

## ALAD

### Abstract

#### Associations of ALAD Genotype with Renal Function Indices in Lead Workers

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**Objective:** -Aminolevulinic acid dehydratase (ALAD) is a polymorphic enzyme that has two alleles, ALAD1 (ALAD1-1 as genotype) and ALAD2 (ALAD1-2 or ALAD2-2 as genotype). ALAD genotype has been reported to modify the pharmacokinetics and toxicity of lead. The authors investigated the influence of ALAD genotype polymorphism on renal function in lead workers

**Methods:** We studied 935 male lead workers and 87 male non-lead workers in the same industries. For cross-sectional renal indices, blood urea nitrogen (BUN), serum creatinine, serum uric acid and urine total protein were selected. Blood lead level was also measured an index of lead exposure. Information on weight, age, job duration, and smoking and drinking habits was collected.

**Results:** Whereas the mean blood lead level of lead workers was  $25.4 \pm 10.9$   $\mu\text{g}/\text{dL}$ , that of non-lead workers in the same premise was  $10.1 \pm 2.8$   $\mu\text{g}/\text{dL}$ , and the difference between the two groups was statistically significant.

Whereas the prevalence of the variant allele, ALAD2 in 935 lead workers was 10.6%, that in 87 non-lead workers was 4.6%. However there was no difference of prevalence between the two groups.

The mean blood lead level of subjects with ALAD1 was  $23.9 \pm 11.4$   $\mu\text{g}/\text{dL}$ , which was slightly lower than that of subjects with ALAD2 ( $25.8 \pm 10.7$   $\mu\text{g}/\text{dL}$ ). However, this difference was not statistically significant.

After adjustment for the covariates, the subjects with ALAD2 allele were 12.8% less likely to have a median value or more of BUN than subject with ALAD1. The adjusted odds ratio was 0.59 (95% confidence interval; 0.38-0.91).

After adjustment for the covariates, the subjects with ALAD2 allele were 9.3% less likely to

have a median value or more of serum creatinine than subject with ALAD1. The adjusted odds ratio was 0.64 (95% confidence interval; 0.41-0.98).

**Conclusions:** From the above results, it was found that the variant allele, ALAD2 appeared to modify the association of lead and renal function, and that ALAD2 genotype may be supportive for the protective effect of lead.

**Key Words:** -Aminolevulinic acid dehydratase (ALAD), Polymorphism, Renal function indices, Lead workers

aminolevulinic acid dehydratase(ALAD) (reserve capacity) blood urea nitrogen(BUN) uric acid가 ALAD ALAD 가 가

-aminolevulinic acid(ALA) porphobilinogen ALA 가 BUN, creatinine 가

ALAD1 ALAD2 2 allele 가 (polymorphism) retinol-binding protein, N-acetyl- D-glucosaminidase, 2-microglobulin 가 (Weaver , 2003)

ALAD1-1, ALAD1-2 ALAD2-2 가 (Wetmur, 1994). ALAD poly- (1993)

가 (Alexander , BUN 1998; Astrin , 1987; Bergdahl , 1997). Wetmur (1991) ALAD 가

ALAD1 (ALAD1-2 ALAD2-2) 가 ALAD1 BUN (ALAD1-1) 가 가

, Schwartz (1995) ALAD2 ALAD

zinc protoporphyrin(ZPP) ALAD2 가

Smith (1995) ALAD 935 ALAD1 가 87

BUN, creatinine, uric acid, ALAD

1. (Hitachi 8100) (Fernandez, 1975). cyanmethemoglobin (Beckman Coulter, Inc., Model Ac-T 8, Fullerton, CA), caillary (, 1987). (BUN) urease (Bertelot), creatinine Folin-W(Jaffe reaction), uricase (, 1987). Tonein-TP (, 1993).

2. ALAD (Wetmur, 1991)

1) DNA 15%-K3EDTA Vacutainer . DNA 200 µ 1.5 ml microcentrifuge tube 25 µ QIAgen protease K 200 µ AL(prepare) buffer 70 10 . isopropanol(96-100%) 210 µ 가 5 , 8,000rpm . 2 ml collection tube QIAamp spin column , 8,000 rpm . filter DNA wash buffer 8,000 rpm 1 2 , 13,000 rpm 2

QIAamp spin column AE(elution) buffer 200 µ 70 incubation

8,000rpm 1 filter DNA (PCR: Polymerase Chain Reaction)

2) (PCR) Bio RAD Thermal Gene Cyclor . primer OPERON (Operon Inc., CA U.S.A) oligonucleotide primer 4 µg/ml . primer

ALAD-A: 5'-CCCAACCATCCCTCTCAGTC-3  
ALAD-B: 5'-CCCAACCTCCCTTCCTTTT-3

10 PCR buffer 5 µ, 0.2 mM-dNTP 1 µ, 1-5 u/100 µ Taq DNA polymerase 0.3 µ (5 unit/µl) . primer A 1 µ (200 µg/µ) primer B 1 µ (200 µg/µ) template DNA 3 µ (<1 µg /100 µ 105-106 copies) 50 µ . PCR 94 3 1 cycle, 94 30 , 60 30 , 72 1 41 cycle 59 1.5 , 72 10 . 3) MspI 2 µ(sequence GGC C, 10 unit/µ) incubation buffer (SURE/Cut 5 buffer L) 5 µ PCR tube DNA 20 µ 가 50 µ 37 24

4) agarose gel 10 µ 0.5 µ/µ ethidium bromide가 1.5% agarose gel TBE buffer(Tris base 0.089 M, boric acid 0.089

M, EDTA 0.002M) 150V 40 25.4±10.9 µg/dℓ  
 gel UV-transillu 10.1±2.8 µg/dℓ  
 minator 139-473 (base pair)  
 DNA DNA 가  
 DNA DNA BUN  
 ALAD 1-1, 1-2, 2-2 14.0±3.3 mg/dℓ 13.1±3.0  
 가 mg/dℓ (p<0.05)  
 ALAD1-1 473, 271, 158, 139 10.1 µg/dℓ  
 (base pair fragment) ALAD1-2 가  
 473, 402, 271, 158, 139  
 ALAD2-2 402, 271, 158, 139

ALAD

3.

Table 2

ALAD 가  
 가  
 SAS 6.12 (SAS Institute Inc.)  
 ALAD1 23.9±11.3 µg/dℓ  
 ALAD2 25.8±10.7 µg/dℓ

Chi-square Student t- ALAD 가  
 p Peason (median) ALAD2 BUN  
 13.3±3.2 mg/dℓ ALAD1  
 14.0±3.3 mg/dℓ (p<0.05), creatinine, 가  
 ( )  
 , ALAD  
 (interaction) 0 , ALAD 1

BUN 13.5 mg/dℓ  
 ALAD2 odds ratio 0.59 (95%  
 ( ) Table 1 0.38-0.91) ALAD2  
 33.0 34.1 ALAD1 BUN  
 가 41% (Table 3).  
 ALAD1-1 89.4% , BUN  
 95.4% ALAD  
 ALAD2  
 가 ALAD1 가

**Table 1.** Characteristics of study subjects

Variables	Exposed (N=935)			Non-exposed (N=87)		
	Mean $\pm$ S.D	Min	Max	Mean $\pm$ S.D	Min	Max
Age (yrs)	33.0 $\pm$ 7.5	20.0	71.0	34.1 $\pm$ 6.6	23.0	55.0
JOBDUR (yrs)	5.6 $\pm$ 4.6	1.0	30.0	6.2 $\pm$ 3.7	1.0	16.0
Weight (kg)	63.4 $\pm$ 8.8	32.0	173.0	64.7 $\pm$ 7.7	49.0	87.0
PBB ( $\mu\text{g}/\text{d}\ell$ )	25.4 $\pm$ 10.9	8.0	65.6	10.1 $\pm$ 2.8	2.3	14.9
HB (g/d $\ell$ )	14.8 $\pm$ 1.0	9.9	18.3	14.9 $\pm$ 1.0	10.7	17.0
HCT (%)	45.0 $\pm$ 3.9	31.0	63.0	45.4 $\pm$ 4.1	33.0	59.0
BUN (mg/d $\ell$ )*	14.0 $\pm$ 3.3	6.8	27.5	13.1 $\pm$ 3.0	6.1	21.9
BCr (mg/d $\ell$ )	1.0 $\pm$ 0.1	0.7	1.5	1.0 $\pm$ 0.1	0.7	1.4
Uric acid (mg/d $\ell$ )	5.4 $\pm$ 1.1	1.0	10.9	5.4 $\pm$ 0.9	3.0	8.0
TP (mg/d $\ell$ )	7.4 $\pm$ 0.4	6.2	9.2	7.5 $\pm$ 0.4	6.7	8.5
<b>Smoking*</b>						
Current, N (%)	713 (76.3)			56 (64.4)		
Never & Ex, N (%)	222 (23.7)			31 (35.6)		
<b>Drinking</b>						
Current, N (%)	741 (79.3)			64 (73.6)		
Never & Ex, N (%)	194 (20.7)			23 (26.4)		
<b>ALAD genotype</b>						
1-1, N (%)	836 (89.4)			83 (95.4)		
1-2 / 2-2, N (%)	99 (10.6)			4 ( 4.6)		

\* : p&lt;0.05

JOBDUR: job duration PBB: blood lead HB: hemoglobin HCT: hematocrit BUN: blood urea nitrogen  
BCr: serum creatinine TP: urine total protein

**Table 2.** The means of lead biomarker variables and renal function indices by gene status of ALAD

Variables	ALAD 1-1 (N=919)			ALAD 1-2/2-2 (N=103)		
	Mean $\pm$ S.D	Min	Max	Mean $\pm$ S.D	Min	Max
Age (yrs)	33.0 $\pm$ 7.4	21.0	71.0	33.4 $\pm$ 7.6	20.0	58.0
JOBDUR (yrs)	5.6 $\pm$ 4.5	1.0	30.0	5.8 $\pm$ 4.4	1.0	17.0
Weight (kg)	63.5 $\pm$ 8.8	32.0	173.0	63.0 $\pm$ 7.6	46.0	85.0
PBB ( $\mu\text{g}/\text{d}\ell$ )	23.9 $\pm$ 11.4	2.3	65.6	25.8 $\pm$ 10.7	9.0	61.4
HB (g/d $\ell$ )	14.8 $\pm$ 1.0	9.9	18.3	14.9 $\pm$ 1.0	10.6	17.9
HCT (%)	45.0 $\pm$ 3.9	31.0	63.0	45.3 $\pm$ 3.9	32.0	59.0
BUN (mg/d $\ell$ )*	14.0 $\pm$ 3.3	6.8	27.5	13.3 $\pm$ 3.2	6.1	22.5
BCr (mg/d $\ell$ )	1.0 $\pm$ 0.1	0.7	1.5	1.0 $\pm$ 0.1	0.8	1.2
Uric acid (mg/d $\ell$ )	5.4 $\pm$ 1.1	1.0	10.9	5.5 $\pm$ 1.1	2.2	8.4
TP (mg/d $\ell$ )	7.4 $\pm$ 0.4	6.2	9.2	7.4 $\pm$ 0.3	6.6	8.3

\* : p&lt;0.05

JOBDUR: job duration PBB: blood lead HB: hemoglobin HCT: hematocrit BUN: blood urea nitrogen  
BCr: serum creatinine TP: total protein

BUN, BUN  
 ALAD  
 ( ).  
 가  
 ALAD2 odds ratio  
 가  
 ALAD  
 가 ( (ALAD) ferrochelatase (Onalaja Claudio, 2000).  
 creatinine 1.0 mg/dl ALAD heme  
 ALAD2 odd ratio 0.64 ALAD metallo-enzyme  
 (95% 0.41-0.98) ALAD2  
 ALAD1 cre- 가  
 atinine 36% (Wetmur,  
 (Table 4). 1994).  
 atinine cre- ALAD1 (ALAD1-1) ALAD2  
 ALAD (ALAD1-1 ALAD2-2)  
 ALAD2 가 ALAD1 9:1 (Wetmur,  
 가 creati- ALAD2 15~20%  
 nine creatinine, ALAD2  
 ALAD ( (Benkmann, 1983; Sousa  
 ). , 1991). ALAD2 가  
 10.6% Weaver (2003)

**Table 3.** Associations of ALAD genotype with BUN (median value=13.5 mg/dl)

ALAD genotype	BUN (mg/dl)		Total	Crude OR	Adjusted OR <sup>#</sup>
	<13.5	13.5			
1-1	445 (48.4)	474 (51.6)	919 (100)	1.0	1.0
1-2 / 2-2	63 (61.2)	40 (38.8)	103 (100.0)	0.59 (0.39, 0.90)	0.59 (0.38, 0.91)
Total	508 (49.7)	514 (50.3)	1,022 (100.0)		

<sup>#</sup> All models controlled for age, job duration, blood lead, smoking and drinking status and weight.

**Table 4.** Associations of ALAD genotype with serum creatinine (median value=1.0 mg/dl)

ALAD genotype	serum creatinine (mg/dl)		Total	Crude OR	Adjusted OR <sup>#</sup>
	<1.0	1.0			
1-1	464 (50.5)	455 (49.5)	919 (100)	1.0	1.0
1-2 / 2-2	63 (60.2)	41 (39.8)	103 (100.0)	0.67 (0.44, 1.02)	0.64 (0.41, 0.98)
Total	527 (51.5)	496 (48.5)	1,022 (100.0)		

<sup>#</sup> All models controlled for age, job duration, blood lead, smoking and drinking status and weight.

9.9% Kim (2004) 9.6% 가 가 ALAD2  
 , 9% 가 가  
 (Benkmann , 1983). BUN creatinine  
 Astrin (1987) New York Lead  
 Screening Program  
 ALAD2 가 BUN ALAD2  
 ALAD2 ALAD1 ALAD1  
 가 가 41% 0.59  
 , Wetmur (1991) ALAD2 가 (95% : 0.38-0.91)  
 ALAD1 가 (odds ratio) , creatinine  
 9-11  $\mu\text{g}/\text{dL}$  ALAD2 creatinine  
 가 ALAD 가 36% 0.64 (95% : 0.41-  
 0.98) (odds ratio) ALAD2  
 ALAD2 가  
 (Wetmur, 1994). BUN creati-  
 Schwartz (1995) 3 nine  
 290 ALAD  
 , 40  $\mu\text{g}/\text{dL}$  ALAD  
 (odds ratio) 가 ALAD1 ALAD2  
 , ALAD2 가 tinine BUN crea-  
 가 ALAD1 가 ALAD2 ALAD1  
 Weaver (2003) 798  
 ALAD2  
 가 ZPP가 ALAD1 ALAD1 creatinine creatinine clearance  
 가 ALAD2 ALAD1  
 ALAD2 ,  
 Smith (1995) 691  
 ALAD 가 BUN retinol-binding pro-  
 ALAD2 ALAD ALAD pharmacody tein, N-acetyl- -D-glucosaminidase, 2-  
 namic microglobulin , ( )  
 Bergdahl (1997) ) 가  
 89 .  
 calcium creatinine ALAD2 ALAD ALAD

ALAD

ALAD ALAD 가 (p<0.05).

ALAD2 가

Onalaja Claudio (2000) 가 99 10.6%

가 ALAD2 가 4

ALAD , 4.6%

ALAD1

가 가 ( ) 23.9±11.4 µg/dl

ALAD ALAD2 ( ) 25.8±

가 가 10.7 µg/dl

가 BUN, creatinine,

uric acid

ALAD 가 ,

가 , , , ,

ALAD2 BUN

가 logistic ALAD2

가 odds ratio가 0.59 (95% : 0.38-0.91)

가 ALAD2 ALAD1

ALAD 41% 가

creatinine

가 logistic ALAD2

odds ratio가 0.64 (95% : 0.40-0.98)

ALAD2 ALAD1

: -aminolevulinic acid dehydratase 36%가 가

(ALAD) (polymorphism) : ALAD2

ALAD1 가

가

935 , , , , .

87

BUN, creati- 1993;5(1):58-75.

nine, uric acid, 1987.

Alexander BH, Checkoway H, Costa-Mallen P, Faustman EM, Woods JS, Kelsey KT, Netten C, Costa LG. Interaction of blood lead and -aminolevulinic acid dehydratase genotype on markers of heme synthesis and sperm production in lead smelter workers. Environ Heal Persp 1998;106(4):213-6.

25.4±10.9 µg/dl , ( )

10.1±2.8 µg/dl



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