

Associations of Renal Function with Polymorphisms in the δ -Aminolevulinic Acid Dehydratase, Vitamin D Receptor, and Nitric Oxide Synthase Genes in Korean Lead Workers

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We analyzed data from 798 lead workers to determine whether polymorphisms in the genes encoding δ -aminolevulinic acid dehydratase (ALAD), endothelial nitric oxide synthase (eNOS), and the vitamin D receptor (VDR) were associated with or modified relations of lead exposure and dose measures with renal outcomes. Lead exposure was assessed with job duration, blood lead, dimercaptosuccinic acid (DMSA)-chelatable lead, and tibia lead. Renal function was assessed with blood urea nitrogen (BUN), serum creatinine, measured creatinine clearance, calculated creatinine clearance and urinary *N*-acetyl- β -D-glucosaminidase (NAG), and retinol-binding protein. Mean (\pm SD) tibia lead, blood lead, and DMSA-chelatable lead levels were 37.2 ± 40.4 μ g/g bone mineral, 32.0 ± 15.0 μ g/dL, and 767.8 ± 862.1 μ g/g creatinine, respectively. After adjustment, participants with the *ALAD*² allele had lower mean serum creatinine and higher calculated creatinine clearance. We observed effect modification by *ALAD* on associations between blood lead and/or DMSA-chelatable lead and three renal outcomes. Among those with the *ALAD*¹⁻² genotype, higher lead measures were associated with lower BUN and serum creatinine and higher calculated creatinine clearance. Participants with the *eNOS* variant allele were found to have higher measured creatinine clearance and BUN. In participants with the *Asp* allele, longer duration working with lead was associated with higher serum creatinine and lower calculated creatinine clearance and NAG; all were significantly different from relations in those with the *Glu/Glu* genotype except NAG ($p = 0.08$). No significant differences were seen in renal outcomes by *VDR* genotype, nor was consistent effect modification observed. The *ALAD* findings could be explained by lead-induced hyperfiltration. **Key words:** δ -aminolevulinic acid dehydratase, endothelial nitric oxide synthase, genetic susceptibility factors, lead exposure, *N*-acetyl- β -D-glucosaminidase (NAG), renal function, retinol-binding protein, vitamin D receptor. *Environ Health Perspect* 111:1613–1619 (2003). doi:10.1289/ehp.6116 available via <http://dx.doi.org/> [Online 12 June 2003]

Genetic susceptibility is one factor that contributes to the wide range of health outcomes occurring among individuals exposed to similar levels of toxicants. The gene that encodes the δ -aminolevulinic acid dehydratase (ALAD) enzyme is a potentially important modifier of relations between lead exposure/dose and renal function. The ALAD enzyme is a principal lead-binding protein with two common alleles, *ALAD*¹ and *ALAD*² (Battistuzzi et al. 1981). A higher percentage of lead is bound to the protein present in those with the *ALAD*² allele compared with those with the *ALAD*¹ allele (Bergdahl et al. 1997b). Several studies have found that similarly exposed participants with the *ALAD*² allele have higher blood lead levels than those who are homozygous for the *ALAD*¹ allele (Kelada et al. 2001; Wetmur et al. 1991; Ziemsen et al. 1986). Other toxicokinetic differences have also been reported, including lower dimercaptosuccinic

acid (DMSA)-chelatable lead levels (controlling for exposure duration and blood lead) (Schwartz et al. 1997) and less efficient uptake of lead into bone, resulting in a lower bone lead level for a given cumulative blood lead (Fleming et al. 1998). The impact of these differences on lead toxicity is not clear. *ALAD*² binding could prevent lead from reaching target organs, thus reducing toxicity. On the other hand, increased blood lead could result in greater potential for toxicity. Two studies have examined the impact of *ALAD* genotype on the renal system; one reported higher unadjusted mean serum creatinine ($p = 0.11$) in participants with the *ALAD*² allele (Bergdahl et al. 1997a). The other found higher mean serum creatinine ($p = 0.11$) and blood urea nitrogen (BUN; $p = 0.03$) in participants with the *ALAD*² allele; however, after adjustment for covariates, the statistical significance of these associations

decreased ($p = 0.16$ for serum creatinine and $p = 0.06$ for BUN) (Smith et al. 1995).

Endothelial nitric oxide synthase (eNOS) catalyzes the transformation of L-arginine to nitric oxide, which is a vasodilator. Animal models of renal disease have demonstrated that administration of L-arginine results in decreased glomerulosclerosis and tubulointerstitial damage; this is thought to be mediated via increased NO (Klahr 2001). The Glu298Asp polymorphism of the *eNOS* gene involves a G-to-T conversion at nucleotide position 894 within exon 7, which results in substitution of aspartic acid for glutamic acid at codon 298. Research in patients with essential hypertension has found both an increased frequency of the *Asp* allele (Miyamoto et al. 1998) and decreased NO production (Klahr 2001). Presence of the *Asp* allele was associated with an earlier age at development of end-stage renal disease in males with autosomal dominant polycystic kidney disease; patients with the *Asp* allele also demonstrated decreased NO synthase activity (Persu et al. 2002). Data on the *eNOS* Glu298Asp polymorphism in diabetic nephropathy are inconsistent; Noiri et al. (2002) found a higher frequency of the *Asp* allele in those with diabetic end-stage renal disease; however, Zanchi et al. (2000) did not. Noiri et al. (2002) also reported decreased NO production with the *Asp* allele

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in an *in vitro* system. In addition to its potential as a renal genetic susceptibility factor, the *eNOS* gene is of interest because animal data suggest that lead exposure also results in decreased NO levels (Vaziri et al. 1997).

The vitamin D receptor (VDR) is important for calcium absorption and bone mineralization and is activated through binding of 1,25-dihydroxyvitamin D₃. The *VDR BsmI* polymorphism has three genotypes resulting from restriction enzyme digestion: *bb*, *Bb*, and *BB*, with the uppercase letter signifying the absence of the restriction site. Decreased bone mineral density has been reported in those with the *BB* compared with the *bb* genotype (Cooper and Umbach 1996). In the lead workers studied here, participants with the *B* allele were found to have significantly higher blood lead, DMSA-chelatable lead, tibia lead (Schwartz et al. 2000a), and systolic and diastolic blood pressure (Lee B-K et al. 2001), compared with participants with the *bb* genotype. In a Caucasian population, the *b* allele was significantly associated with improved survival in renal dialysis patients (Marco et al. 2001). In contrast, an increased risk for renal disease in Japanese systemic lupus erythematosus patients with the *b* allele has also been reported (Ozaki et al. 2000).

Herein, we report associations of *ALAD*, *VDR*, and *eNOS* polymorphisms with six renal outcomes, and effect modification by these polymorphisms on associations among four lead exposure and dose measures and six renal outcomes in a cross-sectional analysis of Korean lead workers.

Materials and Methods

Study design and population. In this article we focus on data obtained from 798 current and former lead workers from the first of three annual visits in a longitudinal study of the neurobehavioral, peripheral nervous system, renal, hematopoietic, and blood pressure effects of inorganic lead exposure. All participants provided written, informed consent. The study protocol was approved by institutional review boards at the Soonchunhyang University School of Medicine and the Johns Hopkins University Bloomberg School of Public Health. As previously described (Schwartz et al. 2001; Weaver et al. 2003), workers were recruited from 26 different facilities, including lead battery, lead oxide, lead crystal, and radiator manufacture, and secondary lead smelting. Participation in the study was voluntary, and workers were paid approximately \$30 U.S. for their time and effort. Participation rates by plant generally exceeded 85%. No medical exclusionary criteria (e.g., blood pressure, renal disease) were applied. Study participants were not currently occupationally exposed to other known renal toxicants.

Data collection. Data collection was completed either at the Institute of Industrial Medicine of the Soonchunhyang University in Chonan, Korea, or at the study plants, using previously reported methods (Schwartz et al. 2001; Weaver et al. 2003). Data and biologic specimens collected included a standardized questionnaire on demographics, medical history, and occupational history; blood pressure measured with a Hawksley random zero sphygmomanometer (Lee B-K et al. 2001); height and weight measurement; a blood specimen (for blood lead, BUN, serum creatinine, and genotyping); and a spot urine sample [for retinol-binding protein (RBP), *N*-acetyl- β -D-glucosaminidase (NAG), and creatinine], both of which were stored at -70°C until analyzed; and tibia lead concentration. A 4-hr urine collection after oral administration of 10 mg/kg DMSA was also obtained to measure DMSA-chelatable lead and creatinine clearance (787 participants completed this collection).

Laboratory methods. The lead biomarkers and renal outcomes were measured using previously reported assays (Schwartz et al. 2001; Weaver et al. 2003). In brief, blood lead was measured with a Hitachi 8100 Zeeman background-corrected atomic absorption spectrophotometer (Hitachi Ltd. Instruments, Tokyo, Japan) with the standard addition method of the National Institute of Occupational Safety and Health (Kneip and Crable 1988) at the Institute of Industrial Medicine, a certified reference laboratory for lead in Korea. Tibia lead was assessed with a 30-min measurement at the left mid-tibia shaft using ^{109}Cd K-shell X-ray fluorescence (Schwartz et al. 1999; Todd and McNeill 1993; Todd et al. 1992). All point estimates, including negative values, were retained in the statistical analyses in order to minimize bias and avoid censoring of data (Kim et al. 1995). Urine lead levels in the 4-hr collection were measured at the Wadsworth Center of the New York State Department of Health (Albany, NY, USA) by electrothermal atomic absorption spectrometry with Zeeman background correction (model 4100ZL; Perkin-Elmer, Norwalk, CT, USA) (Parsons and Slavin 1999).

BUN and serum creatinine were measured via an Automatic Chemical Analyzer (TBA 40FR Biochemical Analyzer; Toshiba, Tokyo, Japan). Urine creatinine was measured in spot samples, for adjustment of NAG and RBP, and in the 4-hr sample after DMSA, for determination of measured creatinine clearance and adjustment of DMSA-chelatable lead levels, using a modification of the Sigma kit (Creatinine test kit 555A; Sigma Chemical Co., St. Louis, MO, USA) (Weaver et al. 2000). Measured creatinine clearance was defined as [(urinary creatinine in milligrams per deciliter \times urine volume in milliliters) \div serum creatinine in milligrams

per deciliter] \times collection time in minutes. Calculated creatinine clearance was obtained from the Cockcroft and Gault (1976) equation. NAG was measured using the P.P.R. NAG Test kit (P.P.R. Diagnostics Ltd., London, UK), which uses 2-methoxy-4-(2'-nitrovinyl)-phenyl 2-acetamido-2-deoxy- β -D-glucopyranoside as the substrate, resulting in 2-methoxy-4-(2'-nitrovinyl)-phenol formation after hydrolysis by NAG (Yuen et al. 1984). RBP was measured using a modification of the method of Topping et al. (1986).

For genotyping, DNA was isolated from whole blood samples using the QIAamp Blood Kit (QIAGEN, Hilden, Germany), and all assays were based on polymerase chain reaction (PCR). The protocol for *ALAD* genotyping has been previously described (Schwartz et al. 1995; Wetmur et al. 1991; Ziemsen et al. 1986). In brief, the initial amplification, using 3' and 5' oligonucleotide primers (5'-AGACAGACATTAGCTCAGTA-3') and (5'-GGCAAAGAACACGTC-CATTTC-3'), generated a 916 base pair (bp) fragment. A second round of amplification using a pair of nested primers [provided by J. Wetmur (Mount Sinai School of Medicine, New York, NY, USA); sequences 5'-CAGAGCTGTTCCAACAGTGGGA-3' and 5'-CCAGCACAATGTGGGAGTGA-3', respectively] generated an 887 bp fragment. The amplified fragment was cleaved at the diagnostic *MspI* site, present only in the *ALAD*² allele.

The *Glu298Asp* polymorphism was determined by a modification of the assay of Hibi et al. (1998). The primer sequences were 5'-TCCCTGAGGAGGGCATGAGGCT-3' and 5'-TGAGGGTCACACAGGTTTCT-3', which resulted in a 457 bp PCR amplification product. Subsequent digestion with *BanII* cleaved this into two fragments (137 bp and 320 bp) in G-variant individuals who have the *BanII* restriction enzyme digest site. Fragments were resolved on a 1.5% agarose gel (with 0.2% Synergel; Diversified Biotech, Boston, MA, USA) and stained with ethidium bromide.

As previously published (Schwartz et al. 2000b), the *VDR BsmI* polymorphic site in intron 8 was amplified using primers originating in exon 7 (primer 1: 5'-CAACCAAGAC-TACAAGTACCGCGTCAGTGA-3') and intron 8 (primer 2: 5'-AACCAGCGGGAA-GAGGTCAAGGG-3'). Participants homozygous for the presence of the *BsmI* restriction site are designated *bb*, heterozygotes are designated *Bb*, and those homozygous for the absence of the site are designated *BB*.

Statistical analysis. The primary goals of the analysis were *a)* to examine associations between *ALAD*, *VDR*, and *eNOS* genotypes and six renal outcomes (BUN, serum creatinine, measured creatinine clearance, calculated creatinine clearance, RBP, and NAG) in

lead workers, while controlling for covariates; and *b*) to evaluate whether *ALAD*, *VDR*, and *eNOS* genotypes modified associations between one lead exposure measure (job duration) and three lead dose biomarkers (tibia lead, blood lead, DMSA-chelatable lead) and the renal outcomes. Statistical analysis was completed using SAS statistical software programs (SAS Institute, Inc., Cary, NC, USA).

Initially, variable distributions were examined. The distributions of NAG and RBP evidenced departures from normality and were thus ln (base 2)-transformed. The adequacy of ln-transformation of these measures was confirmed by verification that distributions of residuals after linear regression modeling were normal. Linear regression modeling with a dichotomous genotype variable was used to compare renal outcome measures by genotype, while controlling for the same covariates used in the final models. For *ALAD* genotype, participants with *ALAD*¹⁻² were compared with participants with *ALAD*¹⁻¹. Because of small numbers, all analyses combined homozygous and heterozygous variant genotype carriers for *VDR* (*BB* and *Bb*) and *eNOS* (*Glu/Asp* and *Asp/Asp*), unless noted otherwise.

Linear regression modeling, with cross-product terms for genotypes and lead variables, was used to evaluate effect modification by genotype on associations between lead measures and renal outcomes. Covariate selection for final regression models used *a priori* variables [age, sex, body mass index (BMI; defined as weight in kilograms divided by the square of height in meters)] and a dichotomous variable for current versus former worker status to adjust for differences between these two groups (the former workers were older, had lower mean blood and DMSA-chelatable lead levels, had longer job durations, and a greater proportion were women) as well as biologically directed stepwise forward modeling to

identify other significant variables, as previously described (Weaver et al. 2003). Covariates in the model for clinical renal outcomes (BUN, serum creatinine, measured creatinine clearance, and calculated creatinine clearance) included age, sex, BMI, hypertension, work status (current vs. former worker), and a dichotomous variable for current smoking. Models of NAG and RBP were adjusted for age, sex, BMI, systolic blood pressure, work status, diabetes mellitus, and alcohol consumption.

Models were evaluated for linear regression assumptions and the presence of outlying points using jackknife residual (Kleinbaum et al. 1998) and added variable plots (Weisberg 1985). The latter plots are graphical summaries of the relation between *Y* and a particular *X* (referred to as *X_a* below) adjusted for all the other covariates. Specifically, the residuals of the regression of *Y* on all the covariates except *X_a* are plotted on the *y*-axis. This is the part of *Y* not explained by those covariates. Next, the residuals from the regression of *X_a* on all the other covariates are plotted on the *x*-axis. This is the part of *X_a* not explained by the other covariates. The regression line and a line determined by a scatter plot smoothing method that calculates a locally weighted least-squares estimate for each point in the scatter plot (Cleveland 1979) were displayed. We used the lowest function in the S-Plus statistical software (MathSoft, Seattle, WA, USA) to produce these plots. When warranted, outliers were removed and the models were repeated.

Results

A total of 79 (9.9%) participants were heterozygous for the *ALAD*² allele, and none was homozygous (Table 1). For *VDR*, 85 (10.7%) were genotype *Bb*, and four (0.5%) were *BB*. For *eNOS*, 114 (14.4%) participants were genotype *Glu/Asp*, and six (0.7%) were

Asp/Asp. Mean (\pm SD) crude values for demographic, exposure, and outcome variables by genotype are presented in Table 1, and adjusted differences are noted below.

ALAD. After removal of previously identified outliers (Weaver et al. 2003) and adjustment for age, sex, BMI, hypertension, current smoking, work status (current vs. former lead worker), and lead dose, workers with the *ALAD*¹⁻² genotype were found to have lower serum creatinine and higher calculated creatinine clearances compared with those with the *ALAD*¹⁻¹ genotype ($p < 0.05$).

Effect modification by *ALAD* on relations between lead and the renal outcomes was observed (Table 2). Among participants with the *ALAD*¹⁻² genotype, higher lead measures were associated with lower BUN, serum creatinine, and RBP and with higher creatinine clearances; these relations were statistically different ($p < 0.1$) compared with those in participants with the *ALAD*¹⁻¹ genotype. Added variable plots of associations between blood lead and the renal outcomes indicate that these relations are not due to influential outliers (Figure 1). These plots also illustrate the magnitude of change in the renal outcomes across the blood lead range in those with the *ALAD*² allele. No blood lead level threshold for these effects is apparent.

To determine whether increased urinary creatinine from increased glomerular filtration contributed to the association between higher blood lead and lower RBP in those with the *ALAD*¹⁻² genotype, the model was repeated using RBP unadjusted by creatinine. The β coefficient was similar (-0.0123), and $p = 0.09$.

eNOS. After removal of previously identified outliers (Weaver et al. 2003) and adjustment, mean measured creatinine clearance was found to be higher in participants with the *eNOS Asp* allele ($p < 0.05$). Mean measured creatinine clearance, adjusted for age, sex,

Table 1. Selected demographic, exposure, and outcome variables by *ALAD*, *eNOS*, and *VDR* genotype in Korean lead workers.^a

Characteristic	<i>ALAD</i> genotype		<i>eNOS</i> genotype		<i>VDR</i> genotype	
	1-1	1-2	<i>Glu/Glu</i>	<i>Asp/Glu</i> or <i>Asp/Asp</i>	<i>bb</i>	<i>Bb</i> or <i>BB</i>
Number (%)	716 (90.1)	79 (9.9)	673 (84.9)	120 (15.1)	709 (88.8)	89 (11.2)
Age, years (mean \pm SD)	40.5 \pm 10.2	40.1 \pm 9.7	40.3 \pm 10.1	41.1 \pm 10.1	40.2 \pm 10.1	42.7 \pm 10.3
Sex, <i>n</i> (%)						
Male	569 (79.5)	62 (78.5)	537 (79.8)	93 (77.5)	572 (80.7)	62 (69.7)
Female	147 (20.5)	17 (21.5)	136 (20.2)	27 (22.5)	137 (19.3)	27 (30.3)
BMI, kg/m ² (mean \pm SD)	23.1 \pm 3.0	22.3 \pm 2.6	23.1 \pm 3.0	22.7 \pm 2.8	22.9 \pm 2.9	23.9 \pm 3.4
Hypertension, <i>n</i> (%)	52 (7.3)	5 (6.3)	51 (7.6)	7 (5.8)	47 (6.6)	11 (12.4)
Lead job duration, years (mean \pm SD)	8.1 \pm 6.6	7.9 \pm 5.8	8.0 \pm 6.6	8.5 \pm 6.5	8.2 \pm 6.7	7.1 \pm 5.6
Blood lead, μ g/dL (mean \pm SD)	31.7 \pm 14.9	34.2 \pm 15.9	32.0 \pm 15.1	31.2 \pm 14.6	31.6 \pm 14.8	34.8 \pm 16.1
Tibia lead, μ g Pb/g bone mineral (mean \pm SD)	37.5 \pm 40.6	31.4 \pm 29.5	37.5 \pm 41.6	35.8 \pm 34.0	37.1 \pm 41.2	38.1 \pm 33.5
DMSA-chelatable lead, μ g Pb/g creatinine (mean \pm SD)	763.2 \pm 822.4	838.2 \pm 1188.3	775.2 \pm 899.7	717.9 \pm 622.8	750.2 \pm 873.9	925.7 \pm 758.5
BUN, mg/dL (mean \pm SD)	14.5 \pm 3.6	13.7 \pm 3.9	14.3 \pm 3.6	14.9 \pm 3.8*	14.4 \pm 3.7	14.4 \pm 3.3
Serum creatinine, mg/dL (mean \pm SD)	0.90 \pm 0.15	0.86 \pm 0.12**	0.90 \pm 0.16	0.89 \pm 0.13	0.90 \pm 0.16	0.89 \pm 0.13
Measured creatinine clearance, mL/min (mean \pm SD)	114.6 \pm 34.2	115.6 \pm 27.6	113.7 \pm 32.9	118.8 \pm 36.3**	115.0 \pm 33.7	111.8 \pm 33.5
Calculated creatinine clearance, mL/min (mean \pm SD)	94.6 \pm 20.7	95.7 \pm 19.7**	94.6 \pm 20.0	94.1 \pm 22.6	94.6 \pm 20.3	94.8 \pm 23.3
NAG, μ mol/hr/g creatinine (mean \pm SD)	229.7 \pm 248.6	204.6 \pm 141.6	221.5 \pm 239.5	259.6 \pm 244.5	226.1 \pm 244.5	229.3 \pm 200.1
RBP, μ g/g creatinine (mean \pm SD)	57.2 \pm 101.1	65.6 \pm 89.5	63.7 \pm 204.7	61.7 \pm 87.8	64.7 \pm 201.7	55.8 \pm 60.3

^a*ALAD*, *eNOS*, and *VDR* genotyping were completed on 795, 793, and 798 lead workers, respectively. * $p < 0.1$ for difference between renal outcomes by genotype after adjustment. ** $p < 0.05$ for difference between renal outcomes by genotype after adjustment.

Table 2. Linear regression modeling of effect modification by genotype on selected associations between renal outcomes and lead dose variables.

Variable	β coefficient	SE β	p-Value
BUN (mg/dL)			
Intercept	9.6098	1.1686	< 0.01
<i>ALAD</i> ¹⁻²	2.0037	1.0164	0.05
Blood lead ($\mu\text{g/dL}$)	0.0094	0.0102	0.36
Blood lead \times <i>ALAD</i> ¹⁻²	-0.0771	0.0278	< 0.01
Serum creatinine (mg/dL)			
Intercept	9.5556	1.1701	< 0.01
<i>ALAD</i> ¹⁻²	0.8670	0.6672	0.19
DMSA-chelatable lead ($\mu\text{g Pb/g creatinine}$)	0.0000	0.0002	0.93
DMSA-chelatable lead \times <i>ALAD</i> ¹⁻²	-0.0022	0.0008	< 0.01
Measured creatinine clearance (mL/min)			
Intercept	0.8472	0.0358	< 0.01
<i>ALAD</i> ¹⁻²	0.0222	0.0316	0.48
Blood lead ($\mu\text{g/dL}$)	0.0003	0.0003	0.41
Blood lead \times <i>ALAD</i> ¹⁻²	-0.0017	0.0009	0.05
Intercept	0.8519	0.0361	< 0.01
<i>ALAD</i> ¹⁻²	0.0076	0.0205	0.71
DMSA-chelatable lead ($\mu\text{g Pb/g creatinine}$)	-0.0000	0.0000	0.02
DMSA-chelatable lead \times <i>ALAD</i> ¹⁻²	-0.0001	0.0000	0.02
Intercept	0.8507	0.0362	< 0.01
<i>eNOS Asp/Glu</i> or <i>Asp/Asp</i>	-0.0364	0.0173	0.04
Lead job duration (years)	-0.0009	0.0007	0.25
Lead job duration \times <i>eNOS Asp/Glu</i> or <i>Asp/Asp</i>	0.0039	0.0016	0.02
Calculated creatinine clearance (mL/min)			
Intercept	74.0404	9.2094	< 0.01
<i>ALAD</i> ¹⁻²	-2.2897	5.2482	0.66
DMSA-chelatable lead ($\mu\text{g Pb/g creatinine}$)	-0.0010	0.0016	0.53
DMSA-chelatable lead \times <i>ALAD</i> ¹⁻²	0.0106	0.0061	0.08
Intercept	77.4254	9.0917	< 0.01
<i>VDR Bb</i> or <i>BB</i>	-9.7894	5.2827	0.06
Tibia lead ($\mu\text{g Pb/g bone mineral}$)	0.0514	0.0290	0.08
Tibia lead \times <i>VDR Bb</i> or <i>BB</i>	0.2419	0.1229	0.05
In NAG [ln ($\mu\text{mol/hr/g creatinine}$)] models			
Intercept	56.6738	4.3455	< 0.01
<i>ALAD</i> ¹⁻²	-6.3221	3.7759	0.09
Blood lead ($\mu\text{g/dL}$)	-0.0567	0.0383	0.14
Blood lead \times <i>ALAD</i> ¹⁻²	0.2875	0.1033	< 0.01
Intercept	56.2870	4.3876	< 0.01
<i>ALAD</i> ¹⁻²	-2.2172	2.4992	0.38
DMSA-chelatable lead ($\mu\text{g Pb/g creatinine}$)	0.0005	0.0007	0.49
DMSA-chelatable lead \times <i>ALAD</i> ¹⁻²	0.0079	0.0029	< 0.01
Intercept	56.6014	4.3343	< 0.01
<i>eNOS Asp/Glu</i> or <i>Asp/Asp</i>	5.0939	2.0672	0.01
Lead job duration (years)	0.2401	0.0898	0.01
Lead job duration \times <i>eNOS Asp/Glu</i> or <i>Asp/Asp</i>	-0.4015	0.1957	0.04
In RBP [ln ($\mu\text{g/g creatinine}$)]			
Intercept	4.0771	0.2156	< 0.01
<i>eNOS Asp/Glu</i> or <i>Asp/Asp</i>	0.2254	0.0931	0.02
Lead job duration (years)	0.0070	0.0040	0.08
Lead job duration \times <i>eNOS Asp/Glu</i> or <i>Asp/Asp</i>	-0.0157	0.0088	0.08
Intercept	4.1184	0.2130	< 0.01
<i>VDR Bb</i> or <i>BB</i>	0.1528	0.1014	0.13
DMSA-chelatable lead ($\mu\text{g Pb/g creatinine}$)	0.0002	0.0000	< 0.01
DMSA-chelatable lead \times <i>VDR Bb</i> or <i>BB</i>	-0.0002	0.0001	0.05

BUN, serum creatinine, measured creatinine clearance, and calculated creatinine clearance models were also adjusted for age, sex, BMI, current/former exposure status, current smoking, and hypertension. NAG and RBP models were adjusted for age, sex, BMI, systolic blood pressure, current/former exposure status, current alcohol ingestion, and diabetes. *p*-Values for the cross-product terms reflect the statistical significance of the difference between the slopes of the regression line for the gene variant group and the regression line for the reference gene group. Slopes in the nonreference category are obtained by adding the β coefficient of the cross-product term to the β coefficient for the reference category. Only outcomes with significant or borderline significant associations ($p < 0.1$) are presented from a total of 24 models for each gene.

BMI, hypertension, work status (current vs. former), current smoking, and blood lead, was 112.3 mL/min in participants with the *Glu/Glu* genotype, 118.2 mL/min in those with *Glu/Asp* genotype, and 125.4 mL/min in the five participants with the *Asp/Asp* genotype ($p = 0.02$ for trend). In contrast, BUN was also higher ($p = 0.04$ – 0.06 depending on lead covariate in the model), and a trend was present for mean BUN by genotype ($p = 0.04$); means after adjustment for the same covariates, except tibia instead of blood lead, were 14.2, 14.9, and 15.8 $\mu\text{g/dL}$ for the *Glu/Glu*, *Glu/Asp*, and *Asp/Asp* genotypes, respectively. Effect modification by *eNOS* on relations between lead and the renal outcomes was observed in only 3 of 24 models (Table 2). Among participants with the *Glu/Glu* genotype, longer lead job duration was associated with higher calculated creatinine clearance but also borderline associated ($p = 0.08$) with higher NAG. In contrast, in those with the *Asp* allele, longer lead job duration was associated with higher serum creatinine and lower calculated creatinine clearance but also lower NAG. These relations were statistically different ($p < 0.05$ except for NAG, where $p < 0.1$) and in opposite directions compared with those in participants with the *Glu/Glu* genotype.

VDR. No main effects of *VDR* genotype on renal outcomes were observed. Effect modification was present in 2 of 24 models (Table 2). Higher tibia lead was associated with higher measured creatinine clearance in all participants, but the slope of the relation was greater in those with *VDR Bb* or *BB* genotypes. DMSA-chelatable lead was directly associated with NAG only in those with the *bb* genotype.

Discussion

In this study, we evaluated whether polymorphisms in three genes (*ALAD*, *VDR*, and *eNOS*) were associated with or modified relations of lead exposure and dose measures with six renal outcomes in a large cross-sectional study of Korean lead workers. To our knowledge, this is the first study to evaluate effect modification by *ALAD*, *VDR*, or *eNOS* genetic polymorphisms on the relations between lead measures and renal outcomes. After adjustment, participants with the *ALAD*² allele had lower mean serum creatinine and higher calculated creatinine clearance. Effect modification by *ALAD* on associations between lead dose and renal outcomes was present. Higher lead dose was associated with lower BUN, serum creatinine, and RBP but higher creatinine clearances in participants with the *ALAD*¹⁻² genotype. Mean renal outcome differences by *ALAD* genotype were relatively small in magnitude, despite being statistically significant. However, examination of effect modification by *ALAD* revealed that, in those with the

*ALAD*² allele, clinical renal outcomes changed by 10% or more across the blood lead range.

The prevalence of the *ALAD*² allele is approximately 20% in Caucasians and 10% in Asians (Kelada et al. 2001; Schwartz et al. 1995). In addition to the toxicokinetic differences mentioned above, lower cortical bone lead (Hu et al. 2001) has been reported. Hu et al. (2001) also noted less efficient uptake of lead into bone when trabecular lead levels > 60 µg/g bone mineral were present. Data on the health implications of these toxicokinetic differences suggest a protective effect of the *ALAD*² allele on the hematologic system

(Alexander et al. 1998; Schwartz et al. 1995; Sithisarankul et al. 1997). In the lead workers studied here, those with the *ALAD*² allele had lower levels of zinc protoporphyrin and plasma aminolevulinic acid, but there was no clear effect modification by *ALAD* genotype on relations between lead dose and hematologic system outcomes (Lee SS et al. 2001). Possibly improved neuropsychologic function has also been reported, based on a few participants with the variant allele (Bellinger et al. 1994).

Two studies have assessed the effect of *ALAD* genotype on the renal system in lead-exposed populations. Smith et al. (1995)

studied 691 volunteers from a construction trade union, of whom 96 had the *ALAD*² allele. Mean blood lead was 7.78 µg/dL. As described above, BUN and serum creatinine were elevated in participants with the *ALAD*² allele compared with those with the *ALAD*¹⁻¹ genotype, but *p*-values for these differences increased after adjustment. Bergdahl et al. (1997a) assessed the impact of *ALAD* genotype on kidney function in 89 lead workers, of whom only seven had the *ALAD*² allele. Serum creatinine was elevated in the latter group, although, again, the difference was not statistically significant and adjusted data were not presented. Thus, our results are not consistent with the data presented in these publications. There are some potentially important differences between our work and these studies. The small number of participants with the *ALAD*² allele limits the power to detect a difference in the study of Bergdahl et al. (1997a). Compared with Smith et al. (1995), our participants had a higher mean blood lead level and a wider range of lead measures and renal outcomes. These factors may play a role in the differences noted.

Our work expands on the existing literature pertaining to the effect of *ALAD* genotype on the renal system in lead-exposed populations by assessing effect modification. Although our data by *ALAD* genotype are unique, inverse associations similar to those we found in participants with *ALAD*² allele (i.e., associations between higher lead measures and lower BUN and serum creatinine and higher creatinine clearances) have been previously reported in lead-exposed populations. Mean measured creatinine clearance was 112.9 mL/min/1.73 m² in 22 adults who had experienced childhood lead poisoning, compared with 88.8 mL/min/1.73 m² in age- and sex-matched controls (*p* < 0.01) (Hu 1991). Roels et al. (1994) also reported a mean creatinine clearance of 121.3 mL/min/1.73 m² in a group of 76 lead workers compared with 115.5 mL/min/1.73 m² in 68 age- and sex-matched controls (*p* < 0.05). More important, they also observed a positive association between tibia lead and creatinine clearance. Similarly, we also found associations in the population studied here between higher lead measures and lower BUN and serum creatinine, and higher creatinine clearance measures, especially in younger workers (Weaver et al. 2003).

A longitudinal study in rodents reported a positive association between glomerular filtration rate (GFR) and blood lead after 3 months of lead acetate ingestion (Khalil-Manesh et al. 1992). However, after 6 months of exposure, tubulointerstitial fibrosis was present, and at 12 months, focal glomerulosclerosis was seen and signs of renal insufficiency had developed. A similar pattern of renal function change has been observed in studies of human diabetics

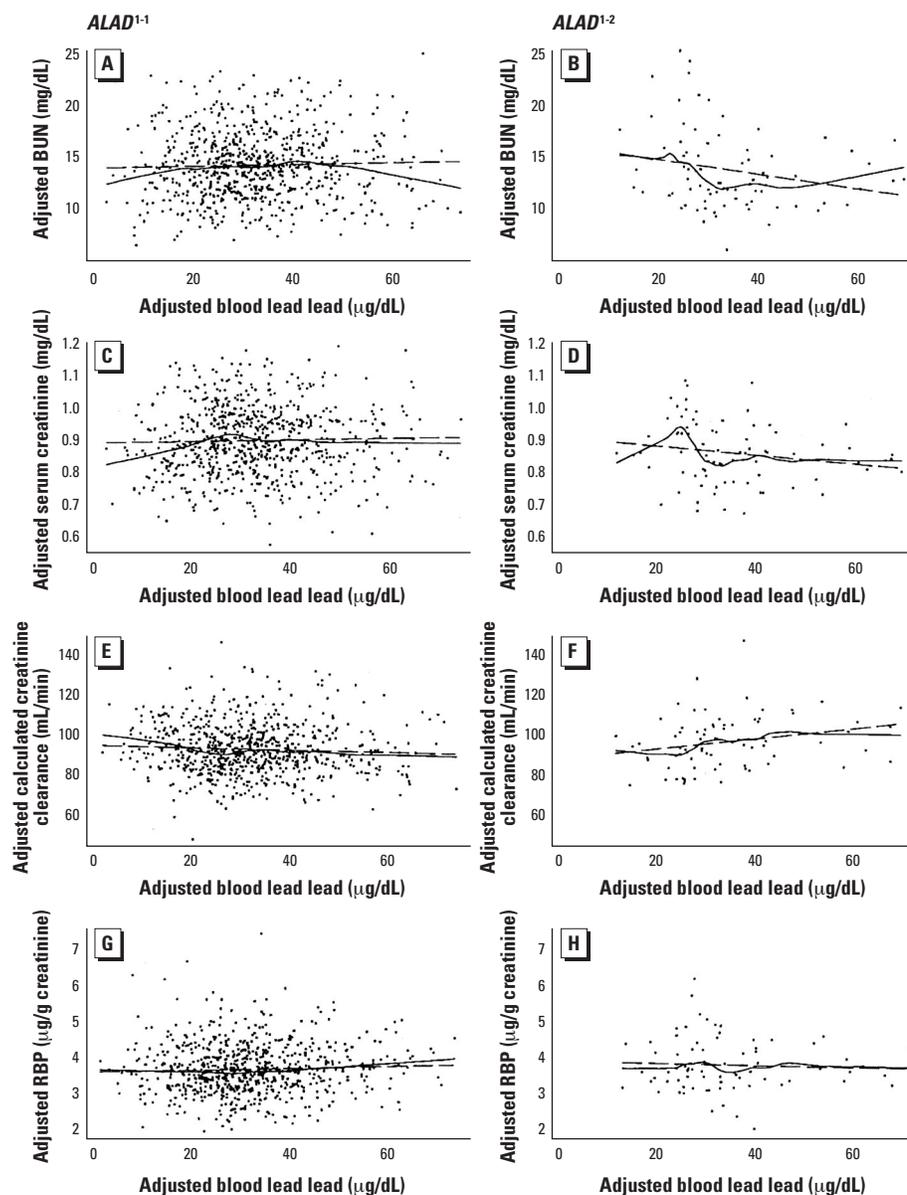


Figure 1. Added variable plots of the effect modification by *ALAD* on relations between blood lead and four renal outcomes in 795 Korean lead workers. (A, C, E, G) Data from participants with the *ALAD*¹⁻¹ genotype. (B, D, F, H) Data from those with *ALAD*¹⁻². (A, B) Adjusted BUN. (C, D) Adjusted serum creatinine. (E, F) Adjusted calculated creatinine clearance. (G, H) Adjusted RBP. For each plot, the adjusted regression line (dashed) and the lowest line (solid; see "Materials and Methods" for details) are shown. Both have been adjusted for covariates. For ease of interpretation, axes have been scaled so that the plotted residuals are centered around the means rather than zero.

followed longitudinally; supranormal creatinine clearance early in the clinical course of type I diabetes is a predictor of subsequent nephropathy (Brenner et al. 1996). Other diseases and conditions in which increased glomerular filtration is present, such as sickle cell disease and obesity, are also associated with an increased risk for subsequent renal abnormalities (Allon 1990; Nenov et al. 2000). The hyperfiltration theory, involving a paradoxical initial increase in GFR associated with glomerular hypertension and ultimately ending in glomerulosclerosis and renal failure, is one mechanistic explanation for this pattern (Brenner et al. 1996). However, kidney donors also have elevated GFR but generally do not have evidence of other renal abnormalities. These individuals are carefully screened to exclude underlying diseases that could result in adverse renal outcomes, a fact that may, in part, explain this observation (Nenov et al. 2000). It is also possible that the significance of increased creatinine clearance may vary depending on the underlying condition.

The hyperfiltration theory generally refers to clinical renal function. The effect modification by *ALAD* genotype on relations between blood lead and RBP, a renal early biologic effect marker, is another novel and intriguing finding in this population. One possible explanation for this finding is that RBP is routinely divided by urinary creatinine to reduce the impact of urinary dilutional variation on its concentration. However, in the setting of increased glomerular filtration, urinary creatinine may also be increased, which adversely affects the accuracy of spot urine creatinine-adjusted biomarker results (Muller et al. 1999). We were able to exclude this as an explanatory factor in our findings by modeling with unadjusted RBP.

After adjustment, we found that participants with the *eNOS Asp* allele had higher measured creatinine clearance but, paradoxically, higher BUN, with significant trend tests for both across the three *eNOS* genotype groups (none, one, and two *Asp* alleles). Effect modification by *eNOS* was present in only 3 of 24 models and, although the two significant associations suggested increased risk for adverse renal outcomes with higher lead dose in those with the *Asp* allele, the borderline association with NAG was inconsistent. Reported *Asp* allele frequencies of the *Glu298Asp* polymorphism in two Japanese populations were 5.0% and 8.7% (Hibi et al. 1998; Miyamoto et al. 1998); a frequency of 32% was reported in a U.S. population of unspecified ethnicity (Zanchi et al. 2000). Data on this polymorphism are still relatively limited; however, as discussed above, recent work suggests that the *Asp* allele may be associated with decreased serum NO levels. Because lead-induced hypertension in rats is

also associated with reduced urinary excretion of NO metabolites (Vaziri et al. 1997), the combination of the *Asp* allele and lead exposure may confer an increased risk for lead-induced renal disease via vasoconstriction. Although some of our findings are consistent with this, our ability to draw firm conclusions based on our data is limited by their lack of consistency. Similarly, we found few significant results for *VDR*, despite the previously mentioned lead measure and blood pressure differences reported in this same population. It is possible that our relative lack of positive findings with *eNOS* and *VDR* genotypes is because the effect of lead dose on the kidney is not mediated through either of these genes. However, it must also be noted that, although the sample size was large, the number of participants with variant alleles was relatively small, leading to unstable estimation of coefficients. Further research is needed to determine which genetic susceptibility factors, other than *ALAD*, are important for the effect of lead on the renal system.

In conclusion, higher lead dose was associated with lower BUN, serum creatinine, and RBP but higher creatinine clearances in participants with the *ALAD*¹⁻² genotype. These findings may represent lead-induced hyperfiltration. Longitudinal data will be important in determining whether subsequent renal function decline differs by genotype.

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