

Low Dose Effect of Chronic Lead Exposure on Neuromotor Response Impairment in Children is Moderated by Genetic Polymorphisms

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ABSTRACT Previous research on children of the Cincinnati Lead Program Project (CCLP) showed a strong correlation of blood lead level with postural balance impairment. Here we investigated whether this association is dependent on genetic polymorphisms that are implicated with lead metabolism and/or neuromotor disorders, suggesting the role of gene-environment interaction in neurotoxicity of lead exposure in early life of children. Genotyping was done for 10 polymorphic sites on 83 children from the CLPP cohort, on whom postural balance measurements and average blood lead levels (PbB05) were available. Analysis of variance and regression analysis were performed to examine genotype-dependency on lead and postural balance. Heterogeneity tests of lead-postural balance regression coefficients were done to examine genotype dependency of lead-balance association. Two loci, Vitamin D Receptor (VDR) and Dopamine Receptor D3 (DRD3), showed suggestive evidence of genotype dependency of toxicokinetics of lead. Regression coefficients of PbB05 on postural sway area (s_A) under all test conditions were significantly heterogeneous for at least one or more of these genes. The three-way link between PbB05, postural sway, and genotypes suggested that at least three genes, Dopamine Receptor D2 (DRD2-A), Vitamin D Receptor (VDR), and N-Acetyltransferase 2 (NAT2), may be involved in moderating the detrimental effect of lead exposure on postural balance response. These observations provide preliminary evidence that toxicokinetic effect of lead on neuromotor response may be moderated by genotypes at several genes.

ABBREVIATIONS USED

ALAD - δ -Aminolevulinic acid dehydratase; **BMI** - Body mass index; **CCLP** - Cincinnati Lead Program Project; **DRD2, DRD3, DRD4** - Dopamine receptor-2, 3, and 4; **EC** - Eyes closed on no-foam platform; **EO** - Eyes closed on no-foam platform; **FC** - Eyes closed on foam platform; **FO** - Eyes open on foam platform; **GSTM** - Glutathione S-transferase mu gene; **HOME** - Home observation of environment; **MAO-A, MAO-B** - Monoamine Oxidase-A and B; **NAT2** - N-acetyltransferase 2 gene; **PbB** - Blood lead level; **PbB05** - Average blood lead level during first 5 years of life; **PIQ** - Performance Intelligence Quotient; **SES** - Socio-economic status; **VDR** - Vitamin D receptor;

INTRODUCTION

Lead has been known as a potent neurotoxicant in humans since ancient times. However, some of the most severe pernicious consequences of exposure to this metal have been described during the last three decades

(Mercetti, 2003). Exposure to even small amounts of this metal during the early stage of development of infants has been shown to be associated with impaired growth in stature (Shukla et al., 1989), deficiency of cognitive development (Bellinger et al., 1991; Dietrich et al., 1991), learning disabilities (Ris et al., 2004), and reduced IQ scores (Baghurst et al., 1992; Canfield et al., 2003) in young children. Elevated levels of lead at any age induce degeneration of cells in the peripheral and central nervous system, leading to loss of neuromuscular coordination and motor control (Schwartz et al., 2005). The Cincinnati Lead Program Project (CLPP), a study initiated in late 1970s in the inner

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Cincinnati neighborhoods to examine the effects of chronic lead exposure on health, behavior, and growth of children, evaluated the effects of chronic early life lead exposure on children's health, neurobiological, and cognitive impairments (Bhattacharya et al., 1995; Bornschein et al., 1985; Dietrich et al., 1987, 1993a, 1993b). One of the key observations from this cohort of subjects is that the ability to maintain upright postural balance in six-year old children is significantly associated with an increased blood lead level, independent of the socio-economic, racial and environmental factors (Bhattacharya et al., 1995).

Studies done on 3- and 6-month old children of the CLPP cohort demonstrated that the inverse relationship between both prenatal and neonatal blood lead levels and neurobehavioral deficits (as measured by Bayley Mental Developmental Index) are partly mediated by lead-related reductions in birth weight and gestation (Dietrich et al., 1987). Further follow-up data on these children (approximately 6.5 yrs old) showed a negative relationship of blood lead levels on the performance IQ (PIQ), to the extent that a 7 point deficit of PIQ was detected in children having an average lifetime blood lead level in excess of 20 μ g/dL (Dietrich et al., 1993a). As the CLPP cohort, established in late 1970s, reached adolescence and adulthood, focus of CLPP studies has now shifted to adverse effects of early childhood lead exposure on neuropsychological deficits and delinquent behavior (Dietrich et al., 2001), and maturation of control of neuromotor functions (Bhattacharya et al., 2006). Nonetheless, observations from other studies suggest that the effects of lead exposure on neuropsychological deficit, as well as neuromotor imbalance may be mediated by genetic factors as well (Chia et al., 2004, 2006; Kelada et al., 2001; Lidsky and Schneider, 2003; Mercetti, 2003; Stroombergen and Waring, 1999).

As of now such suggestions are not substantiated by direct empirical data, since the neurobiological end-points are either measured late in life, lead exposure in affected individuals have not been objectively measured, or genetic factors are not specifically evaluated in such subjects. The CLPP cohort provides opportunity to circumvent these limitations, since this longitudinal study provides serial data on lead levels in blood at several stages of the children's lifetime (including prenatal and maternal data), and several neurobiological parameters have

been measured on them at several time points of their life. In particular, the postural upright balance measurements taken on the children of the CLPP cohort, which had been established as indicators of these subjects' functional health status of the central and peripheral nervous system (see Bhattacharya et al., 1995 and its cited references), can be argued to be legitimate biomarkers for lead-induced modifications of neuromotor systems.

Though direct evidence of influence of genetic polymorphisms on postural balance is still lacking, several studies reported association of specific polymorphisms with neuromotor disorders (such as Parkinson's disease), neuropsychological impairment, and other neurological diseases. For example, Chaudhary et al. (2005) showed that specific single nucleotide polymorphisms (SNPs) and SNP-haplotypes at the N-acetyl transferase 2 (NAT2) gene are associated with young onset Parkinson's disease in Indian patients. Likewise, Fujiwara et al. (1997) showed involvement of Dopamine receptors 2, 3, and 4 (DRD2, DRD3, DRD4) genes with schizophrenia, mood disorder, and neurological diseases. Evidence of statistically significant risk modification for neuropsychological impairment (and reduction of grip strength) depending on genotypes at the NAT2 and Glutathione S-transferase mu 1 (GSTM1) genes has also been reported by Dick et al. (2002). Evidences are also accumulating suggesting that one or more genes, acting singly or in interaction with each other, may modify the toxicokinetics of lead absorption. Polymorphism at the δ -Aminolevulinic Acid Dehydratase (ALAD) gene has been shown to be associated with increased blood lead level and increased risk of lead toxicity by generating a protein that binds lead more tightly than the protein coded by the wild type allele (Kelada et al., 2001). There are suggestions that variant Vitamin D Receptor (VDR) alleles may modify lead concentration either by influencing lead content or calcium content, or both (Schwartz et al., 2000).

From such data, one may hypothesize that certain specific genotypes, singly, or acting in combination with each other, may differentially affect the neurobiological impairment in children induced by lead exposure. This study is an attempt to test this hypothesis of genotype-dependent neurotoxic effect lead exposure with genotyping performed for 10 polymorphic sites

(DRD2-A, DRD2-B, DRD2-D, DRD3, VDR, GSTM, NAT2-*KpnI*, NAT2-*TaqI*, NAT2-*DdeI*, and NAT2-*BamHI*) on the children of the CLPP cohort on whom blood lead level data and postural balance measurements were available.

More specifically, with DNA extracted from frozen and fresh blood samples of 83 children from the CLPP cohort, we obtained genotype data at each of the 10 polymorphic sites mentioned above. These were used to investigate whether any of these genetic loci has any direct influence of blood lead level (designated as PbB05, measured by the average blood lead level between birth and first 5 years of life of the children) and/or postural balance. This was done by conducting association analyses of the genotypes at each locus with each subject's average blood lead levels and postural balance measurements (adjusting for age, height, and body weight effects). Second, we investigated whether genotypic variability at any of these loci influences the extent of the positive association of blood lead level and impairment of postural balance by testing the significance of genotype-dependent differences of the regression coefficient of PbB05 on postural sway. In view of these objectives, this is the first study investigating whether or not any gene loci moderate the relationship of blood lead level and postural sway in young children.

MATERIALS AND METHODS

Subjects, Lead Exposure, and Postural Balance Measurements: The CCLP cohort of children consists of over 202 children from inner city of Cincinnati with history of prenatal and childhood lead exposure. The study design of recruitment and follow-up of postural balance measurements, along with descriptive statistics of major covariates are documented in the earlier publications of findings from the CCLP project (see e.g., Bhattacharya et al., 1995). Inclusion criteria for the present analyses included children having lead and postural balance measurements who could be genotyped at the ten loci studied. Thus, the characteristics of the children from the CCLP cohort that are of specific relevance for the present analyses are: (i) children on whom postural sway measurements were made at least once; (ii) for each child the outcome variables (i.e., postural balance) consist of sway area, s_A ; and sway length, s_L (both analyzed in logarithmic

scale), measured in four conditions (eyes open, EO; and eyes closed, EC – each taken with subjects standing on a no-foam platform; and eyes open, FO; and eyes closed, FC – both taken with subjects standing on a 3 inch foam placed on the platform). The rationale and details for these multi-dimensional sway measurements have been described before (Bhattacharya et al., 1995), which indicate that postural balance involves visual, proprioceptive, and vestibular systems of physiological pathways of upright balance. The four norms of measurements of s_A and s_L were validated with a smaller subset of CLPP cohorts and adults in other independent studies before (Bhattacharya et al., 1988, Sack et al., 1993); (iii) blood lead levels (PbB) were measured for mothers (maternal PbB), and maximum PbB levels for each of the first 5 years of the children (MaxPbB1, MaxPbB2, MaxPbB3, MaxPbB4, and MaxPbB5, respectively) as well as their childhood average (average of the above five yearly maximum measurements for each child, designated as PbB05); (iv) other covariates in the study include age, sex, birth weight and length, together with height, body mass index, and foot length/width data taken at the time of postural balance measurements. Also socio-economic measurement (SES), race, home observation of environment (HOME), maternal IQ data were obtained by specially designed sets of observations for the CLPP study (Bornschein et al., 1985; Dietrich et al., 1987, 1991, 1993a,b; Shukla et al., 1989, Bhattacharya et al., 1995; and Ris et al., 2004). (v) Finally over 88% of the children for whom postural balance measurements were made were African-American. Previous results on the lead-sway relationship showed that the association of lead exposure (measured by PbB05) is independent of the socio-economic, racial and environmental factors (Bhattacharya et al., 1995). Therefore, the principal explanatory variable for the present analyses was PbB05, with the dependent variables being postural sway length (s_L) and area (s_A), both measured in four testing conditions (EO, EC, FO, and FC, as mentioned above), and the analyses were done by categorizing the 83 children by their genotypes at each of the 10 polymorphic sites examined.

Genotyping Methods: DNA was extracted from frozen as well as fresh blood samples from the CLPP study cohort using the Puregene kit from Genetra Systems. Genotyping was performed using

published PCR-based protocols. At the DRD2 locus (located on the chromosomal region 11q23), three bi-allelic TaqI restriction fragment length polymorphisms (RFLPs), TaqI 'A', 'B', and 'D' sites, were typed following protocols described previously (Grandy et al., 1989, Hauge et al., 1991, Parsian et al., 1991). For the DRD3 locus (located on chromosome 3q13.3), the point mutation Ser9Gly, causing a serine (Ser) to glycine (Gly) amino acid substitution in the N-terminal extracellular part of the dopamine D3 receptor results in the creation of a BallI restriction enzyme site. This was typed by using PCR-amplification primers and restriction digestion assays as described in Scharfetter et al. (1999). Vitamin D receptor (VDR) polymorphism (located on chromosome 12q13.11) was recognized by the restriction site polymorphism of TaqI (leading to two alleles T and t), by PCR-amplification and electrophoresis methods of TaqI digested products as described in Fassbender et al. (2002). The glutathione S-transferase M (GSTM) polymorphism (located on chromosome 1p13) is characterized by the presence (+), or absence (-) of a PCR-product of a GSTM1-specific primer, viewed from an agarose gel electrophoresis assay stained with ethidium bromide, as described in Nomura et al. (2000). Four single nucleotide polymorphic sites at the arylamine N-acetyltransferase-2 (NAT2) gene (NAT2-*KpnI*, NAT2-*TaqI*, NAT2-*DdeI*, and NAT2-*BamHI*) were assayed by the method as described in Zielnska et al. (1998).

Statistical Analysis: In terms of studying the three-way link between postural balance, blood lead level, and genotypes of these children, the variables for data analyzed in this study were of three types. The blood lead level, measured by average blood lead level during the first five years of the children's life (PbB05 in units of $\mu\text{g}/\text{dL}$), as used in previous studies (Bhattacharya et al., 1995); and the postural balance measurements, postural sway length (s_L , in cm) and postural sway area (s_A , in cm^2), both measured in four testing conditions (EO, EC, FO, and FC) as described earlier, are quantitative variables. In contrast, the genotypes are all categorical, in which except for GSTM (which detected only the presence or absence of a relevant gene fragment), the other nine represented three classes (genotypes) each, explained by codominant bi-allelic systems. As done in an earlier study (Bhattacharya et al., 1995), we used natural logarithmic transformed values

of PbB05, s_A , and s_L measurements, and regressed out the effects of age, and body mass index ($\text{BMI} = \text{Wt}/\text{Ht}^2$, with Wt, weight in kg, and Ht, height in meter) of each child at the time of postural balance measurement on s_L and s_A before examining the three-way relationships between blood lead level, postural balance, and genotypes.

Relationships between genotypes and (log transformed) blood lead level (PbB05), between genotypes and (log transformed, adjusted for age and BMI) postural balance response, and evaluations of gene x PbB05 interaction effects on postural balance were studied by analysis of variance, with single polymorphic site at a time. Regression analyses of PbB05 on postural balance (both transformed and adjusted for covariates) were conducted within each genotype of every genetic polymorphism to examine heterogeneity of regression effects (i.e., to examine more explicitly the gene \times PbB05 interaction effects on postural balance). All analyses were done using the SAS routines (SAS release 6.03 of 1995). Since the sample size for children with genotype data is almost one-half of that of the earlier study ($n = 162$ in Bhattacharya et al., 1995), its representativeness of the CLPP cohort of children is examined by computing the descriptive statistics of the ages of the children (at the time of postural balance measurements, mean = 6.11 yrs, SD = 1.05 yrs.), PbB05 levels (geometric mean = 11.98, geometric SD = 1.46), and correlation of postural balance measurements with PbB05 levels (correlation of $\log s_A$ with $\log \text{PbB05}$ being 0.12, 0.19, 0.14, and 0.15 under the testing conditions of EO, EC, FO, and FC; and that of $\log s_L$ with $\log \text{PbB05}$ being 0.35, 0.29, 0.34, and 0.32 under the same testing conditions, respectively). With respect to each of these summary statistics, the earlier sample ($n = 162$) is well represented by the 83 children included in this study (see for example data on Tables II through IV of Bhattacharya et al., 1995 for similar data on the larger sample. Thus, this study represents the first effort to examine the influence of genetic variation on the association of blood lead level on postural balance, an objective biomarker for neuromotor function impairment in the CCLP cohort of children.

RESULTS

Table 1 presents the mean and s.d. of PbB05 (log transformed), s_L and s_A (both log transformed

Table 1: Average (± s.e.) of Blood lead levels (PbB05) and Postural sway measurements (s_L and s_A) under four testing conditions by genotype

Locus/Genotype	Sample size	Postural Sway Length (s _L)				Postural Sway Area (s _A)				
		EO	EC	FO	FC	EO	EC	FO	FC	
DRD2-	A:A ₁ A ₁	11	2.62 ± 0.08	0.26 ± 0.30	0.29 ± 0.30	0.20 ± 0.30	-0.48 ± 0.29	-0.44 ± 0.30	-0.27 ± 0.30	-0.30 ± 0.30
	A ₁ A ₂	55	2.45 ± 0.05	-0.10 ± 0.13	-0.11 ± 0.13	-0.10 ± 0.13	-0.02 ± 0.13	-0.03 ± 0.13	-0.08 ± 0.13	-0.03 ± 0.13
	A ₂ A ₂	15	2.50 ± 0.10	0.17 ± 0.26	0.19 ± 0.26	0.24 ± 0.26	0.32 ± 0.25	0.32 ± 0.25	0.36 ± 0.25	0.29 ± 0.25
DRD2-B:	++	57	2.51 ± 0.05	-0.03 ± 0.13	-0.02 ± 0.13	-0.00 ± 0.13	0.03 ± 0.13	0.03 ± 0.13	0.05 ± 0.13	0.05 ± 0.13
	+-	20	2.38 ± 0.09	0.11 ± 0.22	0.08 ± 0.22	0.03 ± 0.23	-0.02 ± 0.22	-0.05 ± 0.23	-0.11 ± 0.22	-0.09 ± 0.23
	--	3	2.71 ± 0.24	-0.51 ± 0.57	-0.54 ± 0.58	-0.54 ± 0.58	-0.22 ± 0.56	-0.21 ± 0.59	0.26 ± 0.58	-0.04 ± 0.59
DRD2-D:	++	51	2.45 ± 0.06	0.06 ± 0.14	0.07 ± 0.14	0.12 ± 0.14	-0.02 ± 0.14	0.05 ± 0.14	0.01 ± 0.14	0.11 ± 0.14
	+-	29	2.51 ± 0.06	-0.05 ± 0.18	-0.06 ± 0.18	-0.14 ± 0.18	0.03 ± 0.19	-0.05 ± 0.19	-0.04 ± 0.19	-0.18 ± 0.18
	--	3	2.70 ± 0.31	-0.58 ± 0.57	-0.69 ± 0.57	-0.61 ± 0.57	0.10 ± 0.58	-0.29 ± 0.58	0.19 ± 0.58	-0.11 ± 0.58
DRD3:	Gly/Gly	45	2.50 ± 0.05	-0.07 ± 0.15	-0.05 ± 0.15	-0.04 ± 0.14	-0.13 ± 0.15	-0.05 ± 0.15	-0.02 ± 0.15	0.02 ± 0.15
	Gly/Ser	32	2.43 ± 0.07	0.02 ± 0.17	0.01 ± 0.17	0.00 ± 0.17	0.11 ± 0.18	0.07 ± 0.18	-0.06 ± 0.18	-0.03 ± 0.18
	Ser/Ser	4	2.71 ± 0.13	0.70 ± 0.49	0.64 ± 0.49	0.60 ± 0.49	0.17 ± 0.50	-0.01 ± 0.51	0.24 ± 0.50	-0.18 ± 0.50
VDR:	++	40	2.41 ± 0.07	-0.16 ± 0.15	-0.17 ± 0.15	-0.13 ± 0.16	0.03 ± 0.15	-0.05 ± 0.16	-0.04 ± 0.16	-0.02 ± 0.16
	Tt	34	2.54 ± 0.06	0.24 ± 0.17	0.24 ± 0.16	0.17 ± 0.17	0.17 ± 0.17	0.20 ± 0.17	0.04 ± 0.17	0.08 ± 0.17
	TT	8	2.60 ± 0.11	0.00 ± 0.34	0.03 ± 0.34	0.13 ± 0.34	-0.75 ± 0.34	-0.50 ± 0.35	0.00 ± 0.36	-0.30 ± 0.36
GSTM:	+	65	2.51 ± 0.05	0.01 ± 0.12	0.01 ± 0.12	0.03 ± 0.12	-0.07 ± 0.12	-0.05 ± 0.12	-0.07 ± 0.12	-0.02 ± 0.12
	-	18	2.39 ± 0.09	-0.02 ± 0.23	-0.02 ± 0.23	-0.10 ± 0.12	0.26 ± 0.23	0.17 ± 0.24	0.26 ± 0.23	0.07 ± 0.24
NAT2-KpnI:	CC	30	2.42 ± 0.07	-0.03 ± 0.18	-0.05 ± 0.18	-0.05 ± 0.18	-0.15 ± 0.18	-0.13 ± 0.18	-0.03 ± 0.18	-0.18 ± 0.18
	CT	43	2.56 ± 0.06	0.01 ± 0.15	0.10 ± 0.15	0.15 ± 0.15	0.02 ± 0.15	-0.02 ± 0.15	0.06 ± 0.15	0.18 ± 0.15
	TT	8	2.37 ± 0.09	-0.14 ± 0.35	-0.11 ± 0.35	-0.20 ± 0.35	0.31 ± 0.35	0.44 ± 0.35	-0.22 ± 0.35	-0.28 ± 0.34
NAT2-TaqI:	AG	34	2.57 ± 0.07	0.05 ± 0.17	0.03 ± 0.17	0.02 ± 0.17	0.01 ± 0.17	-0.18 ± 0.17	-0.10 ± 0.17	-0.23 ± 0.17
	CG	49	2.43 ± 0.05	-0.04 ± 0.14	-0.02 ± 0.14	0.01 ± 0.14	-0.01 ± 0.14	0.13 ± 0.14	0.07 ± 0.14	0.16 ± 0.14
NAT2-DdeI:	AA	21	2.44 ± 0.09	-0.05 ± 0.22	-0.05 ± 0.21	-0.14 ± 0.21	-0.08 ± 0.22	-0.03 ± 0.22	-0.13 ± 0.22	-0.26 ± 0.22
	AG	42	2.51 ± 0.06	-0.03 ± 0.15	-0.06 ± 0.15	-0.03 ± 0.15	0.14 ± 0.15	-0.11 ± 0.16	0.15 ± 0.15	0.10 ± 0.15
	CG	19	2.49 ± 0.06	0.19 ± 0.23	0.27 ± 0.22	0.20 ± 0.17	-0.26 ± 0.23	0.28 ± 0.23	-0.20 ± 0.23	0.10 ± 0.23
NAT2-BamHI:	AG	4	2.34 ± 0.33	-0.69 ± 0.49	-0.70 ± 0.49	-0.62 ± 0.49	-0.47 ± 0.49	-0.13 ± 0.50	-0.45 ± 0.49	0.27 ± 0.50
	CG	79	2.49 ± 0.04	0.03 ± 0.11	0.04 ± 0.11	0.03 ± 0.11	0.02 ± 0.11	0.01 ± 0.11	0.02 ± 0.11	-0.01 ± 0.11

Note: The blood lead level (PbB05) values are log transformed, and the postural sway length (s_L) and area (s_A) values are standardized residuals of their respective log transformed values regressing out the effects of subjects' age and body mass index (BMI). The four testing conditions (EO, EC, FO, and FC) are as explained in the text.

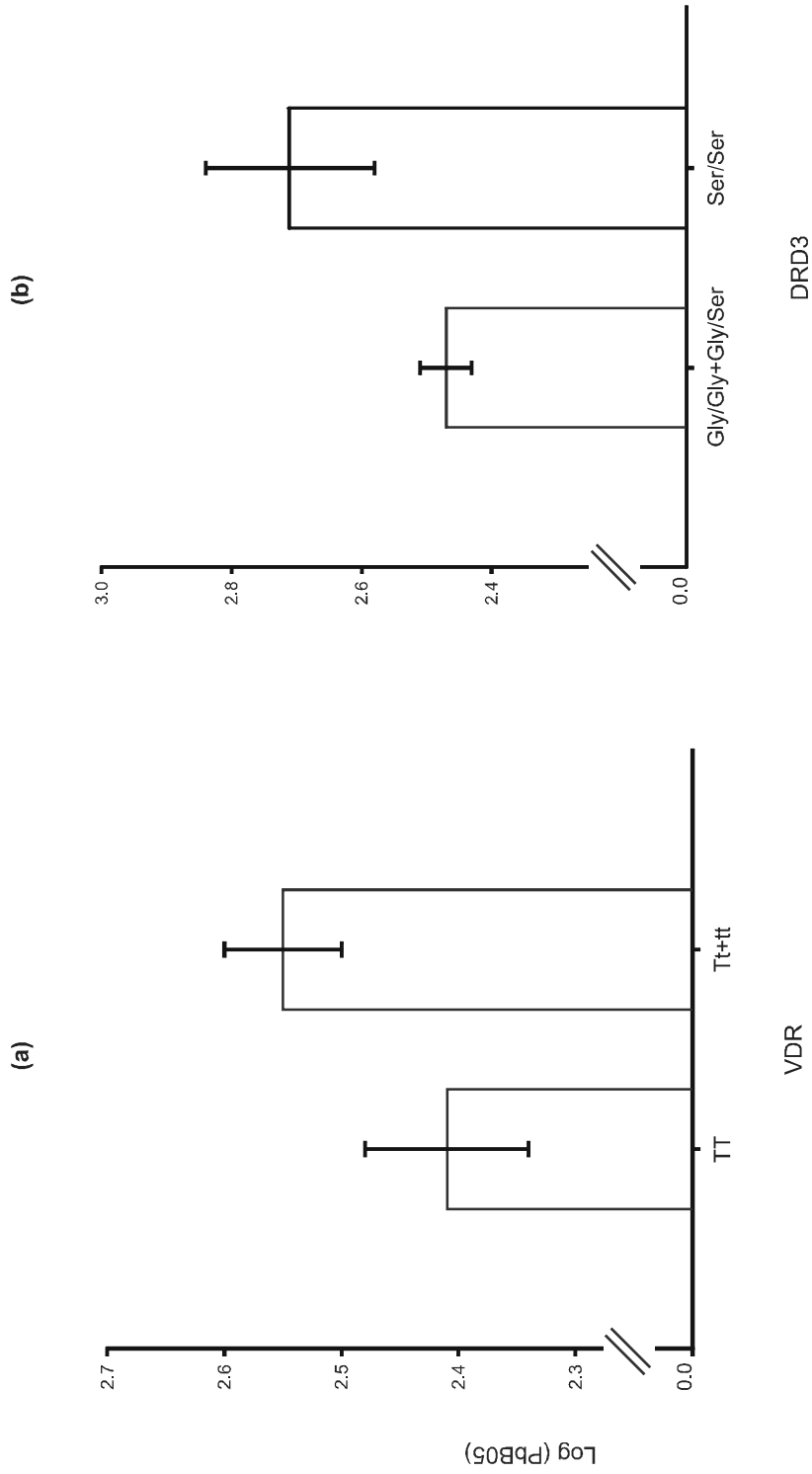


Fig. 1. Genotype-specific values of logarithmic PbB05 with their standard error bars for two loci. Panel (a) for VDR, and Panel (b) is for DRD3.

Table 2: Levels of Significance for Test of PbB05 × Genotype Interaction Effects on logarithmic transformed Postural Sway Measures (s_L and s_A) adjusted for age and BMI

Gene Locus	Postural Sway Length (s_L)				Postural Sway Area (s_A)			
	EO	EC	FO	FC	EO	EC	FO	FC
DRD2-A	0.264	0.331	0.273	0.194	0.018	0.160	0.088	0.047
DRD2-B	0.381	0.317	0.413	0.271	0.017	0.009	0.193	0.039
DRD2-D	0.838	0.696	0.757	0.580	0.676	0.193	0.719	0.278
DRD3	0.329	0.341	0.292	0.070	0.534	0.155	0.755	0.842
VDR	0.166	0.201	0.136	0.058	0.874	0.420	0.838	0.046
GSTM	0.878	0.977	0.809	0.873	0.860	0.993	0.351	0.640
NAT2- <i>KpnI</i>	0.346	0.452	0.566	0.363	0.761	0.857	0.120	0.688
NAT2- <i>TaqI</i>	0.862	0.828	0.859	0.875	0.013	0.539	0.034	0.743
NAT2- <i>DdeI</i>	0.535	0.407	0.484	0.420	0.991	0.850	0.980	0.966
NAT2- <i>BamHI</i>	0.588	0.688	0.579	0.458	0.591	0.957	0.566	0.343

and age, BMI effects removed) measurements (under all four testing conditions) by genotypes at each of the ten loci studied. These data and their ANOVA (results of which are not shown in details) indicate that genotypes at none of these ten loci appear to have any direct effect on lead exposure or postural balance. However, for the VDR locus, individuals carrying the t allele (i.e., genotypes Tt and tt, with the presence of the *TaqI* restriction site) appear to have a higher (logarithmic) PbB05 mean (2.55 ± 0.05), compared to that (2.41 ± 0.07) in individuals who are homozygous with the *TaqI* restriction site absent (i.e., genotype TT). Likewise, for the DRD3 locus, individuals homozygous for the serine (Ser) amino acid substitution (i.e., genotype Ser/Ser) appear to have a higher (logarithmic) PbB05 level (2.71 ± 0.13), compared to that (2.47 ± 0.04) of other genotypes (Gly/Gly or Gly/Ser). However, these differences do not appear to be significant (student's t values with unequal variances within genotypes being 1.61 and 1.79, respectively for the VDR and DRD3 loci, yielding one-sided p-values approximately 0.07 for both), because of the small number of subjects of genotype tt and Ser/Ser. Genotype dependency of blood lead level for these two loci are shown in Figure 1 (VDR in panel a, and DRD3 in panel b). In contrast, the postural sway response (either s_L or s_A), with logarithmic transformation and age, BMI effect removed, did not show any genotype dependency for any of the ten loci examined.

With log PbB05 and genotypes taken simultaneously as predictor variables, ANOVA of postural sway measures (s_L and s_A), adjusted for age and BMI showed significant PbB05 × genotype interaction effects for eight of the total 80 analyses (10 loci, each with 8 dependent postural sway variables). The p-values of this

interaction effect tests are shown in Table 2. All significant PbB05 × genotype interaction effects on postural sway (s_A measured under EC test condition for the DRD2-B locus, s_A measured under EO test condition for DRD2-A, DRD2-B and Nat2-T loci, s_A measured under FO test condition for the NAT2-*TaqI* site, and s_A measured under FC test condition for DRD2-A, DRD2-B, and VDR loci), however, occurred for the sway area (s_A), in all four test conditions (p-values shown in bold italics in Table 2).

As these significant interaction effects imply that the regression effects of PbB05 on postural sway measures differ by genotypes, we explicitly studied the genotype-dependent heterogeneity of PbB05- s_A regression coefficients for all gene loci. Figure 2 shows the results of these analyses. The linear regression coefficients of PbB05 (log transformed) on postural sway area (s_A , log transformed and age, BMI effect adjusted for) were significantly heterogeneous in the above eight cases, showing that there are genotypes at four loci (DRD2-A, DRD2-B, VDR, and NAT2-*TaqI*) for which PbB05 effect of postural sway vary by genotypes. For children of some genotypes, the exposure to lead appears to be more toxic, while for other genotypes the effect is comparatively milder.

In summation, the genotype dependency of toxicokinetics of lead is suggested from the analysis of genotype-lead relationship for two loci (VDR, and possibly DRD3), though they do not reach the traditional level of significance (5%), probably due to small sample sizes for certain genotypes (tt and Ser/Ser, respectively). Furthermore, the analyses of the three-way link between PbB05, postural sway, and genotypes suggest that genotypes at four sites (DRD2-A,

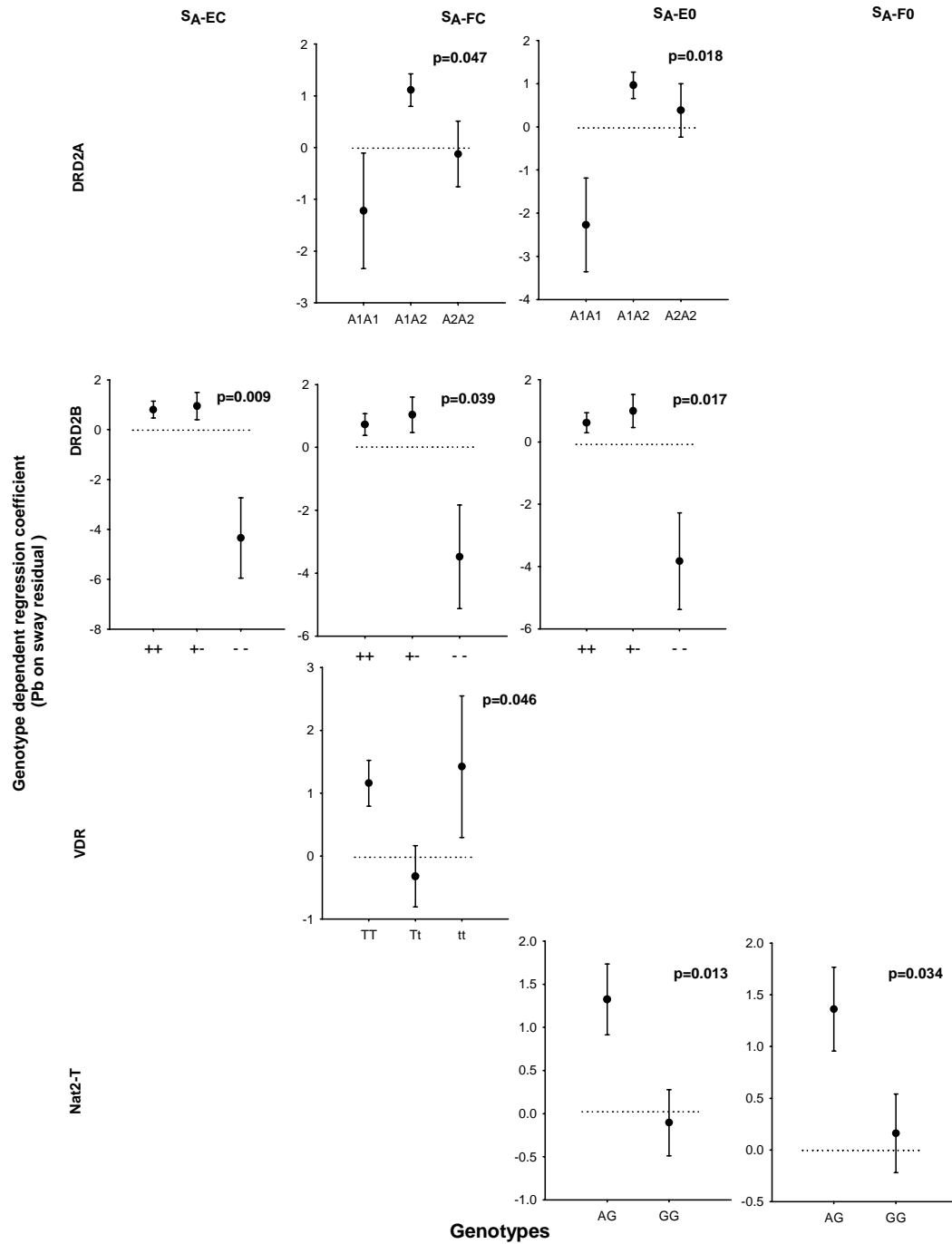


Fig. 2. Genotype-dependent variation of the regression of logarithmic PbB05 on postural sway area (S_A , log transformed and adjusted for age and BMI) under different test conditions for four genetic loci (DRD2-A, DRD2-B, VDR, and NAT2-TaqI).

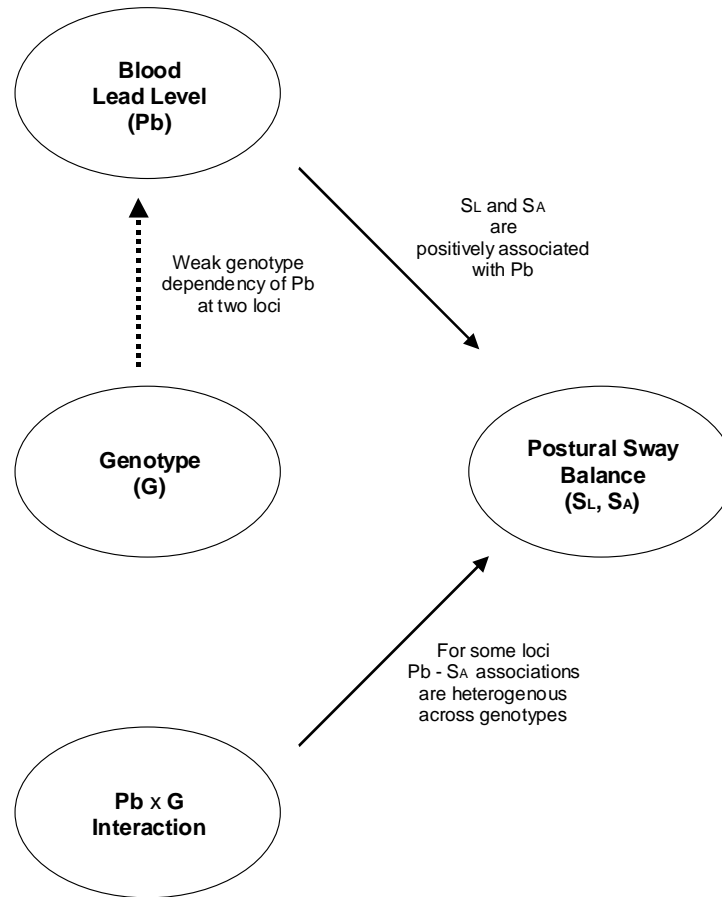


Fig. 3. The model of moderating effects of genotypes on the association of blood lead level on postural sway area.

DRD2-B, VDR, and NAT2-*TaqI*), apparently moderate the detrimental effect of lead exposure on postural balance response, as the regression coefficients of PbB05 on postural sway area (s_A) under all four test conditions (EO, EC, FO, and FC) are significantly heterogeneous at least for one or more of these genes.

DISCUSSION

As mentioned in the introduction, earlier findings from the CLPP cohort of children showed that the early life exposure to lead (as measured by average blood lead level during the first five years of life of the children, PbB05) has a strong detrimental effect on postural balance

response, an objective biomarker of neuromotor response (Bhattacharya et al., 1995). The positive association of PbB05 with both s_L and s_A suggest that lead exposure may influence modifications of functional integration of visual, vestibular, as well as proprioceptive systems.

Since our genotype data are from a subsample of children included in the earlier study (13), we re-examined the descriptive statistics of PbB05 and postural balance (s_A and s_L , at all four test conditions) and correlations among them. This was done to examine if the genotyped children ($n = 83$) produced any systematic bias in showing the detrimental effect of lead exposure (PbB05) on neuromotor response (as measured by s_A and s_L , at all four test conditions). We found no such

systematic bias, since the associations of PbB05 on s_A and s_L , at all four test conditions were almost identical to the values reported earlier (Bhattacharya et al., 1995).

There are two novel findings in this study. First, we found a mild genotype dependency of lead toxicokinetics. The vitamin D receptor (VDR) genotype dependency, found in this study (Fig. 1), is consistent with the results of Schwartz et al. (2000), conducted in adult Koreans with occupational lead exposure. These authors, however, studied another polymorphic site of the VDR gene, the *BsmI* polymorphism, located in the intron separating exons VIII and IX of the gene (Husmyer et al., 1993), while our genotypes refer to the *TaqI* polymorphism, which in due to a single nucleotide substitution T to C, leading to a synonymous change at codon 352 (isoleucine) in exon IX (30). In spite of this difference, our result, namely, PbB05 level is higher in subjects carrying the presence of *TaqI* restriction site (t allele) is consistent with the result of Schwartz et al. (2000), i.e., subjects with VDR B allele (i.e., absence of the *BsmI* restriction site) has higher tibia lead levels. This is so, because, absence of the *BsmI* site (B allele) is at strong positive linkage disequilibrium with the presence of *TaqI* restriction site at the VDR gene locus, and hence, the t allele studied in our study is an appropriate surrogate of the B allele studied by Schwartz et al. (2000).

Second, our finding that the positive association of PbB05 and postural sway area measurements (s_A , which reflect the role of vestibular systems on neuromotor response) vary in magnitude by genotypes at four sites (DRD2-A, DRD2-B, VDR, and NAT2-*TaqI*; as shown in Fig. 2) loci is even more intriguing, suggesting the evidence of gene \times PbB05 interaction effect on postural balance response. Admittedly, for the DRD2-B locus the heterogeneity of PbB05- s_A regression coefficients across genotypes appears to be due to disproportionately smaller values of the regression coefficients at the -/- genotype, which is the rarest (3 out of 80 children genotyped at this locus). However, for the other three sites (DRD2-A, VDR, and NAT2-*TaqI*), the genotype-dependent variation of the regression coefficients appear to be real (Fig. 2). For the DRD2-A locus, children with genotype A1A2 are more prone to postural balance impairment under lead exposure (regression coefficient 1.11 ± 0.31 ,

$p = 0.001$ for s_A under the FC test condition, and 0.96 ± 0.31 , $p = 0.002$ for s_A under EO test condition), compared with those of either homozygotes (A1A1 and A2A2). Likewise, homozygote TT children of the VDR locus showed significantly higher regression coefficient of PbB05 on postural sway area measured with FC test condition (1.16 ± 0.36 , $p = 0.002$), and so is the case with the children of genotype AG at the NAT2-*TaqI* site for two other test conditions (EO, and FO), with regression coefficients 1.32 ± 0.41 ($p = 0.002$) and 1.36 ± 0.41 ($p = 0.001$), respectively. The susceptible genotypes (i.e., genotypes for which the adverse effect of blood lead level on postural balance is more severe) comprise about 41% (for the NAT2-*TaqI* site) to 68% (for the DRD2-A locus) of all children for whom genotype data exists (Table 1). This genotype dependency of lead-postural balance relationship does not totally correspond to the genotypes that are associated with blood lead level (VDR and DRD3), per se (Figure 1). Furthermore, since no genotype appears to have any influence on postural balance directly (Table 3), according to Baron and Kenny's (1986) definition, we characterize our observed interaction effect as moderating effects of genes on lead-balance relationship. Figure 3 depicts this moderating interaction effect.

In summary, our data shows that early life lead exposure associated detrimental effect on neuromotor function is accentuated in children of certain specific genotypes than others. This, in combination with the observation that neurotoxic effects of lead exposure is evident even with blood lead level exceeding $8.8 \mu\text{g/dL}$, suggests that avoidance of even a smaller magnitude of lead exposure would be more critical for these children with genotypes that appear to enhance the detrimental effect of lead toxicity. More importantly, our study shows that the prevalence of genotypes for which lead neurotoxicity appears to be more severe is quite substantial (41% to 68%), suggesting a more urgent need of eliminating lead exposure in early childhood life.

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