δ-Aminolevulinic Acid Dehydratase Single Nucleotide Polymorphism 2 and Peptide Transporter 2*2 Haplotype May Differentially Mediate Lead Exposure in Male Children

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Abstract Child low-level lead (Pb) exposure is an unresolved public health problem and an unaddressed child health disparity. Particularly in cases of low-level exposure, source removal can be impossible to accomplish, and the only practical strategy for reducing risk may be primary prevention. Genetic biomarkers of increased neurotoxic risk could help to identify small subgroups of children for early intervention. Previous studies have suggested that, by way of a distinct mechanism, δ-aminolevulinic acid dehydratase single nucleotide polymorphism 2 (ALAD$_2$) and/or peptide transporter 2*2 haplotype (hPEPT2*2) increase Pb blood burden in children. Studies have not yet examined whether sex mediates the effects of genotype on blood Pb burden. Also, previous studies have not included blood iron (Fe) level in their analyses. Blood and cheek cell samples were obtained from 306 minority children, ages 5.1 to 12.9 years. ^{208}Pb and ^{56}Fe levels were determined with inductively coupled plasma–mass spectrometry. General linear model analyses were used to examine differences in Pb blood burden by genotype and sex while controlling for blood Fe level. The sample geometric mean Pb level was 2.75 μg/dl. Pb blood burden was differentially higher in ALAD$_2$ heterozygous boys and hPEPT2*2 homozygous boys. These results suggest that the effect of ALAD$_2$ and hPEPT2*2 on Pb blood burden may be sexually dimorphic. ALAD$_2$ and hPEPT2*2 may be novel biomarkers of health and mental health risks in male children exposed to low levels of Pb.

Child exposure to environmental lead (Pb) yielding blood levels below the threshold for toxicity (<10 μg/dl) remains an unresolved public health dilemma as well as a child health disparity (Carter-Pokras and Baquet 2002). Childhood exposure to low-level Pb has been associated with diminished function of a specific cluster of neurocognitive abilities associated with frontal and prefrontal cortical regions (Gilbert and Rice 1987; Rice and Karpinski 1988; Levin et al. 1992; Lanphear et al. 2000; Chiodo et al. 2004, 2007; Min et al. 2007; Surkan et al. 2007), adult-onset renal dysfunction (Fadrowski et al. 2010), and metabolic syndrome and cardiac abnormalities (Park et al. 2006). The Centers for Disease Control (CDC) and others (Gilbert and Weiss 2006) have cautioned that the toxicity threshold of 10 μg/dl should not be taken to mean that Pb levels below this threshold are "safe" for children. For children living in lower socioeconomic conditions, multiple potential sources increase the likelihood of exposure. More than 9,000 US industrial facilities emit from 10 to >10,000 lb of lead/y (United States Environmental Protection Agency [USEPA]

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Emissions Inventory 2006); other common sources include (but are not limited to) paint chips and dust in unrenovated housing, ceramic glazes, inexpensive cookware, toy paint, and inexpensive children's jewelry (Rossi 2008; Centers for Disease Control and Prevention 2009). For low-level environmental Pb exposure, the only known intervention is source removal, and this can be impossible to accomplish. Primary prevention may be the only viable approach (Rossi 2008). Identifying genetic factors that mediate Pb blood burden could facilitate the prevention of long-term sequelae by providing a means to identify a subgroup of highest-risk children for early targeted neurocognitive intervention and health monitoring. Genetic findings can also suggest novel mechanisms of action for animal model studies.
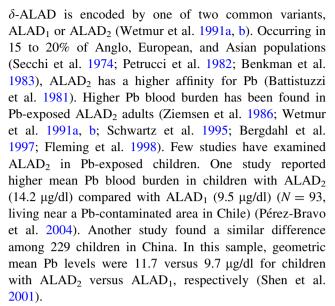
Direct and Indirect Effects of Pb on Brain Function

Neurodevelopmental deficits are the most widely documented childhood outcomes associated with low-level Pb exposure (Lanphear et al. 2000; Chiodo et al. 2004; Chiodo et al. 2007; Min et al. 2007; Surkan et al. 2007). Deleterious brain effects from low-level Pb are likely to result from a dual process that includes the accumulation of Pb molecules in brain tissue and increased brain delta-aminolevulinic acid (δ-ALA) (Kappas et al. 1995). Pb molecules mimic calcium, easily cross the blood–brain barrier, and are preferentially stored in astrocytes (Thomas et al. 1973; Lindahl et al. 1999).

Pb also disrupts heme biosynthesis. In erythrocytes, Pb molecules are bound by delta-aminolevulinic acid dehydratase (δ-ALAD), the enzyme mediating the second step in the heme biosynthesis cascade leading to modulation of the precursor enzyme δ-ALA. Binding inactivates δ-ALAD, resulting in higher brain levels of δ-ALA (Klaassen 2006). Extracellular concentrations of δ-ALA as low as 0.01 pM alter sodium channel activation, suggesting exquisite neuronal sensitivity to small increases in δ-ALA (Wang et al. 2005). Excess brain δ-ALA has a number of potentially deleterious effects. δ-ALA activates $GABA_A$ autoreceptors, directly damages $GABA_A$ receptor sites (Demasi et al. 1996), and stimulates glutamate release (Brennan and Cantrill 1979). δ-ALA also alters glutamate transporter GLT-1 and irreversibly inhibits glutamate uptake by astrocytes (Emanuelli et al. 2003). When chronic, excess δ-ALA decreases NMDA receptor density (Villayandre et al. 2005).

Gene Variants May Mediate Pb Blood Burden

At least two gene variants may exaggerate Pb blood burden in children. Located at chromosome 9q34,

δ-ALAD is encoded by one of two common variants, $ALAD_1$ or $ALAD_2$ (Wetmur et al. 1991a, b). Occurring in 15 to 20% of Anglo, European, and Asian populations (Secchi et al. 1974; Petrucci et al. 1982; Benkman et al. 1983), $ALAD_2$ has a higher affinity for Pb (Battistuzzi et al. 1981). Higher Pb blood burden has been found in Pb-exposed $ALAD_2$ adults (Ziemsen et al. 1986; Wetmur et al. 1991a, b; Schwartz et al. 1995; Bergdahl et al. 1997; Fleming et al. 1998). Few studies have examined $ALAD_2$ in Pb-exposed children. One study reported higher mean Pb blood burden in children with $ALAD_2$ (14.2 µg/dl) compared with $ALAD_1$ (9.5 µg/dl) ($N = 93$, living near a Pb-contaminated area in Chile) (Pérez-Bravo et al. 2004). Another study found a similar difference among 229 children in China. In this sample, geometric mean Pb levels were 11.7 versus 9.7 µg/dl for children with $ALAD_2$ versus $ALAD_1$, respectively (Shen et al. 2001).

Proton-coupled oligopeptide transporter [PEPT2 (SLC15A2)] also may mediate the effects of Pb on brain function. PEPT2 is a protective endogenous transporter that acts on excess peptide-bound amino acids, such as δ-ALA. In kidney, PEPT2 re-absorbs di- and tri-peptides (Shen et al. 1999). At the blood–cerebrospinal fluid barrier, PEPT2 maintains neuropeptide homeostasis and removes potential neurotoxins (Ocheltree et al. 2005). Because PEPT2 lowers δ-ALA in cerebrospinal fluid, it has been suggested that PEPT2 may mediate neurotoxicity in cases of Pb exposure (Hu et al. 2007).

Many single nucleotide polymorphisms of unknown function have been identified in the PEPT2 gene (chromosome 3q13.3); two PEPT2 haplotypes (hPEPT2*1 and hPEPT2*2) overwhelmingly predominate. PEPT2 has been shown to have substantially lower binding potential in the presence of hPEPT2*2 compared with hPEPT2*1 (Ramamoorthy et al. 1995; Pinsonneault et al. 2004). A first study of hPEPT2*2 (and $ALAD_2$) in 116 children suggested that Pb blood burden was higher in hPEPT2*2 homozygotes (Sobin et al. 2009).

When examining the potential modifying effects on Pb blood burden of $ALAD_2$ and hPEPT2*2, two additional factors may be important to consider. Findings have suggested that male children (Gochfeld 2007; Vahter et al. 2007; United States Environmental Protection Agency 2010) and children with low iron (Fe) levels (Wright et al. 1999; Bradman et al. 2001; Choi and Kim 2003; Wright et al. 2003) have increased Pb blood burden. In previous studies of $ALAD_2$ and hPEPT2*2, the possible contribution of sex and Fe level were not included as factors. Also, in the previous study of hPEPT2*2, Pb levels were estimated using an anodic-stripping device (LeadCare System), which can lack precision at lower limits of detection (Sobin et al. 2010).

The goal of the current study was to expand on past findings using improved methods. For this study, Pb and Fe levels were determined from whole blood samples using inductively coupled plasma–mass spectrometry (ICP-MS). Sex was included as an explanatory variable, and Fe was covaried. It was reasoned that $ALAD_2$ and hPEPT2*2 might be expected to synergistically amplify the male tendency toward increased Pb blood burden (see "Discussion"). Higher Pb blood burden in male $ALAD_2$ heterozygotes compared with boys without $ALAD_2$, and all subgroups of girls, was predicted ($ALAD_2$ homozygotes are rare, and none were detected in this sample). Also, higher Pb blood burden in male hPEPT2*2 homozygotes compared with boys without hPEPT2*2, male heterozygotes, and all subgroups of girls was predicted. Possible additive effects of $ALAD_2$ and hPEPT2*2 were explored in secondary analyses.

Materials and Methods

Participants

The project was approved by the Institutional Review Board of the University of Texas in El Paso as well as the El Paso Independent School District Research Board. Participants were local elementary school students. All study forms and materials were available in Spanish and English versions, and parents were asked to complete health and household history information; 249 of 306 (81.4%) parents chose to comply. Written parental informed consent was obtained before all testing sessions. Child verbal assent was obtained immediately before testing.

Procedures

Blood Collection

Universal precautions, including protective barriers and retracting lancets, were used by testers during blood collection. Children washed their hands, and the fingers of the left hand were wiped clean with chelating towelettes specially formulated for industry use to remove Pb as well as nickel, silver, cadmium, and arsenic from the skin surface (D-Wipe; Esca-Tech, Milwaukee, WI). Saf-T-Pro 1.8 mm lancets were used on the forth finger of the left hand. Approximately, 100 µl whole blood was collected into a microvial, refrigerated, and transferred to the chemical analysis laboratory within 72 h of collection.

ICP-MS Analysis of ^{208}Pb and ^{56}Fe

Instrumentation

ICP-MS analyses were performed with an Agilent 7500ce ICP/MS equipped with an octopole reaction system and a CETAC ASX-520 autosampler (Agilent, Santa Clara, CA). Detection of ^{208}Pb was performed without the use of collision gas. Due to spectrometric interferences, the use of helium as collision gas was necessary for the detection of ^{56}Fe. Samples were introduced into the plasma through a MicroMist U-series nebulizer (Glass Expansion, West Melbourne, Australia) and a double-pass quartz spray chamber. Instrument parameters were carrier gas 0.78 l/min, makeup gas 0.15 l/min, radiofrequency power 1420 W, and spray chamber temperature 2°C.

Sample Treatment and Analysis

Certified whole blood standards were analyzed to determine instrument reproducibility (Le Centre de Toxicologie du Quebec, Quebec, Canada). Specifically, ten solutions were prepared as described in later text for each of two standards (4.00 and 6.59 µg/dl), and each of those were analyzed three times by ICP-MS. Standard concentrations were chosen to approximate the low-level Pb values of children.

Samples and blood standards were prepared as previously described (Agilent technical note no. 5988-0533EN). Briefly, 5.58 ml water (18 MΩ DI; Labconco WaterPro PS Station, Kansas City, MO) was placed in a polypropylene tube into which 300 µl whole blood was added, followed by the addition of 60 µl aqueous internal standard solution containing 100 ppb each germanium, yttrium, and terbium in 5% nitric acid (Fisher Optima; ThermoFisher Scientific, Waltham, MA) and 60 µl aqueous 10 ppm gold in 3% hydrochloric acid solution (EMD Chemicals, Gibbstown, NJ). (Internal standards were added to every sample to identify and correct instrument drift. Internal standards were also used when building the calibration curve.) The final dilution was 20-fold; the final internal standard concentration was 1 ppb; and the final gold concentration was 100 ppb. A six-point external calibration curve was prepared from a Pb stock solution in 1% nitric acid. ICP-MS standard solutions containing the elements in 2% nitric acid were obtained from Inorganic Ventures (Christiansburg, VA). Samples were vortexed for a few seconds before a 1 min centrifugation at 2,000 rcf, after which the supernatant was analyzed by ICP-MS.

Genetic Testing

Cheek cell collection, DNA extraction, and polymorphism detection were completed using proprietary technology (TrimGen, Sparks, MD). Cheek cells were collected using Easy-Swab foam collection swabs and DNA extraction was completed with BuccalQuick solution. Children rinsed their mouths with water before collection, and four samples (two from each cheek) were collected from each child. Swabs were labeled and packed in holders for drying. Swab heads were rinsed in extraction buffer, vortexed at high speed (10 s), incubated at 55°C (60 s), and heated at 90°C (180 s). Polymerase chain reaction amplification consisted of one cycle 95°C (5 s); 40 cycles of 95°C (30 s), 53°C (30 s) for ALAD2 or 56°C (30 s) for PEPT2, 72°C (30 s); one cycle 95°C (5 s) using the following gene specific primers: PEPT2, F: 5′ AGGAAAATGGCTGTTGGTAT GATC 3′; R: 5′ CGCAACTGCAAATGCCAG 3′; ALAD, F: 5′ GACCGTTGCCTGGGAC 3′; R: 5′ TCCCTTCTT AGCCCTTCC 3′. Mutation detection was accomplished with a multibase primer extension method (Shifted Termination Assay technology, Mutector Dual Well Test Kit; TrimGen). Labeled nucleotides were examined for color-coded reactions indicating a given genotype.
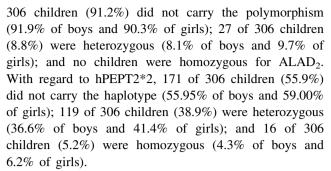
Data Analysis

SAS statistical software (version 9.1) was used for all analyses. General linear model analysis of covariance was used to test for Pb differences in male and female children without and with hPEPT2*2 and ALAD$_2$ (with Fe covaried). Main and interaction effects of ALAD2 × gender and hPEPT2*2 × sex were examined. The interaction term ALAD$_2$ × hPEPT2*2 was examined in secondary analyses. Type III sums of squares were used to determine the significance of main and interaction effects. Dunnett *post hoc* comparisons of least square (LS) means were used to identify the source of significant differences for each effect. LS means indicated the Pb value means for each group after covarying Fe. To estimate precision, 95% confidence limits were calculated for LS means and for significant LS mean differences between groups.

Results

Clinical and Demographic Characteristics

The sample included 306 children. Age, sex, handedness, Pb level, Fe level, ALAD$_2$ and hPEPT2*2 were characterized for all children. The mean age of the sample was 8.2 years ($\pm$1.9), and 47.9% (145 of 306) of the children were female (Table 1). With regard to ALAD$_2$, 270 of

306 children (91.2%) did not carry the polymorphism (91.9% of boys and 90.3% of girls); 27 of 306 children (8.8%) were heterozygous (8.1% of boys and 9.7% of girls); and no children were homozygous for ALAD$_2$. With regard to hPEPT2*2, 171 of 306 children (55.9%) did not carry the haplotype (55.95% of boys and 59.00% of girls); 119 of 306 children (38.9%) were heterozygous (36.6% of boys and 41.4% of girls); and 16 of 306 children (5.2%) were homozygous (4.3% of boys and 6.2% of girls).

Parents of 249 of 306 children studied (81.4%) provided demographic information (57 parents declined completion of demographics forms). The annual household income was ≤ \$20,000 for 91.4% of reporting families; the average household size was 4.94 members; and 49.9% of mothers and 44.3% of fathers had completed high school. The ethnicity of 97.3% of parents was Mexican, Mexican–American, Hispanic, or Latino, and 99.05% of families were white (Table 1).

Table 2 lists the distribution of Pb levels for 306 children. Mean Pb level for the sample was 2.75 μg/dl ($\pm$1.29) (geometric mean = 2.58 and median = 2.57). The interquartile range was 1.35. The mean Fe level for the sample was 5622.70 ng/ml ($\pm$866.13). The range of Fe levels for all children in the sample were within previously reported normal limits (Gulson et al. 2008).

Genetic Predisposition and Pb Blood Burden

Table 3 lists mean and geometric mean blood Pb levels for boys and girls by genotype. The general linear model predicting Pb from ALAD$_2$, hPEPT2*2, and sex, co-varying for Fe, was significant (model degrees of freedom [df] = 10, R^2 = 0.120, F = 4.03, $P < 0.001$). The main effects contributing to model significance, as determined from tests of type III sums of squares, included sex ($df = 1$, $F = 17.88$, $P < 0.001$) and the covariate Fe ($df = 1$, $F = 6.65$, $P = 0.010$). Significant interactions included ALAD$_2$ × sex ($df = 1$, $F = 4.15$, $P = 0.043$) and hPEPT2*2 × sex ($df = 2$, $F = 10.92$, $P < 0.001$). The hPEPT2*2 × ALAD$_2$ interaction was not significant ($df = 2$, $F = 0.18$, $P = 0.834$).

Effect statistics are listed in Table 4. With regard to the main effect of sex, Pb levels in boys were significantly higher compared with girls. A significant interaction of ALAD$_2$ × sex was found, suggesting that ALAD$_2$ exaggerated the effect of sex on Pb level. Two differences accounted for the significance of this interaction. After covarying Fe, the Pb level of ALAD$_2$ hetereozygous boys was significantly greater than the Pb level of girls without ALAD$_2$. The Pb level of heterozygous boys was also greater than the Pb level of heterozygous girls. The Pb level of heterozygous boys was also greater than the Pb

Table 1 Clinical and demographic characteristics of the sample

Clinical and demographic characteristics	Mean (SD) (range) or %		
Age (years)	8.2 (1.8)		
	(5.1–12.9)		
Female	47.9		
Right-handed	91.48		
Pb (µg/dl)	2.75 (1.29)		
	(0.93–12.94)		
Fe (ng/ml)	5622.70 (866.13)		
	(3450.00–8142.92)		
hPEPT2*2	Wild type	Heterozygous	Homozygous
	55.9	38.9	5.2
ALAD$_2$	Wild type	Heterozygous	Homozygous
	91.2	8.8	0
Annual family income			
<10 K	55.1		
10 K–20 K	36.3		
20 K–30 K	4.5		
>30 K	4.1		
Size of household	4.94 (1.53)		
	(2–10)		
Exposed to second-hand smoke in household	4.7		
Parent level of education	Mother	Father	
Completed grades 1–6	32.1	32.3	
Some high school	25.0	22.9	
High school graduate	23.4	29.7	
Attended college	13.1	8.9	
Attended graduate school	6.4	5.7	
Parent ethnicity			
African–American	0.40	1.5	
Mexican/Mexican–American	47.0	50.2	
Hispanic	50.6	44.3	
Latino	2.0	2.5	
Anglo-American	0	1.5	
Parent race			
White	99.6	98.5	
Black	00.4	01.5	

Age, sex, handedness, blood lead level (µg/dl), blood iron level (mcg/l), and ALAD$_2$ and hPEPT2*2 status were available for 306 children; other clinical and demographic data were available for 81.4% (249 of 306) of children studied; and 19% (57 of 306) of parents chose not to provide demographic information

level of boys without ALAD$_2$; however, the statistical significance of the difference was marginal.

The interaction of hPEPT2*2 × sex was also significant, suggesting that hPEPT2*2 exaggerated the effect of sex on Pb level. The Pb level of male homozygotes was greater than the other five subgroups; five paired differences accounted for the significance of the interaction. The Pb level of homozygous boys was significantly greater than that of boys without hPEPT2*2 and heterozygous boys. Also, the Pb level of homozygous boys was greater than the Pb level of girls without hPEPT2*2, heterozygous girls, and homozygous girls.

Discussion

ALAD$_2$ and hPEPT2*2 in Minority Children

Three previous studies suggested that ALAD$_2$ and/or hPEPT2*2 are associated with increased Pb blood burden in Pb-exposed children (Shen et al. 2001; Pérez-Bravo et al. 2004; Sobin et al. 2009). The current study included a unique sample of 306 minority children and improved on previous research by (1) increasing the sample size to allow for the inclusion of sex as an explanatory factor; (2) including Fe as a covariate; and (3) using ICP-MS for

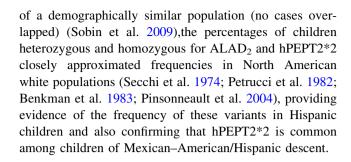**Table 2** Distribution of blood lead levels for 306 children age 5.1 to 12.9 years

Blood lead level in μg/dl ($N = 306$)	n (%)	cumulative frequency (CF) (%)	Age range (years)
10.0[a]	1 (0.32)	306 (100)	(8.1)
9.5–9.9	0		0
9.0–9.4	0		0
8.5–8.9	0		0
8.0–8.4	0		0
7.5–7.9	4 (1.30)	305 (99.6)	6.6–11.4
7.0–7.4	0		0
6.5–6.9	2 (0.65)	301 (98.3)	5.9–7.9
6.0–6.4	0		0
5.5–5.9	4 (1.30)	299 (97.7)	5.8–9.6
5.0–5.4	4 (1.30)	295 (96.4)	5.3–10.3
4.5–4.9	6 (1.96)	291 (95.0)	5.5–10.1
4.0–4.4	14 (4.57)	285 (93.1)	5.1–11.4
3.5–3.9	25 (8.17)	271 (88.5)	5.3–10.6
3.0–3.4	38 (12.41)	246 (80.3)	5.4–11.3
2.5–2.9	58 (18.95)	208 (67.9)	5.4–11.7
2.0–2.4	67 (21.89)	150 (49.0)	5.5–12.9
1.5–1.9	63 (20.58)	83 (27.1)	5.3–11.4
1.0–1.4	19 (6.20)	20 (6.5)	5.3–11.2
0.5–0.9	1 (0.32)	1 (0.3)	(9.3)

[a] 12.94 μg/dl

Table 3 Pb mean (SEM) and geometric mean by genetic predisposition (boys $n = 161$; girls $n = 145$)

	% Genotype (n)		BLL mean (SEM)	
			Geometric mean	
	Boys	Girls	Boys	Girls
PEPT2*2				
No	59 (95)	52.4 (76)	2.8 (.13)	2.8 (.12)
			2.6	2.6
Heterozygous	36.6 (59)	41.3 (60)	2.5 (.09)	2.7 (.15)
			2.4	2.5
Homozygous	4.3 (7)	6.2 (9)	4.9 (1.6)	2.0 (.17)
			3.5	2.0
ALAD₂				
No	91.9 (148)	90.3 (131)	2.7 (.11)	2.7 (.10)
			2.5	2.5
Heterozygous	8.0 (13)	9.6 (14)	3.5 (.51)	2.6 (.20)
			3.1	2.5
Homozygous	0 (0)	0 (0)	–	–

element detection (Pb and Fe). We compared Pb levels in boys and girls with and without ALAD₂ and hPEPT2*2, covarying for blood Fe. Consistent with our previous study

of a demographically similar population (no cases overlapped) (Sobin et al. 2009), the percentages of children heterozygous and homozygous for ALAD₂ and hPEPT2*2 closely approximated frequencies in North American white populations (Secchi et al. 1974; Petrucci et al. 1982; Benkman et al. 1983; Pinsonneault et al. 2004), providing evidence of the frequency of these variants in Hispanic children and also confirming that hPEPT2*2 is common among children of Mexican–American/Hispanic descent.

ALAD₂ and hPEPT2*2 May Exaggerate Pb Blood Burden in Boys

This is the first study to examine the effects of ALAD₂ and hPEPT2*2 in a single model, and it contributes three new findings to the literature. The findings are the first to suggest that the influences of ALAD₂ and hPEPT2*2 on Pb blood burden in children are independent (no apparent additive effects), that the associations are observable at lowest levels of Pb exposure, and that the effects are sexually dimorphic.

By increasing Pb blood burden, ALAD₂ and hPEPT2*2 might be expected to exaggerate the risks associated with low-level Pb exposure in male ALAD₂ heterozygotes and male hPEPT2*2 homozygotes. Results from previous animal studies have suggested that hPEPT2*2 may be a secondary genetic modifier in cases of Pb poisoning (Hu et al. 2007). The current results support the additional suggestion that the modifying effect of hPEPT2*2 on Pb blood burden in cases of low-level Pb exposure is specific to boys.

Pb molecules enter red blood cells where they are bound by δ-ALAD. Pb binding disrupts δ-ALAD function, decreases heme biosynthesis, and causes an increase in brain levels of δ-ALA. Presumably, in children with ALAD₂, Pb binding is reduced, resulting in higher Pb blood burden. Regardless of genotype, boys have higher Pb blood burden, which has been attributed to their higher hematocrit levels (Kameneva et al. 1999). The results of this study may suggest that in ALAD₂ boys, lower Pb binding results in less disruption of heme biosynthesis, increased hematocrit levels, and thus genotype specific increases in Pb blood burden. This speculation requires experimental examination.

Studies exploring the possible role of hPEPT2*2 in Pb blood burden are recent. Pb metabolism is complex and not fully understood. The current literature does not directly suggest why and how hPEPT2*2 may differentially increase Pb blood burden in boys. It may be relevant that Pb exposure has been associated with increased production of at least 100 different proteins (Witzmann et al. 1999). PEPT2 provides the main mechanism for proximal tubular reabsorption of peptide-bound amino acids (Rubio-Aliaga et al. 2003). Previously we suggested that the secondary

Table 4 Summary of associations between genotype and Pb level in 306 children

Effect	Pb least square mean (95% CL)		
Sex	Boys	Girls	Difference (95% CL)
	3.90 (3.26–4.54)	2.53 (2.03–3.02)	1.374 (0.74–2.02)***
$ALAD_2 \times$ sex	Heterozygous boys	Null girls	
	4.42 (3.25 to 5.58)	2.54 (2.21 to 2.86)	1.88 (0.62 to 3.14)**
		Heterozygous girls	
		2.52 (1.59 to 3.46)	1.89 (0.84 to 2.95)**
		Null boys	
		3.39 (3.05 to 3.73)	(1.02)†
hPEPT2*2 $\times$ sex	Homozygous boys	Null boys	
	5.62 (3.96 to 7.28)	3.26 (2.84 to 3.68)	2.37 (0.72 to 4.01)**
		Heterozygous boys	
		2.83 (2.32 to 3.33)	2.80 (1.12 to 4.48)***
		Null girls	
		2.72 (2.31 to 3.14)	2.90 (1.14 to 4.67)***
		Heterozygous girls	
		2.62 (2.08 to 3.16)	3.01 (1.20 to 4.82)**
		Homozygous girls	
		2.24 (0.96 to 3.53)	3.38 (2.03 to 4.73)***

† $P = 0.08$, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

effects of lowered protein reabsorption in children with hPEPT2*2 may partially account for their increased Pb blood burden (Sobin et al. 2009). Boys and girls differ with regard to not only hemoglobin density but also blood viscosity and flow rate. Thus, similar to $ALAD_2$ but by way of other mechanisms, it may be logical to suggest that hPEPT2*2 differentially lowers protein reabsorption rates in boys, thus decreasing Pb clearance. Studies are needed to examine these possibilities.

Limitations

The primary risk associated with low-level Pb exposure is neurodevelopmental disruption as indicated by diminished neurocognitive function at the time of exposure and beyond (Lanphear et al. 2000; Chiodo et al. 2004, 2007; Gilbert and Weiss 2006; Min et al. 2007; Surkan et al. 2007); longer term effects include adult-onset renal (Fadrowski et al. 2010) and cardiovascular dysfunction (Park et al. 2006). Whether the increased Pb blood burden observed in children with $ALAD_2$ and hPEPT2*2 also proves to be associated with previously identified neurobehavioral and physical outcomes has yet to be examined. Consistent with published population rates, the proportions of $ALAD_2$ heterozygotes and hPEPT2*2 homozygotes were small, and no cases of children homozygous for $ALAD_2$ were identified in this sample. Because of random variation, small subgroup size usually results in no significant differences. It may be noteworthy that large differences were identified despite small subgroup sizes. Future studies could select children by genotype to ensure more balanced subgroups.

Conclusion

$ALAD_2$ and hPEPT2*2 mediate Pb blood burden in young boys at levels of exposure previously associated with diminished neurocognitive function. The effects of these genetic mediators appear to be sexually dimorphic. $ALAD_2$ and hPEPT2*2 may provide novel biomarkers of increased health risk in young boys exposed to low-level Pb. Eventually, screening for these genetic variants could be used to identify a subgroup of young boys for early neurocognitive intervention and health monitoring.

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