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

Byung-Kook Lee,[1] Gap-Soo Lee,[1] Walter F. Stewart,[2,3] Kyu-Dong Ahn,[1] David Simon,[2] Karl T. Kelsey,[4] Andrew C. Todd,[5] and Brian S. Schwartz[2,3,6]

[1]Institute of Industrial Medicine, Soonchunhyang University, Chonan, Korea; [2]Department of Epidemiology, Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland, USA; [3]Division of Occupational and Environmental Health, Department of Environmental Health Sciences, Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland, USA; [4]Department of Cancer Cell Biology, Harvard School of Public Health, Boston, Massachusetts, USA; [5]Department of Community and Preventive Medicine, Mount Sinai Medical Center, New York, New York, USA; [6]Department of Medicine, Johns Hopkins School of Medicine, Baltimore, Maryland, USA

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

Address correspondence to B.S. Schwartz, Division of Occupational and Environmental Health, Johns Hopkins School of Hygiene and Public Health, Room 7041, 615 N. Wolfe Street, Baltimore, MD, 21205 USA. Telephone: (410) 955-4158. Fax: (410) 955-1811; E-mail: bschwart@jhsph.edu

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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*I 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