Neurotoxicology and Teratology 47 (2015) 137–145

Contents lists available at ScienceDirect

Neurotoxicology and Teratology

journal homepage: www.elsevier.com/locate/neutera

δ-Aminolevulinic acid dehydratase single nucleotide polymorphism 2 (*ALAD$_2$*) and peptide transporter 2*2 haplotype (*hPEPT2*2*) differently influence neurobehavior in low-level lead exposed children

Christina Sobin [a,b,c,*], Mayra Gisel Flores-Montoya [b,d], Marisela Gutierrez [d], Natali Parisi [e], Tanner Schaub [e]

[a] College of Health Sciences, Department of Public Health Sciences, University of Texas, El Paso, TX, United States
[b] Border Biomedical Research Center, Toxicology Core, University of Texas, El Paso, TX, United States
[c] Laboratory of Neuroendocrinology, The Rockefeller University, New York, NY, United States
[d] Department of Psychology, University of Texas, El Paso, TX, United States
[e] New Mexico State University, Las Cruces, NM, United States

ARTICLE INFO

Article history:
Received 16 July 2014
Received in revised form 6 December 2014
Accepted 8 December 2014
Available online 13 December 2014

Keywords:
Child lead exposure
Neurobehavior
Delta-aminolevulinic acid dehydratase
Proton-coupled oligopeptide transporter
hPEPT2
ALAD

ABSTRACT

Delta-aminolevulinic acid dehydratase single nucleotide polymorphism 2 (*ALAD$_2$*) and peptide transporter haplotype 2*2 (*hPEPT2*2*) through different pathways can increase brain levels of delta-aminolevulinic acid and are associated with higher blood lead burden in young children. Past child and adult findings regarding *ALAD$_2$* and neurobehavior have been inconsistent, and the possible association of *hPEPT2*2* and neurobehavior has not yet been examined. Mean blood lead level (BLL), genotype, and neurobehavioral function (fine motor dexterity, working memory, visual attention and short-term memory) were assessed in 206 males and 215 females ages 5.1–11.8 years. Ninety-six percent of children had BLLs < 5.0 μg/dl. After adjusting for covariates (sex, age and mother's level of education) and sibling exclusion (N = 252), generalized linear mixed model analyses showed opposite effects for the *ALAD$_2$* and *hPEPT2*2* genetic variants. Significant effects for *ALAD$_2$* were observed only as interactions with BLL and the results suggested that *ALAD$_2$* was neuroprotective. As BLL increased, *ALAD$_2$* was associated with enhanced visual attention and enhanced working memory (fewer commission errors). Independent of BLL, *hPEPT2*2* predicted poorer motor dexterity and poorer working memory (more commission errors). BLL alone predicted poorer working memory from increased omission errors. The findings provided further substantiation that (independent of the genetic variants examined) lowest-level lead exposure disrupted early neurobehavioral function, and suggested that common genetic variants alter the neurotoxic potential of low-level lead. *ALAD$_2$* and *hPEPT2*2* may be valuable markers of risk, and indicate novel mechanisms of lead-induced neurotoxicity. Longitudinal studies are needed to examine long-term influences of these genetic variants on neurobehavior.

1. Introduction

Lead poisoning in children has decreased dramatically over the past 40 years while low-level lead exposure continues to impact unknown numbers of children, particularly those living in lowest socio-economic conditions across the United States. (Bernard and McGeehin, 2003). Human and primate studies have suggested that early low-level lead exposure impairs a specific cluster of neurobehavioral functions that are dependent on basal-thalamocortical-striato-pallido loop pathways linking mid-brain and cortical structures (Bolam et al., 2005; Nakano, 2000) including but not limited to fine motor dexterity (Chiodo et al., 2004, 2007; Surkan et al., 2007); visual attention (Chiodo et al., 2004, 2007; Gilbert and Rice, 1987; Min et al., 2007); and working memory and short-term memory (Chiodo et al., 2004; Lanphear et al., 2000; Min et al., 2007).

1.1. Lead-induced neurotoxicity and delta-aminolevulinic acid (δ-ALA)

Although poorly understood, there are multiple mechanisms by which lead exposure can become neurotoxic. For example, lead particles can cross the blood–brain barrier and preferentially accumulate in astroglia (Lindahl et al., 1999; Thomas et al., 1973). In astrocytes lead particles bind to and inactivate the molecular chaperone 78-kDa (glucose-regulated protein 78, GRP78) (Legare et al., 1998; Qian et al., 2000) lowering astrocytic secretion of neuroprotective IL-6, and increasing the likelihood of excitotoxic cell death (White et al., 2007). At low levels of lead exposure however, lead particle accumulation is likely to be minimal.

* Corresponding author at: University of Texas, El Paso, 500 West University, El Paso, TX 79968, United States. Tel.: +1 915 747 8485; fax: +1 915 747 6553.
E-mail address: casobin@utep.edu (C. Sobin).

http://dx.doi.org/10.1016/j.ntt.2014.12.001
0892-0362/

Neurotoxic effects can also be indirect, as in the case of increased brain δ-ALA (Kappas et al., 1995). In erythrocytes, lead particles are bound by δ-aminolevulinic acid dehydratase (δ-ALAD), the second enzyme in the heme biosynthesis pathway. Lead binding inactivates δ-ALAD causing a rise in levels of its substrate, δ-ALA (Klaassen, 2006). Excess brain δ-ALA disrupts the γ-aminobutyric acid (GABA)/glutamate system, in part by blocking GABA receptors and increasing the likelihood of neuroexcitotoxic events and cell death (Brennan and Cantrill, 1979; Demasi et al., 1996b; Emanuelli et al., 2003; Villayandre et al., 2005). Sodium channel activation is altered by extra-cellular concentrations of δ-ALA as low as 0.01 pM (Lindberg et al., 1999; Wang et al., 2005) suggesting an exquisite sensitivity of neurons to very small increases of extra-cellular δ-ALA.

1.2. Two genetic variants are associated with increased blood lead burden

Variants of two genes that contribute to increased blood lead burden and with downstream indirect and direct effects on δ-ALA levels, might be expected to alter the effects of low-level lead exposure on neurobehavior. δ-ALAD (chromosome 9q34) is encoded by the variants $ALAD_1$ and $ALAD_2$ [ID: *rs1800435*] (Wetmur et al., 1991a). $ALAD_2$ [ID: *rs1800435*], is estimated to occur in 10–15% of Anglo, European and Asian populations (Kelada et al., 2001; Wetmur et al., 1991a) and has a higher binding affinity for lead (Battistuzzi et al., 1981). (Homozygotes are relatively rare thus most studies have examined effects of $ALAD_2$ in groups that combine subjects carrying one or two copies of $ALAD_2$.) Lead-exposed adults with $ALAD_2$ [ID: *rs1800435*] had higher blood lead burden (Scinicariello et al., 2007; Wetmur et al., 1991b; Zhao et al., 2007) and child studies revealed similar results. In 93 Chilean children living near a lead-contaminated site, those with $ALAD_2$ [ID: *rs1800435*] had higher BLL (14.2 μg/dl vs. 9.5 μg/dl) (Pérez-Bravo et al., 2004). Similarly, in 229 children in China, mean BLL was higher in children with $ALAD_2$ (11.7 μg/dl vs. 9.7 μg/dl) (Shen et al., 2001). Associations were also observed at lowest levels of exposure in young children (Sobin et al., 2009), and when gender effects were examined differences were found (Sobin et al., 2011b). As compared to other subgroups, mean BLL was highest among males with $ALAD_2$ [ID: *rs1800435*] (3.5 μg/dl vs. 2.7 μg/dl).

Through an entirely different pathway, another genetic variant also impacts blood lead burden and brain δ-ALA. Proton-coupled oligopeptide transporter (PEPT2, aka SLC15A2, chromosome 3q21.1) protects the brain from excess peptide-bound amino acids. In kidney, PEPT2 reabsorbs di- and tri-peptides (Shen et al., 1999), and PEPT2 maintains neuropeptide homeostasis and removes potential neurotoxins at the blood–cerebrospinal fluid barrier (Ocheltree et al., 2005). Relevant to lead exposure, PEPT2 effluxes δ-ALA from cells in cerebrospinal fluid which has suggested to some that PEPT2 may act as a genetic moderator of lead-induced neurotoxicity (Hu et al., 2007).

Several single nucleotide polymorphisms in the *PEPT2* gene with unknown functional impact have been described (Pinsonneault et al., 2004) however two *PEPT2* haplotypes, *hPEPT2*1* and *hPEPT2*2*, overwhelmingly predominate. The *hPEPT2*2* variant has a significantly lower binding potential (Pinsonneault et al., 2004; Ramamoorthy et al., 1995). For example, *hPEPT2*1* and *hPEPT2*2* had significantly different K_m constants (83 ± 16 and 233 ± 38 μM, respectively) with similar V_{max} values for glycyl-sarcosine in hamster ovary cells (Pinsonneault et al., 2004).

Two studies thus far have examined associations between blood lead burden and *hPEPT2*2* (Sobin et al., 2009, 2011b). Similar to the gender effects observed for $ALAD_2$, at lowest levels of lead exposure, males but not females homozygous for *hPEPT2*2* had significantly increased BLL (4.9 μg/dl vs. 2.6 μg/dl) (Sobin et al., 2011b). (Why hPEPT2*2 may be associated with higher blood lead burden in males has not yet been determined; possible explanations are discussed in the referenced manuscript). No interaction or additive effects of these genetic variants were observed which may reflect the broadly different pathways by which these genetic variants are likely to influence blood lead burden. (The mechanisms by which *hPEPT2*2* influences blood lead burden have not been identifiEd.)

1.3. Genetic variants associated with higher blood lead burden could also predict neurobehavior

Several studies have examined associations between $ALAD_2$ [*rs1800435*] and neurobehavior in lead exposed children (Bellinger et al., 1994; Pawlas et al., 2012), adolescents (Krieg et al., 2009) and adults (Gao et al., 2010). Additional studies are needed however. While child studies have suggested $ALAD_2$ is neuroprotective, studies in older adults suggest worse outcomes (Rajan et al., 2008). Moreover, no studies have examined associations between *hPEPT2* genotypes and neurobehavior in low-level lead exposed children; the *ALAD* and *hPEPT2* variants have not yet been considered in a single model; and interactions with BLL have rarely been examined. Understanding how these genetic variants are associated with neurobehavior in low-level lead exposed children could suggest novel hypotheses regarding the mechanisms by which low-level lead exposure disrupts early neurobehavior, and ultimately perhaps, provide a means for identifying subgroups of children at heightened risk for poor outcome (Levin et al., 2009).

The goal of this study was to test the possible moderating effects of *ALAD* and *hPEPT2* genetic variants on motor dexterity, visual attention, working memory and short-term memory in young children tested for lead exposure. Significant main effects of aggregate BLL, *ALAD* and *hPEPT2*, and interaction effects for *ALAD* and *hPEPT2* by BLL on neurobehavior were tested.

2. Materials and methods

2.1. Participants

Permission to conduct these studies was obtained from the local school district and approved by the university institutional review board. Children were tested with the full understanding and prior written consent of parents; child assent was obtained immediately prior to testing. Convenience samples were recruited from two elementary schools (sites) located in lower-income neighborhoods and included children 5.1–11.8 years of age. A participation invitation letter was sent to all parents from the school principal and a copy of the consent form was enclosed in the letter. Interested parents attended informational sessions during which details of the study were explained and informed consent was obtained. Participants represented 27.6–38.4% of enrolled students in each school. All study forms and materials were available in Spanish and English versions. Researchers on this study were fully bilingual and throughout the study interacted with parents and children in their preferred language.

2.2. Procedures

2.2.1. Genetic sample collection

Cheek cell collection, DNA extraction and single-nucleotide polymorphism detection were completed using proprietary technology developed by TrimGen Corporation (Sparks, MD). Detailed procedures were described previously (Sobin et al., 2009).

2.2.2. Blood lead analysis

Blood lead level testing was conducted at two time points, between 53 and 67 days prior to neurobehavioral testing, and at the time of neurobehavioral testing. Detailed procedures were previously described (Sobin et al., 2011a,b). Aggregate blood lead level was the mean of two values obtained an average of 60 days apart for each child. Four hundred twenty-one children were tested (206 males, 215 females). Blood lead level was determined by either inductively coupled plasma