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GENETIC POLYMORPHISMS OF *GRIN2A* AND *GRIN2B* MODIFY THE NEUROBEHAVIORAL EFFECTS OF LOW-LEVEL LEAD EXPOSURE IN CHILDREN

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Abstract

Lead (Pb) is neurotoxic and children are highly susceptible to this effect, particularly within the context of continuous low-level Pb exposure. A current major challenge is identification of children who may be uniquely susceptible to Pb toxicity because of genetic predisposition. Learning and memory are among the neurobehavioral processes that are most notably affected by Pb exposure, and modification of N-methyl-D-aspartate receptors (NMDAR) that regulate these processes during development are postulated to underlie these adverse effects of Pb. We examined the hypothesis that polymorphic variants of genes encoding glutamate receptor, ionotropic, NMDAR subunits 2A and 2B, *GRIN2A* and *GRIN2B*, exacerbate the adverse effects of Pb exposure on these processes in children. Participants were subjects who participated as children in the Casa Pia Dental Amalgam Clinical Trial and for whom baseline blood Pb concentrations and annual neurobehavioral test results over the 7 year course of the clinical trial were available. Genotyping assays were performed for variants of *GRIN2A* (rs727605 and rs1070503) and *GRIN2B* (rs7301328 and rs1806201) on biological samples acquired from 330 of the original 507 trial participants. Regression modeling strategies were employed to evaluate the association between genotype status, Pb exposure, and neurobehavioral test outcomes. Numerous significant adverse interaction effects between variants of both *GRIN2A and GRIN2B*, individually and in combination, and Pb exposure were observed particularly among boys, preferentially within the domains of Learning & Memory and Executive Function. In contrast, very few interaction effects were observed among similarly genotyped girls with comparable Pb exposure. These findings support observations of an essential role of *GRIN2A* and *GRIN2B* on developmental processes

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Conflict of Interest

The authors declare that there are no conflicts of interest.

underlying learning and memory as well as other neurological functions in children and demonstrate, further, modification of Pb effects on these processes by specific variants of both *GRIN2A* and *GRIN2B* genes. These observations highlight the importance of genetic factors in defining susceptibility to Pb neurotoxicity and may have important public health implications for future strategies aimed at protecting children and adolescents from potential health risks associated with low-level Pb exposure.

Keywords

Lead; Children; Neurotoxicity; Cognitive functions; *GRIN2A*; *GRIN2B*; Genetic susceptibility

1. Introduction

Lead (Pb) is a widely disbursed environmental toxicant that adversely affects both central and peripheral nervous systems in humans. Of particular concern are adverse neurobehavioral effects of cumulative, low-level Pb exposure in children and adolescents, in whom impaired cognitive processes including attention, learning and memory, and executive functions are well recognized (Bellinger, 2008; Lanphear et al., 2005; Koller et al., 2004; Skerfving et al., 2015; Toscano and Guilarte, 2005; Weiss, 2000). Although the mechanisms underlying the adverse effects of Pb exposure on these processes have yet to be fully defined, studies strongly implicate the potent antagonistic properties of Pb on N-methyl-D-aspartate receptor (NMDAR) expression and functions, principally in hippocampal neurons, as mediating these effects (Alkondon et al., 1990; Neal et al., 2011; Wang et al., 2016).

NMDARs constitute a family of receptors that are differentially expressed throughout the central nervous system (CNS) and that mediate the excitatory actions of glutamate on synapse formation, plasticity, maintenance and function (Adams et al., 2004; Paoletti et al., 2013). Several subtypes of NMDARs have been identified, each comprised of at least one NR1 (GluN1) subunit, which is required for structural assembly and expression of distinct NMDAR complexes, and one or more of four accessory subunits, NR2A (GluN2) through NR2D (GluN4), which control ion channel kinetics and synaptic signaling (Lau and Zukin, 2007). Although the specific biological and pharmacologic properties differ among each NMDAR subunit, it is well recognized that NMDARs play critical roles in excitatory synaptic transmissions underlying cognitive functions (Paoletti et al., 2013).

Among the NMDAR subunits that have been strongly implicated in Pb neurotoxicity is NR2A, which is encoded by the gene *GRIN2A* (glutamate receptor, ionotropic, N-methyl-D-aspartate 2A), also termed *GluN2A*. Polymorphic variants in *GRIN2A* leading to altered NR2A expression are associated with mental retardation and behavioral anomalies (Endele et al., 2010) as well as with speech and language dysfunction in children (Lesca et al., 2013). A functionally related gene, *GRIN2B* (*GluN2B*), which encodes the NMDAR subunit NR2B, has also been shown to be critical in learning and memory by regulating key aspects of synaptic plasticity in the developing human brain (Endele et al., 2010; Turic et al., 2004). Inasmuch as adverse neurobehavioral effects of Pb exposure are postulated to be associated with altered NMDA receptor signaling (Alkondon et al, 1990; Guilarte et al, 1995; Neal et

al, 2011; Wang et al, 2016), we tested the hypothesis that variants of these genes that adversely affect NMDA receptor processing and/or functioning would increase susceptibility to the adverse effects of Pb exposure in children, particularly on tests of learning & memory and executive functions.

2. Methods

2.1. The study population

The present study included 330 subjects who participated as children in the Casa Pia Clinical Trial of Dental Amalgams in Children (DeRouen et al, 2002, 2006) conducted between 1997 and 2005. Participants in the clinical trial included 279 boys and 228 girls, aged 8–12 years at baseline, who were students of the Casa Pia school system in Lisbon, Portugal (Woods et al., 2014a). All children were developmentally normal with no clinical evidence of preexisting psychological, behavioral, neurologic or immunosuppressive disorders at baseline, and were largely homogeneous with respect to socioeconomic status and geographic region. For the clinical trial, children were initially randomized to Hg amalgam (treatment) or composite resin (control) dental treatment groups. Subjects were evaluated at baseline and at 7 subsequent annual intervals following initiation of the study using an extensive battery of neurobehavioral assessments (Martins et al, 2005). Tests were administered by native Portuguese psychometrists with advanced degrees in psychology and familiarity with standardized testing and research methods. Testing was overseen by a supervising psychometrist and a Ph.D. psychologist, both from the University of Washington with extensive experience in standardized testing of both adults and children in cross-cultural contexts. Follow-up data were obtained on a similar number of subjects in each treatment group. Retention throughout the 7-year course of the clinical trial was approximately 80%, with approximately 20% loss to follow-up largely due to relocation or selective withdrawal. Urine samples were collected from all subjects, irrespective of treatment, at baseline and at each subsequent annual interval through year 7 for assessment of Hg concentrations and other clinical parameters (DeRouen et al., 2006). During the course of the clinical trial, it was demonstrated that the children included in this study had no significant exposure to methyl-Hg from dietary fish consumption (Evens et al., 2001). Blood samples were also collected from all subjects at baseline for determination of blood lead (BPb) levels and other clinical parameters. A detailed description of the study design and methods, including factors measured over the course of the trial and how these factors were considered in constructing analytical models has been published (DeRouen et al., 2002)

Following completion of the clinical trial in 2006, we undertook further studies to determine whether the variant status of specific candidate genes that are reported to adversely affect neurobehavioral processes and/or Hg toxicokinetics would modify the adverse effects of Hg on neurobehavioral functions in the children who had participated in the clinical trial. For those post-trial gene modification studies, we employed Hg urinary concentrations for all participants as a measure of Hg exposure, rather than using the assignment to Hg amalgam or composite treatment groups as in the clinical trial. This change allowed us to capture the effects of all Hg exposure irrespective of source. Detailed descriptions of these studies

including methods employed, analytical outcomes and interpretations of findings are published (Woods et al., 2012; 2013, 2014a, 2014b).

In the present study, we evaluated the same subjects who participated in the post-trial gene modification studies described above, but assessed potential modification of neurobehavioral effects of Pb, rather than Hg, by polymorphisms of genes known to adversely affect neurobehavioral functions in humans (Woods et al., 2014a). The impetus for this assessment was the observation of mean blood lead levels in this population (Martin et al., 2007), which, while very low (4.6 [2.4] μg/dl), have been proposed by other investigators (e.g., Bellinger, 2008; Lanphear et al., 2005; Skerfving et al., 2015) to increase the risk of cognitive deficits in school age children. For these assessments, we conducted initial analyses to examine the possibility of significant genexPb interaction effects on all neurobehavioral tests previously evaluated in the dental amalgam clinical trial. These preliminary analyses revealed significant effect modification of Pb on neurobehavioral functions by genetic variants of *GRIN2A/2B* on tests of Learning & Memory and Executive Function. These specific interactions were, therefore, selected as the principal focus of the present study.

2.2. Neurobehavioral tests

A comprehensive neurobehavioral test battery was used in these analyses, including measures from the Rey Auditory Verbal Learning Test (RAVLT), subtests from the Wide Range Assessment of Visual Motor Abilities (WRAVMA), the Wechsler Adult Intelligence Scale-III (WAIS-III), the Wechsler Memory Scale for Adults-III (WMS-III), Standard Reaction Time (SRT), Finger tapping, Trailmaking A and B, the Wisconsin Card Sorting Tests (WCST), and the Stroop word, color and word-color tests. The validity and rationale underlying the selection and use of these tests in the clinical trial as well as the baseline neuropsychological performance of all subjects have been described (Martins et al., 2005; Townes et al., 2008).

Table 1 lists the neurobehavioral tests that were assessed in the present study and their test abbreviations referenced in subsequent tables. Tests are organized within the 5 behavioral domains (Attention, Learning & Memory, Executive Function, Visual Spatial Acuity and Motor Function) that were evaluated in the clinical trial (DeRouen et al., 2006). All children were evaluated at baseline and at 7 subsequent annual intervals following initiation of the study. Adult versions of child equivalents of some tests were substituted beginning at Year 4 of the clinical trial, as indicated in Table 1. Arrows following test name abbreviations depict whether the test score increases or decreases in magnitude with improved performance. Diminished or adversely affected performance associated with Pb exposure and/or gene variant status is described as occurring in the direction of impaired performance, whereas enhanced or beneficially affected performance associated with any of these variables is described as occurring in the direction of improved performance. The Comprehensive Test Of Nonverbal Intelligence (CTONI) (Portuguese translation) was given to each child at the beginning of the clinical trial to obtain a measure of intelligence quotient (IQ) at baseline (Hammill et al., 1997). We adjusted for nonverbal IQ (CONTI) at baseline in the present study, because it is a nonverbal test developed to minimize the effects of language and culture on the measures of neurobehavioral ability evaluated here. Moreover, adjustment for

nonverbal IQ will facilitate comparison of results from the present studies with those of others in which IQ is employed a primary outcome measure. Social and cultural issues associated with measurements and interpretation of IQ test results among children in cross-cultural contexts have been described (Townes et al., 2003).

2.3. Selection of GRIN2A and GRIN2B Single Nucleotide Polymorphisms (SNPs)

We selected 2 variants of *GRIN2A* that have been postulated to adversely affect cognitive phenotypes of learning and memory in humans (Adams et al., 2004). These include *GRIN2A* rs727605, which is characterized by a C>T transition in intron 5, and rs1070503, a C>T transition in intron 3. We selected two *GRIN2B* SNPs for evaluation based on observations of adverse effects on the same neurobehavioral phenotypes as those postulated for variants of *GRIN2A* (Ohtsuki et al., 2001): rs7301328 (c.366C>G), a C>G transverse substitution in exon 2 and rs1806201 (c.2664C>T), an A>G transition substitution in exon 13. Each of these SNPs has been investigated in a variety of neurobehavioral and/or psychiatric disorders (Adams et al., 2004; Andreoli et al. 2014; Endele et al., 2010; Lesca et al., 2013; Schulz et al., 2012; Taylor et al., 2016), as well as in response to Hg exposure in children (Woods et al., 2014a). All were found to be in Hardy-Weinberg equilibrium. Procedures for genotyping assays performed on DNA in tissue samples acquired from study subjects, including quality control procedures employed, have been described (Woods et al., 2005).

2.4. Blood lead collection and analysis

A sample of venous blood was acquired into a sterile vacuutainer tube from each child at baseline at the University of Lisbon School of Dental Medicine. Samples were collectively shipped in cold storage to the Laboratory of Clinical Chemistry at the University of Washington Medical Center for blood lead determination by flameless atomic absorption spectrometry (Fisher Scientific). Quality control measures included the continuous use of calibration verification standards of 0,1,2,10,20 and 40 μg/dl derived from Pb standard reference material acquired from the National Institute of Standards and Technology, NIST SMR #3128, and use of control blood samples acquired from the Puget Sound Blood Center, currently BloodWorks Northwest, adjusted to 1, 5, 10 and 20 μg/dl, respectively, with Pb standards. Blood lead values were calculated as the average of duplicate analyses of each sample. The detection limit was 1 μg/dl. Duplicate analyses of individual samples that differed by more than 10% were reanalyzed. The coefficient of variation (CV) for sample duplicates was 0.0673 (6.73%) in the blood lead concentration range of 1.0 to 15.0 μg/dl. Repeat analyses of control samples and other procedures were performed weekly to ensure high consistency and accuracy of blood lead concentration assessments.

2.5. Rationale for using baseline blood lead

Baseline blood lead concentrations (BPb) were employed as the measure of Pb exposure for this study as the most widely recognized and generally accepted measure of absorbed dose and predictor of long-term effects (WHO, 2001; Warrington et al., 2015). We acknowledge in this respect potential sources of exposure misclassification associated with use of BPb as a surrogate measure of brain lead levels (Bellinger, 2007), but note, however, the relative homogeneity of the study population with regard to socioeconomic status, and exposure and

assessment settings as factors mitigating this concern. We note further considerable literature supporting the view that Pb exposure in early childhood is associated with neurobehavioral deficits that persist well into adulthood, even in the absence of continued Pb exposure (Mazumdar et al., 2011; Miranda et al., 2007; Reuben et al., 2017; Skerfving et al., 2015; Warrington et al., 2015). Hence, we view baseline BPb as a suitable measure of Pb exposure over the 7-year course of the present study, even if continuous Pb exposure were not assumed. Further consideration of this issue is presented the Discussion (below).

2.6. Study design

We examined whether genotype status of *GRIN2A* and *GRIN2B* variants affected the dose–response relationship between baseline blood lead (lnBPb) concentration and tests of neurobehavioral functions among children who had been evaluated annually from baseline through 7 years of the clinical trial. Because of the wide range in ages of subjects at the beginning of the trial (8-12 years), and the duration of the study (which included passage through puberty and adolescence for most subjects), we evaluated potential genetic modification of Pb effects on neurobehavioral functions at 2 time points of the clinical trial that we considered most pertinent to Pb neurotoxicity: (1) at 2 years after baseline, when most subjects were between puberty and adolescence, with boys aged 12.20(.84) and girls aged 12.16(.95) years(SD); and (2) at 7 years after baseline, when most subjects were between adolescence and young adulthood, with boys aged 17.14(.86) and girls aged 17.08(1.02) years (SD). We define these assessment times as **Year 2** and **Year 7** respectively. These time points correspond to those assessed in the previously described Hg genotyping studies (Woods et al., 2012, 2013, 2014a,b).

2.7. Statistical analyses

The effect of *GRIN2A* and *GRIN2B* variants on the dose-response association between Pb exposure and neurobehavioral performance was the principal focus of this study. Thus, our analytical protocol focused on gene-Pb interactions (genexPb), in which we independently evaluated the impact of genotype status of each genetic variant individually and in combination on performance of each behavioral test. A nested domain Bayesian mixed-model was used to model all outcomes simultaneously (Lam et al. 2013). Such models utilise partial pooling (or shrinkage) of outcome estimates from within a cognitive domain toward each other, thus reducing the risk of errors of size or magnitude (Gelman et al. 2012). Objective Bayesian (or weakly informative) priors were chosen for model parameters with the same specifications as recently described by Lam et al. (2013). Outcome scores which improved with a smaller score were inverted, this ensured that all model coefficients could be interpreted in the same direction (Lam. et al. 2013). Models were estimated across 4 Markov Chain Monte Carlo (MCMC) chains with 1000 iterations for adaption and 35,000 burn-in iterations, after which 25,000 samples thinned every 5 steps to give 5000 samples were saved. Model convergence was assessed via examining plots of posterior samples, and via the Potential Scale Reduction Factor (PSRF). Goodness of fit was compared between models using the Deviance Information Criterion (DIC) and a further 5,000 posterior samples were taken to calculate the DIC.

First, we specified a base model for all outcomes excluding any genetic variants. Age at study entry, race, and nonverbal IQ (CONTI) (niq) determined at baseline were included as fixed effects. These covariates were selected because of their potential to bias neurobehavioral test performance in relation to Pb exposure (Bellinger, 2007; Krieg et al., 2001) and because data pertinent to these specific variables were available from the clinical trial from which subjects in the present study were acquired (Martins et al., 2005; Townes et al., 2003). Adjustment for nonverbal IQ at baseline was also made to minimize the effects of language and culture on the measures of neurobehavioral ability evaluate here (Hammill et al., 1997). Other factors potentially affecting behavioral test performance in relation to Pb exposure, including home environment, parent's socioeconomic status, or medical histories, were comparable among essentially all subjects (DeRouen et al., 2002; Martin et al., 2007) and were, therefore, not included as covariates. Hg exposure was included as a main effect in the present analyses as it has been previously shown only minimal interaction with *GRIN2A* and *2B* variants to affect neurobehavioral performance outcomes in children (Woods et al., 2014a). After construction of the base model, exploratory models were constructed for each genetic variant in turn. Models were specified including an interaction term between Pb, the genetic variant, and sex. In all cases, major genotype status was defined as the homozygous dominant for the major alleles, and minor genotype status was defined as heterozygous plus homozygous recessive for the minor alleles. Statistical analyses were performed using R software® for statistical computing (version 3.2.4) and the "runjags" package (Denwood. 2016) to implement MCMC models in the *JAGS* program for analysis of Bayesian graphical models using Gibbs sampling (Plummer, 2003).

2.8. Human subjects considerations

All parents or guardians of children who participated in the clinical trial gave written consent, and all children provided signed assent, for the treatments and assessments made during the course of the trial, including collection of blood samples. Written consent was also obtained from all participants who provided buccal cell samples for genotyping subsequent to completion of the clinical trial. The study protocols for both the clinical trial and the present genotyping study were approved by the institutional review boards at the University of Lisbon and the University of Washington.

3. Results

The study cohort consisted of 330 children (163 boys and 167 girls) for whom gene variant status was available from among 507 total subjects enrolled at the start of the clinical trial. Table 2 presents the characteristics of the study cohort at Baseline (Entry) and at Year 2 and Year 7 of the study as well as mean values for baseline BPb and natural log calculations. 74.2% of boys and 71.3% of girls were Caucasian at Entry, the remaining being Black (of African heritage). The frequencies of subjects with major and minor genotypes as well as minor allelic frequencies (MAFs) for the genes evaluated are also presented. BPb for all subjects ranged from 1.0 to 15.0 μg/dl at baseline and were presumed to be continuous from common environmental exposure sources throughout the course of the study (discussed in Strengths and Limitations, below). Of note, only 5 of 123 boys (4.1%) and 2 of 119 girls (1.7%) exceeded 10 μg/dl; these were included in the present analyses for cohort continuity.

Regression coefficients for the base model at Year 7 are reported in full in the Supplementary data. In brief, age and non-verbal IQ at entry were associated with improved outcomes across most outcomes of neurobehavioral function *per year of age (except atrlasec, srtmean and atrlbsec)* and *per point of non-verbal IQ (except atrlasec, ravlt1, ravlt6 and atrlbsec)* at entry. For Hg there were no improved outcomes with increased urinary Hg/Cr except for two outcomes, strpcwd1test of the Attention domain and wcscorr test of the Executive Function domain. In the base model there were several impaired outcomes with increased BPb in boys in the learning and memory domain (ravlt1,5,7,8, ravlttot, virede), executive function (wcscatcp, wcscorr), srtmean in the visual-spatial domain, and attention (strpcwd1). For girls, these effects were seen only in srtmean and the motor domain (ftapdom and ftapndom).

3.1. Effects of GRIN2A genotypes on the association between Pb exposure and neurobehavioral functions

3.1.1. GRIN2A rs727605—Among subjects at Year 2 of the clinical trial, no genexPb interaction effects that excluded the null at 95% credible interval were found among boys carrying either the major (CC) or minor (CT/TT) genotype for rs727605. Among Year 2 girls, the srtmean test of Visual Spatial acuity was sufficiently negatively associated with Pb exposure to exclude the null at the 95% credible interval among girls genotyped as homozygous dominant (CC or “wildtype”) for *GRIN2A* rs727605. However, no negative effects of Pb were observed among Year 2 girls carrying the minor (CT/TT) genotype for rs727605.

In contrast, among Year 7 subjects, numerous significant genexPb interactions, specifically within the domain of Learning & Memory, were observed among boys. As shown in Table 3, all outcomes within the domain of Learning and Memory as well as the srtmean test of Visual-Spatial acuity were impaired in relation to Pb exposure among Year 7 boys carrying the minor allele for *GRIN2A* rs727605. Outcomes in the domain of Executive Function also showed negative associations among Year 7 boys carrying the minor allele for *GRIN2A* rs727605, although the credible intervals for these coefficients included the null. Among similarly genotyped Year 7 girls (Table 3), only the srtmean test of Visual Spatial acuity was sufficiently strongly negatively associated with Pb exposure to exclude the null at the 95% credible intervals; however, all coefficients were negative.

3.1.2. GRIN2A rs1070503—Among subjects at Year 2 of the clinical trial, no genexPb interaction effects that excluded the null at 95% credible interval were found among boys. Among Year 2 girls, two tests of Motor Function, ftapdom and ftapndom, were negatively associated with Pb exposure among those genotyped as homozygous dominant (CC) for GRIN2A rs1070503.

Among Year 7 subjects, in contrast, multiple strong genexPb interactions were seen in boys with the minor allele for rs1070503. These include substantial dose-response effects of Pb again predominantly on tests of Learning & Memory (Table 3), as well as in the WCST categories completed (wcscatcp) and number correct (wcscorr) tests of Executive Function. Year 7 girls genotyped as CT or TT for *GRIN2A* rs1070503 showed negative genexPb

effects on the srtmean test of Visual-Spatial acuity and on the WCST categories completed (wcscatcp) test of Executive Function. Estimates in the Learning and Memory domain were also negative for Year 7 girls, but were inclusive of zero within the 95% credible interval range in all cases.

3.1.3. Combined effects of GRIN2A rs727605 and rs1070503—Among Year 7 boys genotyped as CT or TT for both *GRIN2A* variants (Table 5), all tests of Learning & Memory were impaired at 95% credible intervals in relation to Pb exposure. Among Year 7 girls carrying the minor allele for both *GRIN2A* variants, only the srtmean test of Visual Spatial acuity was substantially impaired, comparable to findings observed among girls carrying the minor genotype of either *GRIN2A* SNP alone (Table 3). In contrast, no adverse effects of Pb were observed among Year 7 subjects genotyped as homozygous dominant (CC) for either *GRIN2A* SNP when considered individually or in combination.

3.2. GRIN2B

3.2.1. GRIN2B rs7301328—Similar to findings of multiple significant genexPb interactions on tests on Learning & Memory and Executive Function among subjects with *GRIN2A* variants shown above we observed substantial adverse effects of Pb exposure on tests neurobehavioral performance at both Year 2 and Year 7 among subjects carrying the minor allele (CG/GG) of *GRIN2B* rs7301328.

Among Year 2 subjects, no Pb effects excluding the null at the 95% credible interval were observed among boys. In contrast, among Year 2 girls with the minor *GRIN2B* rs7301328 genotype, substantial adverse effects of Pb were observed for the srtmean test of Visual Spatial acuity, the strpcol test of Attention and the ftapdom and ftapndom tests of Motor Function.

Among Year 7 subjects (Table 4), multiple tests of Learning & Memory as well as the srtmean test of Visual Spatial acuity were significant impaired in relation to Pb exposure among boys carrying the minor allele of *GRIN2B* rs7301328, whereas the srtmean test of Visual Spatial acuity and the Ravlt Tr5 test of Learning & Memory were adversely effected in relation to Pb exposure among similarly genotyped Year 7 girls.

3.2.2. GRIN2B rs1806201—Several genexPb interactions were found among boys or girls at Year 2 of the study. Adverse effects of Pb that excluded the null at the 95% credible interval included ftapdom and ftapndom, both tests of Motor Function, were observed among girls carrying the homozygous dominant (CC, wildtype) genotype for *GRIN2B* rs1806201. Among boys at Year 2, 3 tests of Learning & Memory, Ravlt Trs 5, 7 and 8, were significantly impaired by Pb exposure among those carrying the minor allele of *GRIN2B* rs1806201.

Additionally, as shown in Table 4, all tests of Learning & Memory as well as the wcscatp and wcscorr tests of Executive Function were significantly impaired by Pb exposure among boys carrying the minor allele of *GRIN2B* rs1806201 at Year 7 of the study. Among similarly genotyped Year 7 girls, the srtmean test of Visual Spatial acuity was the only test for which adverse Pb effects excluded the 95% credible interval.

3.2.3. Combined effects of GRIN2B rs1806201 and rs7301238—Similar to findings noted with combined effects of *GRIN2A* variants (3.1.3 above), all tests of Learning & Memory (except Ravlt Tr6) as well as the wcscatcp and wcscorr tests of Executive Function were significantly impaired in relation to Pb exposure among Year 7 boys genotyped as carrying the minor alleles for both *GRIN2B* variants (Table 5). Among similarly genotyped Year 7 girls, the srtmean test of Visual Spatial acuity, the Ravlt Tr5 test of Learning & Memory, and the wcscatcp test of Executive Function were significantly impaired.

3.3. Summary of Findings

The minor genotypes of *GRIN2A* rs727605 and rs1070503, individually and together, substantially modified the effects of Pb exposure on neurobehavioral test performance within the domains of Learning & Memory and Executive Function predominantly among boys in Year 7 of the study. Similar findings were observed among Year 7 boys carrying the minor genotypes of *GRIN2B* rs1806201 and rs7301238. All genexPb effects manifested as impaired performance among subjects carrying the minor alleles of all of the polymorphisms evaluated. After inclusion of genetic polymorphisms of *GRIN2A* and *GRIN2B* main effects of Pb were largely absent (Supplementary Data). The strength and number of significant associations between performance and Pb exposure increased with age and duration of Pb exposure, particularly among boys. The DIC's for all models were very similar indicating that no model is clearly superior to others (Supplementary Data) – likely due to the small magnitude effects of Pb exposure relative to the larger effects of age and non-verbal IQ at entry.

4. Discussion

Numerous studies have demonstrated a component of genetic susceptibility to neurobehavioral disorders associated with Pb exposure (Eum et al., 2013; Krieg et al., 2010; Schneider et al., 2014; Sobin et al., 2015; Taylor et al., 2016; Wagner et al., 2016; Wang et al., 2007; Warrington et al., 2015; Whitfield et al., 2007; Wu et al., 2016). The present studies are the first to our knowledge to describe modification of neurobehavioral effects of low-level Pb exposure in children by genetic variants that are known to be associated with multiple adverse cognitive phenotypes, particularly of learning and memory, in humans. These observations are consistent with the established effect of Pb as a potent antagonist of NMDA receptor expression and functions underlying development and maintenance of learning & memory as well as executive and motor functions (Alkondon et al., 1990; Guilarte et al., 1995; Neal et al., 2011; Wang et al, 2016). The present findings identify specific variants of *GRIN2A and GRIN2B* that exacerbate deficits in learning and memory as well as in executive function through modification of Pb effects on these processes in children.

Mechanisms underlying the modification of Pb effects by *GRIN2A/2B* variants evaluated here remain to be determined, inasmuch as little is yet known about the specific architectural, biochemical or pharmacological properties of these individual variant NMDA receptors. One possibility is that the protein structure of the variant NR2A/2B receptor subunits differ from non-variant receptors in binding site affinities for Zn^{2+} ions, which act

as highly specific endogenous antagonists, providing a competitive advantage to Pb^{2+} for binding and, hence, inhibition of receptor functions at concentrations (< 1 μM) likely to be attained *in vivo* (Neal et al, 2011). Alternatively, sequence variation at the intron or other non-coding regions may alter post-transcriptional control mechanisms affecting regulation of mRNA stability, translation and sub-cellular localization of specific proteins (Hesketh, 2004). Since binding of heavy metals varies depending on the molecular structures and sub-cellular localization of specific proteins, disruption of the post-transcriptional control mechanisms for NMDA receptor synthesis, stability or distribution could lead to alterations in Pb binding and/or tissue levels, subsequently influencing Pb neurotoxicity as observed in the present investigation. Further studies pertinent to the mechanisms underlying the modification of Pb on neurobehavioral functions by the variants of *GRIN2A* and *GRIN2B* evaluated here are required for more complete understanding of these effects.

Most notable of the adverse effects of Pb observed among subjects carrying the minor alleles of either *GRIN2A* or *GRIN2B* are significant deficits on most tests within the domain of Learning and Memory evaluated in these studies. These include sub-tests of the Ravlt series, nearly all of which were significantly impaired among boys carrying the minor allele for these variants, particularly among the older Year 7 subjects. The Ravlts evaluate a wide range of functions associated with short-term auditory and verbal memory, learning, and retention and retrieval of information. Impaired performance reflects deficits in any or all of these functions. Similar findings were observed for WMS-III vireim and virede, which are measures of immediate and delayed working memory, respectively. Also of note, 2 tests of Executive Function, WCST categories completed (wcscatcp) and WCST number correct (wcscorr), which measure ability to adapt to changing rules, were significantly impaired in relation to Pb exposure among Year 7 boys carrying the minor alleles for both *GRIN2A* rs1070503 and *GRIN2B* rs1806201 variants, as well as for boys carrying the minor alleles for both *GRIN2*B SNPs. In contrast, essentially no modification of Pb effects on any test of neurobehavioral function was observed among boys genotyped as homozygous dominant (CC) for either or both *GRIN2A/2B* SNPs. These observations support the view that genetic variants that modify either NR2A or NR2B glutamate receptor functioning and/or expression increase susceptibility to the adverse effects of Pb on a wide range of cognitive processes associated principally with learning and memory and, moreover, particularly among boys. Of note, neither the *GRIN2A* nor the GRIN2B SNPs evaluated here are known to be in linkage disequilibrium (LD) (Adams et al., 2004; Ohtsuki et al, 2001), supporting the view that modification of Pb effects on neurobehavioral functions by each *GRIN2A or 2B* SNP is independently mediated, consistent with the more inclusive effects of Pb on tests of Learning & Memory and Executive Function observed among subjects carrying minor alleles of both *GRIN2A or both GRIN2B* variants. Further studies are required to fully delineate these processes and, in particular, to quantify the magnitude and distribution of increased susceptibility to Pb effects imposed in relation to *GRIN2A* gene modifications as pertains to human populations.

Also of note from the present studies are findings of substantially more robust modification of Pb effects on tests of Learning & Memory and Executive Function by variants of *GRIN2A* and *2B* among boys at Year 7 than were seen at Year 2 of the clinical trial. These findings are consistent with those of other epidemiologic studies (Lanphear et al., 2005;

Mazumdar et al., 2011; Miranda et al., 2007; Skerfving et al., 2015; Wang et al., 2016) demonstrating a strong correlation between Pb exposure in early childhood and deficits in cognitive functions, particularly learning and memory, that extend well into adulthood, even in the absence of continuous Pb exposure. These delayed effects are hypothesized to be attributable to inhibition by Pb of NMDAR-mediated synaptic plasticity that underlies the development of neuronal processes associated with learning & memory and other cognitive functions in hippocampal neurons, even at very low levels of exposure. The findings here of more pronounced effects of Pb exposure at Year 7 versus at Year 2 are consistent with this protracted consequence of early Pb exposure, and, moreover, support the view that subunits of both NR2A and NR2B receptors that are encoded by genetic variants of *GRIN2A or GRIN2B* exacerbate these effects.

Notable also are findings of distinct differences between boys and girls in the effects of Pb exposure and the *GRIN2A/2B* variants on neurobehavioral test performance evaluated in this study, in which genexPb effects were seen to be substantially more prominent among boys than girls in terms of both numbers of neurobehavioral domains as well as tests within such domains affected. In this respect, boys carrying one or both minor alleles of either *GRIN2A* or *GRIN2B* demonstrated significant impairment of most tests of Learning and Memory as well as of Executive Function in relation to Pb exposure, whereas girls of similar age and genotype demonstrated relatively few if any effects of Pb on these domains of neurobehavioral function. These findings are consistent with previous observations of sex-related differences in neurobehavioral responses to numerous drugs and environmental chemicals including Pb (Gochfeld, 2007; Schneider et al, 2016; Vahter et al, 2007; Valentino et al, 2012; Woods et al, 2014a; Wu et al, 2017) and support the view that genetic predisposition is a significant factor in defining sex differences in susceptibility to the adverse neurobehavioral effects of Pb, particularly in children. In this regard, genetic and hormonal differences affecting brain development, morphology and function between boys and girls may potentially underlie the sex differences seen in response to genotype and Pb exposure on neurobehavioral functions (Goel et al, 2014; Schneider et al, 2016). Epigenetic factors that differentially affect the timing, duration and/or magnitude of gene expression are also likely to contribute to the sex differences in susceptibility to Pb-induced neurobehavioral deficits observed (Chung and Auger, 2013; Jirtle and Skinner, 2007; Schneider et al, 2016; Wu et al., 2017). Further studies are required to fully define the specific factors underlying the differential sex responses observed.

The present findings have potentially important implications for the assessment, prediction and management of risks of neurobehavioral dysfunction associated with low-level Pb exposure, particularly among children. Foremost is the observation that the minor allelic frequencies (MAFs) of the SNPs of both *GRIN2A* and GRIN2B evaluated in the present study cohort are in the range of 16 to 37% (Table 2), implying a relatively high prevalence of increased susceptibility to deficits in learning & memory as well as other neurobehavioral functions in response to Pb exposure particularly among boys carrying minor alleles for either SNP. Inasmuch as the present study population was largely of European (Caucasian) ancestry, the findings of susceptibility to Pb toxicity observed here should be predictably similar among all populations of comparable descent, as noted by other investigators (Adams et al., 2004). We note particularly in reference to the SNPs evaluated here that the

present findings demonstrate genetic modification of neurobehavioral test results in relation to Pb exposure, but not specifically by Pb alone. Such findings point to the importance of genetic variation both as an etiologic factor underlying susceptibility to Pb toxicity as well as for assessment, prediction and management of Pb risk. Further studies in larger Caucasian cohorts as well as in populations of different genetic background are encouraged to confirm the present findings and to more precisely define the relative contribution of genetic variability to assessment of population health risks associated with Pb exposure.

Finally, we emphasize that the substantial modification of Pb effects on neurobehavioral functions by genetic variants of *GRIN2A* and *GRIN2B* observed here stands in contrast to previous findings of no or very limited modification of Hg effects on the same functions by the same variants in the same subjects (Woods et al., 2014). In this respect, inclusion of Hg as a covariate in the statistical models used here did not alter the extent of modification of Pb effects by *GRIN2A/2B* variants observed without Hg in the model. These findings suggest that Pb and Hg act via independent mechanisms to individually affect the neurobehavioral processes evaluated. Additionally, these observations support the view (Neal et al., 2011; Wang et al., 2016) that Pb acts with high specificity by impairing NR2A/2B NMDA receptor-mediated processes underlying the development and maintenance of cognitive functions (Woods et al., 2014a), and imply, moreover, that genetic variants of *GRIN2A* and *GRIN2B* significantly exacerbate these effects.

Strengths and Limitations

A pertinent limitation of the present study is the relatively small population in which we sought to evaluate potential modification of effects of low-level Pb exposure on neurobehavioral test performance by *GRIN2A/2B* variants. Despite this limitation, subject participation and retention (~80%) throughout the period of the clinical trial were sufficient to demonstrate significant modification of Pb effects on multiple tests of Learning and Memory and Executive Function particularly among boys carrying either or both variants of either *GRIN2A* or *GRIN2B*, supporting the likelihood of a potential genetic basis underlying differences in susceptibility to Pb neurotoxicity. We note, however, the importance of interpreting these findings with caution until confirmed in larger and more heterogeneous populations.

Another limitation associated with the relatively small population size was our inability to concomitantly evaluate effects of other genetic variants that might significantly modify the genexPb outcomes evaluated here. Of particular note in this respect is δ-aminolevulinic acid dehydratase (*ALAD*), the *ALAD 2* variant (rs1800435) of which has been demonstrated to have a neuroprotective effect on low-level Pb-exposure in children (Sobin et al., 2015), owing possibly to increased sequestration of Pb in red blood cells (Wetmur et al., 1991). Given that only 10-15% of our subjects are likely to carry the *ALAD 2* genotype (Kelada et al., 2001), it is highly unlikely that including *ALAD* as an additional covariate, therefore, would have improved the assessment of modification of Pb effects by *GRIN2A/2B* on neurobehavioral processes, our principal goal in the present study. We emphasize, however, that stratification on genotypes of *ALAD* or other genes reported to modify the neurotoxic effects of Pb (Cantonwine et al, 2010; Engstrom et al, 2007; Krieg et al, 2010; Wagner et al,

2016; Whitfield et al, 2007; Wang et al, 2007) should be considered for evaluation in future studies assessing Pb effects on cognitive functions in larger human populations.

Another limitation of the present study was the availability of baseline blood Pb levels as our sole measure of Pb exposure. We note in this regard that, whereas multiple measures over the course of the study may have been preferable in the context of the longitudinal assessments undertaken, the baseline BPb employed is likely to have remained a relatively constant indicator of Pb exposure throughout the 7-year course of the study, in light of the highly stable home and school environments of essentially all study subjects, and the relative lack of significant sources of environmental Pb exposure throughout the study period. In this respect, schools were found to have no Pb-based paints or measurable Pb from water sources, nor were there any structural or other changes made in any of the schools within the system during the study period that could have affected Pb exposure. In terms of potential chronic exposure sources outside the school or home environments, the city of Lisbon itself has no significant industrial activity, although some Pb exposure could still have occurred from burning of Pb-containing gasoline, which was largely but not entirely eliminated at the time of the study. Other possible sources of Pb exposure were dust and/or soil from schoolyards, parks or other recreational areas that had potentially bioaccessible Pb from motor vehicle emissions. Exposures from these sources, however, were not viewed as posing a health risk for children, even among those who were frequent users of these areas (Reis et al., 2014). Finally, even if Pb exposure were not consistent over the course of the study, prevailing literature supports the view that Pb exposure during early childhood is predictive of long-term health effects even long after exposure has ended (Mazumdar et al., 2011; Miranda et al., 2007; Skerfving et al., 2015; Warrington et al., 2015). In view of these considerations, we find no compelling reasons to assume changes in Pb exposure over the course of the study. We, therefore, view baseline BPb as an appropriate surrogate measure for the assessment of the potential effects of Pb exposure at the times (Years 2 and 7 from baseline) at which neurobehavioral effects were evaluated in this study.

In conclusion, the present findings describe modification of low-level Pb effects on multiple neurobehavioral functions by common variants of genes encoding N-methyl-D-aspartate receptor subunits NR2A and NR2B in children. These findings are consistent with mechanistic hypotheses underlying the long-term neurotoxicity associated with low-level Pb exposure in childhood and underscore the increasing recognition of genetic variability in modifying susceptibility to these effects. These findings may have important implications for future research and regulatory strategies aimed mitigating the social, economic and public health consequences associated with low-level environmental Pb exposure, particularly in children.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

Adams J, Crosbie J, Wigg K, Ickowicz A, Pathare T, Roberts W, et al. Glutamate receptor, ionotropic, N-methyl D-aspartate 2A (GRIN2A) gene as a positional candidate for attention-deficit/hyperactivity disorder in the 16p13 region. Molec Psychiat. 2004; 9:494–499.

Alkondon M, Costa ACS, Radhakrishnan V, Aronstram R, Albuquerque EX. Selective blockade of NMDA-activated channel currents may be implicated in learning deficits caused by lead. FEBS Lett. 1990; 261:124–130. [PubMed: 1689669]

Andreoli V, De Marco EV, Trecroci F, Cittadella R, Di Palma G, Gambardella A. Potential involvement of GRIN2B encoding the NMDA receptor subunit NR2B in the spectrum of Alzheimer's disease. J Neural Transm. 2014; 121:533–542. [PubMed: 24292895]

Bellinger DC. Lead neurotoxicity in children: Decomposing the variability in dose-effect relationships. Am J Ind Med. 2007; 50:720–728. [PubMed: 17290364]

Bellinger DC. Very low lead exposures and children's neurodevelopment. Curr Opin Pediatr. 2008; 20:172–177. [PubMed: 18332714]

Cantonwine D, Hu H, Téllez-Rojo MM, Sánchez BN, Lamadrid-Figueroa H, Ettinger AS, Mercado-García A, Hernández-Avila M, Wright RO. HFE Gene Variants Modify the Association between Maternal Lead Burden and Infant Birthweight: A Prospective Birth Cohort Study in Mexico City, Mexico. Environ Health. 2010; 9:43. 2010. doi: 10.1186/1476-069X-9-43 [PubMed: 20659343]

Chung WCJ, Auger AP. Gender differences in neurodevelopment and epigenetics. Pflugers Arch – Eur J Physiol. 2013; 465:573–584. [PubMed: 23503727]

Denwood MJ. runjags: An R Package Providing Interface Utilities, Model Templates, Parallel Computing Methods and Additional Distributions for MCMC Models in JAGS. J Stat Softw. 2016; 71(9) Available from: http://www.jstatsoft.org/v71/i09/.

DeRouen TA, Leroux BG, Martin MD, Townes BD, Woods JS, Leitão J, Castro-Caldas A, Braveman N. Issues in the design and analysis of a randomized clinical trial to assess the safety of dental amalgam restorations in children. Contr Clin Trials. 2002; 23:301–320.

DeRouen TA, Martin MD, Leroux BG, Townes BD, Woods JS, Leitão J, Castro-Caldas A, Luis H, Bernardo M, Rosenbaum G, Martins IP. Neurobehavioral effects of dental amalgam in children: a randomized clinical trial. JAMA. 2006; 295:1784–1792. [PubMed: 16622140]

Endele S, Rosenberger G, Geider K, Popp B, Tamer C, Stefanova I, et al. Mutations in GRIN2A and GRIN2B encoding regulatory subunit of NMDA receptors cause variable neurodevelopmental phenotypes. Nat Genet. 2010; 42:1021–1028. [PubMed: 20890276]

Eum K-D, Wang FT, Schwartz J, Hersh C, Kelsey K, Wright RO, et al. Modifying roles of glutathione S-transferase polymorphisms on the association between cumulative lead exposure and cognitive functions. Neurotoxicol. 2013; 39:65–71.

Engstrom AK, Snyder JM, Maeda N, Xia Z. Gene-environment interaction between lead and Apolipoprotein E4 causes cognitive behavior deficits in mice. Mol Neurodegener. 2017 Feb 7.12(1):14. 2017. doi: 10.1186/s13024-017-0155-2 [PubMed: 28173832]

Evens CC, Martin MD, Woods JS, Soares HL, Bernardo M, Leitão J, et al. Examination of dietary methylmercury exposure in the Casa Pia study of the health effects of dental amalgams in children. J Toxicol Environ Health. 2001; 64:521–530.

Gelman A, Hill J, Yajima M. Why We (Usually) Don't Have to Worry About Multiple Comparisons. J Res Educ Eff. 2012; 5(2):189–211.

Gochfeld M. Framework for gender differences in human and animal toxicology. Environ Res. 2007; 104:4–21. [PubMed: 16616135]

Goel N, Workman JL, Lee TT, Innala L, Viau V. Sex differences in the HPA axis. Compr Physiol. 2014; 4:1121–1155. [PubMed: 24944032]

Guilarte TR, Miceli RC, Jett DA. Neurochemical aspects of hippocampal and cortical Pb^{2+} neurotoxicity. Neurotoxicol. 1994; 15:459–466.

Guilarte TR, Miceli RC, Jett DA. Biochemical evidence of an interaction of lead at the zinc allosteric sites of the NDMA (*sic*) receptor complex: effects of neuronal development. Neurotoxicol. 1995; 16:63–71.

Hammill, DD., Pearson, NA., Wiederholt, JL. Comprehensive test of nonverbal intelligence – manual. Austin, TX: Pro-Ed; 1997.

Hesketh J. 3′-Untranslated regions are important for mRNA localization and translation: lessons from selenium and metallothionein. Biochem Soc Trans. 2004; 32:990–993. [PubMed: 15506944]

Jirtle RI, Skinner MK. Environmetal epigenomics and disease susceptibility. Nat Rev Genet. 2007; 8:253–262. [PubMed: 17363974]

Kelada SN, Shelton E, Kauffmann RB, Khoury MJ. Delta-aminolevulinic acid dehydratase genotype and lead toxicity: a HuGE review. Am J Epidemiology. 2001; 154:1–13.

Koller K, Brown T, Spurgeon A, Levy L. Recent developments in low-level exposures and intellectual impairment in children. Environ Health Perspex. 2004; 112:987–994.

Krieg EF Jr, Butler MA, Chang M-H, Luis T, Yesupriya A, Dowling N, et al. Lead and cognitive functions in VDR genotypes in the third National Health and Nutrition Examination Survey. Neurotoxicol Teratol. 2010; 32:262–272. [PubMed: 20006704]

Krieg EF Jr, Chrislip DW, Letz RE, Otto DA, Crespo CJ, Brightwell WS, et al. Neurobehavioral test performance in the third National Health and Nutrition Examination Survey. Neurotoxicol Teratol. 2001; 23:569–589. [PubMed: 11792526]

Lam V, Albrecht MA, Takechi R, Giles C, James AP, Foster JK, et al. The serum concentration of the calcium binding protein S100B is positively associated with cognitive performance in older adults. Front Aging Neurosci. 2013 Sep.5:1–6. [PubMed: 23430962]

Lanphear BP, Hornung R, Khoury J, Yolton K, Baghurst P, Bellinger DC, et al. Low-level environmental lead exposure and children's intellectual function: an international pooled analysis. Environ Health Perspect. 2005; 113:894–899. [PubMed: 16002379]

Lau CG, Zukin RS. NMDA receptor trafficking in synaptic plasticity and neuropsychiatric disorders. Nat Rev Neurosci. 2007; 8:413–426. [PubMed: 17514195]

Lesca G, Rudolf G, Bruneau N, Lozovaya N, Labalme A, Boutry-Kryza N, et al. GRIN2A mutations in acquired epileptic aphasia and related childhood focal epilepsies and encephalopathies with speech and language dysfunction. Nat Genet. 2013; 45:1061–1066. [PubMed: 23933820]

Martin MD, Benton T, Bernardo M, Woods JS, Townes BD, Luis H, et al. The association of dental caries with blood lead in children when adjusted for IQ and neurobehavioral performance. Sci Tot Environ. 2007; 377:159–164.

Martins IP, Castro-Caldas A, Townes BD, Ferreira G, Rodrigues P, Marques S, Rosenbaum G. Age and sex differences in neurobehavioral performance: A study of Portuguese elementary school children. Internat J Neurosci. 2005; 115:1687–1709.

Mazumdar M, Bellinger DC, Gregas M, Abanilla K, Bacic J, Needleman HL. Low-level environmental lead exposure in childhood and adult intellectual function: a follow-up study. Environ Health. 2011; 10:24.doi: 10.1186/1476-069X-10-24 [PubMed: 21450073]

Miranda ML, Kim D, Galeano MAO, Paul CJ, Hull AP, Morgan SP. The relationship between early childhood blood lead levels and performance on end-of-grade tests. Environ Health Perspect. 2007; 115:1242–1247. [PubMed: 17687454]

Neal AP, Worley PF, Guilarte TR. Lead exposure during synaptogenesis alters NMDA receptor targeting via NMDA receptor inhibition. Neurotoxicol. 2011; 32:281–289.

Ohtsuki T, Sakurai K, Dou H, Toru M, Yamakawa-Kobayashi K, Arinami T. Mutation analysis of the NMDAR2B (GINR2B) gene in schizophrenia. Molec Psychiatr. 2001; 6:211–216.

Paoletti P, Bellone C, Zhou Q. NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease. Nat Rev Neurosci. 2013; 14:383–399. [PubMed: 23686171]

Plummer M. JAGS: A program for analysis of Bayesian graphical models using Gibbs sampling. Proc 3rd Int Work Distrib Stat Comput. 2003:20–2.

Reis AL, Patinha C, Wragg J, Dias AS, Cave M, Sousa AJ, Batista MJ, Praseres C, Costa C, Ferreira da Silva E, Rocha F. Urban geochemistry of lead in gardens, playgrounds and schoolyards of Lisbon, Portugal: Assessing exposure and risk to human health. Appl Geochem. 2014; 44:45–53.

Reuben MEM, Caspi A, Belsky DW, Broadbnent J, Harrington H, Sugden K, Houts RM, et al. Association of childhood blood-lead levels with cognitive function and socioeconomic status at age 38 years and with IQ change and socioeconomic mobility between childhood and adulthood. JAMA. 2017; 317:1244–1251. [PubMed: 28350927]

Schneider JS, Anderson DW, Kidd SK, Sobolewski M, Cory-Slechta DA. Sex-dependent effects of lead and prenatal stress on post-translational histone modifications in frontal cortex and hippocampus in the early postnatal brain. Neurotoxicol. 2016; 54:65–71.

Schneider JS, Talsania K, Mettil W, Anderson DW. Genetic diversity influences the response of the brain to developmental lead exposure. Toxicol Sci. 2014; 141:29–43. [PubMed: 24913800]

Schulz S, Arning L, Pinnow M, Epplen JT, Beste C. N-methyl-D-aspartate receptor 2B subunit (*GRIN2B*) gene variation is associated with alerting, but not with orienting and conflicting in the Attention Network Test. Neuropharmacol. 2012; 63:259–265.

Skerfving S, Löfmark L, Lundh T, Mikoczy Z, Strömberg U. Late effects of low blood lead concentrations in children on school performance and cognitive functions. Neurotoxicol. 2015; 49:114–120.

Sobin C, Flores-Montoya MG, Gutierrez M, Parisi N, Schaub T. δ-Aminolevulinic acid dehydratase single nucleotide polymorphism 2 ($ALAD_2$) and peptide transporter 2*2 haplotype (*hPEPT2*2*) differentially influence neurobehavior in low-level lead exposed children. Neurotoxicol Teratol. 2015; 47:137–145. [PubMed: 25514583]

Taylor DL, Tiwari AK, Lieberman JA, Potkin SG, Meltzer HY, Knight J, et al. Genetic association analysis of the N-methyl-D-aspartate receptor subunit gene GRIN2B and clinical responses to clozapine. Hum Psychopharmacol Clin Exp. 2016; 31:121–134.

Taylor JY, Wright ML, Houseman D. Lead toxicity and genetics in Flint, MI. NPJ Genom Med. 2016; 1doi: 10.1038/njpgenmed.2016.18

Toscano DC, Guilarte TR. Lead neurotoxicity: from exposure to molecular effects. Brain Res Rev. 2005; 49:529–554. [PubMed: 16269318]

Townes BD, Rosenbaum JG, Martins IP, Castro-Caldas A. Neurobehavioral assessment of children: A cross-cultural perspective. Psychologica. 2003; 34:177–185.

Townes BD, Rosenbaum JG, Martin MD, Martins IP, Luis H, Bernardo MA. A longitudinal factor analytic study of children's neurocognitive abilities. Int J Neurosci. 2008; 118:1009–1023. [PubMed: 18569157]

Turic D, Langley K, Mills S, Stephens M, Lawson D, Govan C, et al. Follow-up of genetic linkage findings on chromosome 16p13: evidence of association of N-methyl-D-aspartate glutamate receptor 2A gene polymorphism with ADHD. Mol Psychiatr. 2004; 9:169–173.

Vahter M, Gochfeld M, Casati B, Thiruchevam M, Falk-Filippson A, Kavlock R, et al. Implications of gender differences for human health risk assessment and toxicology. Environ Res. 2007; 104:70–84. [PubMed: 17098226]

Valentino RJ, Reyes B, Bockstaele EV, Bangasser D. Molecular and cellular sex differences at the intersection of stress and arousal. Neuropharmacology. 2012; 62:13–20. [PubMed: 21712048]

Wagner PJ, Park H-R, Wang Z, Kirchner R, Wei Y, Su L, et al. In vitro effects of lead on gene expression in neural stem cells and associations between upregulated genes and cognitive scores in children. Environ Health Perspect. 2016; doi: 10.1289/EHP265

Wang T, Guan R-L, Liu M-C, Shen X-F, Chen JY, Zhao M-G, et al. Lead exposure impairs hippocampus related learning and memory by altering synaptic plasticity and morphology during juvenile period. Mol Neurobiol. 2016; 53:3740–3752. [PubMed: 26141123]

Wang FT, Hu H, Schwartz J, Weuve J, Spiro IIIAS, Sparrow D, et al. Modifying effects of the *HFE* polymorphisms on the association between lead burden and cognitive decline. Environ Health Perspect. 2007; 115:1210–1215. [PubMed: 17687449]

Warrington NW, Zhu G, Dy V, Heath AC, Madden PAF, Hemani G, Kemp JP, Mcmahon G, et al. Genome-wide association study of blood lead shows multiple associations near ALAD. Hum Molec Genet. 2015; 24:3871–3879. [PubMed: 25820613]

Weiss B. Vulnerability of children and the developing brain to neurotoxic hazards. Environ Health Perspect. 2000; 108(suppl 3):375–381. [PubMed: 10852831]

Wetmur JG, Lehnert G, Desnick RJ. The delta-aminolevulinate dehydratase polymorphism: higher blood lead levels in lead workers and environmentally exposed children with the 1-2 and 2-2 isozymes. Environ Res. 1991; 56:109–119. [PubMed: 1769358]

Whitfield JB, Dy V, McQuilty R, Zhu G, Montgomery GW, Ferreira MAR, et al. Evidence of genetic effects on blood lead concentration. Environ Health Perspect. 2007; 115:1224–1230. [PubMed: 17687451]

WHO Regional Office for Europe. Copenhagen, Denmark: 2001. p. 1-17.Lead, Chapter 6.7

Woods JS, Echeverria D, Heyer NJ, Simmonds PL, Wilkerson J, Farin FM. The association between genetic polymorphisms of coproporphyrinogen oxidase and an atypical porphyrinogenic response to mercury exposure in humans. Toxicol Appl Pharmacol. 2005; 206:113–120. [PubMed: 15967199]

Woods JS, Heyer NJ, Echeverria D, Russo JE, Martin MD, Bernardo MF, Luis HS, Vaz L, Farin FM. Modification of neurobehavioral effects of mercury by a genetic polymorphism of coproporphyrinogen oxidase in children. Neurotoxicol Teratol. 2012; 34:513–521. [PubMed: 22765978]

Woods JS, Heyer NJ, Russo JE, Martin MD, Pillai PB, Farin FM. Modification of neurobehavioral effects of mercury by genetic polymorphisms of metallothionein in children. Neurotoxicol Teratol. 2013; 39:36–44. [PubMed: 23827881]

Woods JS, Heyer NJ, Russo JE, Martin MD, Farin FM. Genetic polymorphisms affecting susceptibility to mercury neurotoxicity in children: Summary findings from the Casa Pia Children's Amalgam Clinical Trial. Neurotoxicol. 2014a; 44:288–302.

Woods JS, Heyer NJ, Russo JE, Martin MD, Pillai PB, Bammler TK, Farin FM. Genetic polymorphisms of catechol-O-methyltransferase modify the neurobehavioral effects of mercury in children. J Toxicol Environ Health Pt A. 2014b; 77:293–312.

Wu S, Hilvert M-F, Cardenas A, Zhong J, Rifas-Shiman S, Agha G, Colicino E, et al. Exposure to low levels of lead in utero and umbilical cord blood DNA methylation in Project Viva: An epigenome-wide association study. Environ Health Perspect. 2017; doi: 10.1289/EHP1246

Wu Y, Wang Y, Miaomiao W, Sun N, Li C. GRIN2A polymorphisms and expression levels are associated with lead-induced neurotoxicity. Toxicol Ind Health. 2016; doi: 10.1177/0748233716647636

Highlights

- Genetic polymorphisms may increase adverse effects of Pb on neurobehavioral processes.
- We studied 330 participants of the Casa Pia Dental Amalgams Trial in Children.
- Adverse interactions were found with variants of *GRIN2A* and *GRIN2B* in boys.
- Our results implicate genetic factors in defining susceptibility to Pb neurotoxicity.

Table 1

Neurobehavioral tests assessed by domain and clinical trial years employed.

YEARS 0-3	YEARS 4-7
ATTENTION	
Stroop word (strpwd) (↑)	Stroop word (strpwd) (↑)
Stroop color (strpcol) (↑)	Stroop color (strpcol) (↑)
Stroop color/word (strpcwd) (↑)	Stroop color/word (strpcwd) (↑)
WISC-III Digitspan (digspn) (↑)	WAIS-III digitspan (adigit) (↑)
—	WMS-III spatialspan (spsn) (↑)
Trailmaking A (trisasec)(↓)	Adult Trailmaking A (atriasec) (↓)
VISUAL-SPATIAL ACUITY	
Standard Reaction Time (SRTmean)(↓)	Standard Reaction Time (SRTmean)(↓)
WISC-III coding (coding) (↑)	WAIS-III digit symbol (adigsym) (↑)
WISC-III symbolsearch (symser) (↑)	WAIS-III symbolsearch ((asymb) (↑)
LEARNING & MEMORY	
RAVLT Tr1-Lsit A (rvlt1) (↑)	RAVLT Tr1-List A (rvlt1) (↑)
RAVLT Tr5-List A (rvlt5) (↑)	RAVLT Tr5-List A (rvlt5) (↑)
RAVLT Tr6-List B (rvlt6) (↑)	RAVLT Tr6-List B (rvlt6) (↑)
RAVLT Tr7 List A/post List B (rvlt7) (↑)	RAVLT Tr7 List A/post List B (rvlt7) (↑)
RAVLT Tr8 List A 20′ (rvlt8) (↑)	RAVLT Tr8 List A 20′ (rvlt8) (↑)
RAVLT Sum Trs1-5 (ravlttot) (↑)	RAVLT Sum Trs1-5 (ravlttot) (↑)
—	WMS-III vis repro immed (vireim) (↑)
—	WMS-III vis repro del (virede) (↑)
—	CMVT d-prime (CMVTdp) (↑)
EXECUTIVE FUNCTION	
Trailmaking B (trisbsec) (↓)	Adult Trailmaking B (atrlbsec) (↓)
—	WCST cats completed (wcsca) (↑)
—	WCST # correct (wcscorr) (↑)
MOTOR FUNCTION	
WRAVMA pegs-dom (pegdom) (↑)	WRAVMA pegs-dom (pegdom) (↑)
WRAVMA pegs-nondom (pegnd) (↑)	WRAVMA pegs-nondom (pegnd) (↑)
Finger Tapping dom (ftapd) (↑)	Finger Tapping dom (ftapd) (↑)
Finger tapping non-dom (ftapndo) (↑)	Finger tapping non-dom (ftapndo) (↑)

Arrows depict direction of improved performance.

Abbreviations employed:
CVMT Continuous Visual Memory Test
RAVLT Rey Auditory Verbal Learning Test
WAIS-III Weschler Adult Intelligence Scale-III
WCST Wisconsin Card Sorting Test
WISC-III Weschler Intelligence Scale for Children-III
WMS-III Weschler Memory Scale for Adults-III
WRAVMA Wide Range Assessment of Visual Motor Abilities

Table 2

Study population characteristics for participants at Entry (Baseline) and in Year 2 and Year 7

Characteristic	BOYS			GIRLS		
	ENTRY	YEAR 2	YEAR 7	ENTRY	YEAR 2	YEAR 7
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Age	10.17 (.82)	12.22 (.84)	17.15 (.85)	10.14 (.93)	12.15 (.96)	17.06 (1.03)
School Year	4.05 (1.05)	5.78 (1.21)	9.40 (2.05)	4.14 (1.07)	5.91 (1.16)	9.86 (1.51)
Non-Verbal IQ (at entry only)	85.96 (9.96)	—	—	85.54 (10.23)	—	—
Blood Lead Concentrations (BPb)						
BPb Mean µg/dl (SD)	5.26(2.73)	—	—	4.42(2.19)	—	—
Calculated lnBPb Mean (SD)	2.53(0.50)	—	—	2.37(0.48)	—	—
Distribution	**% (N)**	**% (N)**	**% (N)**	**% (N)**	**% (N)**	**% (N)**
Total Subjects (N)	163	158	120	167	151	119
Caucasian - % (N)	74.2% (121)	74.2% (118)	71.7% (86)	71.3% (119)	69.1% (105)	69.7% (83)
GRIN2A - rs727605 MAF=37%						
Major (CC)	36% (59)	36% (57)	35% (42)	45% (75)	45% (68)	45% (54)
Minor Heterozygous (CT)	49% (80)	49% (77)	51% (61)	42% (70 %)	41% (62)	40% (48)
Minor Homozygous (TT)	15% (24)	15% (24)	14% (17)	13% (22)	14% (21)	14% (17)
GRIN2A - rs1070503 MAF=16%						
Major (CC)	71% (116)	72% (114)	68% (82)	69% (116)	68% (103)	69% (82)
Minor Heterozygous (CT)	26% (43)	25% (40)	29% (35)	28% (46)	28% (43)	28% (33)
Minor Homozygous (TT)	3% (4)	3% (4)	3% (3)	3% (5)	3% (5)	3% (4)

Characteristic	BOYS			GIRLS		
	ENTRY	YEAR 2	YEAR 7	ENTRY	YEAR 2	YEAR 7
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
GRIN2B - rs7301328						
MAF=42%						
Major (CC)	35% (56)	34% (54)	34% (41)	35% (58)	34% (51)	34% (40)
Minor Heterozygous (CG)	50% (82)	51% (80)	50% (60)	20% (34)	45% (68)	46% (55)
Minor Homozygous (GG)	15% (25)	15% (24)	16% (19)	45% (75)	21% (32)	20% (24)
GRIN2B - rs1806201						
MAF=22%						
Major (CC)	59% (96)	60% (95)	64% (77)	66% (110)	66% (99)	69% (82)
Minor Heterozygous (CT)	35% (57)	34% (54)	31% (37)	29% (49)	30% (45)	26% (31)
Minor Homozygous (TT)	6% (10)	6% (9)	5% (6)	5% (8)	5% (7)	5% (6)

MAF: Minor Allelic Frequency

Table 3

Dose-response Effects of Lead on Neurobehavioral Test Performance after 7 years among BOYS and GIRLS with Minor (CT/TT) Genotype Status in either *GRIN2A* polymorphism

		***GRIN2A* rs727605 Minor (CT/TT)**		***GRIN2A* rs1070503 Minor (CT/TT)**	
Behavioral Test	**Domain**	**Boys**	**Girls**	**Boys**	**Girls**
		Beta (Cred. Int.)	**Beta (Cred. Int.)**	**Beta (Cred. Int.)**	**Beta (Cred. Int.)**
srtmean	Vis-Sp	**−0.01 (−0.020, − 0.002)**	**−0.01 (−0.022, −0.002)**	0.00 (−0.013, 0.008)	**−0.01 (−0.027, −0.001)**
Ravlt Tr1	L&M	**−0.14 (−0.263, − 0.029)**	−0.03 (−0.158, 0.087)	**−0.17 (−0.314, −0.038)**	−0.10 (−0.270, 0.076)
Ravlt Tr5	L&M	**−0.16 (−0.262, − 0.046)**	−0.10 (−0.221, 0.009)	**−0.15 (−0.271, −0.021)**	−0.12 (−0.277, 0.050)
Ravlt Tr6	L&M	**−0.13 (−0.257, − 0.015)**	−0.02 (−0.146, 0.114)	−0.13 (−0.281, 0.004)	−0.05 (−0.239, 0.122)
Ravlt Tr7	L&M	**−0.17 (−0.291, − 0.057)**	−0.08 (−0.209, 0.032)	**−0.19 (−0.329, −0.060)**	−0.09 (−0.261, 0.078)
Ravlt Tr8	L&M	**−0.19 (−0.329, − 0.067)**	−0.05 (−0.175, 0.077)	**−0.25 (−0.392, −0.105)**	−0.06 (−0.231, 0.121)
Ravlttot	L&M	**−0.29 (−0.945, − 0.035)**	−0.06 (−0.321, 0.214)	**−0.37 (−0.746, −0.069)**	−0.07 (−0.417, 0.266)
vireim	L&M	**−0.18 (−0.410, − 0.010)**	−0.01 (−0181, 0.216)	**−0.27 (−0.493, −0.064)**	0.04 (−0.213, 0.322)
Virede	L&M	**−0.18 (−0.431, − 0.005)**	−0.02 (−0.209, 0.215)	**−0.31 (−0.558, −0.074)**	0.06 (−0.201, 0.352)
wcscatcp	Exec Fn	−0.07 (−0.133, 0.005)	−0.03 (−0.106, 0.053)	**−0.10 (−0.186, −0.017)**	**−0.12 (−0.239, −0.023)**
wcscorr	Exec Fn	−0.13 (−0.512, 0.061)	−0.03 (−0.297, 0.231)	**−0.29 (−0.640, −0.010)**	−0.17 (−0.630, 0.182)

Data are presented as Betas, their 95% credible intervals (Cred. Int.). Values in **bold** exclude the null effect at the 95% credible interval. Note that each *GRIN2A* polymorphism is modelled independently.

Table 4

Dose-response Effects of Lead on Neurobehavioral Test Performance after 7 years among BOYS and GIRLS with Minor Genotype Status in either *GRIN2B* polymorphism

		***GRIN2B rs1806201* Minor (CT/TT)**		***GRIN2B rs7301238* Minor (CG/GG)**	
Behavioral Test	**Domain**	**Boys**	**Girls**	**Boys**	**Girls**
		Beta (Cred. Int.)	**Beta (Cred. Int.)**	**Beta (Cred. Int.)**	**Beta (Cred. Int.)**
srtmean	Vis-Sp	−0.01 (−0.021, 0.003)	**−0.02 (−0.029, − 0.001)**	**−0.01 (−0.023, −0.003)**	**−0.01 (− 0.023, − 0.003)**
Ravlt Tr1	L&M	**−0.16 (−0.312, − 0.003)**	−0.08 (−0.241, 0.070)	−0.12 (−0.251, 0.002)	−0.07 (−0.212, 0.069)
Ravlt Tr5	L&M	**−0.17 (−0.311, − 0.032)**	−0.05 (−0.203, 0.086)	**−0.13 (−0.262, −0.020)**	**−0.14 (− 0.266, − 0.005)**
Ravlt Tr6	L&M	**−0.16 (−0.322, − 0.003)**	−0.04 (−0.197, 0.123)	−0.11 (−0.246, 0.013)	0.03 (−0.114, 0.171)
Ravlt Tr7	L&M	**−0.21 (−0.355, − 0.057)**	−0.07 (−0.224, 0.080)	**−0.12 (−0.250, −0.002)**	−0.12 (−0.253, 0.022)
Ravlt Tr8	L&M	**−0.29 (−0.442, − 0.126)**	−0.05 (−0.215, 0.099)	**−0.15 (−0.286, −0.021)**	−0.08 (−0.222, 0.073)
Ravlttot	L&M	**−0.55 (−0.984, − 0.144)**	−0.04 (−0.297, 0.227)	−0.15 (−0.362, 0.001)	−0.15 (−0.520, 0.214)
vireim	L&M	**−0.29 (−0.549, − 0.035)**	0.02 (−0.175, 0.338)	**−0.15 (−0.319, −0.004)**	0.12 (−0.115, 0.346)
Virede	L&M	**−0.51 (−0.831, − 0.210)**	0.01 (−0.184, 0.338)	**−0.15 (−0.318, −0.004)**	0.09 (−0.163, 0.338)
wcscatcp	Exec Fn	**−0.15 (−0.255, − 0.056)**	−0.08 (−0.216, 0.025)	−0.06 (−0.141, 0.036)	−0.08 (−0.167, 0.015)
wcscorr	Exec Fn	**−0.48 (−0.924, − 0.101)**	−0.08 (−0.411, 0.168)	−0.07 (−0.260, 0.051)	−0.24 (−0.616, 0.138)

Data are presented as Betas, their 95% credible intervals (Cred. Int.). Values in **bold** exclude the null effect at the 95% credible interval. Note that each *GRIN2B* polymorphism is modelled independently.

Table 5

Dose-response Effects of Lead on Neurobehavioral Test Performance among Year 7 BOYS carrying Minor alleles for both *GRIN2A* polymorphisms or both *GRIN2B* polymorphisms.

		GRIN2A (1 or 2 × CT/TT)		***GRIN2B ((1 or 2 × CT/TT + 1 or 2 × CG/GG))***	
Behavioral Test	**Domain**	**Boys**	**Girls**	**Boys**	**Girls**
		Beta (Cred. Int.)	**Beta (Cred. Int.)**	**Beta (Cred. Int.)**	**Beta (Cred. Int.)**
srtmean	Vis-Sp	−0.01 (−0.016, 0.002)	**−0.01 (−0.022, − 0.002)**	**−0.01 (−0.021, −0.002)**	**−0.01 (− 0.023, − 0.003)**
Ravlt Tr1	L&M	**−0.14 (−0.255, − 0.028)**	−0.05 (−0.166, 0.072)	**−0.13 (−0.251, −0.003)**	−0.07 (−0.202, 0.060)
Ravlt Tr5	L&M	**−0.14 (−0.242, − 0.031)**	−0.08 (−0.200, 0.027)	**−0.16 (−0.273, −0.044)**	**−0.13 (− 0.254, − 0.012)**
Ravlt Tr6	L&M	**−0.14 (−0.257, − 0.018)**	−0.03 (−0.149, 0.095)	−0.11 (−0.237, 0.021)	−0.00 (−0.140, 0.136)
Ravlt Tr7	L&M	**−0.16 (−0.274, − 0.052)**	−0.07 (−0.195, 0.038)	**−0.16 (−0.282, −0.038)**	−0.12 (−0.247, 0.009)
Ravlt Tr8	L&M	**−0.20 (−0.315, − 0.081)**	−0.04 (−0.167, 0.073)	**−0.24 (−0.372, −0.112)**	−0.13 (−0.262, 0.011)
Ravlttot	L&M	**−0.43 (−0.749, − 0.106)**	−0.05 (−0.288, 0.162)	**−0.47 (−0.823, −0.155)**	−0.19 (−0.511, 0.085)
vireim	L&M	**−0.25 (−0.456, − 0.072)**	−0.02 (−0.185, 0.178)	**−0.34 (−0.547, −0.132)**	0.00 (−0.207, 0.218)
Virede	L&M	**−0.27 (−0.487, − 0.073)**	−0.03 (−0.194, 0.186)	**−0.39 (−0.614, −0.152)**	−0.03 (−0.253, 0.201)
wcscatcp	Exec Fn	−0.07 (−0.148, 0.010)	−0.05 (−0.130, 0.029)	**−0.10 (−0.176, −0.022)**	**−0.08 (− 0.164, − 0.008)**
wcscorr	Exec Fn	−0.23 (−0.530, 0.051)	−0.05 (−0.297, 0.182)	**−0.37 (−0.713, −0.058)**	−0.20 (−0.539, 0.104)

Data are presented as Betas, their 95% credible intervals (Cred. Int.). Values in **bold** exclude the null effect at the 95% credible interval. Note that each *GRIN2B* polymorphism is modelled independently.