ORIGINAL ARTICLE

Association of renal function and δ -aminolevulinic acid dehydratase polymorphism among Vietnamese and Singapore workers exposed to inorganic lead

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Objectives: To investigate the effect of δ -aminolevulinic acid dehydratase (ALAD) polymorphisms on the association between blood lead and renal function among Vietnamese and Singaporean workers who were exposed to low to medium levels of inorganic lead, and to study the distribution of ALAD polymorphism among Vietnamese, Chinese, Malays and Indians.

Methods: A total of 459 male and female workers were studied. Blood and urine were collected for each worker in order to determine ALAD genotype, blood lead, and urinary δ -aminolevulinic acid (ALAU). Renal function tests included urine albumin (Ualb), urine β 2 microglobulin (U β 2m), urinary α 1 microglobulin (U α 1m), N-acetyl-glucosaminidase (NAG), and urine retinol binding protein (RBP). A multiple regression model with interaction term was applied to fit the entire data and to explore the modifying effect of ALAD polymorphism on the relation of blood lead to each renal function parameter.

Results: ALAD1-1 was the predominant genotype for all the ethnic groups while ALAD2-2 was the rarest. The frequency of ALAD2 allele was higher among Malays (8.8%) and Indians (10.6%) compared to the Chinese (5.0%) and Vietnamese (4.3%). The geometric mean of blood lead for all workers was 19.0 μ g/dl. The models for U β 2m, U α 1m, and NAG showed that the ALAD1-2/2-2 group had higher β coefficients than the ALAD1-1 group. Corresponding to 10 μ g/dl blood lead, ALAD1-1 homozygotes had an increment of 1.288 μ g/g Cr, 1.175 mg/g Cr, and 1.995 U/g Cr for U β 2m, U α 1m, and NAG, respectively. ALAD1-2/2-2 subjects had higher increments of 3.802 μ g/g Cr, 2.138 mg/g Cr, and 3.89 U/g Cr for U β 2m, U α 1m, and NAG, respectively.

Conclusion: The frequency of the ALAD2 allele is as low in Vietnamese workers as in Chinese. Workers with the ALAD2 allele appeared more susceptible to the effects of lead (especially at higher levels) on renal function.

Exposure to inorganic lead (hence referred to as lead) in the environmental and occupational settings continues to be a serious public health problem. At high exposure levels, lead causes encephalopathy, kidney damage, anaemia, and toxicity to the reproductive system. Even at lower doses, lead produces alterations in cognitive development in children. A safe level of lead exposure has not been defined, as health risks associated with lead are found at ever lower doses.

Pinpointing the health risks associated with low level exposures to lead will have important implications with respect to its regulation. Health based guidelines limiting occupational and environmental exposures to lead have become more stringent over the past decade and are now thought to protect most of the population against major adverse health effects. However, genetically susceptible individuals may not be fully protected by current regulatory standards. Better understanding of genetic factors that influence susceptibility to lead induced intoxication could have significant ramifications for public health and intervention initiatives.

Delta-aminolevulinic acid dehydratase (ALAD) is an important enzyme in the accumulation and distribution of lead in the blood, bone, and internal organs in humans and animals. The gene that encodes ALAD, the ALAD gene, exists in two polymorphic forms that may modify lead toxicokinetics and ultimately influence individual susceptibility to lead poisoning. The ALAD gene is located on chromosome 9q34, which has two co-dominant alleles, ALAD1 and ALAD2.¹ The ALAD2 allele, which contains a G-C transversion

at nucleotide position 177 in the coding region, results in a non-conservative change in amino acid residue 59 from lysine (Lys) to asparagine (Asn). As the ALAD2 (59Asn) protein is more negatively charged than ALAD1 (59Lys), it has a higher affinity to the lead cation.^{2,3} This increased affinity to lead is supposed to cause the higher binding ability of ALAD2 enzymes to lead. Data to determine exactly how ALAD genotype affects lead toxicokinetics are still limited and the implications for health risks are not entirely clear.

ALAD2 carriers have been found to be associated with higher levels of total lead in blood and of free erythrocyte protoporphyrin levels.^{4–6} Bergdahl *et al* reported that the concentrations of urinary calcium and creatinine were lower in seven workers with ALAD1-2/2-2 compared with 82 ALAD1-1 lead workers.⁷ They suggested that the results indicated the presence of ALAD allele specific differences in kidney function, as well as a possible genetic healthy worker selection. It is therefore thought that because ALAD2 carriers/homozygotes retain lead longer, they sustain more oxidative and structural damage caused by lead. However, the association of ALAD2 with higher PbB was mostly observed in populations with relatively higher exposure level. It is unknown if this association is also involved at normal non-occupational situations. Furthermore, subsequent studies by

Abbreviations: ALAD, δ -aminolevulinic acid dehydratase; ALAU, urinary δ -aminolevulinic acid; NAG, N-acetyl-beta-glucosaminidase; RBP, retinol binding protein; Ualb, urinary albumin; U α 1m, urine α 1 microglobulin; U β 2m, urine β 2 microglobulin; URBP, urine retinol binding protein; UNAG, urinary N-acetyl-beta-glucosaminidase

Schwartz *et al* (2000), using the chelator dimethylsuccinic acid (DMSA), showed that bioavailable lead is actually lower in ALAD2 carriers compared to the ALAD1 homozygotes, suggesting that the ALAD1 allele is the susceptible allele for neurological risk.⁸

The ALAD2 allele is the less common form, occurring in 20% of the Caucasian population and more rarely in populations of African descent. Not much work has been done among the Asian populations except for the Koreans and Japanese, with the ALAD2-2 homozygotes occurring at a frequency less than 1%, and ALAD1-2 heterozygotes between 4% and 15%. A recent study by Hsieh *et al* in Taiwan indicates that the frequency of ALAD1-2/2-2 individuals is low (4.6%).⁹ Generally, Asian populations have a lower reported ALAD2 frequency than other populations.

Lead is known to affect tubular and glomerular function. Functionally, these effects on the kidney may cause leakage of tubular enzymes such as total amount of N-acetyl-beta-glucosaminidase (NAG) in urine, and possibly other proteins with low molecular weights (for example, β 2 microglobulin (β 2m), α 1 microglobulin (α 1m), and retinol binding protein (RBP)) and high molecular weights (for example, albumin) which may result in higher urinary concentration of these proteins.¹⁰ To our knowledge, there are few reports on the association between ALAD polymorphism and such known renal parameters reflecting early renal damage.

In consideration of the above, the present study was conducted with the following objectives: (1) to obtain some insights of the distribution of ALAD polymorphism among Vietnamese, Chinese, Malays, and Indians; and (2) to investigate the modifying effect of δ -aminolevulinic acid dehydratase (ALAD) polymorphisms on the association between blood lead and some renal indices in a cohort of lead exposed workers.

METHODS

The design of the study was cross-sectional in nature. The study was approved by Institutional Review Boards at the National University Hospital, Singapore and the Department of Preventive Medicine, Ministry of Health, Vietnam.

Study population

The study population consisted of two groups of workers, one from Singapore and the other from Vietnam. The Singapore workers consisted of 183 workers (out of a total workforce of 184) from two factories that produces lead stabiliser. One of the workers was not present throughout the study period and was thus excluded from the study, giving a response rate of 99.5%. The main exposure is lead oxide dust. The workers were usually rotated around various sections and had considerable lead exposure throughout their working lives. Each worker was interviewed by a trained technologist using a standard questionnaire. Signed consent was obtained for each worker before blood and urine samples were taken for subsequent analysis. This was followed by a clinical examination by an occupational physician.

The Vietnamese workers were from a battery factory in HaiPhong city, Vietnam. All workers from the production line as well as in the division of management and quality control were recruited into this study. The study population consisted of 323 workers, of which 246 were occupationally exposed to lead, while the remaining 77 were not directly exposed to lead. Signed consent was obtained for each worker before blood and urine samples were taken for subsequent analysis. The workers also completed a questionnaire with the help of a Vietnamese interviewer (the questionnaire is similar to the one used for the Singaporean workers, but had been translated to Vietnamese). After completing the questionnaire, spot urine and 10 ml of blood were collected from each

Table 1 Characteristics of study populations

Characteristic	Mean	SD
Age (y)	38.68	10.74
Exposure duration (y)	12.91	9.75
Haemoglobin (g/l)	151.1	11.7
	Frequency	%
Nationality		
Singaporean	183	39.87
Vietnamese	276	60.13
Gender		
Male	384	83.66
Female	75	16.34
Ethnic group		
Chinese	99	21.57
Malays	44	9.59
Indians	40	8.71
Vietnamese	276	60.13
Smoking history		
Smoker	229	49.9
Non-smoker	230	50.1
Alcohol use		
None	106	23.09
Occasional	205	44.66
Regular	148	32.24
Total	459	

worker during the medical examination. Twenty of the exposed lead workers were not present during the study period while another 17 did not want to participate. Ten of 77 workers who were not directly exposed to lead were unwilling to give their urine and blood for analysis. As the study was voluntary, the workers' decisions in not giving urine and/or blood were respected. Hence the 47 workers were excluded from the study, giving a response rate of 85.4% (276/323).

The overall response rate for the study, which consists of Singaporean and Vietnamese populations, was 90.5% (459/507).

Questionnaire

Information gathered included age, years of education, detailed occupational history, current and previous smoking habits, and alcohol intake. Smoking habit is classified as smoker and non-smoker; alcohol consumption has three categories—non-drinker, and occasional and regular drinkers.

Laboratory analysis

Blood lead (PbB) measurement

Blood sample was obtained by venepuncture using lead free disposable syringes, and stored in lead free bottles. PbB was determined using an atomic absorption spectrometer with a graphite furnace. External quality control was strictly carried out under the External Quality Assessment Scheme (NEQAS) in the United Kingdom. The absolute difference was obtained by calculating the absolute difference between the reference laboratory and our laboratory result. The mean percentage difference between NEQAS values and our results was less than 5% for the last 10 years.

ALA measurement

ALA in urine was measured by the method of Oishi and colleagues.¹¹ In brief, 3.5 ml of acetylacetone reagent, 50 μ l of urine, and 0.45 ml of 10% formaldehyde solution were mixed for approximately 3 seconds. The mixture was cooled in an ice bath after heating at 100°C for 10 minutes. Samples (10 μ l

Table 2 Frequencies of alleles and genotypes by race and nationality

SNP	ALAD1	ALAD2	p values	1-1	1-2	2-2	p values
Race							
Chinese	95	5		90 (81)	10 (9)	0	
Malay	91.2	8.8		82.5 (33)	17.5 (7)	0	
Indian	89.4	10.6		81.8 (27)	15.2 (5)	3.0 (1)	
Vietnamese	95.7	4.3		92.2 (226)	7.0 (17)	0.8 (2)	
			0.182				0.098
Nationality							
Singaporean	92.9	7.1		86.5 (141)	12.9 (21)	0.6 (1)	
Vietnamese	95.7	4.3		92.3 (226)	6.9 (17)	0.8 (2)	
			0.352				0.059

Numbers are percentages. Numbers within parentheses are actual numbers.

The distribution of genotype and allele has been proven to follow the Hardy-Weinberg equilibrium.

aliquots) were analysed by high performance liquid chromatography with fluorescence detection.

Genotyping of ALAD polymorphisms

The G177C polymorphism responsible for ALAD1 and 2 isozymes were genotyped using a rapid PCR-RFLP method. Genomic DNA was extracted from 1 ml of buffy coat using the conventional phenol-chloroform method. The purity and yield of the extracted DNA were quantitated spectrometrically and then subjected to PCR amplification. The G177C polymorphism generates a *MspI* restriction site (C ↓ CGG) and the digested products were separated by agarose gel electrophoresis and thereafter genotyped accordingly.²

Renal function tests

Urine creatinine concentration was determined using standard laboratory techniques. Urinary albumin (Ualb), urine α 1 microglobulin (U α 1m), urine β 2 microglobulin (U β 2m), and urine retinol binding protein (URBP) were measured by enzyme linked immunosorbent assay (ELISA) using commercially available polyclonal antibodies. Urinary activity of N-acetyl- β -D-glucosaminidase (UNAG) was determined using Noto's method. The details of these methods have been reported previously.^{12 13}

Statistical analysis

Fifty one workers did not have any genotype measurements and had to be excluded from the analysis, leaving a sample of 408 workers. The data set of these 408 workers was used in all subsequent analyses. For some of the biological samples we were not able to analyse all the parameters as some samples ran out before we could complete the test.

Statistical analysis was carried out using SPSS (version 12.0, Chicago, IL) on a personal computer. Renal parameters, U α 1m, U β 2m, URBP, Ualb, UNAG, PbB, and ALAU were skewed and were logarithmically transformed to normalise the distribution. These data were reported as geometric means. Analysis of covariance (ANCOVA) was used to test for

significant differences in the renal parameters among the ALAD genotypes, adjusting for possible confounders (age, gender, race, and exposure duration). A linear regression model of each renal parameter with a log_PbB*ALADg interaction term was used to evaluate the effect of ALAD polymorphism on the relationship of blood lead to renal outcomes. Covariates in the regression model were the confounders adjusted in ANCOVA (age, gender, race, and exposure duration). The x axis consisted of the log transformed blood lead level while the y axis was the log transformed predicted values of renal parameters based on the linear regression equation (table 4). Separate regression lines were plotted for the two genotypes in each figure.

The observed genotype frequencies for *MspI* locus was compared with the expected genotype frequencies according to the Hardy-Weinberg law. Based on the test of conformance to the Hardy-Weinberg equilibrium, insignificant differences was noted in the expected and observed frequencies for the three Mendelian genotypes ($p > 0.05$).

RESULTS

Table 1 shows the basic characteristic of the study population. Generally, the two groups of workers were fairly similar. The mean exposure duration was 12.9 years with a wide range of 0.1–41 years. The geometric mean of blood lead was 19 μ g/dl; 78% of the workers had blood lead levels less than 30 μ g/dl.

There were no significant differences between the distribution of ALAD polymorphisms between Singaporean and Vietnamese workers or among ethnic groups (table 2).

ALAD1-1 was the predominant genotype for all the ethnic groups while ALAD2-2 was the rarest. The percentage of ALAD2 allele was higher among the Malays and Indians compared to the Vietnamese and Chinese (table 2).

As there were few workers with ALAD2-2, they were grouped together with ALAD1-2 heterozygotes. Table 3 shows the crude and adjusted mean concentration of exposure biomarkers and renal parameters by ALAD genotype.

Table 3 Crude and adjusted* geometric mean concentration of biological tests by ALAD genotypes

	Sample size		Crude means			Adjusted means		
	1-1	1-2/2-2	1-1	1-2/2-2	p values	1-1	1-2/2-2	p values
PbB (μ g/dl)	364	40	19.07	14.75	0.053	17.31	12.86	0.002
NAG (U/g Cr)	344	35	2.39	1.93	0.123	2.13	1.82	0.232
U α 1m (mg/g Cr)	350	36	6.55	5.41	0.102	5.25	4.77	0.391
Ualb (mg/g Cr)	347	36	7.1	5.69	0.088	6.85	6.05	0.324
U β 2m (μ g/g Cr)	349	35	0.13	0.09	0.089	0.08	0.07	0.464
URBP (mg/g Cr)	349	35	0.12	0.08	0.009	0.06	0.05	0.157
ALAU (mg/g Cr)	354	40	0.94	0.7	0.001	0.91	0.78	0.047

*Adjusted for gender, age, race, and exposure duration.

Sample sizes differ between tests as there were insufficient samples left for all the tests.

Table 4 Linear regression modelling of effect modification by ALAD genotypes on some renal parameters and blood lead levels

	β coefficient	SE β	p value
Urinary β2 microglobulin (log_Uβ2m)			
R ² = 0.326			
Intercept	-0.96	0.17	<0.001
ALADg	-0.60	0.24	0.014
Log_PbB (μ g/dl)	0.11	0.08	0.181
Log_PbB*ALADg	0.47	0.20	0.020
Urinary α1 microglobulin (log_Uα1m)			
R ² = 0.160			
Intercept	0.55	0.12	<0.001
ALADg	-0.35	0.06	0.040
Log_PbB (μ g/dl)	0.07	0.06	0.208
Log_PbB*ALADg	0.26	0.14	0.068
Urinary N-acetyl-glucosaminidase (log_UNAG)			
R ² = 0.138			
Intercept	-0.23	0.14	0.094
ALADg	-0.37	0.20	0.065
Log_PbB (μ g/dl)	0.30	0.07	<0.001
Log_PbB*ALADg	0.29	0.17	0.083
Urinary aminolevulinic acid (log_ALAU)			
R ² = 0.215			
Intercept	-0.63	0.10	<0.001
ALADg	-0.05	0.14	0.731
Log_PbB (μ g/dl)	0.40	0.05	<0.001
Log_PbB*ALADg	-0.02	0.12	0.870
Urinary retinol binding protein (log_URBP)			
R ² = 0.387			
Intercept	-0.78	0.15	<0.001
ALADg	-0.40	0.21	0.058
Log_PbB (μ g/dl)	-0.03	0.07	0.685
Log_PbB*ALADg	0.26	0.18	0.132
Urinary albumin (log_Ualb)			
R ² = 0.09			
Intercept	0.62	0.14	<0.001
ALADg	-0.42	0.20	0.035
Log_PbB (μ g/dl)	-0.04	0.07	0.541
Log_PbB*ALADg	0.30	0.16	0.068

The model adjusts for age, gender, race, and exposure duration. ALADg refers to the ALAD genotypes. ALADg has two categories, ALAD1-1 = 0, ALAD1-2/2-2 = 1.

The reference group is ALAD1-1; the β coefficient of log_PbB is the slope for the association between PbB and renal parameters in participants with this genotype. The corresponding slope in participants with ALAD1-2/2-2 is the sum of the beta coefficient of log_PbB and that of log_PbB*ALADg in each model. (i.e. in the U α 1m model, the β coefficient of log_PbB for ALAD1-1 group is 0.07, the corresponding β for ALAD1-2/2-2 is 0.33 = 0.07 + 0.26). P values for the log_PbB*ALADg reflect the statistical significance of the difference between the slopes of the regression line for ALAD1-1 participants and for ALAD1-2/2-2 participants.

Workers with ALAD1-1 genotype had significantly higher mean PbB and ALAU compared to workers with ALAD1-2 and 2-2 genotypes, even after correcting for possible confounders.

The effects of lead on some of the measured renal outcomes differed depending on the ALAD genotypes (table 4). PbB had a significant effect on UNAG even after adjusting for age, gender, race, and exposure duration. The types of ALAD allele were significantly associated with U β 2m ($p = 0.014$), U α 1m ($p = 0.040$), and Ualb ($p = 0.035$) levels. There were significant interactions between ALAD genotypes and PbB for U β 2m (table 4). Workers with ALAD1-2/2-2 genotype had larger β coefficients for blood lead in the regression equation for U β 2m ($p = 0.02$), U α 1m ($p = 0.068$), and UNAG ($p = 0.083$) compared to workers with the ALAD1-1 genotype. Workers with the ALAD1-2/2-2 genotype also had larger β coefficients for blood lead in the regression equation for URBP and Ualb. However, log_PbB β coefficients for these two renal parameters were negative (table 4).

The association of blood lead and renal parameters are presented graphically in figs 1–3. Figure 1 shows the linear relationship of blood lead to predicted U β 2m (adjusted for

age, exposure duration, gender, and race by the regression model) by ALAD genotypes. At a given concentration of lead (below 40 μ g/dl), its effects on U β 2m appeared to be greater on workers with ALAD1-1 genotype compared to workers with ALAD1-2/2-2 genotypes. When blood lead concentration was above 40 μ g/dl the reverse was true (fig 1). This observation was also true for U α 1m and UNAG with lead concentrations below 30 μ g/dl; when lead concentration was above 30 μ g/dl the reverse applied (figs 2 and 3). The gradients of the regression lines for ALAD1-2/2-2 (for U β 2m, U α 1m, and UNAG) were greater than for ALAD1-1. An one unit increase in the log_PbB (10 μ g/dl PbB) for ALAD1-1 homozygotes corresponded to an increment of 1.288 μ g/g Cr, 1.175 mg/g Cr, and 1.995 U/g Cr for U β 2m, U α 1m, and UNAG, respectively, while ALAD1-2/2-2 variant subjects had a higher increment of 3.802 μ g/g Cr, 2.138 mg/g Cr, and 3.89 U/g Cr.

DISCUSSION

Most of the Chinese population in Singapore expressed the ALAD1-1 genotype (90.0%). Only 10% of the Chinese subjects who were studied expressed the ALAD1-2 genotype, and

Figure 1 Regression lines of urinary $\beta 2$ microglobulin ($U\beta 2m$) versus blood lead (PbB) by ALAD genotypes adjusted for age, gender, race, and exposure duration.

Figure 2 Regression lines of urinary $\alpha 1$ microglobulin ($U\alpha 1m$) versus blood lead (PbB) by ALAD genotypes adjusted for age, gender, race, and exposure duration.

none had the ALAD2-2 genotype. Similarly, most Vietnamese had the ALAD1-1 (92.2%) genotype; 7.0% and 0.8% respectively had the ALAD1-2 and ALAD2-2 genotypes. The frequencies of ALAD genotypes for the Singaporean Chinese and Vietnamese were similar to that of the mainland Chinese (ALAD 1-1 of 0.92; 1-2 of 0.08; 2-2 of 0),⁵ the Taiwanese (ALAD 1-1 of 0.955; 1-2 of 0.044; 2-2 of 0.002), and Thai populations (ALAD 1-1 of 0.941; 1-2 of 0.058; 2-2 of 0.001).¹⁴ In this study, the frequency of ALAD1-2 was higher among the Malays (17.5%) and Indians (15.2%) compared to the Vietnamese (7.0%) and Chinese (10%) (table 2).

Most of the studies published so far did not examine the contribution of individuals' lead levels to the outcomes by separate genotypes. Comparing the mean of the measured outcomes with the different genotypes may give too crude an estimate to an association. In fact, the findings of this study showed the inadequacy of just comparing means. When we examined the relationship of blood lead to the adjusted measured outcome, the interpretation of the findings was slightly different. Workers with the ALAD1-1 genotype had significantly higher mean ALAU and PbB than workers with ALAD1-2/2-2 genotypes, even after adjusting for possible confounders (table 3). But when we plot the relationship between PbB and $U\beta 2m$, $U\alpha 1m$, and UNAG (figs 1–3), ALAD1-2/2-2 was likely to be the more “susceptible” group because of its higher gradient in the regression lines of renal parameters on blood lead. Here we take the view that susceptibility is shown by a greater increment of renal outcomes in response to the unit ($\log_{10}$ PbB) increase of blood lead for ALAD1-2/2-2 compared to ALAD1-1. We found that one unit increase in $\log_{10}$ PbB corresponded to a greater increase in $U\beta 2m$ (3.0 times), $U\alpha 1m$ (1.82 times), and UNAG (1.95 times) levels for subjects with ALAD1-2/2-2 genotypes compared with those with ALAD1-1.

In figs 1–3, which show the relationship between PbB and renal parameters, the two regression lines (ALAD1-1 and ALAD1-2/2-2) intersected at around 30 $\mu\text{g/dl}$ PbB for $U\alpha 1m$ and UNAG and at around 40 $\mu\text{g/dl}$ PbB for $U\beta 2m$. This highlights the importance of not just examining outcomes by

mean result. Our findings may explain, to some degree, why there have been contradicting reports on the association between blood lead levels and ALAD alleles in some studies. Wetmur *et al* found a significant overrepresentation of ALAD2 isozymes among individuals with PbB in excess of 30 $\mu\text{g/dl}$.⁴ Schwartz *et al* reported that the overrepresentation of ALAD2 allele can only be present when the PbB is over 40 $\mu\text{g/dl}$.¹⁵ Smith *et al* failed to show any association of ALAD2 to PbB and ascribed the non-association to the low lead exposure of the study population (mean PbB was 7.78 $\mu\text{g/dl}$).¹⁶

Figure 3 Regression lines of urinary N-acetyl-glucosaminidase (UNAG) versus blood lead (PbB) by ALAD genotypes adjusted for age, gender, race, and exposure duration.

Lead is known to affect tubular and glomerular function. Functionally, these effects on the kidney may cause leakage of tubular enzymes into the urine and blood, such as N-acetyl- β -glucosaminidase (NAG), and possibly other proteins with low molecular weights (for example, β 2 microglobulin) and high molecular weights (for example, albumin).¹⁰ Several studies have shown that urinary RBP,¹⁷ urinary α 1m,^{10,18} and urinary β 2m¹⁹ are good indicators of early renal effects due to lead exposure. A study of 128 lead workers in Singapore showed that U α 1m appears to be the most sensitive parameter compared to U β 2m and URBP.¹⁰ Activities of NAG, a lysosomal enzyme present in the brush borders of the proximal tubular cells, have been shown to increase in urine during the early stages of renal injury, before abnormalities in excretory function take place.²⁰ Urinary NAG has been shown in many studies to be an early marker of lead nephrotoxicity,¹⁰ but its predictivity for lead nephropathy is not known.

As far as can be ascertained, most studies on the effects of ALAD polymorphism and lead exposure on renal function did not (except for one study²¹) use any of these more sensitive indicators—that is, RBP, β 2m, and NAG. Urinary creatinine and calcium levels,⁷ blood urea nitrogen, creatinine, and uric acid,¹⁶ and creatinine clearance²² were used. Only two of these studies^{16,21} showed any significant differences in the measured renal parameters between the ALAD1-1 and ALAD1-2/2-2 individuals. These findings were not surprising given that creatinine, calcium, uric acid, and blood urea nitrogen levels are neither specific nor sensitive markers of renal damage. Considerable renal impairment would be needed before there are significant changes to these parameters. However, Wu *et al* did suggest that "...ALAD status modifies the relationship between lead and hyperuricemia with ALAD1-2/2-2 individuals having a higher risk of lead induced hyperuricemia".²² Weaver *et al* reported "effect modification by ALAD on associations between blood lead and/or DMSA-chelatable lead and three renal outcomes"—that is, blood urea nitrogen, serum creatinine, and creatinine clearance. They reported that "among those with the ALAD1-2 genotypes, higher lead measures were associated with lower blood urea nitrogen, serum creatinine and higher calculated creatinine clearance".²¹ This inverse association was attributed to a possible "lead induced hyperfiltration" which was explained in the study.²¹ Unfortunately, we did not measure serum creatinine or blood urea nitrogen levels in our study and thus were not able to examine this possible effect (lead induced hyperfiltration).

ALAD genotype has been found to affect urinary lead excretion following oral administration of dimercaptosuccinic acid. ALAD1-2 subjects excreted less lead during a four hour collection period than subjects with ALAD1-1.²³ It may be possible that the presence of ALAD2 allele may increase the retention of lead in blood and therefore decrease the amount of chelatable lead. It has been postulated that "the difference between the ALAD2 and ALAD1 polypeptides is a substitution of asparagine for lysine...this substitution changes the electrical charge of the molecule resulting in ALAD2 having a higher affinity for lead than ALAD1".¹ It was further postulated that "carriers of the ALAD2 allele who are exposed to lead might retain it in their blood and tissues longer, increasing the chance of an adverse effect due to inhibition of ALAD and the consequent buildup of aminolevulinic acid or perhaps due to lead itself, which can initiate oxidative damage and change the structure of cellular components".¹⁴

In workers exposed to low levels of lead, subclinical kidney effects have been found to be more prominent in heterozygote individuals compared to ALAD1 homozygotes. Smith *et al* studied a group of carpenters (n = 688) with a mean

Main messages

- The frequency of the ALAD2 allele is as low in Vietnamese workers as in Chinese workers.
- Workers with the ALAD2 allele appear more susceptible to the effects of lead (especially at higher levels) on renal function.

blood lead of 7.8 μ g/dl. They reported that "comparison of blood urea nitrogen (BUN) and uric acid by genotype indicated elevated levels among ALAD2 individuals ($p = 0.03$ and 0.07 , respectively)".¹⁶ In logistic regression models adjusting for other possible confounders, BUN and uric acid levels with ALAD2 were of borderline significance in the model ($p = 0.06$ and 0.07). In our study, we too found that workers with ALAD1-2/2-2 genotype had larger β coefficients for blood lead in the models of U β 2m, U α 1m, and NAG, compared to workers with the ALAD1-1 genotype with p values of 0.02, 0.068, and 0.083, respectively (table 4, figs 1–3).

Some limitations are inherent in this study. The ALAD alleles' frequency for Malays and Indians were not so representative and cannot be generalised to Malays or Indian as ethnic groups. We did not measure the body burden of lead and thus could not examine the effects of the ALAD genotype in relation to lead accumulation in the body. We did use exposure duration as a surrogate and this factor has been adjusted for in our analysis. As we did not measure other reported ALAD genotypes, we are not able to explore other possible gene-gene interactions. The possible roles of the vitamin D receptor, hemochromatosis-major histocompatibility complex class I protein, and ALAD gene interactions were also not examined.¹⁴

Conclusion

The frequency of the ALAD2 allele is as low in Vietnamese workers as in Chinese workers. The effects of blood lead on some renal parameters (U β 2m, U α 1m, and UNAG) were greater in workers with ALAD1-2/2-2 genotype than in those with ALAD1-1 genotype. Workers with ALAD2 allele appear to be more susceptible to the effects of lead (especially at higher levels) on renal function. Further investigations are needed to confirm this inference.

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