Associations of Blood Pressure and Hypertension with Lead Dose Measures and Polymorphisms in the Vitamin D Receptor and δ-Aminolevulinic Acid Dehydratase Genes

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Evidence suggests that lead and selected genes known to modify the toxicokinetics of lead—namely, those for the vitamin D receptor (VDR) and δ-aminolevulinic acid dehydratase (ALAD)—may independently influence blood pressure and hypertension risk. We report the relations among ALAD and VDR genotypes, three lead dose measures, and blood pressure and hypertension status in 798 Korean lead workers and 135 controls without occupational exposure to lead. Lead dose was assessed by blood lead, tibia lead measured by X-ray fluorescence, and dimercaptosuccinic acid (DMSA)-chelatable lead. Among lead workers, 9.9% (n = 79) were heterozygous for the *ALAD*[2] allele, and there were no *ALAD*[2] homozygotes; 11.2% (n = 89) had at least one copy of the VDR *B* allele, and 0.5% (n = 4) had the *BB* genotype. In linear regression models to control for covariates, VDR genotype (*BB* and *Bb* vs. *bb*), blood lead, tibia lead, and DMSA-chelatable lead were all positive predictors of systolic blood pressure. On average, lead workers with the VDR *B* allele, mainly heterozygotes, had systolic blood pressures that were 2.7–3.7 mm Hg higher than did workers with the *bb* genotype. VDR genotype was also associated with diastolic blood pressure; on average, lead workers with the VDR *B* allele had diastolic blood pressures that were 1.9–2.5 mm Hg higher than did lead workers with the VDR *bb* genotype (p = 0.04). VDR genotype modified the relation of age with systolic blood pressure; compared to lead workers with the VDR *bb* genotype, workers with the VDR *B* allele had larger elevations in blood pressure with increasing age. Lead workers with the VDR *B* allele also had a higher prevalence of hypertension compared to lead workers with the *bb* genotype [adjusted odds ratio (95% confidence interval) = 2.1 (1.0, 4.4), p = 0.05]. None of the lead biomarkers was associated with diastolic blood pressure, and tibia lead was the only lead dose measure that was a significant predictor of hypertension status. In contrast to VDR, ALAD genotype was not associated with the blood pressure measures and did not modify associations of the lead dose measures with any of the blood pressure measures. To our knowledge, these are the first data to suggest that the common genetic polymorphism in the VDR is associated with blood pressure and hypertension risk. We speculate that the *Bsm*I polymorphism may be in linkage disequilibrium with another functional variant at the VDR locus or with a nearby gene. *Key words*: δ-aminolevulinic acid dehydratase, blood pressure, hypertension, lead, polymorphisms, vitamin D receptor, X-ray fluorescence. *Environ Health Perspect* 109:383–389 (2001). [Online 22 March 2001]
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Lead absorption increases blood pressure, especially systolic blood pressure, at blood lead levels as low as 5 μg/dL (*1*,*2*). Little is known, however, about genetic variation in risk of elevated blood pressure from lead. In particular, two polymorphic genes known to modify the toxicokinetics of lead—those for the vitamin D receptor (VDR) (*3*–*5*) and δ-aminolevulinic acid dehydratase (ALAD) (*5*–*17*)—could influence the effect of lead on blood pressure and hypertension.

ALAD is a principal erythrocytic binding site for lead, and such binding differs for the three isoforms of the ALAD protein (*17*). Thus, the polymorphism could influence the effect of lead on blood pressure by, for example, modifying the deposition of lead at the critical cellular or molecular targets through which lead acts to cause elevations in blood pressure. VDR genotype is also of particular interest not only because it has been implicated to modify the absorption of lead and the uptake and release of lead from bone (*3*,*4*), but also because alterations in calcium metabolism have been implicated in the risk of elevations in blood pressure and essential hypertension. These alterations include such factors as calcium intake, calcium absorption, bone calcium metabolism, serum calcium levels, and cytosolic free calcium (*18*–*21*). Vasoactive, neural, hormonal, and renal effects of calcium also play a role in blood pressure regulation (*22*,*23*). Polymorphisms in the VDR gene could thus have a direct influence on blood pressure and hypertension risk, independent of lead, but this possibility has not been investigated.

The prevalence of the ALAD and VDR polymorphisms differs by race/ethnicity. Although reported prevalence estimates differ from study to study, approximately 15–25% of Australian, U.S., and European whites are homozygous for the absence of the *Bsm*I restriction site (*BB* genotype); in contrast, 0–13% of African Americans and 1–3% of Asians have the *BB* genotype (*24*–*28*). Similarly, the prevalence of the *ALAD*[2] allele varies by race/ethnicity. Approximately 20% of Caucasians, 5–10% of Asians, and 0–2% of Africans or African Americans have the allele (*5*,*7*,*9*,*10*).

Here we we report a cross-sectional evaluation of the relations among the two polymorphic genes, three lead dose measures, and blood pressure and hypertension status in 798 Korean lead workers and 135 controls without occupational exposure to lead.

Materials and Methods

Study overview and design. The results presented here are a cross-sectional analysis of data from the first year of a 3-year longitudinal study of the health effects of occupational inorganic lead exposure (*29*,*30*). Enrollment began in October 1997 with the first of three annual evaluations for each study subject. The current report is an analysis of data obtained during the first study

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visit from 933 subjects enrolled between 24 October 1997 and 19 August 1999. The study was reviewed and approved by institutional review boards at the Johns Hopkins School of Hygiene and Public Health and the Soonchunhyang University School of Medicine.

Study population. Participation in the study was voluntary, and all participants provided written, informed consent. Subjects were paid approximately $30 for their participation. Lead workers were recruited from 24 different lead-using facilities, with participation in most facilities exceeding 80% (*29*). Retired workers from three facilities who had received medical surveillance services by Soonchunhyang University for several years were also recruited to participate in the study. Routine, governmentally mandated industrial hygiene sampling revealed that the study plants did not have significant amounts of other heavy metals such as cadmium. Controls without occupational lead exposure were recruited from an air conditioner assembly plant that did not use lead or other heavy metals and from hourly-wage workers of Soonchunhyang University.

Data collection. Data collection methods have been reported previously (*29*). In brief, data were collected either at the Institute of Industrial Medicine at Soonchunhyang University in Chonan or on the premises of the study's lead-using facilities. The following were collected or measured on all study subjects: a standardized interview for demographics, medical history, and occupational history; a neurobehavioral test battery consisting of examiner-administered tests; blood pressure; peripheral vibration threshold and pinch and grip strength; a 10-mL blood specimen taken by venipuncture that was stored at –70°C as whole blood, plasma, and red blood cells; a spot urine sample; tibia lead concentration assessed by X-ray fluorescence (XRF); and a urine sample collected for 4 hr after oral administration of dimercaptosuccinic acid (DMSA) (in lead workers only). Blood pressure—systolic and fifth Korotkoff diastolic—was measured using a Hawksley random zero sphygmomanometer (Hawksley, Sussex, England) according to the Johns Hopkins Welch Center for Prevention, Epidemiology, and Clinical Trials protocol. Three measurements, using an appropriately sized cuff, were taken 5 min apart with the subject sitting by a physician trained in the method.

Laboratory methods. We assayed hemoglobin by the cyanmethemoglobin method (Model Ac-T 8; Beckman Coulter, Inc., Fullerton, CA, USA), and measured hematocrit by the capillary centrifugation method (*31*). We measured urinary creatinine from the 4-hr urine sample after oral administration of DMSA, using the Sigma kit (St. Louis, MO, USA) and a Beckman DU-7 spectrophotometer (Beckman Instruments, Inc., Fullerton, CA, USA) (*32*). To ensure that DMSA in urine did not interfere with the creatinine assay, we spiked 12 urine samples with 15 mg/dL of a creatinine standard (Sigma), and compared the assay results with expected values. A scatterplot of the relation of the measured to the expected values had a Pearson's r of 0.998, a slope of 1.0, and an intercept of 0, within error. There was also no evidence that DMSA interfered with creatinine excretion *in vivo*, because the measured 4-hr creatinine clearances were within the range of normal expected values for both males and females [determined by multiplying published values for 24-hr creatinine excretion (milligrams per kilogram) by the weight of study subjects and dividing to adjust for a 4-hr collection period].

We measured zinc protoporphyrin levels with a portable hematofluorimeter (*33*) and blood lead levels with a Zeeman background-corrected atomic absorption spectrophotometer (Z-8100 model; Hitachi, Tokyo, Japan) using the standard addition method of the National Institute of Occupational Safety and Health (*34*) at Soonchunhyang University Institute of Industrial Medicine, a certified reference laboratory for lead in Korea. We assessed tibia lead, in units of micrograms lead per gram bone mineral (hereafter referred to as μg/g), with a 30-min measurement at the left mid-tibial shaft using ^{109}Cd-induced K-shell XRF, as previously described (*30,35,36*). XRF can provide negative point estimates of bone lead concentrations; however, all point estimates were retained in the statistical analyses, including negative values, because this method minimizes bias and does not require censoring of data (*37*).

We used 4-hr urinary lead excretion after oral administration of 10 mg/kg DMSA to measure DMSA-chelatable lead (*38*). We measured urine lead levels in the laboratories of the Wadsworth Center at the New York State Department of Health, Albany, New York. We determined urinary lead concentrations by electrothermal atomization atomic absorption spectrometry (Model 4100ZL; PerkinElmer, Norwalk, CT, USA) using previously published methods (*39*). Urinary lead excretion was highly correlated with lead excretion adjusted for differences, generally small, in urine collection times (Pearson's $r = 0.98$), so we presented only the unadjusted data.

ALAD and VDR genotyping. We completed ALAD and VDR genotyping on 795 and 798 lead workers, respectively, and 135 nonexposed control subjects. VDR genotyping was completed using previously published methods (*4,40*). In brief, we extracted genomic DNA from whole blood using the QIAamp Blood Kit (QIAGEN, Hilden, Germany), and the *Bsm*I polymorphic site in intron 8 was amplified by polymerase chain reaction (PCR) using the primers originating in exon 7 (primer 1: 5´-CAACCAAGACTACAAGTACCGCGTCAGTGA-3´) and intron 8 (primer 2: 5´-AACCAGCGGGAAGAGGTCAAGGG-3´). Subjects homozygous for the presence of the *Bsm*I restriction site are designated *bb*, heterozygotes are designated *Bb*, and those homozygous for the absence of the site are designated *BB*.

We used a modified PCR-based protocol for ALAD genotyping, as described previously (*6–9*). The ALAD gene has two alleles, $ALAD^1$ and $ALAD^2$, producing three isozymes, ALAD1-1, ALAD1-2, and ALAD2-2. In brief, the initial amplification, using 3´ and 5´ oligonucleotide primers [(5´-AGACAGACATTAGCTCAGTA-3´) and (5´-GGCAAAGAACACGTCCATTC-3´)] generates a 916 base-pair fragment. A second round of amplification using a pair of nested primers (flanking DNA sequence kindly provided by J. Wetmur), sequences (5´-CAGAGCTGTTCCAAC-AGTGGA-3´) and (5´-CCAGCACAATGTGGGAGTGA-3´), respectively, and generates an 887 base-pair fragment. The amplified fragment was cleaved at the diagnostic *Msp*1 site, present only in the $ALAD^2$ allele.

Statistical analysis. The primary goals of the analysis were to examine relations of ALAD and VDR genotype with systolic blood pressure, diastolic blood pressure, and hypertension status, controlling for covariates, and to determine if ALAD and VDR genotype modified the relations of age, blood lead, tibia lead, and DMSA-chelatable lead with systolic blood pressure, diastolic blood pressure, or hypertension status.

We used linear regression to model separately systolic and diastolic blood pressure, controlling for confounding variables, using SAS software programs (SAS Institute, Inc., Cary, NC, USA). First, we compared lead workers to controls without occupational lead exposure. Next, we evaluated associations of the lead dose measures and genetic factors in the lead workers only. Covariates examined in linear regression models included age, gender, creatinine clearance (4 hr), hemoglobin, hematocrit, weight, height, body mass index, job duration, tobacco and alcohol consumption (never, previous, and current use for each), lifetime tobacco consumption (in pack-years), and cumulative lifetime alcohol drinks in current alcohol users [divided into quartiles of lifetime cumulative drinks (one glass of beer or wine or one shot of distilled spirits)]. Covariates were retained in the final regression models if they were either a significant predictor of blood pressure or a confounder of the relations between predictor variables and

blood pressure (i.e, there were substantive changes in the coefficients of predictor variables after inclusion of potential confounding variables). To evaluate effect modification by ALAD and VDR genotype, we added cross-product terms of the genetic factors and the lead dose measures and age to the models of systolic and diastolic blood pressure, one cross-product term at a time.

We defined hypertension as systolic blood pressure > 160 mm Hg or diastolic blood pressure > 96 mm Hg or a patient's currently taking medications for high blood pressure, to increase the specificity of the categorization and to be consistent with prior research (*2,41*). We evaluated associations between ALAD and VDR genotype and hypertension in contingency tables using odds ratios and 95% exact confidence limits calculated with Epi Info version 6.04b (Centers for Disease Control and Prevention, Atlanta, GA). We used logistic regression to model hypertension status, controlling for confounding variables, after evaluating the potential covariates described above for systolic and diastolic blood pressure. We added cross-product terms to the logistic regression models of hypertension one at a time to evaluate effect modification by ALAD or VDR genotype.

Results

Demographics and dose measures. Compared to nonexposed controls, lead-exposed subjects were older (40.5 vs. 34.5 years), had lower education levels (49.9% vs. 19.2% did not complete high school), and had a lower proportion of male subjects [79.4% vs. 91.9% (Table 1)]. The majority of both nonexposed and exposed subjects were current users of tobacco and alcohol products. There was a wide range of blood lead (4–86 μg/dL), tibia lead (–7–338 μg/g), and DMSA-chelatable lead (4.8–2,103 μg) levels among lead workers (Table 1). The corresponding values among nonexposed control subjects were low (Table 1).

Prevalence and associations of genotypes. Among lead workers, 9.9% (n = 79) were heterozygous for the *ALAD*^{2} allele and there were no *ALAD*^{2} homozygotes. A total of 11.2% (n = 89) had at least one copy of the VDR *B* allele and 0.5% (n = 4) had the *BB* genotype. The corresponding values for nonexposed controls were 8.1% (n = 11) for the *ALAD*^{2} allele and 8.9% (n = 12) and 0.7% (n = 1) for one and two copies of the VDR *B* allele, respectively. Because of the small number of subjects with the *BB* genotype, they were combined with the heterozygous variant allele carriers for all subsequent analysis.

There were no differences in age, job duration, lead dose measures, or systolic or diastolic blood pressure by ALAD genotype. In contrast, subjects with the VDR *B* allele were older, had higher DMSA-chelatable lead levels, and had higher systolic and diastolic blood pressures (all p-values < 0.05; Table 2).

Analysis comparing lead workers and controls. Compared to lead workers, control subjects without occupational exposure to lead evidenced no average difference in systolic or diastolic blood pressure after adjustment for age, sex, body mass index, antihypertensive medication use, current alcohol use, blood lead, and ALAD and VDR genotypes (data not shown). There was

Table 2. Selected study variables (mean ± SD) by gene status in 798 lead-exposed subjects, October 1997–August 1999, Republic of Korea.

Characteristic	ALAD genotype (n = 795)		VDR genotype (n = 798)	
	1-1	*1-2*	*bb*	*Bb* or *BB*
Number (%)	716 (90.1)	79 (9.9)	709 (88.8)	89 (11.2)
Age, years	40.5 ± 10.2	40.1 ± 9.7	40.2 ± 10.0*	42.7 ± 10.3*
Job duration, years	8.2 ± 6.6	8.2 ± 5.8	8.4 ± 6.6	7.2 ± 5.6
Blood lead, μg/dL	31.7 ± 14.9	34.2 ± 15.9	31.6 ± 14.8	34.8 ± 16.1
Tibia lead, μg/g	37.5 ± 40.6	31.4 ± 29.5	37.1 ± 41.2	38.1 ± 33.5
DMSA-chelatable lead, μg	180.3 ± 181.2	161.7 ± 143.0	173.5 ± 176.8*	217.2 ± 179.7*
Systolic blood pressure, mm Hg	123.4 ± 16.5	122.3 ± 14.5	122.6 ± 15.5*	129.1 ± 20.6*
Diastolic blood pressure, mm Hg	75.9 ± 11.9	73.9 ± 12.5	75.3 ± 11.7*	79.4 ± 13.5*

*p < 0.05 comparing VDR *Bb* or *BB* to VDR *bb*.

Table 1. Description of study subjects, October 1997–August 1999, Republic of Korea.

Characteristic	Lead-exposed subjects (n = 798)			Nonexposed controls (n = 135)		
	Mean	SD	Range	Mean	SD	Range
Age, years	40.5	10.1	17.8–64.8	34.5	9.1	22.0–60.2
Lead work job duration, years	8.2	6.5	0.1–36.2	NA		
Height, cm	164.7	8.1	127.8–186.0	167.9	6.2	148.0–183.4
Weight, kg	62.5	9.1	37.4–92.7	66.9	9.0	48.0–93.5
Body mass index, kg/cm^2	23.0	3.0	15.7–34.2	23.7	2.8	18.5–30.1
Blood lead, μg/dL	32.0	15.0	4–86	5.3	1.8	2–10
Tibia lead, μg Pb/g bone mineral	37.2	40.4	–7–338	5.8	7.0	–11–27
DMSA-chelatable lead, μg[a]	186.0	208.4	4.8–2,103	NA		
Hemoglobin, g/dL	14.2	1.4	6.5–17.9	15.3	1.2	11.1–18.2
Creatinine clearance, 4-hr, mL/min	114.3	33.9	11.2–351.6	NA		
Educational level, n (%)						
Lower school (≤ 6 years)	183 (23.0)			10 (7.4)		
Some middle school (7–8 years)	29 (3.6)			3 (2.2)		
Middle school graduate (9 years)	155 (19.4)			12 (8.9)		
Some high school (10–11 years)	31 (3.9)			1 (0.7)		
High school graduate (12 years)	335 (42.0)			93 (68.9)		
One or two years college (13–14 years)	37 (4.6)			11 (8.1)		
College graduate or more	27 (3.3)			5 (3.7)		
Missing	1 (< 0.1)			0 (0.0)		
Sex, male, n (%)	634 (79.4)			124 (91.9)		
Tobacco use, n (%)						
Never	254 (31.9)			35 (25.9)		
Current use	455 (57.1)			87 (64.4)		
Past use	88 (11.0)			13 (9.6)		
Alcohol use, n (%)						
Never	231 (29.0)			31 (23.0)		
Current use	518 (65.0)			95 (70.4)		
Past use	48 (6.0)			9 (6.7)		

NA, not applicable. The 4-hr urine collection was performed only in subjects who received DMSA.
[a]DMSA-chelatable lead (μg) was estimated as 4-hr urinary lead excretion after oral administration of 10 mg/kg DMSA, in lead-exposed subjects only (784 subjects completed the urine collection).

no significant association of hypertension status by current occupational lead exposure status (lead workers vs. controls without occupational exposure to lead), in crude [odds ratio (OR) (95% CI) = 0.7 (0.4, 1.3)] or adjusted analyses controlling for age, sex, body mass index, current alcohol use, and ALAD and VDR genotype [OR (95% CI) = 1.8 (0.9, 3.9)]. In controls, there was no effect modification by ALAD or VDR genotype on the relations of blood lead or tibia lead with the blood pressure measures, but the numbers of controls with the less prevalent genotypes were small (data not shown).

Predictors of systolic blood pressure. In linear regression models in lead workers only, VDR genotype (*BB* and *Bb* vs. *bb*), blood lead, tibia lead, and DMSA-chelatable lead were all positive predictors of systolic blood pressure, controlling for age (linear and quadratic terms), sex, body mass index, antihypertensive medication use, and cumulative lifetime alcoholic drinks (divided into quartiles) (Table 3). In the model with tibia lead (model 1, Table 3), adding blood urea nitrogen to the model increased the β coefficient for VDR genotype to 3.732 ($p = 0.02$). On average, lead workers with the VDR *B* allele had systolic blood pressure that was 2.7–3.7 mm Hg higher than did workers with the *bb* genotype (depending on the model). Models in which two lead dose measures were included at a time suggested that DMSA-chelatable lead was the best predictor of systolic blood pressure (models 4 and 5, Table 3). Neither ALAD nor VDR genotype was associated with systolic blood pressure in linear regression models with controls only, but there were only 11 and 13 controls with the *ALAD*[2] or VDR *B* alleles, respectively.

VDR genotype modified the relation of age with systolic blood pressure (model 6, Table 3 and Figure 1). Lead workers with the VDR *B* allele had larger elevations in blood pressure with increasing age than did lead workers with the VDR *bb* genotype. These elevations in blood pressure were also observed at younger ages. In contrast, ALAD genotype, VDR genotype, and age did not modify the relations of blood lead, tibia lead, DMSA-chelatable lead, sex, body mass index, or job duration with systolic blood pressure.

Predictors of diastolic blood pressure. On average, lead workers with VDR *BB* or *Bb* genotype had diastolic blood pressures that were 1.9 mm Hg higher than did lead workers with VDR *bb*, controlling for age, sex, body mass index, antihypertensive medication use, cumulative alcohol consumption, and blood lead levels ($p = 0.09$). After addition of 4-hr creatinine clearance to this model, lead workers with VDR *BB* or *Bb* genotypes had diastolic blood pressures that were 2.5 mm Hg higher than lead workers with the VDR *bb* genotype ($p = 0.04$). There were no significant associations of tibia lead, blood lead, DMSA-chelatable lead, job duration, or ALAD genotype with diastolic blood pressure (data not shown). ALAD and VDR genotype did not modify the relations of blood lead, tibia lead, DMSA-chelatable lead, or age with diastolic blood pressure.

Predictors of hypertension. In crude analysis, VDR genotype was associated with hypertension status; lead workers with the VDR *B* allele had a higher prevalence of hypertension compared to lead workers with the *bb* genotype [OR (95% CI) = 2.0 (1.1, 3.9); Table 4]. This association persisted after adjustment, using logistic regression, for age, sex, body mass index, tibia lead, and current alcohol use [OR = 2.1 (1.0, 4.4)]. In this model (Table 4), tibia lead was also a predictor of hypertension status [OR = 1.005 (1.000, 1.011) for tibia lead as a continuous variable, $p = 0.05$]. Blood lead, DMSA-chelatable lead, and ALAD genotype were not associated with hypertension status in the lead workers. ALAD genotype, VDR genotype, and age did not modify the relations of the three lead dose measures with hypertension status.

Discussion

In the lead workers under study, blood lead, tibia lead, and DMSA-chelatable lead were all predictors of systolic blood pressure; none of these three lead dose measures was associated with diastolic blood pressure; and tibia lead was the only predictor of hypertension status. Taken as a whole, the associations of the lead biomarkers with the blood pressure measures and hypertension suggest that both acute and chronic mechanisms may be involved in the relations of lead exposure and blood pressure (*2*,*41*). An interesting new observation was that, on average, lead workers with the VDR *B* allele, compared to lead workers with the VDR *bb* genotype, had higher systolic blood pressure, diastolic blood pressure, and prevalence of hypertension, even after adjustment for important confounding variables. Furthermore, VDR genotype modified the relation of age with systolic blood pressure; lead workers with the *B* allele had earlier and larger elevations of systolic blood pressure with increasing age at the study visit. This suggests that release of lead from bone stores, which may be influenced by VDR genotype (*4*), may play a role in the observed blood pressure elevations.

Our *a priori* expectation was that ALAD and VDR genotypes would directly influence blood lead, tibia lead, and DMSA-chelatable lead levels (*3*,*4*). We thus did not expect that the two genotypes would modify the relations among the lead biomarkers and the blood pressure measures because, first, the three lead biomarkers were measured

Table 3. Linear regression modeling of systolic blood pressure, Korean lead workers, 1997–1999.[a]

Independent variable	Units of coefficient	Coefficient	SE	*p*-Value	Model r^2
Model 1					0.32
Tibia lead	mm Hg per µg/g	0.024	0.013	0.07	
VDR, *Bx* vs. *bb*	mm Hg	3.236	1.555	0.04	
ALAD, 12 vs. 11	mm Hg	0.554	1.624	0.73	
Model 2					0.32
Blood lead	mm Hg per µg/dL	0.069	0.037	0.06	
VDR, *Bx* vs. *bb*	mm Hg	2.858	1.570	0.07	
ALAD, 12 vs. 11	mm Hg	0.234	1.633	0.89	
Model 3					0.31
DMSA-chelatable lead	mm Hg per µg	0.006	0.003	0.04	
VDR, *Bx* vs. *bb*	mm Hg	2.720	1.581	0.09	
ALAD, 12 vs. 11	mm Hg	0.737	1.653	0.66	
Model 4					0.30
Tibia lead	mm Hg per µg/g	0.017	0.014	0.22	
Blood lead	mm Hg per µg/dL	0.058	0.040	0.15	
VDR, *Bx* vs. *bb*	mm Hg	3.513	1.575	0.03	
Model 5					0.29
Tibia lead	mm Hg per µg/g	0.010	0.014	0.46	
DMSA-chelatable lead	mm Hg per µg	0.007	0.003	0.04	
VDR, *Bx* vs. *bb*	mm Hg	3.293	1.596	0.04	
Model 6					0.32
VDR, *Bx* vs. *bb*	mm Hg	2.411	2.212	0.27	
Age	mm Hg per year	0.259	0.061	< 0.01	
Age^2	mm Hg per year^2	0.027	0.005	< 0.01	
Age × VDR × *Bx* vs. *bb*[b]	mm Hg per year	0.363	0.151	0.02	
Age × VDR × *Bx* vs. *bb*[b]	mm Hg per year^2	0.001	0.015	0.94	

[a]In addition to variables listed under each model, models also controlled for age (linear and quadratic terms), sex, body mass index, antihypertensive medication use, and cumulative lifetime drinks in current alcohol users [one glass of beer or wine or one shot of distilled spirits, divided into quartiles (0–1,612 drinks, 1,613–4,160 drinks, 4,161–10,920 drinks, and > 10,920 drinks)]. [b]Cross-product terms between age (linear and quadratic terms) and VDR genotype. The relation of age with systolic blood pressure in subjects with the VDR *bb* genotype is described by the coefficients for age and age^2. In subjects with the VDR *B* allele, the coefficients for the cross-product terms must be added to the respective coefficients for age and age^2 to describe the relation of age with systolic blood pressure. This relation is plotted in Figure 1.

directly, thus accounting for any genetic influence, and, second, lead is not likely to be influencing blood pressure via the ALAD or VDR gene products. Our data suggest that lead and VDR genotype are each independently associated with blood pressure; VDR modifies the toxicokinetics of lead, not the direct influence of lead on blood pressure.

The magnitude of the VDR genotype effect was relatively large. On average and after controlling for covariates, subjects with the *B* allele, mainly heterozygotes, had systolic blood pressures that were approximately 2.7–3.7 mm Hg higher than those of lead workers with the *bb* genotype; and had a 2-fold increased risk of hypertension. The magnitude of the VDR association was largest when blood urea nitrogen was added to the regression models of systolic blood pressure, raising the possibility that lead, the VDR, and the kidney independently contribute to elevations in blood pressure. Prior studies of bone mineral density suggest that the *B* allele has a dose effect, in that the influence of the allele increases with more copies (*25,40,42,43*). If this is the case with blood pressure, then the average effect of the *B* allele may be even larger in populations with larger numbers of *BB* homozygotes. The *BB* genotype has a prevalence of 7–32% in Caucasians (*25*), but only 0.5% of the Korean lead workers had that genotype.

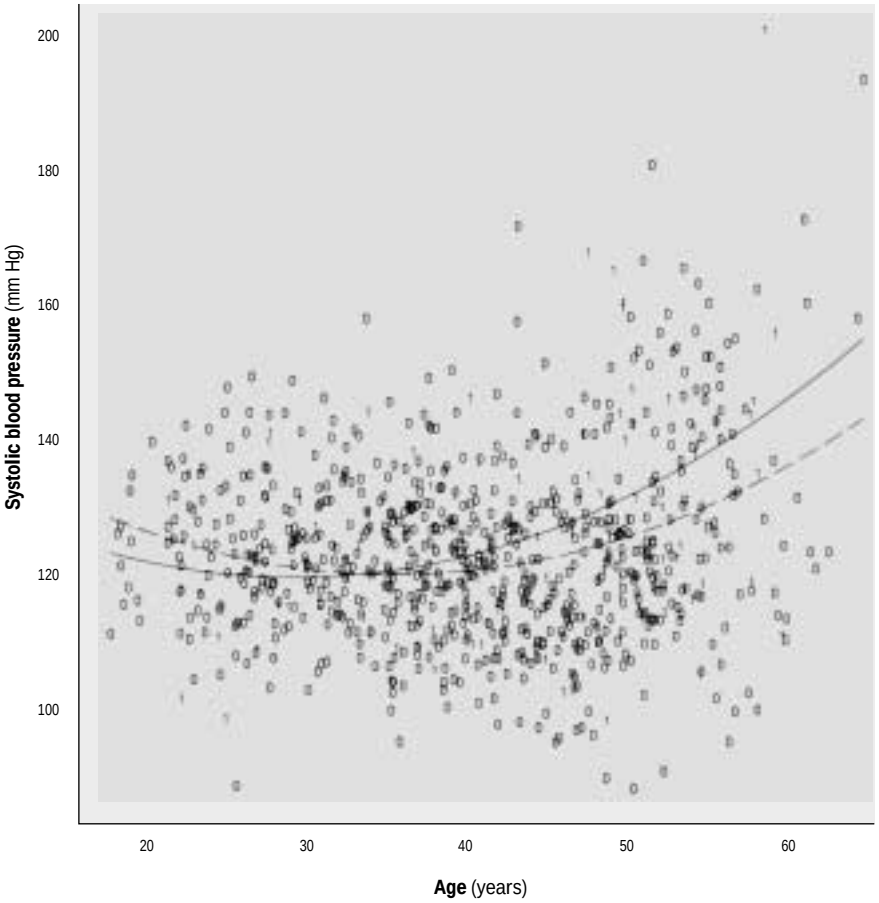

Figure 1. Effect modification by VDR genotype on the relation between age and systolic blood pressure, controlling for age (linear and quadratic terms), sex, body mass index, antihypertensive medication use, and cumulative lifetime alcohol dose (divided into quartiles), in 794 Korean lead workers. The solid line and "1" are for subjects with the VDR *B* allele; the dashed line and "0" are for subjects with the VDR *bb* genotype. This is a plot of the results of model 6, Table 3.

Table 4. Associations of VDR genotype with hypertension status in Korean lead workers.

VDR genotype	Hypertension, *n* (%)[a] Yes	No	Total	Crude OR (95% CI)[b]	Adjusted OR (95% CI)[c]
bb	64 (9.0)	645 (91.0)	709 (100)	1.0	1.0
Bb or *BB*	15 (16.9)	74 (83.1)	89 (100)	2.0 (1.1, 3.9)	2.1 (1.0, 4.4)*
Total	79 (9.9)	719 (90.1)	798 (100)		

[a]Hypertension defined as systolic blood pressure > 160 mm Hg or diastolic blood pressure > 96 mm Hg or currently taking medications for high blood pressure. [b]Subjects with VDR *bb* are the reference group. [c]Adjusted for age, sex, body mass index, tibia lead, and current alcohol use. *$p < 0.05$.

A large body of literature reveals that the ALAD genotype modifies the toxicokinetics of lead (*5*); we were thus interested in evaluating whether these toxicokinetic modifications could influence lead's effect on blood pressure. Human ALAD is encoded by a single gene on chromosome 9p34 (*6,7*). The prevalence of the *ALAD*^{2} allele is approximately 10% in Asians and 20% in Caucasians (*8–10*). Subjects who have at least one copy of the *ALAD*^{2} allele, compared to subjects with none, have higher blood lead levels (*8–10*), lower DMSA-chelatable lead levels (*11*), lower plasma aminolevulinic acid levels (*12*), a larger difference between trabecular and cortical bone lead levels (*13*), higher blood urea nitrogen and serum creatinine levels (*13*), less efficient uptake of lead into bone, especially trabecular (*14*), lower zinc protoporphyrin levels for given levels of blood lead (*15*), and lower urinary calcium and creatinine levels (*16*). ALAD has been identified as a principal lead-binding protein, and the proportion of lead bound to ALAD was greater for subjects with *ALAD*^{2} (*17*). These observations suggested to us that ALAD genotype could modify the influence of lead on blood pressure, but the current data did not support this hypothesis. It is possible that in populations with a higher prevalence of the *ALAD*^{2} allele and with different distributions of other important genes such as VDR, ALAD genotype may modify the influence of lead on blood pressure and other health outcomes.

A second gene that has recently been the focus of lead research is that for VDR, located at chromosome 12cen-12 (*44*). Most studies of the VDR gene have focused on the *Bsm*I polymorphism and the three resulting genotypes termed *bb*, *Bb*, and *BB* (although the *Fok*I polymorphism has been receiving increasing attention). Study subjects (mainly women) with the *BB* genotype have bone mineral densities up to 10–15% lower than subjects with the *bb* genotype (*25,40,42, 43,45*), with an overall difference across studies of 2–2.5% reported in a recent meta-analysis (*25*). We recently reported that subjects with the *B* allele had larger tibia lead concentrations with increasing age and lower tibia lead concentrations with increasing duration since last exposure to lead than did subjects without the *B* allele (*4*). In another study of VDR genotype in lead workers, of the subjects reported here, lead workers with the VDR *B* allele had significantly ($p < 0.05$) higher blood lead levels (on average, 4.2 µg/dL), chelatable lead levels (on average, 37.3 µg), and tibia lead levels (on average, 6.4 µg/g) than did workers with the VDR *bb* genotype, controlling for covariates (*3*). Now we provide evidence that VDR genotype also had a direct effect on blood pressure and

modified the elevations in blood pressure associated with age. VDR genotype did not modify the relations of the lead dose measures with blood pressure.

The most critical role of the active form of vitamin D, 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$], is the activation of genes involved in intestinal calcium transport (*46*). $1,25(OH)_2D_3$ binds to the VDR, and the activated VDR regulates the rate of transcription of vitamin D-responsive genes (*46*). The VDR is found in intestine, bone, kidney, parathyroid glands, hematopoietic tissues, immune tissues, muscle, heart, skin, pancreas, and other sites, and $1,25(OH)_2D_3$ has recognized actions in all these tissues (*46*). Several genetic polymorphisms have been found within the VDR, and these have been implicated to influence bone mineral density (*25*), the risk of primary hyperparathyroidism (*47*), parathyroid hormone levels and tubular resorption of phosphate (*48*), the response of psoriasis to vitamin D therapy (*49*), urinary calcium excretion and the risk of nephrolithiasis (*50*), and serum osteocalcin levels (*40*).

The links among calcium, lead, and blood pressure have been increasingly recognized. Calcium supplementation lowers blood pressure (*51*); several studies have reported that increased dietary calcium, especially from dairy products, is associated with lower blood pressure (*52–54*); and lower vitamin D intake has been associated with higher blood pressure in women (*55*). Moderate lead levels can cause elevations in serum $1,25(OH)_2D_3$ and parathyroid hormone levels (*56,57*). Lower dietary intake of calcium has been associated with higher serum parathyroid levels, and women with these higher levels had significantly higher blood pressures (*58*).

The functional significance of the *Bsm*I polymorphism is unclear because it is not located at exon–intron boundaries, would not influence the structure of the VDR, and is not known to produce splicing errors; and recent *in vitro* studies have not demonstrated differences in VDR expression or cellular responsiveness to vitamin D treatment by genotype (*24,59*). This suggests that the *Bsm*I polymorphism may be in linkage disequilibrium with another functional variant at the VDR locus or with a nearby bone metabolism gene (*24*). It is not known how the VDR polymorphism could influence blood pressure, but the links among lead, calcium, VDR, and blood pressure would clearly suggest biologic plausibility.

An important question is whether selection bias could account for the study results. Evidence from this study and prior ones (*3,8*) suggests that there may be selective movement of workers, by gentoypes, out of lead-using workplaces. For selection by genotype or other factors to account for the observed association of the VDR *B* allele with blood pressure and hypertension, such a factor would have to increase the prevalence of VDR *B* and elevated blood pressure. While this type of selection is possible, it requires a complex interplay of a number of factors. First, there would have to be a behavioral response to lead exposure, perhaps mediated by development of symptoms, that would motivate persons to leave the workplace. Second, the behavioral response would have to be associated with both the VDR *B* allele and elevated blood pressure. We think this complex model of selection bias is unlikely to explain the association we observed. However, longitudinal analysis is less susceptible to these biases, and will be used in the future, after the completion of data collection, to evaluate these associations further.

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