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SCIENCE DIRECT
Environmental Research 102 (2006) 61–69
Environmental Research
www.elsevier.com/locate/envres

Effect modification by δ-aminolevulinic acid dehydratase, vitamin D receptor, and nitric oxide synthase gene polymorphisms on associations between patella lead and renal function in lead workers

Virginia M. Weaver[a,b,*], Byung-Kook Lee[c], Andrew C. Todd[d], Kyu-Dong Ahn[c], Weiping Shi[a], Bernard G. Jaar[b,e], Karl T. Kelsey[f], Mark E. Lustberg[g,1], Ellen K. Silbergeld[h], Patrick J. Parsons[i], Jiayu Wen[a], Brian S. Schwartz[a,b,e]

[a]*Division of Occupational and Environmental Health, Department of Environmental Health Sciences, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD 21205, USA*
[b]*Department of Medicine, Johns Hopkins University School of Medicine*
[c]*Institute of Industrial Medicine, SoonChunHyang University, Asan, Korea*
[d]*Department of Community and Preventive Medicine, Mount Sinai School of Medicine, New York, NY, USA*
[e]*Department of Epidemiology, Johns Hopkins University Bloomberg School of Public Health*
[f]*Department of Cancer Cell Biology, Harvard School of Public Health, Boston, MA, USA*
[g]*Department of Epidemiology and Preventive Medicine, University of Maryland School of Medicine, Baltimore, MD, USA*
[h]*Division of Environmental Health Engineering, Department of Environmental Health Sciences, Johns Hopkins University Bloomberg School of Public Health*
[i]*Lead Poisoning/Trace Elements Laboratory, Wadsworth Center, New York State Department of Health, Albany, NY, USA*

Received 15 August 2005; received in revised form 19 December 2005; accepted 4 January 2006
Available online 17 February 2006

Abstract

Genetic polymorphisms that affect lead toxicokinetics or toxicodynamics may be important modifiers of risk for adverse outcomes in lead-exposed populations. We recently reported associations between higher patella lead, which is hypothesized to represent a lead pool that is both bioavailable and cumulative, and adverse renal outcomes in current and former Korean lead workers. In the present study, we assessed effect modification by polymorphisms in the genes encoding for δ-aminolevulinic acid dehydratase (ALAD), the vitamin D receptor (VDR), and endothelial nitric oxide synthase on those associations. Similar analyses were conducted with three other lead biomarkers. Renal function was assessed via blood urea nitrogen, serum creatinine, measured and calculated creatinine clearances, urinary *N*-acetyl-β-D-glucosaminidase, and retinol-binding protein. Mean (SD) blood, patella, tibia, and dimercaptosuccinic acid-chelatable lead values were 30.9 (16.7) μg/dl, 75.1 (101.1) and 33.6 (43.4) μg Pb/g bone mineral, and 0.63 (0.75) μg Pb/mg creatinine, respectively, in 647 lead workers. Little evidence of effect modification by genotype on associations between patella lead and renal outcomes was observed. The VDR polymorphism did modify associations between the other lead biomarkers and the serum creatinine and calculated creatinine clearance. Higher lead dose was associated with worse renal function in participants with the variant B allele. Models in two groups, dichotomized by median age, showed that this effect was present in the younger half of the population. Limited evidence of effect modification by ALAD genotype was observed; higher blood lead levels were associated with higher calculated creatinine clearance among participants with the $ALAD^{1\text{-}2}$ genotype. In conclusion, VDR and/or ALAD genotypes modified associations between all the lead biomarkers, except patella lead, and the renal outcomes.

Keywords: δ-aminolevulinic acid dehydratase; Endothelial nitric oxide synthase; Renal function; Lead exposure; Patella lead; Tibia lead; Vitamin D receptor

*Corresponding author. Division of Occupational and Environmental Health, Johns Hopkins University Bloomberg School of Public Health, 615 North Wolfe Street, Room 7041, Baltimore, MD, 21205, USA. Fax: 410 955 1811.
E-mail address: vweaver@jhsph.edu (V.M. Weaver).
[1]*Current address*: Department of Medicine, York Hospital, York, PA.

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doi:10.1016/j.envres.2006.01.001

1. Introduction

Genetic polymorphisms that affect lead toxicokinetics or toxicodynamics may be important modifiers of risk for adverse outcomes in lead-exposed populations. Identification of genetic polymorphisms that modify relations of trabecular bone lead (commonly measured in the patella or calcaneus) with adverse health outcomes is of particular interest. Bone is both a repository and a source of lead. Thus, although trabecular and cortical bone lead (measured in the mid-tibia) are both cumulative lead dose measures, the shorter half-life of trabecular bone lead suggests that it is more bioavailable and thus may be of particular relevance to health (Hu et al., 1998). We recently reported associations between higher patella lead levels and adverse renal outcomes in data from the third annual evaluation in a longitudinal study on the adverse health effects of inorganic lead in current and former Korean lead workers (Weaver et al., 2005a). We performed the current analysis to determine whether the G177C polymorphism of the δ-aminolevulinic acid dehydratase (ALAD) gene, the *Bsm*I polymorphism of the vitamin D receptor (VDR) gene, or the Glu298Asp polymorphism of the endothelial nitric oxide synthase (eNOS) gene modified associations between patella lead and adverse renal outcomes in these workers.

Previously, we reported that ALAD genotype modified relations of blood lead and dimercaptosuccinic acid (DMSA)-chelatable lead with renal outcomes in data from the first evaluation in this study (performed a mean of 2.2 years earlier in 798 workers) (Weaver et al., 2003b). In contrast, neither VDR nor eNOS genotype consistently modified relations of lead biomarkers with renal outcomes in those data. Since patella lead was obtained only at the third evaluation, we analyzed data from that evaluation to determine whether these genotypes modified associations between patella lead and renal function. Results were compared with those for blood, tibia, and DMSA-chelatable lead.

2. Materials and methods

2.1. Study design and population

We performed a cross-sectional analysis of data from 647 current and former lead workers who completed the third annual evaluation in an ongoing longitudinal study on the health effects of inorganic lead exposure. As previously described (Schwartz et al., 2001; Weaver et al., 2003a), workers were recruited from 26 different facilities including lead battery, lead oxide, lead crystal, and radiator manufacture and secondary lead smelting. No medical exclusionary criteria (e.g., hypertension, renal disease) were applied. Study participants were not occupationally exposed to other known renal toxicants at the time of the study. All participants provided written, informed consent. The study protocol was approved by Institutional Review Boards at the SoonChunHyang University School of Medicine and the Johns Hopkins University Bloomberg School of Public Health. Participation in the study was voluntary.

2.2. Data collection

Data collection was completed either at the Institute of Industrial Medicine of the SoonChunHyang University in Chonan or at the study plants using previously reported methods (Schwartz et al., 2001; Weaver et al., 2003a). Data and biologic specimens collected included a standardized questionnaire on demographics and medical and occupational history, blood pressure, height and weight measurement, a blood specimen (for blood lead, blood urea nitrogen (BUN), serum creatinine, and genotyping), a spot urine sample (for *N*-acetyl-β-D-glucosaminidase (NAG), retinol-binding protein (RBP), and creatinine), and patella lead concentration. A 4-h urine collection after oral administration of 10 mg/kg DMSA was also obtained to measure DMSA-chelatable lead and creatinine clearance. Tibia lead concentration was not obtained at the third evaluation. Therefore, since tibia lead has a long half-life and thus is unlikely to change substantially in 1 year, tibia lead from the second evaluation was modeled in the subset of workers ($n = 574$) who participated in both the second and the third evaluations (mean = 398 days between the two evaluations in those participants).

2.3. Laboratory methods

The lead biomarkers and renal outcomes were measured using previously reported assays (Schwartz et al., 2001; Weaver et al., 2003a). In brief, blood lead was measured (Fernandez, 1975) with a Hitachi 8100 Zeeman background-corrected atomic absorption spectrophotometer (Hitachi Ltd. Instruments, Tokyo, Japan) at the Institute of Industrial Medicine in Chonan, a certified reference laboratory for lead in Korea. Patella and tibia lead levels were assessed via a 30-min measurement of the left medial patella and mid-tibia diaphysis, respectively, using ^{109}Cd to fluoresce the K-shell X-rays of lead. The lead X-rays were recorded with a radiation detector and then quantified and compared to calibration data to estimate the concentration of lead in bone (Todd and McNeill, 1993; Todd, 2000a, b; Todd et al., 2002; Todd and Chettle, 2003). All point estimates, including negative values, were retained in the statistical analyses to minimize bias and avoid censoring of data (Kim et al., 1995). Urine lead levels in the 4-h collection were measured at the Wadsworth Center of the New York State Department of Health (Albany, NY, USA) by electrothermal atomic absorption spectrometry with Zeeman background correction (Model 4100ZL; Perkin–Elmer, Norwalk, CT, USA) (Parsons and Slavin, 1999).

BUN and serum creatinine were measured via an automatic chemical analyzer (Model TBA 40FR Biochemical Analyzer; Toshiba, Tokyo, Japan). Urine creatinine was measured with the Sigma kit (Sigma Chemical Co., St. Louis, MO, USA). Measured creatinine clearance was defined as ([urinary creatinine in mg/dl × urine volume in ml]/serum creatinine in mg/dl)/collection time in minutes. Calculated creatinine clearance was obtained from the Cockcroft–Gault equation (Cockcroft and Gault, 1976). NAG activity (expressed in μmol substrate converted per hour) was measured using the PPR NAG Test kit (PPR Diagnostics, Ltd., London, UK) and RBP was measured using a modification of the method of Topping and co-workers (Topping et al., 1986).

Genotyping was performed as previously described (Weaver et al., 2003b). In brief, the ALAD polymorphism assayed identifies two alleles: $ALAD^1$ and the variant, $ALAD^2$, which has a G to C transversion at codon 177 and is cleaved by *Msp*1. The Glu298Asp polymorphism of the eNOS gene was measured. This involves a G to T transversion at nucleotide position 894 within exon 7 which results in substitution of aspartic acid for glutamic acid at codon 298; the variant allele is referred to as the Asp or T allele. Genotype was determined by a modification (Lustberg et al., 2004) of the assay of Hibi and colleagues (1998). The VDR *Bsm*I polymorphism in intron 8 includes a common allele, denoted b, and a variant, denoted B, in which the *Bsm*I restriction enzyme site is absent. Amplification used primers originating in exon 7 and intron 8 as previously published (Schwartz et al., 2000b).

2.4. Statistical analysis

The primary goals of our analysis were to (1) examine effect modification by ALAD, VDR, and eNOS genotypes on associations between patella lead and six renal outcomes (BUN, serum creatinine, measured and calculated creatinine clearances, RBP, and NAG) in current and former lead workers and (2) compare and contrast this with effect modification by these genotypes on associations between three other lead biomarkers (tibia lead [from the second evaluation], blood, and DMSA-chelatable lead) and the same renal outcomes. This allowed comparisons among the group of 647 workers using data collected at the same time as patella lead (except for tibia lead in $n = 574$, measured a mean of 398 days earlier as noted previously). Statistical analysis was conducted using SAS software (SAS Institute, Inc., Cary, NC, USA).

Initially, we examined variable distributions. The distributions of NAG and RBP showed departures from normality and were thus ln-transformed; the adequacy of transformation was subsequently confirmed by examination of residuals from the final regression models. For ALAD, participants with the $ALAD^{1\text{-}2}$ genotype (no $ALAD^{2\text{-}2}$ homozygotes were identified) were compared to the reference group of participants with the $ALAD^{1\text{-}1}$ genotype. Due to small numbers, all analyses combined homozygous and heterozygous variant genotype carriers for VDR (BB and Bb) and eNOS (Glu/Asp and Asp/Asp).

Linear regression modeling, with cross-product terms for genotypes and lead variables, was used to assess effect modification by genotype on associations between lead dose biomarkers and renal outcomes. Covariate selection for final regression models used a priori variables (age, gender, body mass index [BMI; defined as weight in kilograms divided by the square of height in meters]) in modeling that initially included other biologically relevant variables (diabetes and hypertension [both based on participant report of physician diagnosis], use of analgesics [based on questionnaire data on medication usage], work status [current vs. former lead worker], systolic and diastolic blood pressure, tobacco use, and alcohol consumption) in separate models. Variables with P values < 0.1 were then modeled together and those with P values < 0.1 in the combined model were retained. Genotype interaction terms were then added to the model. Continuous independent variables were centered at the mean. Final BUN, serum creatinine, and measured and calculated creatinine clearance models were adjusted for age, gender, BMI, work status (current or former lead worker), hypertension, diabetes, use of analgesics, smoking status (current, ex, never), and genotype. Final NAG and RBP models were adjusted for age, gender, BMI, work status, systolic blood pressure, smoking status, diabetes, and genotype. Since renal function declines with age, older workers comprise a susceptible population. We have previously observed effect modification by age on associations between lead biomarkers and renal outcomes in this population that are consistent with increased risk in older workers (Weaver et al., 2005a). Therefore, we dichotomized the population by median age and repeated the gene interaction models in each group.

As in previous analyses (Weaver et al., 2003a), we evaluated models for linear regression assumptions and presence of outlying points using added variable plots (Weisberg, 1985) which are graphical representations of the relation between Y and a particular X adjusted for all the other covariates. The function "lowess" of the S-plus statistical software program was used to produce these plots (MathSoft, Seattle, WA, USA). For each plot, two lines were overlaid: the regression line and a line determined by a scatter plot smoothing method (lowess) that calculates a locally weighted least-squares estimate for each point in the scatter plot (Cleveland, 1979). This allows an examination of the data for outliers that are overly influential, as evidenced by inconsistency between the lowess and the regression lines. When inconsistency was observed, models were repeated without outliers. Patella and tibia lead range maximas were higher in common allele groups than in those with the variant alleles. Therefore, lowest lines of relations between bone lead and renal outcomes were examined to determine the influence of the highest bone lead concentrations on models in which statistically significant effect modification was observed. Models were also assessed for collinearity by examining variance inflation factors and conditional indices.

3. Results

3.1. Selected demographics, exposure, and health outcome measures

Information on demographics, lead biomarkers, renal function, and selected covariates from the third evaluation is shown by genotype in Table 1. Mean values for the clinical renal outcomes (BUN, serum creatinine, measured and estimated creatinine clearances) were normal, although the range for each outcome included abnormal values. A total of 63 (9.8%) participants were heterozygous for the $ALAD^{2}$ allele (none was homozygous) (Table 1). For VDR, 75 (11.6%) were genotype Bb and 3 (0.5%) were BB. For eNOS, 98 (15.2%) and 5 (0.8%) participants had eNOS genotypes Glu/Asp and Asp/Asp, respectively. Differences in exposure and outcome measures by genotype were previously reported using data from the first evaluation (Schwartz et al., 2000a; Lee et al., 2001; Weaver et al., 2003b; Lustberg et al., 2004).

3.2. VDR

No effect modification by VDR genotype on associations between the patella lead and the six renal outcomes was observed after removal of outliers and adjustment for covariates. However, VDR genotype did modify associations between the other three lead biomarkers (tibia, blood, and DMSA-chelatable lead) and two of the six renal measures (Table 2). Among participants with the variant VDR B allele, higher lead measures were associated with worse renal function. Models in two separate groups, dichotomized by median age (43.5 years), showed that this effect was due to participants in the younger half of the population (models of calculated creatinine clearance are shown by age in Table 3). VDR genotype also modified the relation of tibia lead and measured creatinine clearance in the younger group in the same direction ($P < 0.05$ for the difference between the slopes of the associations by genotype; data not shown).

3.3. ALAD

Effect modification by ALAD genotype on associations between the patella lead and the six renal outcomes was not observed. Effect modification by ALAD genotype was observed only for the association between blood lead and calculated creatinine clearance and, of borderline significance, for associations between DMSA-chelatable lead and NAG and RBP (Table 4). Inverse associations (higher lead measures with higher calculated creatinine clearance and lower RBP and NAG) were observed among participants with the $ALAD^{1\text{-}2}$ genotype. This effect was not age dependent, based on an analysis of calculated

Table 1
Selected demographic, exposure, and outcome variables by ALAD, eNOS, and VDR genotype in third evaluation study data from 647 Korean lead workers

Characteristic	ALAD genotype				eNOS genotype				VDR genotype			
	1-1		1-2		Glu/Glu		Asp/Glu or Asp/Asp		bb		Bb or BB	
	N	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%
Number	582	90.2	63	9.8	540	84.0	103	16.0	569	87.9	78	12.1
Gender												
Male	448	77.0	48	76.2	415	76.9	80	77.7	445	78.2	53	68.0
Female	134	23.0	15	23.8	125	23.2	23	22.3	124	21.8	25	32.1
Work status												
Current lead worker	407	69.9	41	65.1	373	69.1	73	70.9	402	70.7	48	61.5
Former lead worker	175	30.1	22	34.9	167	30.9	30	29.1	167	29.4	30	38.5
Diabetes	7	1.2	1	1.6	7	1.3	1	1.0	6	1.1	2	2.6
Hypertension	46	7.9	6	9.5	46	8.5	7	6.8	41	7.2	12	15.4
Regular analgesic use	24	4.1	2	3.2	23	4.3	3	2.9	24	4.2	2	2.6
Smoking												
Never smokers	202	34.7	19	30.2	191	35.4	29	28.2	191	33.6	30	38.5
Current smokers	289	49.7	34	54.0	264	48.9	59	57.3	287	50.4	37	47.4
Ex-smokers	91	15.6	10	15.9	85	15.7	15	14.6	91	16.0	11	14.1
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age, years	43.4	9.8	42.9	9.1	43.3	9.7	43.7	10.1	43.1	9.6	45.5	10.4
BMI, kg/m^2	23.6	3.0	22.8	2.7	23.6	3.0	23.1	3.1	23.4	2.9	24.3	3.6
Systolic blood pressure, mm Hg	120.8	16.4	120.0	13.8	120.8	16.1	120.1	16.7	120.1	15.3	124.5	21.5
Diastolic blood pressure, mm Hg	74.1	12.7	73.7	11.1	74.3	12.4	72.9	13.4	73.8	12.4	75.8	13.6
Lead job duration, years	10.0	6.6	9.5	5.5	10.0	6.5	10.4	6.8	10.2	6.6	8.9	5.5
Blood lead, μg/dl	30.7	16.4	31.5	16.5	30.6	16.2	30.9	17.4	30.5	16.2	33.2	18.5
Patella lead, μg Pb/g bone mineral	75.7	103.0	63.0	61.7	75.6	102.2	72.2	96.8	74.7	99.2	79.2	115.3
Tibia lead, μg Pb/g bone mineral	33.8	43.9	25.4	21.9	33.8	44.5	31.8	37.9	33.7	44.3	32.8	37.2
DMSA-chelatable lead, μg Pb/mg creat.	0.59	0.65	0.59	0.66	0.58	0.63	0.63	0.73	0.59	0.64	0.66	0.71
BUN, mg/dl	14.2	3.6	14.3	3.6	14.2	3.6	14.2	3.5	14.3	3.5	14.2	3.7
Serum creatinine, mg/dl	0.87	0.14	0.85	0.11	0.86	0.13	0.87	0.14	0.86	0.13	0.89	0.15
Measured creatinine clearance, ml/min	109.0	30.9	104.1	31.7	108.7	30.6	108.9	32.2	109.6	31.1	100.7	29.0
Calculated creatinine clearance, ml/min	97.3	22.4	95.5	20.5	97.4	21.5	95.9	25.4	97.5	21.7	94.1	25.5
NAG, μmol/h/g creatinine	205.8	166.8	224.3	229.2	206.7	176.9	209.9	159.3	206.5	177.9	217.5	144.5
RBP, μg/g creatinine	47.3	60.7	42.6	31.2	46.8	61.8	45.6	37.1	47.0	60.4	45.0	42.4

Means and SDs are presented after removal of previously identified outliers (Weaver et al., 2005a).

creatinine clearance in two separate groups, dichotomized by median age.

3.4. eNOS

Effect modification by eNOS genotype was observed in only 1 of 24 models (Table 5). Higher tibia lead was associated with higher RBP in participants with the variant Asp allele although the difference between the slopes of the associations in the two gene groups was of only borderline significance ($P = 0.07$). Models in two separate groups, dichotomized by median age, showed that this effect was limited to participants in the older half of the population in whom effect modification of the association between patella lead and RBP was also observed. However, added variable plots of these models indicated that effect modification was due to data from participants with the variant allele and the highest bone lead values.

4. Discussion

In this cross-sectional analysis of data from the third evaluation in a longitudinal study of Korean lead workers, we determined whether polymorphisms in three genes (ALAD, VDR, or eNOS) modified associations of patella lead with renal function. We compared the results to effect modification by genotype on associations between three other lead biomarkers and the same outcomes. Little evidence of effect modification by genotype on associations between the patella lead and the renal outcomes was observed. However, effect modification by VDR on associations between the three other lead biomarkers and the serum creatinine and calculated creatinine clearance was observed. Higher lead dose was associated with worse renal function in those with the variant B allele; the effect was confined to younger workers. Some evidence of effect modification by ALAD genotype was observed. In contrast

Table 2
Linear regression models evaluating effect modification by VDR genotype on associations between lead biomarkers and serum creatinine and calculated creatinine clearance in 647 South Korean lead workers

	Serum creatinine (mg/dl) models				Calc. creatinine clearance (ml/min) models			
Variable	β coeff	SE β	P value	Model r^2	β coeff	SE β	P value	Model r^2
Intercept	0.9043	0.0133	<0.01	0.28	97.42	1.7015	<0.01	0.58
Patella lead, μg Pb/g bone mineral	*0.0001*	*0.0001*	*0.28*		*−0.0050*	*0.0079*	*0.53*	
Patella lead × VDR BB or Bb	−0.0001	0.0003	0.67		−0.0312	0.0360	0.39	
Intercept	0.9061	0.0133	<0.01	0.29	97.39	1.6904	<0.01	0.58
Blood lead, μg/dl	*−0.0000*	*0.0004*	*0.94*		*0.0104*	*0.0504*	*0.84*	
Blood lead × VDR BB or Bb	0.0017	0.0008	0.03		−0.1850	0.0980	0.06	
Intercept	0.9078	0.0133	<0.01	0.30	97.07	1.6758	<0.01	0.59
Chelatable lead, μg Pb/mg creatinine	*−0.0303*	*0.0085*	*<0.01*		*4.0055*	*1.0706*	*<0.01*	
Chelatable lead × VDR BB or Bb	0.0421	0.0244	0.08		−7.0115	3.0715	0.02	
Intercept	0.9173	0.0143	<0.01	0.29	95.91	1.7748	<0.01	0.59
Tibia lead, μg Pb/g bone mineral	*0.0002*	*0.0002*	*0.24*		*−0.0095*	*0.0192*	*0.62*	
Tibia lead × VDR BB or Bb	0.0014	0.0008	0.08		−0.2125	0.0951	0.03	

Models were also adjusted for age, gender, BMI, work status, hypertension, diabetes, use of analgesics, smoking status (current, ex, never), and genotype. Homozygotes for the common gene allele (VDR bb) are the reference category (italic). P values for the cross-product terms reflect the statistical significance of the difference between the slopes of the regression line for the gene variant group and the regression line for the reference gene group. Slopes for the gene variant group are obtained by adding the beta coefficient of the cross-product term to the beta coefficient for the reference category (i.e., the slope of the relation between blood lead and serum creatinine in those with Bb or BB genotypes is 0.0017 [0.0000 + 0.0017]).

Table 3
Linear regression models evaluating effect modification by VDR genotype on associations between lead biomarkers and calculated creatinine clearance in two groups of lead workers, dichotomized by median age

Variable	Age ⩽ 43.5 years				Age > 43.5 years			
	β coeff	SE β	P value	Model r^2	β coeff	SE β	P value	Model r^2
Intercept	105.78	2.1923	<0.01	0.49	88.69	2.7282	<0.01	0.41
Patella lead, μg Pb/g bone mineral	*0.0111*	*0.0234*	*0.64*		*−0.0076*	*0.0083*	*0.36*	
Patella lead × VDR BB or Bb	−0.0693	0.1105	0.53		−0.0042	0.0410	0.92	
Intercept	105.76	2.1625	<0.01	0.50	88.50	2.6798	<0.01	0.41
Blood lead, μg/dl	*0.0512*	*0.0738*	*0.49*		*−0.0326*	*0.0680*	*0.63*	
Blood lead × VDR BB or Bb	−0.4606	0.1649	<0.01		−0.0022	0.1186	0.99	
Intercept	105.66	2.1297	<0.01	0.51	88.38	2.7036	<0.01	0.41
Chelatable lead, μg Pb/mg creatinine	*6.4818*	*1.9237*	*<0.01*		*2.6201*	*1.2893*	*0.04*	
Chelatable lead × VDR BB or Bb	−14.0318	4.9054	<0.01		−1.0235	3.8976	0.79	
Intercept	103.94	2.2472	<0.01	0.50	88.70	2.9141	<0.01	0.44
Tibia lead, μg Pb/g bone mineral	*0.0444*	*0.0463*	*0.34*		*−0.0238*	*0.0215*	*0.27*	
Tibia lead × VDR BB or Bb	−0.5672	0.1645	<0.01		0.0279	0.1223	0.82	

Models were also adjusted for age, gender, BMI, work status, hypertension, diabetes, use of analgesics, smoking status (current, ex, never), and genotype. Homozygotes for the common gene allele (VDR bb) are the reference category (italic). P values for the cross-product terms reflect the statistical significance of the difference between the slopes of the regression line for the gene variant group and the regression line for the reference gene group. Slopes for the gene variant group are obtained by adding the beta coefficient of the cross-product term to the beta coefficient for the reference category (i.e., the slope of the relation between patella lead and calculated creatinine clearance in those with Bb or BB genotypes is –0.0582 [0.0111 + –0.0693]).

to VDR, higher lead measures were associated with higher calculated creatinine clearance and lower RBP and NAG among participants with the ALAD$^{1\text{-}2}$ genotype. Little evidence of effect modification by eNOS genotype was observed.

Polymorphisms of the VDR gene are of interest in studies of human lead exposure, because of the role of vitamin D and its receptor in regulating both intestinal calcium absorption and bone mineralization. These pathways impact lead absorption from the gastrointestinal tract and may impact lead storage and/or release from bone (Onalaja and Claudio, 2000). Analysis of data from the first evaluation of this lead worker cohort found that participants with the B allele had significantly higher blood, DMSA-chelatable, and tibia lead levels than those with the bb genotype (Schwartz et al., 2000a); significantly

Table 4
Linear regression models evaluating effect modification by ALAD genotype on associations between lead biomarkers and calculated creatinine clearance in 645 South Korean lead workers

	Calc. Creatinine clearance (ml/min)			RBP (ln [μg/g creatinine])			NAG (ln [μmol/h/g creatinine])		
Variable	β coeff	SE β	*P* value	β coeff	SE β	*P* value	β coeff	SE β	*P* value
Intercept	97.37	1.7046	<0.01	3.3411	0.0786	<0.01	4.9049	0.0730	<0.01
Patella lead, μg Pb/g bone mineral	*−0.0097*	*0.0080*	*0.22*	*−0.0001*	*0.0004*	*0.76*	*0.0010*	*0.0004*	*<0.01*
Patella lead × ALAD$^{1\text{-}2}$	0.0501	0.0419	0.23	−0.0019	0.0014	0.19	−0.0015	0.0013	0.25
Intercept	97.38	1.6932	<0.01	3.3764	0.0794	<0.01	4.9235	0.0733	<0.01
Blood lead, μg/dl	*−0.0558*	*0.0491*	*0.26*	*0.0007*	*0.0022*	*0.75*	*0.0050*	*0.0021*	*0.02*
Blood lead × ALAD$^{1\text{-}2}$	0.2507	0.1184	0.03	−0.0070	0.0057	0.22	−0.0043	0.0051	0.40
Intercept	96.82	1.6772	<0.01	3.3724	0.0791	<0.01	4.9262	0.0731	<0.01
Chelatable lead, μg Pb/mg creatinine	*3.0013*	*1.0553*	*<0.01*	*0.0223*	*0.0440*	*0.61*	*0.1146*	*0.0407*	*<0.01*
Chelatable lead × ALAD$^{1\text{-}2}$	4.6365	3.5250	0.19	−0.2565	0.1429	0.07	−0.2367	0.1313	0.07
Intercept	96.02	1.7623	<0.01	3.3437	0.0822	<0.01	4.9324	0.0795	<0.01
Tibia lead, μg Pb/g bone mineral	*−0.0252*	*0.0183*	*0.17*	*−0.0008*	*0.0008*	*0.37*	*0.0004*	*0.0008*	*0.59*
Tibia lead × ALAD$^{1\text{-}2}$	0.0916	0.0928	0.32	0.0011	0.0044	0.80	0.0043	0.0042	0.30

Calculated creatinine clearance models were also adjusted for age, gender, BMI, work status, hypertension, diabetes, use of analgesics, smoking status (current, ex, never), and genotype. NAG and RBP models were also adjusted for age, gender, BMI, work status, systolic blood pressure, smoking status, diabetes, and genotype. Homozygotes for the common gene allele (ALAD$^{1\text{-}1}$) are the reference category (italic). *P* values for the cross-product terms reflect the statistical significance of the difference between the slopes of the regression line for the variant gene group and the regression line for the reference gene group. Slopes for the variant gene group are obtained by adding the beta coefficient of the cross-product term to the beta coefficient for the reference category (i.e., the slope of the relation between patella lead and calculated creatinine clearance in those with ALAD$^{1\text{-}2}$ genotype is 0.0404 [−0.0097 + 0.0501]).

Table 5
Linear regression models evaluating effect modification by eNOS genotype on associations between lead biomarkers and RBP (ln [μg/g creatinine])

	All Lead workers			Age ⩽ 43.5 years			Age > 43.5 years		
Variable	β coeff	SE β	*P* value	β coeff	SE β	*P* value	β coeff	SE β	*P* value
Intercept	3.3413	0.0784	<0.01	3.2057	0.0950	<0.01	3.5250	0.1364	<0.01
Patella lead, μg Pb/g bone mineral	*−0.0006*	*0.0004*	*0.17*	*0.0004*	*0.0010*	*0.65*	*−0.0008*	*0.0005*	*0.09*
Patella lead × eNOS Asp	0.0013	0.0008	0.10	−0.0033	0.0034	0.34	0.0019	0.0009	0.04
Intercept	3.3408	0.0819	<0.01	3.2049	0.1014	<0.01	3.5471	0.1420	<0.01
Tibia lead, μg Pb/g bone mineral	*−0.0013*	*0.0009*	*0.13*	*0.0018*	*0.0020*	*0.40*	*−0.0021*	*0.0011*	*0.07*
Tibia lead × eNOS Asp	0.0037	0.0020	0.07	−0.0023	0.0091	0.80	0.0048	0.0023	0.04

Models were also adjusted for age, gender, BMI, work status, systolic blood pressure, smoking status, diabetes, and genotype. Homozygotes for the common gene allele (eNOS Glu/Glu) are the reference category (italic). *P* values for the cross-product terms reflect the statistical significance of the difference between the slopes of the regression line for the variant gene group and the regression line for the reference gene group. Slopes for the variant gene group are obtained by adding the beta coefficient of the cross-product term to the beta coefficient for the reference category (i.e., the slope of the relation between patella lead and RBP in all lead workers with eNOS Asp/Glu or eNOS Asp/Asp genotyps is 0.0007 [−0.0006 + 0.0013]).

higher patella lead in workers with the B allele was reported in data from the third evaluation (same participants as in this report) (Theppeang et al., 2004). A study of 216 lead workers also reported higher blood lead levels in workers with the B allele ($n = 20$), after adjustment for age, gender, smoking, and alcohol ingestion (Ye et al., 2003). In a study of 504 former organolead manufacturing workers, with an average of almost 2 decades since last occupational exposure, the slope of the positive association between age and tibia lead concentration was steeper in participants with the B allele and tibia lead declined with years since last exposure in participants with the bb genotype but increased in those with the B allele (Schwartz et al., 2000b). In contrast, Chuang and colleagues (2004) found no difference in current or cumulative blood lead in 544 lead workers and Kamel and colleagues (2003) reported no significant differences in blood, tibia, or patella lead by VDR genotype although the number of participants ($n = 38$) was quite small.

We are aware of only one other population in which effect modification by this VDR polymorphism on associations between lead dose and renal outcomes has been studied (Ye et al., 2003). No difference in associations between lead dose and urinary albumin and NAG by VDR genotype was observed, although the population was stratified by genotype which decreased the power to detect

a difference. Similarly, we did not observe consistent evidence of effect modification by VDR genotype on relations between lead dose and renal function in data from the first evaluation of our lead worker cohort (Weaver et al., 2003b, 2005b). The inconsistency between our earlier analysis and our current results could be due to a different subset of workers ($n = 647$ in evaluation three compared to 798 in evaluation one) or to changes in renal function that occurred over the 2-year follow-up period. We excluded the former explanation by repeating the models of effect modification by VDR on associations of blood and DMSA-chelatable lead with serum creatinine and calculated creatinine clearance using data from the first evaluation of the 647 workers who completed the third evaluation. No evidence of effect modification by VDR was observed (data not shown).

The difference in effect modification by VDR on patella lead associations compared to those of the other lead biomarkers may be related to the unique nature of lead in trabecular bone, particularly given the potential for this polymorphism to affect bone lead. However, greater measurement uncertainty for patella lead compared to tibia lead (Hu et al., 1998) is also a consideration. Other explanations seem less likely. In the third evaluation of data from our Korean lead worker population, all the lead biomarkers were highly correlated and associations between patella lead and renal function were similar to those with the other biomarkers (Weaver et al., 2005a). Therefore, the inconsistency cannot be explained by different patella lead correlations or by different associations of this biomarker with the renal outcomes. Further, as noted above, the toxicokinetic difference by VDR that we observed for patella lead in this population was similar to those for the other three lead biomarkers. The difference in effect modification between the two bone lead measures is not due to different population subsets ($n = 647$ for patella compared to 574 for tibia). Interaction models with a cross-product term for VDR genotype and patella lead in models of serum creatinine and calculated creatinine clearance in the same subset of 574 workers as in the tibia models also revealed no evidence of effect modification by VDR (data not shown).

Conclusions that may be drawn with regard to the public health significance of the observed effect modification by the *Bsm*I polymorphism on associations between the blood, tibia, and DMSA-chelatable lead and the renal outcomes and the lack of an effect on patella lead associations in data from the third evaluation in our study are limited by two factors. First, the functional significance of the *Bsm*I polymorphism remains uncertain (Uitterlinden et al., 2004) and, second, few data on the interaction between VDR genotype and lead dose on renal function have been published. Studies of effect modification by VDR on associations between lead dose, particularly patella lead, and renal function in other populations and in our longitudinal data will be helpful in understanding the significance of the difference that we observed.

Steeper slopes for one or more associations between lead dose and adverse renal function in participants with the $ALAD^{2}$ allele compared to those with the $ALAD^{1\text{-}1}$ genotype have been reported in the two other populations in which effect modification by ALAD genotype on associations between lead dose and renal outcomes has been studied (Wu et al., 2003; Ye et al., 2003). This suggests that the variant ALAD gene confers additional risk for adverse renal outcomes in lead-exposed populations. In our analysis of data from the first evaluation of this cohort, higher blood and/or DMSA-chelatable lead were associated with lower BUN and serum creatinine and higher calculated creatinine clearance among those with the $ALAD^{1\text{-}2}$ genotype (Weaver et al., 2003b). The current data, from the third evaluation conducted a mean of 2.2 years after the first evaluation, also reveal inverse associations although fewer were significant compared to the first evaluation. Hyperfiltration is a process in which initial supranormal renal function is paradoxically associated with an increased risk for subsequent renal dysfunction (Nenov et al., 2000), If our data represent lead-induced hyperfiltration, our results suggest increased risk from the variant ALAD gene also. Only two longitudinal studies have reported hyperfiltration from lead exposure; both were in rodents (Khalil-Manesh et al., 1992, 1993). Severity of subsequent renal dysfunction was greater in the high-lead-dose animals although initial hyperfiltration was observed earlier in the low-lead-dose animals which suggests that additional mechanisms contributed to the renal damage in the high-lead-dose animals. Ultimately, analysis of longitudinal data in our population will be required to elucidate these complex relations.

The product of the eNOS gene catalyzes L-arginine to the renoprotective vasodilator, nitric oxide (NO). However, the functional significance of this polymorphism remains uncertain. Some data suggest that the Asp allele is associated with decreased NO (Sofowora et al., 2001; Noiri et al., 2002; Tanus-Santos et al., 2002; Veldman et al., 2002). This could be related to increased proteolytic cleavage of the eNOS protein which was reported by Persu and colleagues (2002) in renal artery specimens from participants with the Asp allele (including under nonacidic pH conditions). In contrast, other authors have reported no difference in various functional measures in participants with the Asp allele compared to those with the Glu–Glu genotype (Moon et al., 2002; Golser et al., 2003; Li et al., 2004; Hoffmann et al., 2005). Lack of knowledge of the functional significance of this polymorphism coupled with limited evidence of effect modification by eNOS on relations between lead dose and renal outcomes in data analysis in this population to date (Weaver et al., 2003b, 2005b) greatly limit any conclusions that can be made with regard to the public health significance of the effect modification noted in this analysis.

In conclusion, little evidence of effect modification on associations between patella lead and renal outcomes was observed. However, VDR and, to a lesser extent, ALAD

genotypes did modify associations between the other lead biomarkers and the renal outcomes. Higher lead dose was associated with worse renal function in younger participants with the variant VDR B allele. In contrast, higher lead dose was inversely associated with worse renal function in participants with the $ALAD^{1\text{-}2}$ genotype. Compared to the initial evaluation of this population (Weaver et al., 2003b), results from this evaluation provide new genetic information in three areas. The first is the limited effect modification on patella lead associations. The second is the presence of effect modification by VDR on the other lead dose measures which was not observed in the earlier evaluation. The third is the fact that effect modification by ALAD, reported in the original evaluation, is still present. A review of the current literature on this topic indicates that individuals with the ALAD variant allele may be at increased risk for adverse renal outcomes from lead exposure.

Acknowledgments

We thank Drs. Yong-Bae Kim, Bong-Ki Jang, Gap-Soo Lee, and Sung-Soo Lee for assistance in data collection in South Korea and Professor James Wetmur of The Mount Sinai School of Medicine for providing the nested primers for ALAD genotyping. This research was supported by NIEHS Grants ES07198 (Dr. Schwartz), 2 ES07198 (Dr. Weaver), and ES00002 (Dr. Kelsey), National Research Service Award F30-ES05922-02 (Dr. Lustberg), Korea Research Foundation Grant KRF-2000-00545 (Dr. Lee), and Centers for Disease Control and Prevention Grant ATPM TS288-14/14 (Drs. Lustberg and Silbergeld).

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