CME Article #2

Associations of Patella Lead With Polymorphisms in the Vitamin D Receptor, δ-Aminolevulinic Acid Dehydratase and Endothelial Nitric Oxide Synthase Genes

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Learning Objectives

- Summarize the demographic factors that influenced the patella lead concentration in this study of lead-exposed workers.
- Examine the association between genotypes for three genes and patella lead concentration after adjusting for potential confounding factors, as well as the relationship between patella and blood lead levels.
- Explain how genotype and age interact in determining patella lead concentration

Abstract

*A cross-sectional analysis was performed to evaluate associations of polymorphisms in the vitamin D receptor (VDR), δ-aminolevulinic acid dehydratase (ALAD), and endothelial nitric oxide synthase (eNOS) genes with patella lead concentrations in 652 lead workers in the Republic of Korea. There was a wide range of patella lead (from below detection limit to 946 μg Pb/g bone mineral), with a mean (standard deviation) of 75.2 (101.0). There were no associations of ALAD or eNOS genotypes with patella lead, but workers with the VDR B allele had significantly (*P *value <0.05) higher patella lead (on average, 25% or approximately 6.6 μg Pb/g bone mineral) than lead workers with the VDR bb genotype. There was evidence that the relation between age and patella lead was modified by both the VDR and eNOS genotypes.* (J Occup Environ Med. *2004;46:528–537*)

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Lead accumulates in bone tissue by substituting for calcium in hydroxyapatite crystal, $Ca_{10}(PO_4)_6(OH)_2$, during all stages of bone remodeling and bone growth.[1] Although absorbed lead deposits in the skeleton represent approximately 90% to 95% of an adult's total lead burden,[2] the distribution and toxicokinetics of lead in the skeleton are influenced by bone type (ie, the relative amounts of cortical and trabecular bone), age, gender, and genetic polymorphisms.[3–10] Data suggest that trabecular bone lead concentrations are higher with current exposure but have a shorter clearance half-time after the cessation of exposure. (It should be noted, however, that for the ^{109}Cd-based XRF technique, trabecular lead concentrations can be higher than cortical lead concentrations because the bone mineral content of trabecular bone is lower.) The estimated residence time of lead has been reported to be 27 years in tibia (consisting mainly of cortical bone) and a significantly shorter time in trabecular bone, ranging from several months to years in the various models.[11,12]

It has now been well demonstrated that genetic factors modify the toxicokinetics of lead. The δ-aminolevulinic acid dehydratase (ALAD) gene has been of primary interest. ALAD is encoded by a single gene located on chromosome 9p34 that has 2 alleles, ALAD 1 and ALAD 2, responsible for 3 isozymes, $ALAD^{1-1}$

$ALAD^{1-2}$ and $ALAD^{2-2}$, all of which display similar activities but different charges.[3–8] The prevalence of the ALAD 2 allele is approximately 10% in Asians and 20% in whites.[3–6] Current data suggest that the ALAD 2 allele is associated with higher blood lead, lower dimercaptosuccinic acid (DMSA)-chelatable lead, less efficient uptake of lead into cortical bone, and apparent increased mobilization of lead from trabecular bone compared with the $ALAD^{1-1}$ genotype.[3–8] ALAD has a high affinity for metal and is considered the most sensitive enzyme to lead in the heme pathway.[6] The ALAD 2 gene product has higher affinity for lead than does the $ALAD^{1-1}$-encoded isozyme,[4,6,8] but current data suggest that there is no association between the ALAD genotype and bone lead concentrations in cross-sectional studies.[3,4,7,8]

The vitamin D receptor (VDR) gene regulates the production of calcium-binding proteins and accounts for up to 75% of the total genetic effect on bone density.[6] A prior study suggested that the VDR gene influenced the toxicokinetics of tibia lead concentrations.[3] Most studies of the VDR gene (which is located on chromosome 12cen-12) have focused on the *Bsm*I polymorphism; restriction enzyme digest results in 3 genotypes, which are designated by bb when the restriction site is present, BB when the restriction site is absent, and Bb when both alleles are present.[3,6,9,10] The BB genotype has a prevalence of 17.2%, 4.9%, and 2.3% in studies of whites, blacks and Asians, respectively.[9] A study of predominantly female subjects found that females with the BB genotype have bone mineral densities 2% to 10% lower than subjects with the bb genotype.[6] Previous investigators have also found that subjects with the BB genotype have higher bone lead concentration than subjects with the bb genotype.[10]

The endothelial nitric oxide synthase (eNOS) gene is also of interest with respect to lead; it has a common polymorphism and studies have reported associations among eNOS genotype, hypertension, lead exposure, and intracellular calcium concentrations.[13–18] Endothelial NOS is responsible for the conversion of L-arginine into nitric oxide in the endothelium, resulting in the relaxation of vascular smooth muscle.[13,14] The activity of eNOS is partly regulated by a change in intracellular calcium.[15,16] The eNOS gene is located on chromosome 7 and has 26 exons.[13] A polymorphism of the eNOS gene in exon 7 involves a G to T conversion at nucleotide position 298 (Glu298ASP).[13,14] The 3 genotypes, denoted GG, GT, and TT, result in 3 phenotypes (Glu-Glu, Glu-Asp, and Asp-Asp, respectively). The Asp allele is associated with a reduction in the amount or activity of eNOS.[13,14] Current data in animals suggest that exposure to lead causes hypertension by depressing nitric oxide activity.[17,18] No prior studies have evaluated associations of the eNOS genotype with bone lead concentrations.

We report associations between patella lead concentrations and ALAD, VDR, and eNOS genotypes in a cross-sectional analysis of data from a longitudinal study. To our knowledge, this is the first study to examine the influence of the VDR and eNOS genotypes on patella lead concentration and is the largest study to evaluate the influence of the ALAD genotype on patella lead in currently exposed lead workers.

Materials and Methods

Study Overview and Design

We performed a cross-sectional analysis of the data from the third visit of a 3-year longitudinal study of the health effects of occupational inorganic lead exposure in Korea, focused on the influence of the VDR, ALAD, and eNOS genetic polymorphisms on patella lead concentration (which was measured at the third study visit only). A total of 652 lead workers who were enrolled (between October 24, 1997, and August 19, 1999) returned for the third study visit (between December 19, 1999, and June 24, 2002) and underwent a patella lead measurement. The study was reviewed and approved by Institutional Review Boards at The Johns Hopkins University, Baltimore, Maryland, and the Soonchunhyang University School of Medicine, Chonan, Korea.[3,19] Participation in the study was voluntary, and all participants provided written informed consent. Subjects were paid approximately $30 for their participation.

Study Population

A description of the study population has been previously published.[3,19] In brief, lead workers were recruited from 24 different lead-using facilities, with participation in most facilities exceeding 80%. Former lead workers from 3 facilities who had received medical surveillance services by Soonchunhyang University for several years were also recruited to participate in the study.

Data Collection

Data collection methods have also been previously reported.[3,19] 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 primarily 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; and a 4-hour urine sample after oral administration of DMSA. Tibia lead was measured at the first and second study visits and patella lead at the third study visit.

Laboratory Methods

Laboratory methods for hemoglobin, hematocrit, zinc protoporphyrin, urinary creatinine, blood lead, and tibia lead have already been reported.[3,19] In brief, blood lead concentrations were measured with a Zeeman background-corrected atomic absorption spectrophotometer at Soonchunhyang University Institute of Industrial Medicine, a certified reference laboratory for lead in Korea.[20] Tibia and patella lead were assessed, in units of micrograms of lead per gram of bone mineral, with a 30-minute measurement at the left midtibia shaft and left-center patella, respectively, using ^{109}Cd-based K-shell x-ray fluorescence (XRF).[19] 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.

Four-hour urinary lead excretion after oral administration of 10 mg/kg DMSA was used to measure DMSA-chelatable lead.[19] Urine lead concentrations 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 using previously published methods.[3,19]

VDR, ALAD, and eNOS Genotyping

Genomic DNA was extracted from whole blood by using the QIAamp Blood Kit (QIAGEN, Hilden, Germany). Genotyping methods for ALAD and VDR have been previously reported.[3] For VDR genotyping, 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′-CAACCAAGACTACAAG-TACC-GCGTCAGTGA-3′) and intron 8 (primer 2: 5′-AACCAGCGG-GAAGAGGTCAAGGG-3′).

For ALAD genotyping, the initial amplification, using 3′ and 5′ oligonucleotide primers (5′-AGACAGA-CATTAGCTCAGTA-3′) and (5′-GGCAAAGAACACGTCC-ATTC-3′), generates a 916-basepair fragment. A second round of amplification using a pair of nested primers (provided by Dr. J. Wetmur) sequences (5′-CAGAGCTGTTC-CAACAGTGGA-3′) and (5′-CCA GCACAATGTGGGAGTGA-3′), respectively, generates an 887-basepair fragment. The amplified fragment was cleaved at the diagnostic *Msp*1 site, only present in the ALAD 2 allele, and 3 isozymes are observed, designated $ALAD^{1-1}$, $ALAD^{1-2}$, and $ALAD^{2-2}$.

For eNOS genotyping, the Glu298Asp polymorphism was determined by a modification of the assay of Hibi et al.[21] The primer sequences, 5′-TCCCTGAGGAGG GCATGAGGCT-3′ and 5′-TGAG GGTCACACAGGTTCCT-3′, resulted in a 457-basepair PCR amplification product. Subsequent digestion with *Ban*II cleaved this into 2 fragments (137 bp and 320 bp) in G-variant individuals who have the *Ban*II enzyme digest restriction site. Fragments were resolved on a 1.5% agarose gel (with 0.2% synergel) and stained with ethidium bromide, thus identifying GG homozygotes, GT heterozygotes, and TT homozygotes.

Quality control procedures consisted of repeating the analyses of every tenth sample and including both positive and negative controls in each batch of samples.

Statistical Analysis

The 2 primary goals of the analysis were 1) to examine patella lead concentration by VDR, ALAD, and eNOS genotypes, controlling for covariates; and 2) to determine whether the genotypes modified the relations of age, gender, and other factors with patella lead concentration.

Statistical analyses were performed using the software programs of STATA Corporation version 7 (College Station, TX). Linear regression was used to control for confounding variables; the covariates examined in linear regression models included age, gender, weight, height, job duration, working status (current or former worker), 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 patella lead concentrations or if they were a confounder of the relations between predictor variables and patella lead concentration. Covariates were also retained in the final regression models to allow us to compare the patella lead models with models of tibia lead, blood lead, and DMSA-chelatable lead that have been previously published from earlier study visits in the same subjects.[3,19]

Because of departures from normality, patella lead was natural logarithm (*ln*)-transformed before regressing on covariates. The adequacy of *ln* transformation was confirmed by examination of the distributions of the residuals after determination of the final regression models. To estimate the mean adjusted difference between genotypes in the untransformed scale, the beta coefficients were exponentiated. To evaluate nonlinear relations, quadratic terms for age and job duration were evaluated. Effect modification was evaluated by genotype by including cross-product terms between the genetic variable and age, gender, and other relevant predictor variables.

Student's *t* tests were used to evaluate differences in mean *ln* patella lead concentrations by genotype and differences in mean *ln* patella lead concentrations from the third study visit and *ln* tibia lead concentrations from the second study visit.

Pearson's correlation coefficients were used to assess the associations of covariates with *ln* patella lead concentrations from the third study visit and *ln* tibia lead concentrations from the second study visit for lead workers who had both bone lead concentrations measured.

Results

Description of Demographics

The ages of the 652 lead workers ranged from 20 to 68 years, with a mean (standard deviation) of 43.3 (9.8) years (Table 1). Job durations ranged from 1 month to 36 years, with a mean of 10.0 (6.5) years. Current workers were younger than former workers but had worked longer (Table 1). The majority of both current and former workers were male (77%; Table 2), but the proportion of females among former workers was significantly higher than among current workers. More than half the current and former workers currently consumed alcoholic beverages (Table 2), but the proportion of females among former workers was significantly higher than among current workers. In contrast, current workers had a higher prevalence of current tobacco use than did former workers (55.4% and 38.2%, respectively; Table 2), possibly a cohort effect (but there is only 4 years' difference between the mean age of the current and former workers) or a result of sampling error.

Prevalence and Associations of Patella Lead and Genotypes

Among all lead workers, the prevalence of the $ALAD^{1-1}$, VDR bb, and eNOS GG genotypes were 90.2%, 87.9%, and 84.0%, respectively. There were small differences in genotype prevalence comparing current and former workers, but none achieved statistical significance ($P > 0.1$).

Current workers had higher patella lead concentrations than former workers (80.1 μg/g vs. 63.8 μg/g; $P < 0.001$; Table 1). There was, however, a wide range of patella lead concentrations in each group, with the highest values observed in the former workers. Although there was a higher proportion of female workers among the current workers, regression analysis showed that the interaction term between gender and working status was not significant ($P = 0.7$) (as were the interaction terms between gender and age, age,^{2} [VDR, ALAD, and eNOS] genotype, and job duration).

There was no statistically significant difference between the average patella lead concentrations for current workers with the ALAD 2 allele and current workers with the $ALAD^{1-1}$ genotype (71.3 vs. 81.1 μg/g, respectively; Table 3; $P = 0.95$). There was also no significant difference between average patella lead for former workers with the ALAD 2 allele and former workers with the $ALAD^{1-1}$ genotype (66.2 and 63.4 μg/g, respectively; $P = 0.32$).

There was no significant difference between the average patella lead for current workers with the eNOS GT or TT genotypes and current workers with the GG genotype (86.1 and 78.9 μg/g, respectively; $P = 0.88$; Table 3), and there was likewise no significant difference for former workers with the GT or TT genotypes and former workers with the GG genotype (38.5 and 68.2 μg/g, respectively; $P = 0.18$).

TABLE 1

Characteristics and Concentrations of Selected Lead Biomarkers Among Study Subjects Who Participated in the Third Study Visit,‡ December 1999 to June 2002, Republic of Korea

	Current Lead Workers (n = 452)			Former Lead Workers (n = 200)			All Lead Workers (n = 652)		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
Age (years)*	42.2	8.7	22.3–65.0	46.0	11.4	20.0–67.7	43.3	9.8	20.0–67.7
Job duration (years)*	10.8	6.3	1.8–34.4	8.2	6.7	0.0–36.3	10.0	6.5	0.0–36.3
Blood lead (μg/dL)									
Visit 1*	34.3	12.8	6.7–76.1	26.4	17.3	4.3–78.5	31.9	14.8	4.3–78.5
Visit 2†	35.6	12.8	7.2–83.5	24.0	17.5	3.3–82.8	32.5	15.0	3.3–83.5
Visit 3‡	37.0	14.3	8.7–89.2	17.0	13.0	4.0–83.8	30.9	16.7	4.0–89.2
Tibia lead (μg/g bone mineral)									
Visit 1*	37.1	38.2	−6.5–290.8	38.4	49.1	−7.4–337.6	37.5	41.8	−7.4–337.6
Visit 2†	33.0	38.9	−17.8–289.7	35.2	54.4	−11.0–334.0	35.6	43.4	−17.8–334.0
Patella lead (μg/g bone mineral)									
Visit 3‡	80.1	94.3	−8.7–756.0	63.8	114.3	−11.8–946.1	75.2	101.0	−11.8–946.1
DMSA-Pb (μg)									
Visit 1*	200.6	196.4	4.8–1794.9	150.2	230.3	4.8–2102.9	185.4	208.3	4.8–2102.9
Visit 3‡	175.9	169.3	2.2–868.6	62.1	150.9	1.1–1284.3	140.2	171.9	1.1–1284.3
Weight (kg)*	64.2	9.2	41.0–98.0	62.3	8.9	41.0–91.3	63.6	9.1	41.0–98.0
Height (cm)*	166.2	7.5	128.3–186.9	161.5	8.8	140.8–179.9	164.7	8.2	128.3–186.9

* Information from the first study visit (at baseline) only for lead workers who returned for the third study visit.
† Information from the second study visit only for lead workers who returned for the third study visit.
‡ Information from the third study visit only for lead workers who returned for the third study visit.
SD, standard deviation.

TABLE 2

Characteristics and Genetic Polymorphisms Among Study Subjects Who Participated in the Third Study Visit,* December 1999 to June 2002, Republic of Korea

	Current Lead Workers (n = 452) No. (%)	Former Lead Workers (n = 200) No. (%)	All Lead Workers (n = 652) No. (%)
Gender			
Male	394 (87.2%)	109 (54.4%)	503 (77.2%)
Female	58 (12.8%)	91 (45.5%)	149 (22.8%)
ALAD genotype			
$ALAD^{1-1}$	407 (90.8%)	175 (88.8%)	582 (90.2%)
$ALAD^{1-2}$	41 (9.2%)	22 (11.2%)	63 (9.8%)
VDR genotype			
VDR bb	402 (89.3%)	167 (84.8%)	569 (87.9%)
VDR Bb or BB	48 (10.7%)	30 (15.2%)	78 (12.1%)
eNOS genotype			
GG	373 (83.6%)	167 (84.8%)	540 (84.0%)
GT or TT	73 (16.4%)	30 (15.2%)	103 (16.0%)
Tobacco			
Never	117 (25.9%)	105 (52.8%)	222 (34.2%)
Current	250 (55.4%)	76 (38.2%)	326 (50.2%)
Previous	84 (18.6%)	18 (9.0%)	102 (15.7%)
Drink			
Never	122 (27.0%)	76 (38.2%)	198 (30.5%)
Current	300 (66.5%)	108 (54.3%)	408 (62.8%)
Previous	29 (6.4%)	15 (7.5%)	44 (6.8%)

ALAD, δ-aminolevulinic acid dehydratase; VDR, vitamin D receptor; eNOS, endothelial nitric oxide synthase.

TABLE 3

Mean Patella Lead Concentrations† (μg/g) Among ALAD, eNOS, and VDR Genotype Groups by Working Status

	Current Lead Workers (n = 452)					Former Lead Workers (n = 200)					All Lead Workers (n = 652)				
	No.	Mean	SD	Range	*P* Value‡	No.	Mean	SD	Range	*P* Value‡	No.	Mean	SD	Range	*P* Value‡
ALAD genotype*															
1-1	407	81.1	95.6	−8.7–756.0	0.95	175	63.4	117.8	−11.8–946.1	0.32	582	75.7	103.0	−11.8–946.1	0.65
1-2	45	71.3	82.3	10.0–533.8		25	66.2	88.2	0.9–393.7		70	69.5	83.9	0.9–533.8	
eNOS genotype*															
GG	373	78.9	91.8	−8.7–756.0	0.88	167	68.2	122.4	−8.5–946.1	0.18	540	75.6	102.2	−8.7–946.1	0.39
GT or TT	73	86.1	108.0	−5.6–592.9		30	38.5	48.5	−11.8–946.1		103	72.2	96.8	−11.8–592.9	
VDR genotype*															
bb	402	79.6	91.9	−8.7–592.9	0.39	167	63.0	114.6	−11.8–946.1	0.26	569	74.7	99.2	−11.8–946.1	0.29
BB or Bb	48	86.8	114.7	3.6–756.0		30	67.2	117.2	−7.4–664.3		78	79.2	115.3	−7.4–756.0	

* Information from the first study visit (at baseline) only for lead workers who returned for the third study visit.

† Information from the third study visit only for lead workers who returned for the third study visit.

‡ From *t* tests to compare the difference in mean ln patella lead concentrations between: 1) $ALAD^{1-1}$ and $ALAD^{1-2}$; 2) eNOS GG and eNOS GT or TT; and 3) VDR bb and VDR BB or Bb.

ALAD, δ-aminolevulinic acid dehydratase; VDR, vitamin D receptor; eNOS, endothelial nitric oxide synthase; SD, standard deviation.

Both current and former workers with the VDR BB or Bb genotypes had higher patella lead concentrations than those with the bb genotypes, but again, in neither group of workers did the difference achieve statistical significance (*P* values were 0.39 and 0.26 for current and former workers, respectively; Table 3).

Comparisons of Correlations With *ln* Bone Lead

For all workers, patella lead concentration was moderately correlated with: blood lead concentrations for visits 1, 2, and 3 (Pearson's $r = 0.55$, 0.52, and 0.62, respectively); DMSA-chelatable lead from visits 1 and 3 (Pearson's $r = 0.53$ and 0.52, respectively); job duration (Pearson's $r = 0.53$); and tibia lead concentrations from the first and second study visits (Pearson's $r = 0.58$ and 0.65, respectively). These correlations were slightly lower among former workers (Table 4). Tables 4 and 5 show the correlations for 1) current, 2) retired, and 3) all lead workers between each of 1) blood

TABLE 4
Pearson's Correlation Coefficient for Associations of Covariates With ln Patella Lead Concentrations‡§

Covariates	Current Lead Workers (n = 452)	Former Lead Workers (n = 200)	All Lead Workers (n = 652)
Blood Lead			
Visit 1*	0.60	0.44	0.55
Visit 2†	0.54	0.43	0.52
Visit 3‡	0.65	0.63	0.62
ln tibia			
Visit 1*	0.60	0.59	0.58
Visit 2†	0.70	0.54	0.65
ln DMSA-lead			
Visit 1*	0.56	0.43	0.53
Visit 3‡	0.48	0.59	0.52
Age*	0.49	0.38	0.34
Job duration*	0.57	0.54	0.57

* Information from the first study visit (at baseline) only for lead workers who returned for the third study visit.
† Information from the second study visit only for lead workers who returned for the third study visit.
‡ Information from the third study visit only for lead workers who returned for the third study visit.
§ Only lead workers who returned for the third study visit and who had information on both tibia lead from the second study visit and patella lead from the third study visit.
DMSA, dimercaptosuccinic acid.

TABLE 5
Pearson's Correlation Coefficient for Associations of Covariates With ln Tibia Lead Concentrations From the Second Study Visit†§

Covariates	Current Lead Workers (n = 452)	Former Lead Workers (n = 200)	All Lead Workers (n = 652)
Blood Lead			
Visit 1*	0.55	0.54	0.54
Visit 2†	0.54	0.55	0.53
Visit 3‡	0.59	0.63	0.54
ln DMSA-lead			
Visit 1*	0.54	0.55	0.53
Visit 3‡	0.44	0.65	0.47
Age*	0.33	0.34	0.31
Job duration*	0.53	0.50	0.52

* Information from the first study visit (at baseline) only for lead workers who returned for the third study visit.
† Information from the second study visit only for lead workers who returned for the third study visit.
‡ Information from the third study visit only for lead workers who returned for the third study visit.
§ Only lead workers who returned for the third study visit and who had information on both tibia lead from the second study visit and patella lead from the third study visit.
DMSA, dimercaptosuccinic acid.

lead (at each of 3 visits), 2) (*ln*) DMSA-chelatable lead (at visits 1 and 3), 3) age, and 4) job duration and patella lead (Table 4) and tibia lead (Table 5). Table 4 also shows the correlation between patella lead and tibia lead for both visit 1 and visit 2 tibia lead. Tables 4 and 5 together show no striking differences between the correlations for each of the 2 bones measured.

Predictors of Patella Lead Concentrations in Adjusted Analyses

After the associations between patella lead and each covariate were examined by simple linear regression, the statistically significant predictors of patella lead that remained were age, gender, job duration, smoking status, and working status (current vs. former) (P values $<$ 0.01; Table 6). On average, lead workers who were older, male, had longer working durations, were current workers, or were current or former users of tobacco had higher patella lead concentrations.

In simple linear regression, associations of patella lead with weight, height, drinking status, VDR, ALAD, and eNOS genotypes did not achieve statistical significance. However, after adjusting for age, gender, job duration, working status, and alcohol and tobacco consumption, lead workers with the VDR BB or Bb genotypes had higher patella lead concentrations (P value = 0.03; Table 6); lead workers with the VDR B allele had patella lead concentrations that were, on average, 25% (approximately 6.6 μg/g after exponentiating the beta coefficient for patella lead concentration) higher than workers with the VDR bb genotypes. In contrast, after adjusting for the same covariates, there was no statistically significant difference in mean patella lead concentration between lead workers with the ALAD 2 allele and lead workers with the $ALAD^{1-1}$ genotype. Similarly, there was no statistically significant difference in mean patella lead concentrations by eNOS genotype.

Prediction of Patella Lead by Genetic and Age Interaction

The relation between age and patella lead was modified by the VDR and the eNOS genotypes (Table 6, models 2 and 3, respectively).

Figure 1 shows that lead workers with the VDR B allele had higher patella lead concentrations than lead workers with the VDR bb genotype when they were younger than 45 years old. In contrast, when comparing (all) workers older than 55, workers with the VDR B allele had

TABLE 6
Linear Regression Modeling Results Identifying Predictors of Patella Lead Concentration (ln transformed) in 652 Korean Lead Workers†

Independent Variables (units of β coefficient)	β Coefficient	β Standard Error	*P* Value
Model 1 (adjusted $r^2 = 37.9\%$)‡§			
Intercept	3.946	0.113	<0.01
Age (μg/g/y)*	0.036	0.005	<0.01
Female (μg/g)*	−0.402	0.136	<0.01
Job duration (μg/g/y)*	0.069	0.006	<0.01
Former lead worker (μg/g)*	−0.359	0.082	<0.01
VDR (BB or Bb vs. bb) (μg/g)*	0.226	0.106	0.03
Model 2 (adjusted $r^2 = 38.4\%$)‡§‖			
Intercept	3.961	0.112	<0.01
Age (μg/g/y)*	0.040	0.005	<0.01
Female (μg/g)*	−0.424	0.136	<0.01
Job duration (μg/g/y)*	0.067	0.006	<0.01
Former lead worker (μg/g)*	−0.351	0.082	<0.01
VDR (BB or Bb vs. bb) (μg/g)*	0.264	0.107	0.01
Age × VDR (μg/g)*	−0.023	0.010	0.03
Model 3 (adjusted $r^2 = 39.0\%$)‡¶			
Intercept	3.965	0.112	<0.01
Age (μg/g/y)*	0.032	0.005	<0.01
Female (μg/g)*	−0.374	0.136	0.01
Job duration (μg/g/y)*	0.069	0.006	<0.01
Former lead worker (μg/g)*	−0.334	0.082	<0.01
NOS ([GT or TT] *vs.* GG) (μg/g)*	−0.173	0.094	0.06
Age × NOS (μg/g)*	0.020	0.009	0.04

* μg/g lead *per* gram bone mineral, ln-transformed.
† Korean lead workers who returned for the third study visit only.
‡ Models controlled for age, gender, job duration, working status (current vs. former), categories of lifetime tobacco consumption, categories of lifetime alcohol consumption.
§ Model controlled for VDR genotypes.
‖ Model evaluated interaction between age and VDR genotypes.
¶ Models evaluated interaction between age and eNOS genotype.
VDR, vitamin D receptor; eNOS, endothelial nitric oxide synthase.

lower patella lead than lead workers with the VDR bb genotype. Both worker age -groups had similar patella lead concentrations between the ages of 45 and 50. On average, lead workers with the VDR B allele had lower increases in patella lead with increasing age than lead workers with the VDR bb genotype (1.7% and 4.1% average increases in patella lead with each increasing year of age for the VDR B allele and the VDR bb genotype, respectively; $P = 0.03$).

Figure 2 shows that lead workers with the eNOS GT or TT genotypes had, on average, lower patella lead concentrations than lead workers with the eNOS GG genotype. However, patella lead concentrations were similar in both groups when age exceeded 50. On average, lead workers with the eNOS GT or TT genotype had greater increases in patella lead with increasing age than lead workers with the eNOS GG genotype (average increases of 5.3% per year and 3.3% per year, respectively; $P = 0.04$).

Discussion

Our study examined patella lead concentrations by VDR, ALAD, and eNOS genotypes, controlling for covariates; and determined whether the genotypes modified the relations of age, gender, and other factors with patella lead concentrations. In unadjusted analyses, we found that there were 4 main predictors of patella lead: age, gender, job duration, and working status (all P values < 0.001). In contrast, weight, height, VDR, ALAD, and eNOS genotypes were not associated with patella lead concentration. In adjusted analysis, the VDR B allele was the only genotype associated with higher patella lead (compared with the VDR bb genotype; P value $= 0.03$). In addition, the association between patella lead and age was modified by VDR (the rate of increase of patella lead was lower for lead workers with the VDR B allele) and eNOS (the rate of increase of patella lead was lower for lead workers with the eNOS GG genotype) (P values of 0.03 and 0.04, respectively).

This study found that patella lead concentration increases with age and job duration, findings consistent with a broad literature. Furthermore, current workers had higher patella lead concentrations than former workers, a finding again consistent with previous data that patella lead can decrease after occupational lead exposure ceases.[22] This study also found

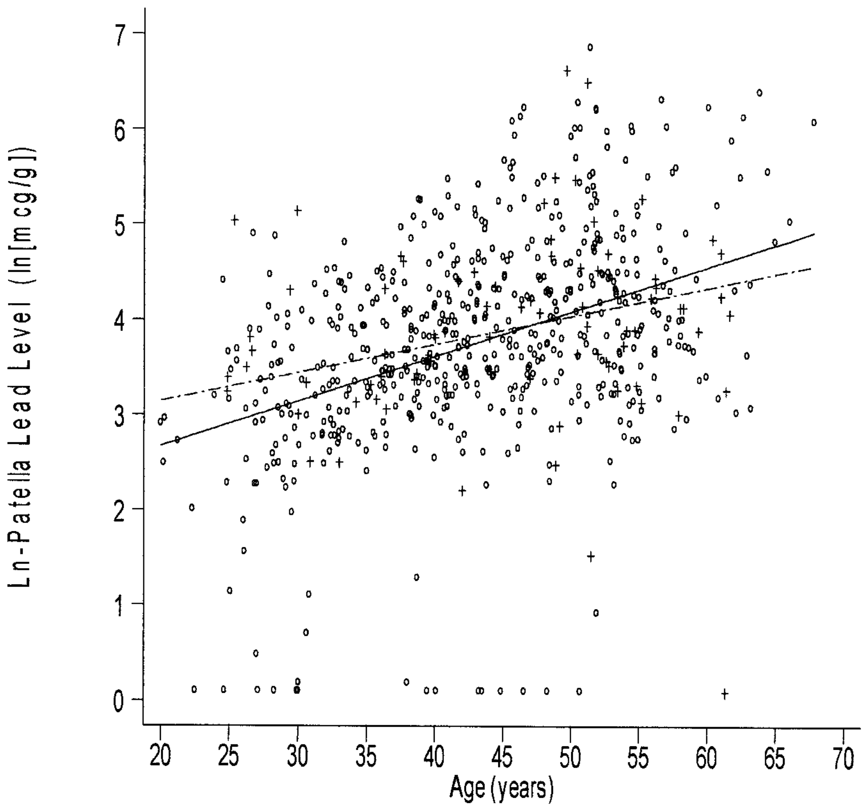

Fig. 1. Relation of patella lead (*ln* transformed) with subject age in VDR genotype groups (BB and Bb compared with bb groups) after controlling for gender, job duration, working status, smoking, and alcohol consumption for 652 current and former lead workers in South Korea, 1999–2002.
° *In* paatella lead concentrations for VDR bb genotype + *In* patella lead concentrations for VDR B allele ——— Fitted Values for VDR bb genotype − − − − Fitted Values for VDR B allele.

that patella lead was higher in male workers than in female workers. This particular finding is also consistent with previous data that show that lead concentration in patella (a predominantly trabecular bone) is age- and gender-dependent.[2,23,24]

In an adjusted model (model 1, Table 6), lead workers with the VDR B allele had significantly higher patella lead concentrations (*P* value $<$ 0.05) than lead workers with the VDR bb genotype by, on average, 25% (approximately 6.6 μg/g bone mineral). Previous data have suggested that the VDR B allele is also associated with tibia lead concentrations higher than those found in subjects with the VDR bb genotype.[3,6,10] The association between patella lead and VDR genotype therefore seems to be similar to the association between tibia lead and VDR genotype.

In another adjusted model with an interaction term (model 2, Table 6), VDR genotype modified the relation between patella lead and age in 2 ways: first, lead workers with the VDR B allele had higher patella lead concentrations before the age of 45 and lower patella lead after age 50 (Fig. 1) compared with lead workers with the VDR bb genotype. Second, patella lead in lead workers with the VDR B allele increased less with increasing age than it did for lead workers without the VDR B allele (1.7% per year and 4.1% per year, respectively; *P* value $<$ 0.05). A prior study reported that the VDR B allele was associated with a higher rate of increase in tibia lead than was observed with the VDR bb genotype,[10] a result that is in contrast to that of the present study but which applies to a predominantly cortical bone. It seems possible, therefore, that the way in which the VDR genotype modifies the association between bone lead concentration and age depends on the cortical or trabecular nature of the bone under consideration.

The effect on health outcomes of our finding of a 25% difference in the patella lead concentration (age-related) increase for VDR is unknown but potentially substantial given the growing body of literature on the adverse effects of low blood lead concentrations and the bone's role as a source of endogenous lead exposure, especially after the cessation of occupational exposure (eg, Gerhardsson et al.[11] found that blood lead was significantly associated with calcaneus lead, a trabecular bone, like the patella, in retired smelter workers).

No association was observed between either the ALAD or the eNOS polymorphisms and patella lead concentration. However, in adjusted analysis (model 3, Table 6), we found that eNOS genotype modifies the relation between age and patella lead: before the age of 50, lead workers with the eNOS GT or TT genotypes had lower patella lead than those with the eNOS GG genotype, but after the age of 50, both groups had similar patella lead concentrations (Fig. 2). Furthermore, lead workers with the eNOS GT or TT genotypes tended to have patella lead concentrations that increased at a greater rate with increasing age than the rate of increase in lead workers with the eNOS GG genotype (5.3% per year and 3.3% per year, respectively; *P* value $<$ 0.05).

Conclusions

In conclusion, age, gender, job duration, and working status were found to be predictors of patella lead concentration. After controlling for these predictors, VDR genotype was associated with patella lead concen-

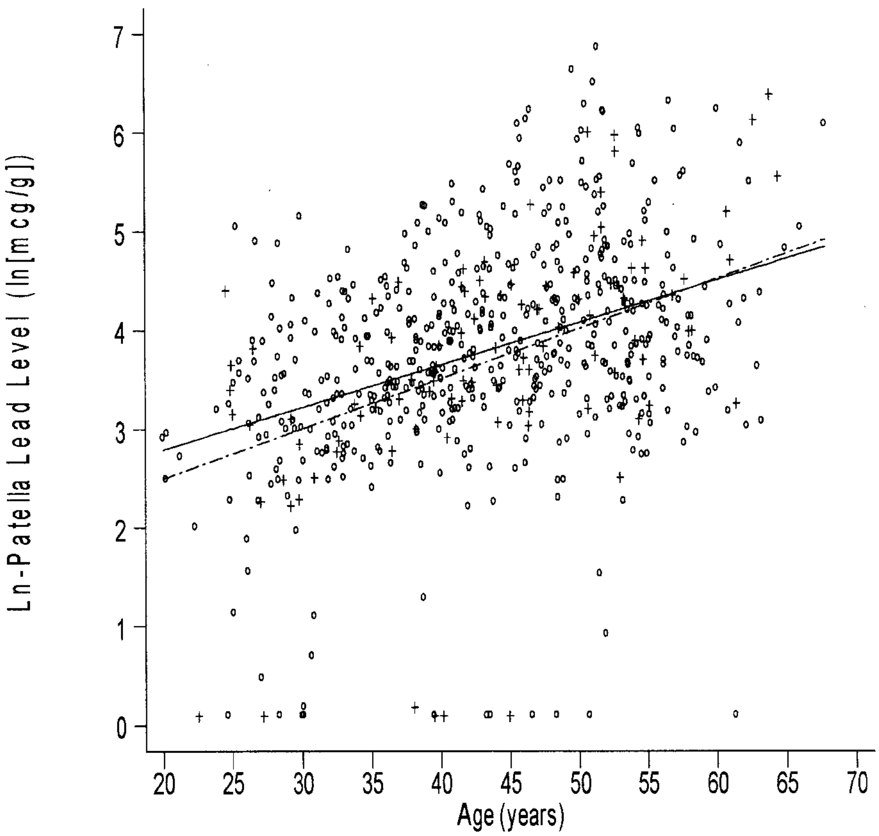

Fig. 2. Relation of patella lead (*ln* transformed) with subject age in eNOS genotype groups (TT and GT compared with GG groups) after controlling for gender, job duration, working status, smoking, and alcohol consumption for 652 current and former lead workers in South Korea, 1999–2002.

° *In* paatella lead concentrations for eNOS GG genotype + *In* patella lead concentrations for eNOS GT or TT genotype ——— Fitted Values for eNOS GG genotype − − − − Fitted Values for eNOS GT or TT genotype.

tration, and in addition, VDR and eNOS genotypes modified the association between patella lead and age. It is possible that VDR and eNOS genotypes modify bone mineral density or the kinetics of lead in bone (ie, the accumulation in or release of lead from bone). These data suggest that the VDR B allele could be associated with higher patella lead concentrations in younger people, compared with the VDR bb genotype, but lower patella lead concentrations in the elderly. In addition, the eNOS GT or TT genotypes could be associated with lower patella lead concentrations in younger people, compared with the eNOS GG genotype, but higher patella lead concentrations in older people. These data imply that an individual's susceptibility to lead toxicity could be a function of age, VDR, and eNOS genotypes.

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References

1. Castellino N, Castellino P, Sannola N. Inorganic lead exposure: metabolism and intoxication. *Lead Metabolism.* Boca Raton, FL: CRC Press; 1995:168–180.
2. Barry PSI. A comparison of concentrations of lead in human tissues. *Br J Ind Med*. 1975;32:119–139.
3. Schwartz BS, Lee BK, Lee GS, et al. Associations of blood lead, DMSA-chelatable lead, and tibial lead with polymorphisms in the vitamin D receptor and δ-aminolevulinic acid dehydratase genes. *Environ Health Perspect*. 2000;108:949–954.
4. Bergdahl IA, Gerhardsson L, Schütz A, et al. Delta-aminolevulinic acid dehydratase polymorphism: influence on lead levels and kidney function in humans. *Arch Environ Health*. 1997;52:91–96.
5. Kelada SN, Shelton E, Kaufmann RB, et al. δ-aminolevulinic acid dehydratase genotype and lead toxicity: a HuGE review. *Am J Epidemiol.* 2001;154:1–13.
6. Onalaja AO, Claudio L. Genetic susceptibility to lead poisoning. *Environ Health Perspect*. 2000;108(suppl 1):23–28.
7. Fleming DEB, Chettle DR, Wetmur JG, et al. Effect of the δ-aminolevulinate dehydratase polymorphism on the accumulation of lead in bone and blood in lead smelter workers. *Environ Res*. 1998; 77:49–61.
8. Hu H, Wu MT, Cheng Y, et al. The δ-aminolevulinic acid dehydratase (ALAD) polymorphism and bone and blood lead levels in community-exposed men: the Normative Aging Study. *Environ Health Perspect*. 2001;109:827–832.
9. Cooper GS, Umbach DM. Are vitamin D receptor polymorphisms associated with bone mineral density? A meta-analysis. *J Bone Miner Res*. 1996;11:1841–1849.
10. Schwartz BS, Stewart WF, Kelsey KT, et al. Associations of tibial lead levels with *Bsm*I polymorphisms in the vitamin D receptor in former organolead manufacturing workers. *Environ Health Perspect.* 2000;108:199–203.
11. Gerhardsson L, Attewell R, Chettle DR, et al. In vivo measurements of lead in bone in long-term exposed lead smelter workers. *Arch Environ Health.* 1993;48: 147–156.
12. Chettle DR. *In vivo* x-ray fluorescence of lead and other toxic trace elements. *Adv X-Ray Anal*. 1995;38:563–572.
13. Columbo MG, Andreassi MG, Paradossi U, et al. Evidence for association of a common variant of the endothelial nitric oxide synthase gene (Glu^{298} - Asp polymorphism) to the presence, extent, and severity of coronary artery disease. *Heart*. 2002;87:525–528.
14. Sofowora G, Dishy V, Xie HG, et al. In vivo effect of Glu298Asp endothelial nitric oxide synthase polymorphism. *Pharmacogenetics*. 2001;11:809–814.
15. Schneider JC, El Kebir D, Chereau C, et al. Involvement of Na(+)/Ca(2+) exchanger in endothelial NO production and endothelium-dependent relaxation. *Am J Physiol Heart Circ Physiol*. 2002; 283:H837–H844.
16. Wagnaer S, Groschner K, Mayer B, et al. Desensitization of endothelial nitric ox-

ide synthase by receptor agonists. *Biochem J*. 2002;364:863–868.

17. Vaziri ND, Ding Y, Ni Z. Compensatory up-regulation of nitric-oxide synthase isoforms in lead-induced hypertension; reversal by a superoxide dismutase-mimetic drug. *JPET*. 2001;298:679–685.
18. Vaziri ND, Ding Y. Effect of lead on nitric oxide synthase expression in coronary endothelial cells: role of superoxide. *Hypertension*. 2001;37:223–226.
19. Todd AC, Lee BK, Lee GS, et al. Predictors of DMSA-chelatable lead, tibia lead and blood lead in 802 Korean lead workers. *Occup Environ Med*. 2001;58:73–80.
20. Fernandez FJ. Micromethod for lead determination in whole blood by atomic absorption, with use of the graphite furnace. *Clin Chem*. 1975;21:558–561.
21. Hibi K, Ishigami T, Tamura K, et al. Endothelial nitric oxide synthase gene polymorphism and acute myocardial infarction. *Hypertension*. 1998;32:521–526.
22. Kim R, Landrigan C, Mossman P, et al. Age and secular trends in bone lead levels in middle-aged and elderly men: three-year longitudinal follow-up in the Normative Aging Study. *Am J Epidemiol*. 1997;146:586–591.
23. Wittmers LJ, Wallgren JE, Alich A, et al. Lead in bone. IV. Distribution of lead in the human skeleton. *Arch Environ Health*. 1988;43:381–391.
24. Watanabe H, Hu H, Rotnitzky A. Correlates of bone and blood lead levels in carpenters. *Am J Ind Med*. 1994;26:255–264.