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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 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.

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

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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