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The Association of Metallothionein-4 Gene Polymorphism and Renal Function in Long-Term Lead-Exposed Workers

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Abstract The goal of this study is to investigate if metallothionein (MT) gene polymorphism affects the susceptibility to lead as well as renal function parameters and blood pressures (BP) in workers exposed to lead for extended period of time. By means of real-time polymerase chain reaction, the MT4-216 A/G genotypes classified as rs396230 in the single nucleotide polymorphism database of the National Center for Biotechnology Information (database) were analyzed on 113 workers of a lead battery-recycling factory. Workers with G (mutant) allele were more susceptible to the toxic effects of lead on their systolic BP and serum renal function parameters. Their BP was 10 mmHg higher than those with wild-type (AA type) allele. Among subjects with the 3-genome, the GG mutant type subjects appear to be more susceptible to lead. Regression models of serum creatinine and BUN showed significant differences between the GG and GA types compared to AA type subjects. This cross-sectional study shows that workers with different MT-4 genotypes have different lead-induced adverse health effects. Those with the G allele have the greater susceptibility to lead so their exposure should be reduced.

Keywords Lead · Metallothionein · Gene polymorphism · Renal function

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Introduction

Although leaded gasoline was phased out since 2000 in Taiwan, lead continues to be a public health concern due to its widely industrial uses. Blood lead concentrations in the 20–50 ug/dL range have been reported in exposed workers [1]. At this level, lead can affect the hematopoietic, nervous, cardiovascular, and reproductive systems. Chronic occupational exposure to lead has also been linked to a high incidence of renal dysfunction, which is characterized by glomerular and tubulointerstitial changes resulting in chronic renal failure, hypertension, hyperuricemia, and gout [2].

Lead has high affinity to proteins, in particular to metallothionein, which is a low molecular weight, cystein-rich protein that binds and detoxifies heavy metals [3–7]. Hence, MT plays an important role on protecting against lead toxicity by binding lead and making it a non-bioavailable compound.

Animal studies showed that MT-bound lead interacts with enzymes on the renal proximal tubular membranes disrupting energy production, calcium metabolism, glucose homeostasis, ion transport processes, and renin–angiotensin system [4]. Although the correlation between lead and MT has been established, the true mechanism of lead detoxification by MT is still not fully elucidated.

In both animals and human, MT consists of four classes of a multigene family designated MT-1 to MT-4 [8–14]. Various MT-1 and MT-2 are present in all organs, whereas MT-3 is expressed predominantly in the brain and MT-4 in specific organs, such as the gastrointestinal (GI) and renal systems [15, 16].

As such, MT-4 is involved in detoxification of lead in its main routes of absorption and excretion, the GI tract and renal system, respectively. However, studies on MT-4 in human are relatively scarce, in particular those on the renal effects of chronic lead exposure. For that reason, this study was dedicated to investigate MT-4 gene polymorphism and the effects of lead on renal function.

Briefly, we conducted a cross-sectional study to evaluate the relationship between blood lead levels and renal function in a cohort of workers in a lead-acid battery recycling plant and studied whether the relationship modified by MT-4 polymorphism. The specific objectives of this study were (1) to investigate MT-4 gene polymorphisms that may affect susceptibility to lead in exposed workers and (2) to compare renal function parameters and related influences such as blood pressure of subjects with different MT-4 polymorphisms.

Materials and Methods

Participants and Health Examination

Under the regulations of labor health protection in Taiwan, lead workers should undergo annual health examinations that includes physical examination, blood lead tests, hemoglobin, hematocrit, red blood count, liver function test, and renal function tests, including serum creatinine, urea nitrogen (BUN), and routine urine tests.

A total of 113 volunteers working in a lead-battery recycling factory were initially participated for the study. After informed consent and following the guidelines of the Institutional Review Board of Kaohsiung Medical University for experimentation with human subjects, blood and urine samples were obtained for analysis of genotypes, and a full medical checkup was given to each worker. All the serum and urine samples were

measured in the central laboratory in Kaohsiung Medical University Hospital. In addition, a short questionnaire to inquire about job title, medical and working history, family history, use of oral contraceptives or other medications, menstrual cycle, and alcohol and cigarette consumptions as well as potential confounders was part of the health examination.

Measurements of Blood Pressure, Weight, and Height

The participants' weight and height were measured without shoes or shirt on an industrial balance scale fitted with a vertical ruler. Using a calibrated mercury sphygmomanometer, a registered nurse recorded the systolic and diastolic blood pressure in triplicate, with the participant resting for 5 min before the first recording and 1 min between each measurement. The average of the three readings was used in the analysis.

Renal Function Parameters

Serum creatinine, BUN, and uric acid levels were measured as indicators of renal function. Urine uric acid, *N*-acetyl-beta-D-glucosaminidase (NAG), and *r*-glutamyl transpeptidase (*r*-GT) concentrations corrected for urine creatinine levels were also measured.

MT Genotyping Methods

The databases from the National Center for Biotechnology Information and the Hapmap database were used to search for the single nucleotide polymorphism (SNP). It was found that genotype rs396230 (n.t. 216 on human MT-4 gene) has a minor allele frequency higher than 10% in Han-Chinese population so it was selected for the analysis of samples.

The samples were subjected to real-time PCR with a GeneAmp 5700 Sequence Detection System (Applied Biosystems, Foster City, LA, USA). The PCR mixture consisted of 5 μl of template DNA, 25 μl of 2× Taqman master mix (Applied Biosystems, Foster City, LA, USA), 0.5 μM of primers, and 0.5 μM of Taqman MGB probe (Applied Biosystems, Foster City, LA, USA). A reporter dye FAM (6-carboxy-fluorescein) was covalently attached to the 5′-end and a non-fluorescent quencher attached to the 3′ end. When in the intact form, the probe contained these two dyes whose physical proximity to each other suppresses any light emitted by the reporter. During PCR, the probe annealed specifically to complementary sequence between the forward and reverse primer site. The 5′>3′ nuclease activity of the DNA polymerase released the reporter dye from the probe and resulted in an increase in fluorescence indicating the allele present in the sample. Mismatch between the probe and allele reduced the efficiency of probe hybridization; the probe was more likely displaced rather than cleaved to release reporter dye.

The primers and probes were designed utilizing Primer Express software (version 1.0; Applied Biosystems). The forward primer sequence was 5′-CCC ACT ACT TAG AGG AAG TAG GAA AAG AG-3′, and the reverse primer sequence was 5′-CTT CTG TGG GCT TCT GAT GGA-3′. Two probes were designed for discrimination of single nucleotide polymorphism. Both of them shared the same sequence (5′-TTA GCA AAA ATG CTG RGA TAG GAG GTT GAG-3′) except the nucleotide 216. The MT4-216A probe was substituted by A and the MT4-216G probe was substituted by G. Each sample was amplified by the same primer set, but it was detected by MT4-216A and MT4-216G, respectively. Amplification was done with initial denaturation for 10 min at 95°C, followed by 45 cycles of denaturation at 95°C for 30 s and annealing/extension at 64°C for 45 s.

Statistical Analysis

Descriptive statistics was used to calculate the means of continuous variables, including blood lead levels, age, blood pressures, and biochemistry data. It also presented the dispersion of these data. For category variables, such as gender, and smoking and alcohol consumption, proportions were used. Chi-square test can present the difference of nominal variables among groups. Depending on genotypes, participants can be divided into three groups. ANOVA was performed to examine the differences of blood lead levels, renal function parameters, blood pressure, as well as other confounders among the three groups. Regression analysis could give the association of biomarkers and blood lead levels with adjustment of genotype and other variables such as age, gender, smoking status, BMI, and drinking status. The statistic software, SPSS v. 14, was used for treatment of the data, setting α at 0.05, two-tailed.

Results

The MT4 216 A/G genotypes were analyzed in 113 workers. Seventy-four workers (65.5%) had AA type; 18 (15.9%) and 21 (18.6%) had AG, GG types, respectively. The data are shown on the Table 1, revealing no significant differences of blood lead levels and demographic data among the three genotypes. Comparison of these groups using one-way ANOVA and the nonparametric Kruskal–Wallis revealed that workers with AG type had higher serum creatinine and BUN as well as being among those with more years on the job.

Table 1 Characteristics of Workers with Long-Term Exposure to Lead Involved in the Study (n=113)

	AA (n=74)	AG (n=18)	GG (n=21)	p Value[a]	p Value[c]
Gender male (%)	62 (83.8%)	17 (94.4%)	16 (76.2%)	0.33 [b]	–
Smoker (%)	29 (39.2%)	10 (55.6%)	10 (47.6%)	0.41 [b]	–
Drinking twice and more/week (%)	19 (25.7%)	7 (38.9%)	7 (33.3%)	0.49 [b]	–
Age (year)	39.04 (9.06)	39.77 (7.37)	37.20 (10.27)	0.633	0.296
Work duration (year)	8.68 (3.81)	9.17 (4.07)	5.85 (3.91)	0.008	0.008
Body mass index (kg/cm^2)	23.76 (3.22)	23.94 (3.22)	23.32 (3.19)	0.810	0.856
Blood lead (ug/dL)	17.03 (11.82)	24.17 (13.43)	21.27 (16.69)	0.082	0.142
Systolic BP (mmHg)	113.49 (11.68)	128.13 (14.88)	131.24 (14.35)	<0.001	<0.001
Diastolic BP (mmHg)	69.20 (7.80)	80.46 (8.86)	80.14 (9.80)	<0.001	<0.001
Creatinine (mg/dL)	1.02 (0.16)	1.22 (0.47)	1.11 (0.19)	0.006	0.010
Uric acid (mg/dL)	6.10 (1.41)	7.03 (1.69)	7.46 (1.38)	<0.001	0.001
BUN (mg/dL)	11.21 (2.40)	14.81 (4.70)	14.58 (2.72)	<0.001	<0.001
Urine NAG (mg/dL Cr)	2.32 (1.31)	2.93 (1.75)	2.92 (1.93)	0.136	0.219
Urine r-GT (mg/dL Cr)	6.81 (6.71)	6.78 (5.86)	7.28 (9.62)	0.963	0.651
Urine uric acid (mg/dL Cr)	0.46 (0.17)	0.43 (0.19)	0.36 (0.14)	0.044	0.110

Data are expressed as mean (SD) or number of subjects (%)

[a] One-way ANOVA

[b] Chi-square test

[c] Nonparametric (Kruskal–Wallis) test

Blood pressure and serum uric acid values showed a positive trend among AA, AG, and GG types, in that order.

After adjusting potential confounders, regression models showed that workers with AG and GG types have significantly higher blood pressures than those with AA type. The effect of lead on blood pressure was significant only on systolic pressure with a regression coefficient of 0.23±0.11 (Table 2).

In case of serum creatinine, uric acid, and BUN as renal function indicators, workers with GA and GG types had significantly higher levels than those with AA type. The regression coefficients of these parameters were all significant in GG and GA types compared to wild-type subjects, having 3.1 versus 3.4 mg/dL BUN, 0.76 versus 1.51 mg/dL uric acid, and 0.11 versus 0.16 mg/dL creatinine. Blood lead levels did not significantly affect these parameters after adjusting MT-4 genotypes and potential confounders (Table 3).

Regression models of urine renal function parameters showed there was weak effect of M4-216 A/G types as well as blood lead on urine renal function parameters. Only workers with GG type have significantly lower urinary uric acid excretion than those with AA type (Table 4).

Discussion

Analysis of MT4-216 A/G genotypes in 113 lead-exposed workers revealed that the susceptibility to lead toxicity and its effects on blood pressure, serum renal function parameters, and urine uric acid excretion depends on the individual's genotype. Workers with G allele had blood pressures >10 mmHg higher than those with AA type. Workers with the GG mutant type might be susceptible to lead, because with some alterations of their blood pressure and the measured indicators of renal function.

Metallothionein was first found in kidney tissue [3, 5]. It plays roles in the intracellular fixation of zinc and copper, neutralizing harmful elements such as cadmium, mercury, and lead, and in protection from of a variety of stress conditions [15, 17, 18]. Chronic lead exposure irreversibly damages the kidneys and may be associated with hypertension and

Table 2 Regression Models of Blood Pressures Predicted by Blood Lead Concentrations, MT4-216A/G Types, and Other Potential Confounders

	Systolic blood pressure		Diastolic blood pressure	
	Coefficient (SE)	*p* Value	Coefficient (SE)	*p* Value
MT4 AG vs AA	13.08 (3.21)	<0.0001	10.85 (2.18)	<0.0001
GG vs AA	17.85 (3.03)	<0.0001	11.34 (2.06)	<0.0001
Blood lead	0.23 (0.11)	0.03	0.03 (0.07)	0.69
Gender (F vs M)	−1.62 (3.95)	0.68	−1.21 (2.68)	0.65
Smoking (yes vs. no)	−4.92 (2.85)	0.09	−3.56 (1.93)	0.07
Drinking twice or more/week	1.92 (2.84)	0.50	3.68 (1.92)	0.06
BMI	0.52 (0.41)	0.21	0.43 (0.28)	0.13
Age	0.25 (0.14)	0.07	0.12 (0.09)	0.20
(Constant)	89.06 (11.25)	<0.0001	54.44 (7.63)	<0.0001
R^2	0.403		0.373	

Table 3 Regression Models of Serum Renal Function Parameters Predicted by Blood Lead Concentrations, MT4-216A/G Types, and Other Potential Confounders

	BUN		Uric acid		Creatinine	
	Coefficient (SE)	*p* Value	Coefficient (SE)	*p* Value	Coefficient (SE)	*p* Value
MT4 AG vs AA	3.06 (0.72)	<0.0001	0.76 (0.32)	0.02	0.16 (0.06)	0.005
GG vs AA	3.42 (0.68)	<0.0001	1.51 (0.31)	<0.0001	0.11 (0.05)	0.04
Blood lead	0.04 (0.02)	0.15	0.01 (0.01)	0.54	0.003 (0.002)	0.12
Gender (F vs M)	−1.78 (0.89)	0.05	−1.46 (0.40)	0.0004	−0.28 (0.07)	0.0001
Smoking (yes vs. no)	−0.14 (0.64)	0.83	−0.46 (0.29)	0.11	−0.08 (0.05)	0.13
Drinking twice or more/week	0.61 (0.64)	0.34	0.19 (0.29)	0.51	−0.03 (0.05)	0.55
BMI	−0.01 (0.09)	0.88	0.17 (0.04)	0.0001	0.001 (0.01)	0.91
Age	0.06 (0.03)	0.07	−0.02 (0.01)	0.23	0.002 (0.002)	0.34
(Constant)	8.89 (2.54)	0.001	3.09 (1.14)	0.01	0.94 (0.20)	<0.0001
R^2	0.399		0.439		0.331	

renal insufficiency at sub-clinically toxic levels [4]. For these reasons, MT can be considered of importance in lead toxicity.

Human possess four subfamilies of MT genes: the ubiquitous MT-1 and MT-2, the brain-specific MT-3, and the squamous epithelium-specific MT-4. All are located on a single chromosome 16 [19]. The squamous epithelium is characterized by having a single or multiple layers of cells in its surface, referred to as stratified squamous epithelium. Each type performs different functions, ranging from nutrient exchange to protection of other tissues. In addition to skin, it is found in capillaries, alveoli, glomeruli, and other tissues where rapid diffusion is required such as lungs, gastrointestinal tract, and kidney. These organs are involved in lead absorption and excretion, so the specific type MT-4 of metallothionein would be of importance in the case of this toxic metal.

Table 4 Regression Models of Urine Renal Function Parameters Predicted by Blood Lead Concentrations, MT4-216A/G Types, and Other Potential Confounders

	Urine uric acid (mg/dL Cr)		Urine NAG (mg/dL Cr)		Urine r-GT (mg/dL Cr)	
	Coefficient (SE)	*p* Value	Coefficient (SE)	*p* Value	Coefficient (SE)	*p* Value
MT4 AG vs AA	−0.01 (0.04)	0.83	0.53 (0.40)	0.19	0.19 (1.97)	0.92
GG vs AA	−0.09 (0.04)	0.03	0.58 (0.38)	0.13	0.54 (1.86)	0.77
Blood lead	−0.002 (0.001)	0.07	0.02 (0.01)	0.08	0.04 (0.07)	0.59
Gender (F vs M)	0.05 (0.05)	0.40	0.50 (0.49)	0.31	−0.58 (2.42)	0.81
Smoking (yes vs. no)	0.04 (0.04)	0.33	−0.23 (0.36)	0.52	−1.49 (1.75)	0.40
Drinking twice or more/week	−0.03 (0.04)	0.39	0.10 (0.35)	0.77	−1.81 (1.74)	0.30
BMI	−0.001 (0.01)	0.82	0.12 (0.05)	0.02	0.00 3(0.25)	0.99
Age	0.005 (0.002)	0.01	0.01 (0.02)	0.41	−0.05 (0.08)	0.54
(Constant)	0.33 (0.15)	0.03	−1.44 (1.40)	0.31	9.32 (6.89)	0.18
R^2	0.137		0.129		0.032	

The expression of MT-4 gene occurs specifically in epithelia where it has an essential but poorly defined role in regulating the metabolism of zinc or other metals. However, regulated MT-4 expression together with expression of entire MT gene locus has been reported in mouse, i.e., all of the known mouse MT genes are co-expressed in at least some of the cells in mouse [20]. This might explain our finding that MT-4 SNP may influence the lead toxicity, though few studies reported gene polymorphism in MT-4.

One of the limitations of the study is that the MT activity has not been assessed in different genotypes due to the difficulty involved in its measurement. Because the genetically polymorphous proteins are most abundant in parenchymatous tissues, i.e., liver, kidney, pancreas, and intestines, there are wide variations in MT concentrations in different species and tissues, depending on age, stage of development, dietary regimen, and other not yet fully identified factors. The lack of activity and concentrations of MT in this study resulted in the finding focusing on the lead effects on blood pressures and renal function in the different MT SNP types. We have no evidence on the activity of MT or mechanism of lead toxic effect modified by the genotypes.

In conclusion, in this cross-sectional study, it is shown that workers with different MT genotypes would have different susceptibilities to lead-induced adverse health effects. Workers with G allele might be more susceptible to lead toxicity and should be more closely monitored. The MT activity or concentration still needs to be determined in different tissues and polymorphisms to elucidate its role on metal toxicity, oxidative status, and tumor formation.

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