Associations of Blood Lead, Dimercaptosuccinic Acid-Chelatable Lead, and Tibia Lead with Polymorphisms in the Vitamin D Receptor and δ-Aminolevulinic Acid Dehydratase Genes

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A cross-sectional study was performed to evaluate the influence of polymorphisms in the δ-aminolevulinic acid dehydratase (ALAD) and vitamin D receptor (VDR) genes on blood lead, tibia lead, and dimercaptosuccinic acid (DMSA)-chelatable lead levels in 798 lead workers and 135 controls without occupational lead exposure in the Republic of Korea. Tibia lead was assessed with a 30-min measurement by ^{109}Cd-induced K-shell X-ray fluorescence, and DMSA-chelatable lead was estimated as 4-hr urinary lead excretion after oral administration of 10 mg/kg DMSA. The primary goals of the analysis were to examine blood lead, tibia lead, and DMSA-chelatable lead levels by ALAD and VDR genotypes, controlling for covariates; and to evaluate whether ALAD and VDR genotype modified relations among the different lead biomarkers. There was a wide range of blood lead (4–86 μg/dL), tibia lead (−7–338 μg Pb/g bone mineral), and DMSA-chelatable lead (4.8–2,103 μg) levels among lead workers. Among lead workers, 9.9% (n = 79) were heterozygous for the ALAD^2 allele and there were no homozygotes. For VDR, 10.7% (n = 85) had the Bb genotype, and 0.5% (n = 4) had the BB genotype. Although the ALAD and VDR genes are located on different chromosomes, lead workers homozygous for the ALAD^1 allele were much less likely to have the VDR bb genotype (crude odds ratio = 0.29, 95% exact confidence interval = 0.06–0.91). In adjusted analyses, subjects with the ALAD^2 allele had higher blood lead levels (on average, 2.9 μg/dL, p = 0.07) but no difference in tibia lead levels compared with subjects without the allele. In adjusted analyses, 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. The current data confirm past observations that the ALAD gene modifies the toxicokinetics of lead and also provides new evidence that the VDR gene does so as well. *Key words*: δ-aminolevulinic acid dehydratase, bone lead, cross-sectional study, lead, polymorphisms, vitamin D receptor, X-ray fluorescence. *Environ Health Perspect* 108:949–954 (2000). [Online 31 August 2000]
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An increasing body of evidence suggests that genetic factors modify the toxicokinetics of lead. The gene for the δ-aminolevulinic acid dehydratase (ALAD) enzyme has been a focus of primary interest. Human ALAD is encoded by a single gene on chromosome 9p34 that has two alleles, ALAD^1 and ALAD^2, resulting in three isozymes, ALAD1-1, ALAD1-2, and ALAD2-2 (*1*,*2*). The prevalence of the ALAD^2 allele is approximately 10% in Asians and 20% in Caucasians (*3*–*5*). Subjects who have at least one copy of the ALAD^2 allele, compared to subjects with none, have higher blood lead levels (*3*–*5*); lower dimercaptosuccinic acid (DMSA)-chelatable lead levels (*6*); lower plasma aminolevulinic acid levels (*7*); a larger difference between trabecular and cortical bone lead levels (*8*); higher blood urea nitrogen and serum creatinine levels (*8*); less efficient uptake of lead into bone, especially trabecular bone (*9*); lower zinc protoporphyrin (ZPP) levels for given levels of blood lead (*10*); and lower urinary calcium and creatinine levels (*11*). 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 (*12*).

Recent data suggest that polymorphisms in the vitamin D receptor (VDR) gene influence tibia lead levels (*13*). The VDR gene is located at chromosome 12cen-12 (*14*), and thus variant VDR alleles would not be expected to be linked with variant ALAD alleles. Most studies of the VDR gene have focused on the *Bsm*I polymorphism; restriction enzyme digestion produces three genotypes commonly termed bb, Bb, and BB. The BB allele (defined by the absence of the polymorphic restriction site) has a prevalence of 7–32% in Caucasians (*15*). Study subjects (mainly women) with the BB genotype have bone mineral densities up to 10–15% lower than subjects with the bb genotype, with an overall difference across studies of 2–2.5% reported in a recent meta-analysis (*15*). As lead and calcium are known to behave similarly in biologic systems, these findings have motivated investigations of VDR genotype and bone lead levels. 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 (*13*).

No prior studies have evaluated the joint influence of the VDR and ALAD genes on bone, blood, and chelatable lead levels. Here we report the largest such study to date in a cross-sectional analysis of 798 Korean lead workers and 135 controls without occupational lead exposure.

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 (*16*). Enrollment began in October 1997 with the first of three annual evaluations for each study subject. The current report is focused on the influence of the ALAD and VDR polymorphisms on blood lead, tibia lead, and dimercaptosuccinic acid (DMSA)-chelatable lead levels measured during this first study evaluation and is based on the 798 lead workers and 135 controls without occupational lead exposure who were 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

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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% (*16*). 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, government-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 employees of Soonchunhyang University.

Data collection. Data collection methods have been previously reported (*16*). In brief, data were collected either at the Institute of Industrial Medicine at Soonchunhyang University in Chonan or on the premises of the lead-using facilities. The following data 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 by venipuncture that was stored at −70°C as whole blood, plasma, and red blood cells; a spot urine sample; tibia lead concentration by X-ray fluorescence; and a 4-hour urine sample after oral administration of DMSA (in lead workers only).

Laboratory methods. Hemoglobin was assayed by the cyanmethemoglobin method (Beckman Coulter, Inc., Model Ac-T 8, Fullerton, CA), and hematocrit was measured by the capillary centrifugation method (*17*). Zinc protoporphyrin levels were measured with a portable hematofluorimeter (*18*). Urinary creatinine was measured using a Sigma kit (St. Louis, MO) (*19*). Blood lead levels were measured with a Zeeman background-corrected atomic absorption spectrophotometer (Hitachi Z-8100 model; Hitachi, Ltd., Tokyo, Japan) with the standard addition method of the National Institute of Occupational Safety and Health (*20*) at Soonchunhyang University Institute of Industrial Medicine, a certified reference laboratory for lead in Korea. Tibia lead was assessed, in units of micrograms lead per gram bone mineral, with a 30 min measurement at the left mid-tibial shaft using ^{109}Cd-induced K-shell X-ray fluorescence (XRF), as previously described (*21–23*). 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 (*24*).

We used 4-hour urinary lead excretion after oral administration of 10 mg/kg DMSA to measure DMSA-chelatable lead (*25*). Urine lead levels were measured in the laboratories of the Wadsworth Center at the New York State Department of Health, Albany, New York. Urinary lead concentrations were determined by electrothermal atomization atomic absorption spectrometry (Perkin-Elmer Model 4100ZL, Norwalk, CT) using previously published methods (*26*). Urinary lead excretion was highly correlated with lead excretion adjusted for differences, generally small, in urine collection times (Pearson's $r = 0.98$), so only the unadjusted data are presented.

ALAD and VDR genotyping. We completed ALAD and VDR genotyping on 798 and 795 subjects, respectively. VDR genotyping was completed using previously published methods (*13*). In brief, genomic DNA was extracted from whole blood by 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′-CAACCAAGACTACAAGTACC-GCGTCAGTGA-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.

A modified PCR-based protocol was used for ALAD genotyping and has been previously described (*3–5*). 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 uses a pair of nested primers (provided by J. Wetmur), sequences (5′-CAGAGCTGTTCCAACAGTGGA-3′) and (5′-CCAGCACAATGTGGGAGTGA-3′), respectively, and generates an 887 base pair fragment. The amplified fragment was cleaved at the diagnostic *Msp*1 site, only present in the $ALAD^2$ allele, and three isozymes are observed, designated ALAD1-1, ALAD1-2, and ALAD2-2.

Statistical analysis. The primary goals of the analysis were to examine blood lead, tibia lead, and DMSA-chelatable lead levels by ALAD and VDR genotypes, controlling for covariates; and to evaluate whether ALAD and VDR genotype modified relations among the different lead biomarkers. Associations between ALAD and VDR genotype were evaluated in contingency tables using odds ratios (ORs) and 95% exact confidence intervals (CIs) calculated with Epi Info version 6.04b (Centers for Disease Control and Prevention, Atlanta, GA).

We used linear regression to separately model blood lead, tibia lead, and DMSA-chelatable lead levels, controlling for confounding variables, using statistical software programs of SAS Institute, Inc. (Cary, NC). In these regression models, only lead workers, not controls, were included. Covariates examined in linear regression models included age, sex, creatinine clearance (4-hr), hemoglobin, hematocrit, weight, height, body mass index, job duration, and tobacco and alcohol consumption (never, previous, and current use for each). Covariates were retained in the final regression models if they were either a significant predictor of blood lead, tibia lead, or DMSA-chelatable lead levels, or if they were a confounder of the relations between predictor variables and the lead biomarkers. The decisions regarding the variables in the final regression models were also made to be consistent with prior analyses of the data on the subjects presented here (*23*). Blood lead was modeled with and without adjustment for hematocrit; as this adjustment did not influence regression results, only unadjusted model results are presented.

DMSA-chelatable lead and tibia lead were log-transformed before regressing on covariates because of departures from normality. To estimate the mean adjusted differences between genotypes in the original scale of each lead measure, we exponentiated the predicted value from the regression, separately for each genotype, at the mean value of all continuous covariates and the reference value of all dichotomous covariates. To evaluate nonlinear relations, quadratic terms for continuous variables (i.e., age, job duration, weight, height) were evaluated. We evaluated effect modification by genotype by including cross-product terms between the genetic variables and relevant predictor variables (i.e., age, sex, tibia lead, creatinine clearance).

Results

Demographics and dose measures. Compared to controls without occupational lead exposure, 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 a lower proportion were male (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. Among lead workers, tibia lead was moderately correlated with blood lead (Pearson's $r = 0.42$), DMSA-chelatable lead ($r = 0.43$), and job duration ($r = 0.40$) (all p-values < 0.01). The correlations of blood lead ($r = 0.13$) and DMSA-chelatable lead ($r = 0.17$) with job duration were much lower than were the correlations of these variables with tibia lead. Blood lead was highly correlated with DMSA-chelatable lead ($r = 0.82$).

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; 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 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, all subsequent analysis combined homozygous and heterozygous variant allele carriers.

In unadjusted (crude) analyses, there were no differences in age, job duration, hemoglobin, blood lead, tibia lead, or DMSA-chelatable lead by ALAD genotype (Table 2). In contrast, lead workers with the Bb or BB genotypes, compared to those with bb, were older and had higher DMSA-chelatable lead levels (both p-values < 0.05, Table 2).

The ALAD gene is located on chromosome 9, and the VDR gene is on chromosome 12. Nonetheless, an association was observed between the ALAD and VDR genotypes (Table 3). Among lead workers, subjects homozygous for the $ALAD^1$ allele were much less likely to have the VDR bb genotype (crude OR = 0.28; 95% CI, 0.06–0.89). In contrast, among controls, subjects homozygous for the $ALAD^1$ allele were more likely to have the VDR bb genotype (crude OR = 2.53; 95% CI, 0.23–14.84). Although there were only two controls with ALAD1-2 and VDR Bb or BB, in the stratified analysis, the ORs between the two genes among lead workers and controls were significantly different (test for homogeneity of stratum-specific ORs, $p = 0.04$).

Predictors of blood lead levels in lead-exposed subjects. After adjustment for age, sex, and current tobacco use using linear regression, lead workers with the VDR B polymorphism had higher blood lead levels ($p < 0.01$), in models with or without control for ALAD genotype (model with ALAD genotype; Table 4). Subjects with the $ALAD^2$ allele also had higher blood lead levels ($p < 0.05$) in models without and with control for VDR genotype (model with VDR genotype; Table 4). On average, after controlling for the two genotypes, lead workers with the VDR B polymorphism had blood lead levels 4.2 µg/dL higher than subjects with bb, and lead workers with the $ALAD^2$ allele had blood lead levels 3.6 µg/dL higher than subjects without the allele. There was no evidence of gene–gene interaction in these models (evaluated by

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

Characteristic	Lead-exposed subjects ($n = 798$)	Controls ($n = 135$)
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[a]
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)[b]	186.0 ± 208.4 (4.8–2,103)	NA[a]
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[a]
Educational level[c]		
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[c]	634 (79.4)	124 (91.9)
Tobacco use[c]		
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[c]		
Never	231 (29.0)	31 (23.0)
Current use	518 (65.0)	95 (70.4)
Past use	48 (6.0)	9 (6.7)

Values shown are mean ± SD except where indicated.
[a]The 4-hr urine collection was performed only in subjects who received DMSA. [b]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).[c]Values shown are number (%)

Table 2. Selected demographic and lead biomarker variables (mean ± SD) by gene status in 798 lead-exposed subjects, October 1997 to August 1999, Republic of Korea.[a]

Characteristic	ALAD genotype		VDR genotype	
	1-1	1-2	bb	Bb or BB
Number	716	79	709	89
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
Hemoglobin (g/dL)	14.2 ± 1.4	14.2 ± 1.6	14.2 ± 1.4	14.1 ± 1.4
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*

[a]ALAD and VDR genotyping were completed on 795 and 798 lead workers, respectively. *$p < 0.05$.

Table 3. Association of VDR genotype status by ALAD genotype status.

ALAD	VDR genotype: bb, n (%)[a]	VDR genotype: Bb or BB, n (%)[a]	Total	OR (95% CI)[b]
All study participants				
1-1	743 (80.0)	96 (10.3)	839 (90.3)	0.46 (0.14–1.15)
1-2	85 (9.2)	5 (0.5)	90 (9.7)	
Total	828 (89.1)	101 (10.9)	929 (100)	
Lead workers				
1-1	629 (79.2)	86 (10.8)	715 (90.1)	0.29 (0.06–0.91)
1-2	76 (9.6)	3 (0.4)	79 (10.0)	
Total	705 (88.8)	89 (11.2)	794 (100)	
Controls without occupational lead exposure				
1-1	114 (84.4)	10 (7.4)	124 (91.9)	2.53 (0.23–14.84)
1-2	9 (6.7)	2 (1.5)	11 (8.1)	
Total	123 (91.1)	12 (8.9)	135 (100)	

[a]Percentage of table totals. [b]Test for homogeneity of stratum-specific ORs, $p = 0.04$, indicating that the association of the two genotypes in lead workers and controls was different.

inclusion of an ALAD–VDR cross-product term). There was also no evidence of effect odification by ALAD or VDR genotype on the relations of the predictor variables with blood lead levels. The final linear regression model (Table 4) accounted for 35% of the variance in blood lead levels.

Predictors of DMSA-chelatable lead levels in lead-exposed subjects. After adjustment for covariates (age, sex, current tobacco use, body mass index, and 4-hr creatinine clearance), subjects with the VDR B polymorphism had higher DMSA-chelatable lead levels (on average, 32%, or 37.3 µg higher than subjects with VDR bb, $p < 0.01$). In contrast, the $ALAD^2$ allele was not significantly associated with chelatable lead levels ($p = 0.69$). The relation between creatinine clearance and DMSA-chelatable lead was modified by ALAD genotype (Table 5, model 3). The intercept for lead workers with the $ALAD^2$ allele was 59% lower than for lead workers with the $ALAD^1$ allele ($p = 0.05$). Among lead workers with only the $ALAD^1$ allele, chelatable lead levels increased 5.8 µg for each increase of 10 mL/min in creatinine clearance near its mean value ($p < 0.01$); in contrast, among lead workers with the $ALAD^2$ allele, chelatable lead levels increased 15.5 µg for each increase of 10 mL/min in creatinine clearance near its mean value (difference in two slopes, $p = 0.04$). The final linear regression models accounted for 25–26% of the variance in DMSA-chelatable lead levels. Addition of blood lead to the models of DMSA-chelatable lead increased the model r^2 to 79–80%. There were no interactions in these models between blood lead and either of the two genes.

Predictors of tibia lead levels in lead-exposed subjects. After adjustment for age (linear and quadratic terms), sex, job duration, and body mass index, ALAD genotype was not associated with tibia lead levels (Table 6, model 1, $p = 0.73$), but VDR genotype was associated (Table 6, model 2, $p = 0.03$). On average, subjects with the VDR B allele had tibia lead levels that were 29%, or 6.4 µg/g, higher than did subjects without the allele. The final regression models accounted for 15% of the variance in tibia lead levels.

VDR genotype reportedly influences bone mineral density, so we examined the influence of VDR genotype on age- and sex-associated differences in tibia lead levels. There were no interactions observed between VDR genotype and age on tibia lead levels in all lead workers or in specific subgroups (i.e., males, females, older subjects), and no interaction between VDR genotype and sex on tibia lead levels.

Predictors of lead biomarkers in controls without occupational lead exposure. In linear regression models including only controls, none of these genetic associations with blood lead, DMSA-chelatable lead, or tibia lead levels were observed.

Discussion

The current study confirms past observations that the ALAD gene modifies the toxicokinetics of lead and also provides new evidence that the VDR gene does so as well. In fact, the influence of the VDR B allele on blood lead levels was larger than was the influence of the $ALAD^2$ allele. The mechanism by which these genes influence blood lead levels may differ. $ALAD^2$ and VDR B were associated with higher blood lead levels; however, only VDR B was associated with higher tibia lead levels ($p = 0.03$).

In adjusted analyses, subjects with the VDR B allele had significantly ($p < 0.05$) higher blood lead levels (on average, 4.2

Table 4. Linear regression modeling of blood lead, Korean lead workers, 1997–1999.[a]

Independent variable	Units of β coefficient	β coefficient	β SE	*p*-Value
Age	µg/dL/year	0.286	0.049	< 0.001
Female	µg/dL	−13.782	1.382	< 0.001
Current smoker	µg/dL	3.406	1.081	0.002
Tibia lead	µg/dL/µg/g	0.131	0.011	< 0.00
VDR, Bx vs. bb	µg/dL	4.183	1.376	0.002
ALAD, 12 vs. 11	µg/dL	3.627	1.445	0.01

[a]Model $r^2 = 0.35$.

Table 5. Linear regression modeling of DMSA-chelatable lead, Korean lead workers, 1997–1999.

Independent variable	Units of β coefficient	β coefficient	β SE	*p*-Value	Model r^2
Model 1					0.26
Age	µg/year	0.033	0.004	< 0.001	
Female	µg	–0.958	0.105	< 0.001	
Current smoker	µg	0.299	0.080	< 0.001	
Body mass index	µg/kg/cm^2	0.010	0.012	0.40	
Creatinine clearance	µg/mL/min	0.006	0.001	< 0.001	
VDR, Bx vs. bb	µg	0.282	0.103	0.006	
Model 2					0.25
Age	µg/year	0.034	0.004	< 0.001	
Female	µg	–0.946	0.106	< 0.001	
Current smoker	µg	0.310	0.081	< 0.001	
Body mass index	µg/kg/cm^2	0.013	0.012	0.26	
Creatinine clearance	µg/mL/min	0.006	0.001	< 0.001	
ALAD, 12 vs. 11	µg	0.044	0.108	0.69	
Model 3					0.26
Age	µg/year	0.034	0.004	< 0.001	
Female	µg	–0.937	0.106	< 0.001	
Current smoker	µg	0.307	0.081	< 0.001	
Body mass index	µg/kg/cm^2	0.012	0.012	0.29	
Creatinine clearance	µg/mL/min	0.005	0.001	< 0.001	
ALAD, 12 vs. 11	µg	–0.890	0.458	0.05	
ALAD × creatinine clearance[a]	µg/mL/min	0.008	0.004	0.04	

[a]DMSA-chelatable lead was log-transformed for these regressions because of departure from normality.

Table 6. Linear regression modeling of tibia lead, Korean lead workers, 1997–1999.[a]

Independent variable	Units of β coefficient	β coefficient	β SE	*p*-Value
Model 1[b]				
Age	µg/g/ year	0.014	0.005	0.002
Age^2	µg/g/year^2	0.001	0.0003	0.003
Female	µg/g	–0.407	0.104	< 0.001
Job duration	µg/g/year	0.048	0.007	< 0.001
Body mass index	µg/g/kg/cm^2	0.033	0.013	0.01
ALAD (12 vs. 11)	µg/g	0.042	0.122	0.73
Model 2[b]				
Age	µg/g/year	0.013	0.005	0.006
Age^2	µg/g/year^2	0.001	0.0003	0.002
Female	µg/g	–0.412	0.103	< 0.001
Job duration	µg/g/year	0.050	0.007	< 0.001
Body mass index	µg/g/kg/cm^2	0.030	0.013	0.02
VDR (Bx vs. bb)	µg/g	0.254	0.117	0.03

[a]Tibia lead was log-transformed for these regressions because of departure from normality. [b]Model $r^2 = 0.15$.

µg/dL), chelatable lead levels (on average, 37.3 µg), and tibia lead levels (on average, 6.4 µg/g) than did subjects with the VDb genotype. VDR genotype did not modify relations between such factors as age, sex, and renal function and any of the lead dose measures. In part, these observations may be explained by the greater intestinal absorption of lead, or greater uptake and subsequent release of lead from bone, in individuals with VDR B (*13*,*27*–*32*). Vitamin D, after binding to the VDR receptor, increases intestinal absorption of calcium and lead. The VDR B allele has been associated with lower bone mineral densities and higher tibia lead levels (*13*,*15*), but the mechanism underlying these observations is not currently known. Interpretation of the observation that lead workers with the VDR B allele have higher tibia lead levels than do workers with VDR bb is complicated by the fact that VDR genotype is likely to influence the content of both calcium and lead in bone and tibia lead concentration as measured by XRF is standardized to bone mineral content. Thus, higher tibia lead concentrations can be due to higher lead content, lower calcium content, or both.

In adjusted analyses, subjects with the $ALAD^2$ allele had higher blood lead levels (on average, 3.6 µg/dL; $p = 0.01$) but no differences in tibia or chelatable lead levels compared to subjects without the allele. Creatinine clearance was an important predictor of chelatable lead levels and ALAD genotpe modified the relation between creatinine clearance and chelatable lead. Subjects with the $ALAD^2$ allele had larger increases in chelatable lead levels with increasing creatinine clearance than did subjects without the allele. We previously reported that subjects with $ALAD^2$ had lower DMSA-chelatable lead levels than did lead workers with $ALAD^1$ (*6*). It is important to note that in the previous study, DMSA was administered at 5 mg/kg, and mean DMSA-chelatable lead levels were approximately half of those in the current study, in which workers were administered 10 mg/kg DMSA. These data are consistent with earlier observations that the $ALAD^2$ allele increases erythrocytic binding of lead (*3*–*12*). This increase in intra-erythrocytic lead may decrease the relative deposition of lead in critical target organs and thus protect against the toxicity of lead.

An unexpected observation was that ALAD and VDR genotypes were associated. These genes are located on different chromosomes. Lead workers with the ALAD1-1 genotype were much less likely to have the VDR bb genotype (OR = 0.29, $p < 0.05$). Although this observation has to be interpreted with caution because only three lead workers had the ALAD1-2 and VDR Bb or BB genotypes, the exact confidence interval did not include 1.0. The data also suggested that the association between the two genotypes in the controls without occupational lead exposure was different, in that controls with the ALAD1-1 genotype were more likely to have the VDR bb genotype (OR = 2.5), and the stratum-specific ORs were significantly different. This is not a stable estimate, however, due to small cell sizes, and requires confirmation. One study reported that differential selection in the lead industry may occur by ALAD genotype (*3*), and the current data are further evidence that genetic factors may influence the duration of work in the lead industry.

To date, data would suggest that the $ALAD^1$ allele is more likely to confer health risks associated with lead exposure. For the VDR genotype, data are insufficient to determine whether the polymorphisms are likely to modify health risks due to lead and which allele is the allele of risk. We speculate that the alleles for either ALAD or VDR that confer health risk should become less prevalent with increasing duration of occupational exposure to lead (*3*); this could occur, for example, if the at-risk alleles are associated with the development of acute symptoms that increase the probability of quitting jobs with lead exposure. We have no information in former lead workers on either symptoms or work duration by ALAD or VDR genotype, and serial blood lead measurements from the start of employment are not available for the majority of lead workers. These limitations weaken the inferences that we can make at this time. However, compared to the controls, it appears that lead workers have higher prevalences of both the $ALAD^2$ and VDR B alleles (10.3% vs. 8.1% and 11.4% vs. 8.9%, respectively). This observation would support the inference that the $ALAD^2$ allele is "protective," as is the VDR B allele, and that there may be selection by genotype among lead workers, but this speculation requires further study.

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