* in separate models. To assess effect modification, we fitted repeated measures regression models of MMSE score with the correlation matrix determined by that which gave the best Akaike Information Criteria for the model. For *GSTP1* analyses this was autoregressive and for *GSTM1* it was unstructured. We included in models a term for the lead biomarker, indicator variables for the respective *GST* polymorphisms and cross-product term between the variants and lead biomarkers, along with terms for age, years of education, smoking status (current, never, past), pack-years smoked, nondrinker, alcohol consumption (grams/day), English as first language (yes, no), computer experience (yes, no) and diabetes (diagnosis or fasting glucose >126 mg/dl). Values of covariates as reported at the time of cognitive assessment were used in the analyses. To assess whether the lead and cognition relation may differ between *GSTP1* Val105 heterozygotes and homozygotes, we also conducted analyses to evaluate the association of lead on MMSE score by the number of *GSTP1* Val105 alleles.

All statistical analyses were conducted using SAS version 9.3 (SAS Institute Inc, Cary, NC). We used partial F-tests and likelihood ratio tests for statistical hypothesis testing. The p-value of statistical significance was considered to be <0.05.

3. Results

3.1 Participants characteristics and bone lead

Median concentrations of lead biomarkers in our study population were 19 (interquartile range [IQR], 13-28) μg/g, 25 (IQR, 17-36) μg/g, and 5(IQR, 3-6) μg/dL for tibia, patella, and blood lead respectively. On average, participants were 69 years of age at first cognitive testing. Median MMSE score at first cognitive assessment was 27 (range: 16-29). Participants who were younger, more educated, native English speakers, or had computer experience had lower bone lead levels on average (Table 1). Bone lead concentrations were higher among men with lower baseline cognitive scores. Forty-five % of men were homozygous for the *GSTM1* deletion polymorphism.

3.2 Participant characteristics and glutathione S-transferase polymorphisms

Characteristics of *GSTM1* non-null men were similar to those with the *GSTM1* deletion polymorphism with the exception of alcohol consumption level, which was higher among the *GSTM1* null group (Table 2). Of the subset of participants who were genotyped for *GSTP1*, 52% had at least one *GSTP1* Val105 variant. The distribution of *GSTP1* Ile105Val polymorphisms conformed to Hardy-Weinberg expected frequencies (df=2, χ^2=0.5796, p=0.75). Among members with genotyping data from both 2003 and 2005 cycles, concordance of *GSTP1* IleVal105 genotyping data was over 98%. *GSTP1* wildtype men and *GSTP1* Val105 carriers had comparable baseline characteristics (Table 3). Neither *GSTP1* nor *GSTM1* genotype was associated with lead biomarker measures or cognitive scores.

3.3 Effect Modification by GST genotypes of the association between cumulative lead exposure and cognitive function

Table 4 shows the association between tibia lead and MMSE score by *GST* genotype. Adjustment for age and education (known strong predictors of both lead exposure and cognitive function) substantially changed the effect estimates, while further adjustment had

little additional effect. Among participants with any *GSTP1* Val105 variant, an IQR increment in tibia lead (15μg/g) was associated with a 0.24-point decrement in MMSE score (95% CI: −0.39, −0.09) in the fully-adjusted model (Table 4). This difference in MMSE score was approximately equivalent to the difference in baseline MMSE scores between men in our study who were 3 years apart in age. This association among *GSTP1* Val105 variant carriers was significantly different (p=0.01) from men with wildtype alleles.

The association between higher tibia lead and lower MMSE score appeared progressively stronger with increasing number of *GSTP1* Val105 alleles: a 15μg/g higher tibia lead was associated with a 0.23-point lower MMSE score for men with one copy of *GSTP1* Val105 variant, and 0.30-point lower MMSE score for men with two copies of *GSTP1* Val105 variants (Table 4: Fully-adjusted model). The interaction between tibia lead and the *GSTP1* allele count (0/1/2) was significant (p=0.01).

Among *GSTM1* null men, a 15μg/g higher tibia lead was associated with a 0.16 points lower (95% CI: −0.32, 0.00) MMSE score in the fully-adjusted model. However, this was not significantly different from *GSTM1* non-null men (p=0.81, Table 4).

Overall, the associations with patella lead were similar to those with tibia lead (data not shown). Associations with blood lead were similar, but weaker. When blood lead and tibia lead were included together in models, blood lead was not associated with MMSE in any group, while the results for tibia were similar.

4. Discussion

In our study population of older men, the deleterious association between cumulative lead exposure and poorer cognitive function was significantly worse among men with *GSTP1* Val105 polymorphisms than among *GSTP1* wildtype participants. Moreover, the detrimental association of lead with cognition was greater among participants with more *GSTP1* Val105 alleles. Although lead burden was associated with slightly worse cognition among men with the *GSTM1* deletion polymorphism, the interaction between bone lead and *GSTM1* null did not reach statistical significance.

Several studies have found worse cognitive function amongst adults with higher cumulative low-level lead exposure as measured by lead in bone (Muldoon et al. 1996; Nordberg et al. 2000; Wright et al. 2003; Weisskopf et al. 2004, 2007; Shih et al. 2006, 2007; Bandeen-Roche et al. 2009; Weuve et al. 2009). The only studies to examine genetic modification of this association looked at polymorphisms in hemochromatosis (*HFE*) (Wang et al. 2007), δ-aminolevulinic acid dehydratase (*ALAD*) (Weuve et al. 2006; Rajan et al. 2008; Krieg Jr. et al. 2009), and vitamin D receptor (VDR) (Krieg et al. 2010) genes. We previously found a strong modification of the association between bone lead and MMSE score in the NAS cohort by *HFE* genotype, with those carrying either the H63D or C282Y single nucleotide polymorphism (SNP) showing a steeply inverse association, while those with wildtype alleles showed little association (Wang et al. 2007). Less consistent modifications by *ALAD* genotype have also been reported. We found in the NAS cohort that *ALAD-2* carriers showed a slightly steeper dose–response relation between blood lead, but not bone lead, and MMSE (Weuve et al. 2006), and between tibia lead, but not blood lead, and performance on a spatial copying task (Rajan et al. 2007). In contrast, analyses of National Health And Nutrition Examination Survey (NHANES) data found significantly faster response times on a simple reaction task with increasing blood lead among *ALAD-2* carriers 20-59 years of age, but not among older or younger groups (Krieg Jr. et al. 2009). Slight variation in the relation between blood lead and performance on some cognitive tests was also seen by VDR

genotype among groups 20-59 years old, and 60 years and older in NHANES data (Krieg Jr. et al. 2010).

We are not aware of any other study that has evaluated modification by *GST* polymorphisms of the association between lead and cognitive function. Lead is capable of promoting oxidative damage, a purported mechanism in the pathogenesis of neurodegenerative disease (Adonaylo and Oteiza 1999; Landrigan et al. 2000; Samson and Nelson 2000). GSTs play an essential role in the defense against oxidative stress as they catalyze the conjugation of glutathione with electrophilic compounds and also display glutathione peroxidase activities (Hayes and Strange 2000). Although it is not known how the *GST* variants modify the lead-cognition relation, differences in phenotypic expression of GST isoenzymes may alter the clearance rate of lead-related electrophilic substrates and thereby influence lead-associated cognitive changes. GSTs are also involved in the metabolism of dopamine (Menegon et al. 1998), which is capable of auto-oxidizing to 6-hydroxydopamine and causing oxidative stress (Borisenko et al. 2000; Soto-Otero et al. 2000). Interestingly, lead has also been shown to inhibit GST (Neal et al. 1999). GSTP1 proteins are widely expressed in the brain and blood brain barrier (Menegon et al. 1998). The GSTP1 Val105 isoenzyme has been shown to be more efficient for metabolizing compounds including diol epoxide than the wildtype GSTP1 isoenzyme while the wildtype GSTP1 isoenzyme is more effective in clearance of other compounds including 1-chloro-1,2-dinitrobenzene (Hayes and Strange 2000). It is unknown, however, which electrophilic substrates are the most detrimental in causing cognitive problems.

Approximately 50% of most populations are found to be homozygous for the *GSTM1* deletion polymorphism and do not express this enzyme, the most widely expressed of the GST *mu* class proteins (Geisler and Olshan 2001). These individuals are not capable of conjugating GSTM1-specific substrates and therefore, are thought to be more susceptible to particular forms of oxidative stress. We did not observe the *GSTM1* deletion polymorphism to significantly modify the lead-cognitive function association in our study population. This is not surprising as the GST isoenzymes show differing efficiencies in clearance of different electrophiles and differing localizations (Hayes and Strange 2000). GSTM1 may play a lesser role in neuronal oxidative stress defense as compared to GSTP1 because it is not as prevalently expressed in the brain as GSTP1 (Menegon et al. 1998). Furthermore, it is plausible that lead-related oxidative stress is more efficiently cleared by GSTP1 than GSTM1 enzymes. Lastly, as no substrate is cleared by all GSTs (Hayes and Pulford 1995), it is possible that the predominant electrophilic substrates generated by lead burden are not cleared by GSTM1 enzymes.

There were several limitations to this study. As with any aging cohort, there is potential for selection bias. We were somewhat reassured as there was no difference in baseline characteristics and MMSE scores between participants with and without bone lead measures. It is possible that the modifications observed were caused by other polymorphisms in the *GSTP1* gene or variants in a proximal gene that is in tight linkage disequilibrium with the *GSTP1* Val105 variant, though we believe this is unlikely. Although a polymorphism at amino acid 114 in the *GSTP1* gene has been identified, it is thought to be a non-functional polymorphism (Spiteri et al. 2000). As in any observational study, we cannot rule out unmeasured confounding, although we did account for known strong predictors of lead and cognitive function. Stroke and Alzheimer's disease predict MMSE score, but as only six men in our study population had suffered a stroke and none had been diagnosed with Alzheimer's disease, we did not adjust for these factors in our final model. Likewise, we did not adjust for blood pressure or hypertension, as these conditions may mediate lead's effect on cognition. Ninety-eight % of the men in our study population were white, making population stratification an unlikely confounder.

The MMSE is widely used to screen for dementia and has frequently been used to assess cognitive status and track longitudinal changes in cognitive function (Farmer et al. 1995; Izaks et al. 1995; Christensen et al. 1997; Knopman et al. 2003). However, it is a relatively easy test for which a learning effect has been reported (Izaks et al. 1995). A low degree of variability in MMSE scores among more highly educated persons is often observed (Crum et al. 1993). Therefore, the MMSE may have had low sensitivity in detecting cognitive impairment in our participants. Despite these limitations, the MMSE is among the most extensively characterized and most widely used tests of cognitive status for older adults, and performs with a reasonable degree of reproducibility and validity. Past studies have found high correlations between MMSE scores and scores on other well-described cognitive tests, such as the Blessed Information-Memory-Concentration test, and reasonable sensitivity and specificity in delineating individuals with and without dementia (Tombaugh and McIntyre 1992; Stuss et al. 1996). Finally, persons with mild cognitive impairment are found to have significantly worse MMSE score than cognitively normal individuals (Bennett et al. 2002).

Although we were not yet able to assess development of dementia in our population, cognitive decline is a strong predictor of subsequent dementia (Bischkopf et al. 2002; DeCarli 2003; Ingles et al. 2003; Knopman et al. 2003; Palmer et al. 2003a; Tuokko et al. 2003). Cognitively impaired individuals have a 10-15% annual risk of developing dementia compared with a 1-2% annual risk among healthy controls (Morris et al. 2001; Bischkopf et al. 2002; Knopman et al. 2003). Cognitive impairment has also been associated with a 3.1 to 5-fold increase in risk of developing Alzheimer's disease, the most common cause of age-related dementia (Tuokko et al. 2003). Moreover, the repeated findings of associations of lead burden with cognitive function in aging populations indicate that increased attention should turn to older adults as a population of concern. In addition, given the high prevalence of *GSTP1* Val105 carriers in North American populations and the long retention of lead in the body, our results suggest the lead-related cognitive impairment experienced by a large subset of older adults is likely more substantial than currently recognized (Miller et al. 2002). Lastly, as long-term chronic lead exposure appears to be most detrimental to cognitive health in the later years, our findings stress the continued importance of public health interventions, including lead abatement efforts and lead exposure prevention programs, aimed at reducing occupational and environmental exposure to lead in younger populations.

In summary, we have found the *GSTP1* Val105 polymorphism to significantly modify the association between lead burden and poorer cognitive function. Persons with more copies of the *GSTP1* Val105 allele were observed to perform worse on cognitive assessments per unit increase in bone lead biomarker level. We have also found lead burden, as measured by bone lead levels, to be marginally associated with worse cognition among individuals with the *GSTM1* deletion polymorphism.

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Table 1

Bone lead concentrations by baseline characteristics of study participants (N=719)

	Tibia lead median ug/g [IQR][a]	Patella lead median ug/g [IQR][a]
Age		
<65 years	15 [10,21]	20 [14,30]
65-70	20 [13,27]	25 [17,36]
≥70	23 [17,34]	29 [19,46]
Education		
Never finished high school	27 [18,36]	33 [24,45]
High school graduate	21 [14,30]	27 [18,42]
Some college	19 [13,28]	25 [16,37]
College graduate	17 [11,24]	22 [15,31]
Smoking status		
Never	18 [12,27]	24 [16,37]
Former	20 [13,28]	25 [17,36]
Current	18 [13,29]	30 [19,36]
Alcohol consumption		
Yes	19 [13,27]	24 [16,35]
No	20 [13,28]	25 [17,37]
History of diabetes[b]		
Yes	21 [16,31]	27 [19,38]
No	19 [13,27]	24 [16,36]
English as first language		
Yes	19 [13,27]	24 [16,36]
No	22 [14,32]	26 [19,39]
Computer experience		
Yes	16 [11,23]	21 [15,30]
No	21 [15,30]	27 [19,40]
Baseline MMSE score[c]		
<26	21 [14,31]	30 [18,45]
26-27	20 [13,27]	25 [17,36]
≥28	17 [12,25]	22 [15,32]
GSTM1 genotype		
Non-null allele	19 [12,29]	24 [16,37]
Null allele	20 [14,27]	25 [17,36]
GSTP1 genotype		
Wildtype *GSTP1* lle105	19 [13,27]	25 [16,36]
GSTP1 Val105 heterozygote	20 [13,28]	25 [17,37]
GSTP1 Val105 homozygote	20 [13,28]	22 [17,37]

[a] IQR: interquartile range.

[b] History of diabetes defined as having reported diagnosis of diabetes or having fasting glucose above 126 mg/dl.

[c] Highest possible MMSE score in our analysis was 29 due to deletion of the question “what county are we in?”

Table 2

Baseline characteristics of study participants by *GSTM1* null genotype (N=684)

	***GSTM1* non-null N=306**	***GSTM1* null N=378**
Age, median years [IQR][a]	68.7 [63.4,73.7]	68.7 [63.2,72.6]
Education, n (%)		
Never finished high school	20 (6.5)	24 (6.4)
High school graduate	87 (28.4)	107 (28.3)
Some college	83 (27.l)	101 (26.7)
College graduate	116 (37.9)	146 (38.6)
Smoking status, n (%)		
Never	94 (30.7)	117(31.0)
Former	199 (65.0)	232(61.4)
Current	13 (4.3)	29 (7.7)
Alcohol consumption, median g/day [IQR][a]	4.2 [0,13.9]	6.9 [0,19.3]
History of diabetes, n (%)	37(12.1)	49 (13.0)
English as first language, n (%)	270 (88.2)	342 (90.5)
Computer experience , n (%)	131 (42.8)	143 (37.8)
Blood lead, median ug/g [IQR][a]	5 [3,6]	5 [3,6]
Patella lead, median ug/g [IQRa][a,b]	24 [16,37]	25 [17,36]
Tibia lead, median ug/g [IQR][a,b]	19 [12,29]	20 [14,27]
Baseline MMSE score, median [IQR][a,c]	27 [26,28]	27 [26,28]

[a] IQR: interquartile range.

[b] Patella lead measures with uncertainty value >15 and tibia lead measures with uncertainty value >10 were excluded.

[c] Highest possible MMSE score in our analysis was 29 due to deletion of the question “what county are we in?”.

Table 3

Baseline characteristics of study participants by *GSTP1* 105 genotype (N=582)

	Wildtype (*GSTP1* Ile105) N=270	**Variant (*GSTP1* Val105) N=312**
Age, median years [IQR][a]	68.0 [62.9,72.7]	68.0 [63.0,72.4]
Education, n (%)		
Never finished high school	14 (5.2)	20 (6.4)
High school graduate	66 (24.4)	93 (29.8)
Some college	75 (27.8)	79 (25.3)
College graduate	115 (42.6)	120 (38.5)
Smoking status, n (%)		
Never	75 (27.8)	97 (31.1)
Former	175 (64.8)	199 (63.8)
Current	20 (7.4)	16(5.1)
Alcohol consumption, median g/day [IQR][a]	5.5 [0,17.6]	5.2 [0,15.4]
History of diabetes, n (%)	29(10.7)	41 (13.1)
English as first language, n (%)	241 (89.3)	279 (89.4)
Computer experience , n (%)	120 (44.4)	127 (40.7)
Blood lead, median ug/g [IQR][a]	4 [3, 6]	5 [3, 7]
Patella lead, median ug/g [IQR.][a,b]	25 [16,36]	24 [17,37]
Tibia lead, median ug/g [IQR][a,b]	19 [13,27]	20 [13,28]
Baseline MMSE score, median [IQR][a,c]	27 [26,28]	27 [26,28]

[a] IQR: interquartile range.

[b] Patella lead measures with uncertainty value >15 and tibia lead measures with uncertainty value >10 were excluded.

[c] Highest possible MMSE score in our analysis was 29 due to deletion of the question “what county are we in?”.

Table 4

Difference in MMSE score per an interquartile range (15 ug/g) increase in tibia lead biomarker by *GST* genotypes.

***GST* genotypes**	**Unadjusted**	**Age and education adjusted[a]**	**Fully adjusted[b]**	
	Mean Difference in MMSE score (95% CI)	**Mean Difference in MMSE score (95% CI)**	**Mean Difference in MMSE score (95% CI)**	**P for interaction**
Model 1: *GSTP1* genotype				
Wildtype	−0.18 (−0.37, 0.02)	0.09 (−0.10,0.29)	0.07 (−0.12, 0.26)	
Any *GSTP1* Val105	−0.50 (−0.65,−0.35)	−0.25 (−0.40, −0.10)	−0.24 (−0.39, −0.09)	0.01
Model 2: *GSTP1* genotype				
Wildtype	−0.18 (−0.37, 0.02)	0.09 (−0.10, 0.29)	0.07 (−0.12, 0.26)	
GSTP1 Val105 heterozygote	−0.49 (−0.66, −0.33)	−0.25 (−0.41, −0.09)	−0.23 (−0.39, −0.07)	0.01[c]
GSTP1 Val105 homozygote	−0.55 (−0.93, −0.17)	−0.27 (−0.63, 0.10)	−0.30 (−0.67, 0.06)	0.07[d]
Model 3: *GSTM1* genotype				
GSTM1 non-null	−0.39 (−0.56, −0.23)	−0.12 (−0.29, 0.04)	−0.13 (−0.29, 0.03)	
GSTM1 null	−0.41 (−0.57, −0.25)	−0.16 (−0.32, 0.00)	−0.16 (−0.32,0.00)	0.81

[a] Adjusted for age, years of education, and visits.

[b] Adjusted for age, years of education, nonsmoker, former smoker, packyears, nondrinker, alcohol consumption, english as first language, computer experience, diabetes, and visits.

[c] P-value for tibia lead and *GSTP1* Val105 heterozygote interaction.

[d] P-value for tibia lead and *GSTP1* Val105 homozygote interaction.