Journal of Toxicology and Environmental Health, Part A, 68:1471–1484, 2005

ISSN: 1528–7394 print / 1087–2620 online
DOI: 10.1080/15287390590967414

GENETIC POLYMORPHISMS OF OXIDATIVE AND ANTIOXIDANT ENZYMES AND ARSENIC-RELATED HYPERTENSION

Yu-Mei Hsueh[1], Pinpin Lin[2], Hui-Wen Chen[1], Horng-Sheng Shiue[3], Chi-Jung Chung[4], Chiao-Tzu Tsai[1], Yung-Kay Huang[5], Hung-Yi Chiou[6], Chien-Jen Chen[7]

[1]Department of Public Health, School of Medicine, Taipei Medical University, Taipei,
[2]Institute of Toxicology, Chung-Shan Medical University, Taichung,
[3]Graduate Institute of Medical Sciences, Taipei Medical University and, Department of Chinese Medicine, Chang Gung Memorial Hospital, Taipei,
[4]Graduate Institute of Public Health, Taipei Medical University, Taipei,
[5]Graduate Institute of Medicine Science, Taipei Medical University, Taipei,
[6]School of Public Health, Taipei Medical University, Taipei, and
[7]Graduate Institute of Epidemiology, College of Public Health, National Taiwan University, Taipei, Taiwan

The association of 4 genetic polymorphisms, NAD(P)H oxidase, manganese superoxide dismutase (MnSOD), catalase, and endothelial nitric oxide synthase (e-NOS), was assessed with arsenic-related hypertension risk among 79 hypertensive cases and 213 controls in an arseniasis-hyperendemic area of Taiwan. Overall, MnSOD polymorphism significantly increased the risk of hypertension regardless of arsenic exposure. NADPH oxidase and eNOS polymorphisms were significantly associated with hypertension risk in the high arsenic exposure group; however, catalase polymorphism was not associated with hypertension. Groups were further stratified by triglyceride levels to evaluate whether the cumulative arsenic exposure combined the three polymorphisms together. The adjusted adds ratios (ORs) of at least two risk factors of the cumulative arsenic exposure and MnSOD, NADPH oxidase, and eNOS three-polymorphism combination versus any one risk factor of them were 0.8 (95% CI 0.3–2.3) for individuals with low triglyceride levels (<110 mg/dl) and 2.5 (95% CI 1.0–6.01) for high-triglyceride groups (>110 mg/dl), respectively. These results suggested that the NADPH oxidase, MnSOD, and e-NOS polymorphisms, but not catalase, might play a role in the development of arsenic-related hypertension, especially in subjects with high triglyceride levels.

This study was supported by grants NSC-86-2314-B-038-038, NSC-87-2314-B-038-029, NSC-88-2314-B-038-112, NSC-88-2318-B-038-002-M51, NSC-89-2320-B-038-013, NSC-89-2318-B-038-M51, NSC-89-2314-B-038-049, NSC-90-2320-B-038-021, NSC-90-2320-B-002-197, NSC-90-2320-B-038-021, NSC-91-3112-B-038-001, NSC-92-2320-B-002-156, NSC-92-3112-B-038-001, and NSC-92-2321-B-038-004 from the National Science Council of the ROC.

Address correspondence to Yu-Mei Hsueh, PhD, Department of Public Health, School of Medicine, Taipei Medical University, Taipei, No. 250 Wu-Hsin Street, Taipei , 110, Taiwan. E-mail: ymhsueh@tmu.edu.tw

Approximately 40 million people in different parts of the world are exposed to arsenic through drinking water (Nordstrom, 2002). The chemical form of most of the arsenic in artesian well water is inorganic arsenic (Lin et al., 1998). Epidemiological studies have documented that long-term inorganic arsenic exposure is associated with an increased risk of cancers and atherosclerotic lesions at several anatomic sites (Tsai et al., 1998; Wu et al., 1989). Ingested arsenic has long been associated with the development of blackfoot disease (BFD), a unique peripheral vascular disease that was endemic in the southwestern coastal area of Taiwan where residents used high-arsenic artesian well water for more than 50 yr (Tseng, 1989). In addition, cardiovascular disease, such as ischemic heart disease (Chen et al., 1996), coronary heart disease (Wu et al., 1989), cerebrovascular accidents (Chiou et al., 1997), diabetes mellitus (Lai et al., 1994), and hypertension (Chen et al., 1995; Rahman et al., 1999), is closely related to long-term ingestion of high-arsenic drinking water, but the effect on the development of hypertension from long-term exposure to inorganic arsenic has rarely been studied; it is worthwhile to examine the mechanism underlying the ability of inorganic arsenic to induce hypertension.

Oxidative stress, a state of excessive reactive oxidative species (ROS) generation, is associated with vascular disease states such as hypertension (Berry et al., 2001). Previous studies demonstrated that nicotinamide adenine dinucleotide phosphate (NADPH) oxidase was responsible for superoxide production within the vascular wall (Mohazzab & Wolin, 1994). Recently, an animal study also showed that expression of the vascular NADPH oxidase was increased in hypertensive rats (Morawietz et al., 2001). A study demonstrated that arsenite activates NADPH oxidase P22phox subunit to produce superoxide, which then produces oxidative DNA damage in vascular smooth muscle cells (Lynn et al., 2000). In contrast, arsenite suppresses the relaxation in blood vessels by inhibiting eNOS activity in endothelial cells (Lee et al., 2003). The *eNOS* gene Glu298Asp variant is found in association with coronary artery disease (Hingorani et al., 1999) and hypertension (Miyamoto et al., 1998). Whether or not *NADPH oxidase* or *eNOS* gene polymorphism is associated with arsenic-related hypertension remains unclear.

Endogenous defenses against ROS include glutathione peroxidase, catalase, and superoxide dismutase (SOD) (Oberley & Oberley, 1997). A study found that high concentrations of arsenite have an inhibitory effect on the accumulation of catalase and SOD mRNA in maize (Mylona et al., 1998). An in vitro study showed that catalase and SOD modulated arsenic-induced DNA damage (Wang et al., 2001). In addition, a study reported that *MnSOD* polymorphism was associated with increased breast cancer risk (Ambrosone et al., 1999). A study demonstrated that subjects carrying the common variant (TT allele) of the *catalase* gene had significantly higher catalase activity levels than those carrying the CC allele (Forsberg et al., 2001), but until now, this variant has only been reported to be associated with acatalasemia (Goth et al., 2000). Based on these findings, *MnSOD* and *catalase* genotypes may affect arsenic-induced ROS and alter the risk of hypertension. Therefore, this study attempted to evaluate the

relationships among *NADPH oxidase*, *MnSOD*, *catalase*, and *e-NOS* genetic polymorphisms and correlate them to the risk of arsenic-related hypertension.

MATERIALS AND METHODS

Study Area

The study area included Homei, Fuhsing, and Hsinming Villages in Putai Township, Chayi Count, located in southwestern Taiwan. Residents in this study area exhibited the highest prevalence of BFD in Taiwan (Wu et al., 1961). Due to the high salinity of shallow well water, residents used water from artesian wells for more than 50 yr before the mid-1970s. The median arsenic concentration of the artesian well water ranged from 0.7 to 0.93 mg/L (Kuo, 1964). A tap water supply system was implemented in the study area in the early 1960s, but its usage remained low until the early 1970s. After the mid-1970s, artesian well water was no longer used for drinking and cooking.

Study Subjects

The recruitment of study subjects was previously described in detail (Chen et al., 1995). In brief, all adult residents who lived >6 mo in the study area were selected from records of the local household registration bureau, where demographic status and events including birth, marriage, education, migration, employment, and death of all family members in every household are mandatory to register and update annually. Household visits were carried out to interview residents who lived in the study area ≥ 5 d/wk and invited them to participate in a health examination. In first health examination during January and February 1989, 898 subjects participated. Biannual health examinations were then carried out. During these examinations, 79 hypertensive patients and 213 healthy subjects, who had buffy coat samples, were evaluated for polymorphism of 4 enzyme genes.

Blood Pressure Measurements and Hypertension Status

The standard protocol for measuring blood pressure recommended by the World Health Organization (WHO) (Rose et al., 1982) was used in this study. The WHO used the diagnostic standard of hypertension of on average SBP/DBP of 140/90 mm Hg or greater in 1999. Hypertension status was diagnosed at first health examination. Some subjects who had a history of hypertension and were regularly being treated with antihypertensive drugs were also defined as having hypertension.

Questionnaire Interview and Arsenic Exposure

Well-trained public health nurses carried out a standardized personal interview of study subjects based on a structured questionnaire. Information obtained from the interview included residential and water consumption history, socioeconomic and demographic characteristics, and lifestyle variables

including alcohol consumption, cigarette smoking, and dietary consumption frequency, as well as personal and family histories of hypertension, diabetes, and cardiovascular diseases.

The arsenic concentration of the artesian well water for each village in the BFD endemic area was obtained from a previous study carried out in the 1960s (Wu et al., 1961). A detailed residential history, including villages of residence and duration of residence, and a history of water consumption, including water source and duration of consumption, were obtained on the basis of the questionnaire interview. An index of cumulative arsenic exposure (CAE) was derived to reflect the overall exposure to arsenic for each study subject. The CAE (in mg/L-yr) was defined as the sum of the products derived by multiplying the arsenic concentration in well water (mg/L) by the duration of consuming the artesian well water (yr) during consecutive periods of living in different villages as previously described (Chen et al., 1995). The CAE of a given subject was considered unknown if the arsenic concentration of artesian well water in any one or more villages where the subject lived was unknown.

Biospecimen Collection and Laboratory Examinations

Fasting blood samples were collected from study subjects at first health examination for testing serum levels of total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides by standardized autoanalyzers. Moreover, DNA was extracted using proteinase K digestion following phenol and chloroform extraction from the buffy coat to analyze the genotype of *NADPH oxidase*, *MnSOD*, *catalase*, and *eNOS*.

MnSOD Genetic Polymorphism Determination

Genotyping of *MnSOD* polymorphism (a T-to-C substitution in the mitochondria targeting sequence) was performed by polymerase chain reaction (PCR) amplification following digestion with Turbo *NaeI* (Promega), as previously described (Lin et al., 2003).

NADPH Oxidase Genetic Polymorphism Determination

Genotyping of *NADPH oxidase* polymorphism (a C-to-T substitution of the C242T polymorphic site) was performed by polymerase chain reaction (PCR) amplification following digestion with *Rsa* I, as previously described (Inoue et al., 1998). A 348-bp fragment was characterized as the wild-type allele, while 160- and 188-bp fragments were considered mutant alleles.

Catalase Genetic Polymorphism Determination

Genotyping of *catalase* polymorphism (a C-to-T substitution of the C262T polymorphic site located on chromosome 11 p 13) was performed by PCR amplification following digestion with *Sma* I, and analyzed by 4% agarose gel electrophoresis as previously described (Forsberg et al., 2001). Two fragments of 155 and 30 bp were characterized as the wild-type allele and a 185-bp fragment as the mutant allele.

e-NOS Genetic Polymorphism Determination

Genotyping of *e-NOS* polymorphism (a G-to-T substitution of the G894T polymorphic site is located on chromosome 7q35-36, exon7) was performed by PCR amplification following digestion with *Ban* II, as previously described (Miyamoto et al., 1998). Two fragments of 92 and 62 bp were characterized as the wild-type allele, and a 154-bp fragment was characterized as the mutant allele.

All genotypes were validated by DNA sequence, and 10% of DNA samples were genotyped a second time and the concordance was 100%. The disease status of study subjects was blind when technicians examined four genotypes.

Data Analysis

Mean and standard error (SE) of age and body mass index (BMI) were calculated and analyzed by Student's *t*-test to determine the difference between hypertensive cases and healthy controls. In the multivariate analysis of associations between hypertension and various risk factors, age- and gender-adjusted odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated using multiple logistic regression models. Arsenic exposure indices, BMI, and lipid profiles were categorized according to the tertile levels in healthy controls. Analysis of the combination of variables used the median to categorize CAE, and stratification analysis also used the median to stratify triglyceride.

RESULTS

In total, 292 adult residents including 115 men and 177 women participated in this study. According to the former diagnostic criteria of SBP/DBP (160/95 mm Hg), the prevalences in the age groups of 30 to 39, 40 to 49, 50 to 59, and more than 60 yr were 6.25%, 13.64%, 25%, and 26.32% in male subjects, and 0%, 14.89%, 26.15%, and 31.82% in female subjects, respectively, which are the same as our former data (Chen et al., 1995).

Patient Characteristics

Table 1 shows that the prevalence of hypertension significantly increased with age. No significant associations with the prevalence of hypertension were observed for gender, educational level, cigarette smoking, or alcohol consumption. BMI was significantly associated with an increased hypertension risk in a dose-related manner. A BMI of 27 kg/m^2 or greater is defined as obesity in Taiwan, and the OR was 2.2 compared to a BMI of less 24 kg/m^2. This suggests that the propensity to accumulate abdominal adipose tissue is greater in subjects genetically predisposed to the development of hypertension (Allemann et al., 2001).

Long-Term Arsenic Exposure, Lipid Profiles, and Hypertension

There were significant dose-response relationships between hypertension prevalence and chronic arsenic exposure indicated by duration of living in a BFD area, duration of artesian well water consumption, and CAE (Table 2).

TABLE 1. Sociodemographic Characteristics with Age- and Gender-Adjusted Odds Ratios in Hypertensive Cases and Healthy Controls

Variable	Healthy controls, number (%)	Hypertensive cases, number (%)	Odds ratio[a] (95% CI)
Total number	213 (73.0)	79 (27.1)	
Age (yr)	46.2 ± 0.7[d,f]	54.2 ± 0.8[d,f]	1.1 (1.1–1.2)[f]
<39	66 (31.0)	2 (2.5)	1.0[b,e,f]
39–50	60 (28.1)	16 (20.3)	8.8 (1.9–39.8)[f]
≥50	87 (40.9)	61 (77.2)	28.1 (5.5–98.1)[f]
Gender			
Male	84 (39.4)	31 (39.2)	1.0[c]
Female	129 (60.6)	48 (60.8)	1.2 (0.7–2.1)
Cigarette smoking			
No	175 (82.2)	66 (83.5)	1.0
Yes	38 (17.8)	13 (16.5)	0.7 (0.3–1.7)
Alcohol consumption			
No	191 (89.7)	73 (92.4)	1.0
Yes	22 (10.3)	6 (7.6)	0.7 (0.2–2.0)
Educational level			
Illiterate	62 (29.1)	25 (31.7)	1.0
Elementary school	95 (44.6)	46 (58.2)	1.7 (0.9–3.1)
Junior high school and above	56 (26.3)	8 (10.1)	0.8 (0.3–2.2)
Body mass index (kg/m^2)	24.3 ± 0.2[d,f]	25.9 ± 0.4[d,f]	1.1 (1.0–1.2)[f]
<24	99 (46.5)	20 (25.3)	1.0[e,f]
24–26	58 (27.2)	25 (31.7)	1.7 (0.8–3.4)
≥26	56 (26.3)	34 (43.0)	2.2 (1.1–4.5)[f]

[a]Adjusted for age and gender.
[b]Adjusted for gender.
[c]Adjusted for age.
[d]*t*-Test.
[e]Test for the statistical significance of a trend.
[f]Significant at $p < .05$.

A dose-response relationship between triglyceride and hypertension was noted. The OR of hypertension for LDL cholesterol of 96–130 mg/dl was significantly higher than that for low-density lipoprotein (LDL) cholesterol of less than 96 mg/dl (Table 2). In spite of statistically significant differences between hypertensive patients and healthy controls, the mean values of these lipid measurements were almost within normal range.

Relationship Between *NADPH Oxidase*, *MnSOD*, *Catalase*, and *e-NOS* Genotypes and Hypertension

The distributions of *NADPH oxidase*, *MnSOD*, *catalase*, and *e-NOS* genotypes are summarized in Table 3. The *MnSOD* and *e-NOS* genotypic distributions for hypertensive cases and the control groups fit the Hardy–Weinberg equilibrium. The allelic frequency of the four genotypes in hypertensive cases

TABLE 2. Prevalence of Hypertension by Arsenic Exposure Indices and Lipid Profiles

Variable	Healthy controls; number	Hypertensive cases; number	Age/gender-adjusted OR (95% CI)	Multivariate OR (95% CI)
Duration of living in a blackfoot disease area (yr)				
<31	73	8	1.0[e,f]	1.0[a,e,f]
31–43	67	18	1.9 (0.7–4.8)	1.8 (0.7–4.8)
≥43	73	53	3.5 (1.5–8.3)[f]	3.4 (1.4–8.2)[f]
Duration of artesian well water consumption (yr)				
<3	76	9	1.0[e,f]	1.0[a,e,f]
3–15	62	15	1.6 (0.6–4.1)	1.7 (0.6–4.3)
≥15	75	55	3.2 (1.4–7.4)[f]	3.3 (1.4–7.8)[f]
CAE (mg/L-yr)[c]				
<4.1	52	2	1.0[e,f]	1.0[a,e,f]
4.1–14.7	57	19	7.5 (1.6–34.4)[f]	6.0 (1.3–27.9)[f]
≥14.7	55	42	10.0 (2.2–45.6)[f]	11.6 (2.5–54.9)[f]
Cholesterol[d] (mg/dl)				
<220	71	28	1.0	1.0[b]
220–282	66	17	0.6 (0.3–1.3)	0.7(0.3–1.8)
≥282	74	34	0.8 (0.4–1.5)	1.2 (0.6–2.6)
Triglyceride[d] (mg/dl)				
<88	73	16	1.0[e,f]	1.0[b,e,f]
88–138	65	25	1.6 (0.7–3.5)	1.5 (0.6–3.7)
≥138	73	38	1.9 (0.9–3.8)	2.1 (0.9–5.0)
HDL-cholesterol (mg/dl)				
<53	71	30	1.0	1.0[b]
53–66	70	25	0.8 (0.4–1.5)	0.9 (0.4–2.1)
≥66	72	24	0.7 (0.4–1.5)	1.3 (0.5–2.9)
LDL-cholesterol (mg/dl)				
<96	75	13	1.0	1.0[b]
96–130	67	36	3.9 (1.8–8.5)[f]	3.3 (1.3–8.4)[f]
≥130	71	30	2.1 (1.0–4. 7)	1.9 (0.8–4.7)

[a]Multivariate OR adjusted for age, gender, BMI, and triglyceride.
[b]Multivariate OR adjusted for age, gender, BMI, and cumulative arsenic exposure.
[c]Data on 65 subjects with no information on cumulative arsenic exposure were not included in the table.
[d]Data on two subjects with no information on cholesterol and triglyceride were not included in the table.
[e]Test for the statistical significance of a trend.
[f]Significant at $p < .05$.

did not significantly differ from that in the controls. The four genotypes were divided into two groups, wild types and genotypes carrying variant alleles, for statistical analysis. There were no associations between the *NADPH oxidase*, *catalase*, and *e-NOS* genotypes and hypertension. After being adjusted for risk factors, individuals carrying the C allele of *MnSOD* polymorphism were at a significantly higher risk of hypertension (OR, 2.0; 95% CI, 1.0–3.9).

TABLE 3. Allelic Frequencies and Risk Associated With *NADPH oxidase*, *MnSOD*, *catalase*, and *e-NOS* Polymorphisms Among Hypertensive Cases and Healthy Controls

Genotype	Hypertensive cases, number (%)	Healthy controls, number (%)	OR[a] (95% CI)	OR[b] (95% CI)	OR[c] (95% CI)
NADPH oxidase					
CC	68 (86.1)	193 (90.6)	1.0	1.0	1.0
CT	9 (11.4)	17 (8.0)	1.1 (0.4–2.8)	1.1 (0.4–3.3)	1.0 (0.3–3.0)
TT	2 (2.5)	3 (1.4)	3.2 (0.5–22.3)	2.8 (0.3–25.2)	2.4 (0.3–21.1)
CT/TT vs. CC	11	20	1.3 (0.6–3.1)	1.3 (0.5–3.5)	1.2 (0.4–3.2)
MnSOD[d]					
TT	45 (57.0)	141 (66.8)	1.0	1.0	1.0
TC	32 (40.5)	67 (31.8)	1.8 (1.0–3.2)	2.0 (1.0–3.0)[f]	2.1 (1.1–4.2)[f]
CC	2 (1.4)	3 (1.4)	1.7 (0.2–11.9)	0.9 (0.1–10.1)	0.6 (0.1–7.2)
TC/CC vs. TT	34	70	1.8 (1.0–3.2)	1.9 (1.0–3.7)	2.0 (1.0–3.9)[f]
Catalase					
CC	74 (93.7)	196 (92.0)	1.0	1.0	1.0
CT	5 (6.3)	17 (8.0)	1.1 (0.4–3.2)	0.7 (0.2–2.2)	0.7 (0.2–2.4)
e-NOS[e]					
GG	66 (83.5)	170 (82.1)	1.0	1.0	1.0
GT/TT	13 or 0 (16.5)	37 or 3(17.9)	0.9 (0.4–1.9)	1.2 (0.5–2.8)	1.2 (0.5–2.7)

[a]Adjusted for age and gender.

[b]Adjusted for age, gender, BMI, and cumulative arsenic exposure.

[c]Adjusted for age, gender, BMI, triglyceride, LDL, and cumulative arsenic exposure.

[d]Data on two healthy controls with no information on *MnSOD* polymorphism were not included in the table.

[e]Data on three healthy controls with no information on *e-NOS* polymorphism were not included in the table.

[f]Significant at $p < .05$.

Long-Term Arsenic Exposure, Genotypes, and Hypertension

To understand the interactions between CAE and genotypes for hypertension risk, CAE and the four genotypes were analyzed together (Table 4). Regardless of *eNOS* genotypes, subjects with CAE ≥ 10.5 mg/L-yr had a significantly higher risk than those with CAE < 10.5 mg/L-yr. Subjects with a lower CAE and the TT genotype of *MnSOD* had the lowest risk among the four groups. Subjects carrying the CC genotype of *catalase* with a CAE of ≥10.5 mg/L-yr had a significantly higher risk than those with a CAE of < 10.5 mg/L-yr. Subjects with a CAE of <10.5 mg/L-yr and the NADPH oxidase CC genotype served as the reference group, and subjects with the CT/TT genotype and either a high or low CAE had higher risks than the reference group.

Combined Effects of the Cumulative Arsenic Exposure and Three Genes on Hypertension Risk

The population was dichotomized into three groups based on the cumulative arsenic exposure and the number of variant genotypes of *NADPH oxidase*,

TABLE 4. Multiple Logistical Regression Analysis of Effects of the Combination of Cumulative Arsenic Exposure and *NADPH oxidase*, *MnSOD*, *catalase*, and *e-NOS* Gene Polymorphisms on Hypertension

Genotype	CAE[a] (mg/L-yr)	Number of hypertensive cases/healthy controls	OR[b] (95% CI)	OR[c] (95% CI)
NADPH oxidase				
CC	<10.5	7/73	1.0	1.0
CC	≥10.5	47/76	3.8 (1.5–9.5)[g]	3.8 (1.5–9.6)[g]
CT/TT	<10.5	2/8	2.8 (0.5–16.8)	3.0 (0.5–19.2)
CT/TT	≥10.5	7/7	4.6 (1.1–18.6)[g]	3.7 (0.9–15.6)
MnSOD[d]				
TT	<10.5	3/57	1.0	1.0
TT	≥10.5	33/56	6.2 (1.7–22.2)[g]	5.7 (1.6–20.9)[g]
TC/CC	<10.5	6/24	5.6 (1.2–5.3)[g]	4.5 (1.0–21.4)
TC/CC	≥10.5	21/25	9.8 (2.6–37.2)[g]	9.0 (2.3–35.0)[g]
Catalase				
CC	<10.5	8/73	1.0	1.0
CC	≥10.5	51/75	3.5 (1.5–8.5)[g]	3.5 (1.4–8.6)[g]
CT	<10.5	1/8	1.2 (0.1–11.8)	1.5 (0.2–14.9)
CT	≥10.5	3/8	2.3 (0.5–10.8)	2.4 (0.5–11.6)
e-NOS[e]				
GG	<10.5	8/65	1.0[f,g]	1.0[f,g]
GG	≥10.5	44/69	2.7 (1.1–6.8)[g]	2.7 (1.1–6.7)[g]
GT/TT	<10.5	1/14	0.5 (0.1–4.3)	0.4 (0.1–4.1)
GT/TT	≥10.5	10/13	3.9 (1.2–12.2)[g]	3.7 (1.2–11.7)[g]

[a]Data on 65 subjects with no information on cumulative arsenic exposure were not included in the table.
[b]Adjusted for age and gender.
[c]Adjusted for age, gender, triglyceride, and LDL-cholesterol.
[d]Data on two healthy controls with no information on *MnSOD* polymorphism were not included in the table.
[e]Data on three healthy controls with no information on *e-NOS* polymorphism were not included in the table.
[f]Test for trend.
[g]Significant at $p < .05$.

MnSOD, and *e-NOS* polymorphisms: Group 1, the reference group, consisted of individuals with CAE = 0 and no variant genotype among the three genes or CAE = 0 and at least one variant allele of three polymorphisms; group 2, individuals with CAE > 0 and no variant variant allele of three polymorphisms; group 3, individuals with CAE > 0 and at least one variant allele of three polymorphisms or CAE = 0 and at least two variant allele of three polymorphisms. In the logistic regression analyses, groups 1 and 2 were combined into one group because of the small sample sizes when stratified by triglyceride. The multivariate adjusted ORs of the extreme risk group 3 versus group 1 showed a significant risk of hypertension; OR was 9 (95% CI 1.1–75.1), stratified by triglyceride, in the triglyceride >110 mg/dl group, group 3 still found a higher risk than group 1–2, and the OR was 2.5 (95% CI 1.0–6.0) (Table 5).

TABLE 5. Adjusted Odds Ratios (ORs) and 95% Confidence Intervals (CIs) for the Joint Effect of Cumulative Arsenic Exposure and Three Gene Polymorphisms

Variable	Number of hypertensive cases / healthy controls	Model I OR (95% CI)	Model II OR (95% CI)
Group 1	30/31	1.0[a,c]	1.0[a]
Group 2	66/94	7.0 (0.9–57.5)	7.9 (0.9–66.9)
Group 3	63/97	9.3 (1.1–75.3)[c]	9.0 (1.1–75.1)[c]
Triglyceride < 110			
Group 1–2	47/60	1.0	1.0[b]
Group 3	34/44	0.8 (0.3–2.1)	0.8 (0.3–2.3)
Triglyceride ≥ 110			
Group 1–2	49/65	1.0	1.0[b]
Group 3	29/53	2.9 (1.2–6.8)[c]	2.5 (1.0–6.0)[c]

Note. Combined variable group 1 consisted of individuals with CAE (mg/L-yr) = 0 and no variant alleles of *MnSOD*, *NADPH oxidase*, and *e-NOS* polymorphisms or CAE (mg/L-yr) = 0 and at least one variant allele of the three polymorphisms; group 2 consisted of individuals with CAE (mg/L-yr) > 0 and no variant alleles of the three polymorphisms; group 3 consisted of the individuals with CAE (mg/L-yr) > 0 and at least one variant alleles of the three polymorphisms or CAE (mg/L-yr) = 0 and at least two variant alleles of the three polymorphisms. Model I adjusted for age and gender. Model II adjusted for age, gender, triglyceride, and BMI.

[a]Test for the statistical significance of a trend.

[b]Multivariate OR adjusted for age, gender, and BMI.

[c]Significant at $p < .05$.

DISCUSSION

A dose-response relationship between long-term arsenic exposure and the prevalence of hypertension was quite evident in this study as well as in our previous study (Chen et al., 1995). Recently, a study reported that arsenite altered vascular tone by decreasing vasorelaxation (Lee et al., 2003), which may be a contributing factor in the development of hypertension in populations exposed to arsenic. Frequencies of *NADPH oxidase* 242T in this study were 5%; these data and those from studies from Japan (Inoue et al., 1998) were lower than those for Caucasians—for example, 34% for Americans (Li et al., 1999) and 28% for Australians (Cai et al., 1999). The frequency of *MnSOD* 47C of this study was 17%, the same as for Japanese, but lower than those for Caucasian-Americans at 49% (Ambrosone et al., 1999), French at 34% (Mitrunen et al., 2001), and Finlanders at 48% (Hirvonen et al., 2002). The frequency of *catalase* 262T in this study was lower than that of Finlanders (4% vs. 43%) (Forsberg et al., 2001), and the cause might not be related with the risk of arsenic-related hypertension. The frequency of *eNOS* (894T) of this study (10%) was the same as that of African-Americans but lower than that of whites (32.4%) (Chen et al., 2001). Channon and Guzik (2002) reported that people with the CC genotype showed increased NADPH oxidase activity in blood vessels. Schachinger et al. (2001) also noted that the endothelium-dependent

dilator response was significantly blunted in carriers of the CC genotype of *NADPH oxidase*, and the prevalence of the CT/TT genotype was significantly more frequent in control subjects than in patients with coronary artery disease (Inoue et al. 1998). In contrast, in our present study, data showed that individuals with the CT/TT genotype of *NADPH oxidase* had a 1.3-fold higher hypertension risk compared to those with the wild CC genotype. The risk further increased to 4.6-fold among the high arsenic exposure group. This finding was consistent with results that Korean males carrying the 242T allele in the *NADPH oxidase* p22phox subunit had significantly increased risk of coronary artery disease (Lee et al., 2001), and a Japanese study reported that individuals with the CT/TT genotype had significantly higher risk of cerebrovascular disease than those with the CC genotype (Ito et al., 2000). In addition, arsenic treatment was shown to stimulate superoxide accumulation in vascular endothelial cells, which was attenuated by the inhibitors of NADPH oxidase (Smith et al., 2001). This suggests that arsenic may activate NADPH oxidase to produce ROS in endothelial cells (Smith et al., 2001), resulting in hypertension. Determining whether the TT or CT genotype of NADPH oxidase polymorphism increases ROS during arsenic exposure, thus resulting in hypertension, requires further investigation.

The *eNOS* is responsible for the conversion of l-arginine to NO in the endothelium (Moncada et al., 1991), and is involved in the regulation of blood pressure (Tseng et al., 1996). It was found that the hypertension risk for subjects carrying the T allele of *eNOS* was 1.2-fold higher than for those carrying the GG genotype. Furthermore, a high CAE had a significantly higher risk than those with low CAE for carrying the GG genotype (OR = 2.7, 95% CI 1.1–6.7). Subjects carrying the T allele of *eNOS* with a high CAE had 3.7 times greater risk than those carrying the GG genotype with a low CAE. These findings suggest that subjects carrying the GT/TT genotype might have higher blood pressure (Chen et al., 2001) and lower eNOS activity (Wang et al., 2000) than those with the GG genotype. Furthermore, systemic NO generation might be reduced in subjects carrying the *eNOS* GT/TT genotype, which was associated with enhanced ROS production, such as superoxide, with arsenic exposure (Kao et al., 2003; Pi et al., 2003).

In this study, data showed a significant association between the TC/CC genotype of *MnSOD* and arsenic-related hypertension. In addition, it was also found that subjects carrying the C allele of *MnSOD* had 4.5 times the hypertension risk of those with the T/T genotype at low CAE. However with a higher CAE, the risk decreased to 1.6 times the hypertension risk of those with low CAE in the subjects carrying the C allele of *MnSOD*. It was shown that when seedlings were treated with arsenate, *MnSOD* mRNA levels increased at 0.1 m*M* and then dropped at higher concentrations (Kwon & An, 1999). These findings suggest that *MnSOD* gene polymorphism may modify the ability of mitochondria to defend against low-dose arsenic-induced oxidative stress, but plays a less important role with high arsenic exposure.

However, this study has some limitations. Not all eligible study subjects displayed buffy coat. It was found that the hypertension prevalent in these subjects who have buffy coat according to the former diagnostic criteria of SBP/DBP (160/95 mm Hg) was the same as in our previous study. It is difficult to explain whether the distributions of four genotypes between former and latter study subjects are similar. The information on medication history, to determine whether other medication influenced the blood pressure, is not available, but it might underestimate the hypertension risk. Although 65 subjects (20%) did not have the information of cumulative arsenic exposure, the OR of hypertension for those without arsenic exposure levels was between the OR for the lowest and highest exposure groups (data not shown).

Overall, *MnSOD* polymorphism significantly increased the risk of hypertension. *NADPH oxidase* and *eNOS* polymorphisms were significantly related to hypertension risk with high arsenic exposure. C*atalase* polymorphism was not associated with hypertension. These findings suggest that the *NADPH oxidase* variant genotype at high triglyceride levels (Ceriello et al., 2002) and high cumulative arsenic exposure produces oxidative stress, and that the *MnSOD* and *eNOS* variant genotype cannot compensate for the antioxidant enzyme increment. These results suggest that the combination of variant genotypes may increase the risk of arsenic-associated hypertension especially in subjects with higher triglyceride levels.

REFERENCES

Allemann, Y., Hutter, D., Aeschbacher, B. C., Fuhrer, J., Delacretaz, E., and Weidmann, P. 2001. Increased central body fat deposition precedes a significant rise in resting blood pressure in male offspring of essential hypertensive parents: a 5 year follow-up study. *J. Hypertens.* 19:2143–2148.

Ambrosone, C. B., Freudenheim, J. L., Thompson, P. A., Bowman, E., Vena, J. E., Marshall, J. R., Graham, S., Laughlin, R., Nemoto, T., and Shields, P. G. 1999. Manganese superoxide dismutase (MnSOD) genetic polymorphisms, dietary antioxidants, and risk of breast cancer. *Cancer Res.* 59:602–606.

Berry, C., Brosnan, M. J., Fennell, J., Hamilton, C. A., and Dominiczak, A. F. 2001. Oxidative stress and vascular damage in hypertension. *Curr. Opin. Nephrol. Hypertens.* 10:247–255.

Cai, H., Duarte, N., Wilcken, D. E., and Wang, X. L. 1999. NADH/NADPH oxidase p22 phox c242t polymorphism and coronary artery disease in the australian population. *Eur. J. Clin. Invest.* 29:744–748.

Ceriello, A., Taboga, C., Tonutti, L., Quagliaro, L., Piconi, L., Bais, B., Da Ros, R., and Motz, E. 2002. Evidence for an independent and cumulative effect of postprandial hypertriglyceridemia and hyperglycemia on endothelial dysfunction and oxidative stress generation: Effects of short- and long-term simvastatin treatment. *Circulation* 106:1211–1218.

Channon, K. M., and Guzik, T. J. 2002. Mechanisms of superoxide production in human blood vessels: Relationship to endothelial dysfunction, clinical and genetic risk factors. *J. Physiol. Pharmacol.* 53:515–524.

Chen, C. J., Hsueh, Y. M., Lai, M. S., Shyu, M. P., Chen, S. Y., Wu, M. M., Kuo, T. L., and Tai, T. Y. 1995. Increased prevalence of hypertension and long-term arsenic exposure. *Hypertension* 25:53–60.

Chen, C. J., Chiou, H. Y., Chiang, M. H., Lin, L. J., and Tai, T. Y. 1996. Dose-response relationship between ischemic heart disease mortality and long-term arsenic exposure. *Arterioscler Thromb. Vasc. Biol.* 16:504–510.

Chen, W., Srinivasan, S. R., Elkasabany, A., Ellsworth, D. L., Boerwinkle, E., and Berenson, G. S. 2001. Combined effects of endothelial nitric oxide synthase gene polymorphism (G894T) and insulin resistance status on blood pressure and familial risk of hypertension in young adults: The Bogalusa heart study. *Am. J. Hypertens.* 14:1046–1052.

Chiou, H. Y., Huang, W. I., Su, C. L., Chang, S. F., Hsu, Y. H., and Chen, C. J. 1997. Dose-response relationship between prevalence of cerebrovascular disease and ingested inorganic arsenic. *Stroke* 28:1717–1723.

Forsberg, L., Lyrenas, L., de Faire, U., and Morgenstern, R. 2001. A common functional C-T substitution polymorphism in the promoter region of the human catalase gene influences transcription factor binding, reporter gene transcription and is correlated to blood catalase levels. *Free Radical Biol. Med.* 30:500–505.

Goth, L., Shemirani, A., and Kalmar, T. 2000. A novel catalase mutation (a GA insertion) causes the hungarian type of acatalasemia. *Blood Cells Mol. Dis.* 26:151–154.

Hingorani, A. D., Liang, C. F., Fatibene, J., Lyon, A., Monteith, S., Parsons, A., Haydock, S., Hopper, R. V., Stephens, N. G., O'Shaughnessy, K. M, and Brown, M., J, 1999. A common variant of the endothelial nitric oxide synthase (Glu298→ Asp) is a major risk factor for coronary artery disease in the UK. *Circulation* 100:1515–1520.

Hirvonen, A., Tuimala, J., Ollikainen, T., Linnainmaa, K., and Kinnula, V. 2002. Manganese superoxide dismutase genotypes and asbestos-associated pulmonary disorders. *Cancer Lett.* 178:71–74.

Inoue, N., Kawashima, S., Kanazawa, K., Yamada, S., Akita, H., and Yokoyama, M. 1998. Polymorphism of the NADH/NADPH oxidase p22 phox gene in patients with coronary artery disease. *Circulation* 97:135–137.

Ito, D., Murata, M., Watanabe, K., Yoshida, T., Saito, I., Tanahashi, N., and Fukuuchi, Y. 2000. C242T polymorphism of NADPH oxidase p22 PHOX gene and ischemic cerebrovascular disease in the Japanese population. *Stroke* 31:936–939.

Kao, Y. H., Yu, C. L., Chang, L. W., and Yu, H. S. 2003. Low concentrations of arsenic induce vascular endothelial growth factor and nitric oxide release and stimulate angiogenesis in vitro. *Chem.Res.Toxicol.* 16:460–468.

Kuo, T. L. 1964. *Arsenic content of artesian well water in endemic area of chronic arsenic poisoning* , 20th ed. , Taiwan: Institute of Pathology, National Taiwan University.

Kwon, S. I., and An, C. S. 1999. Isolation and characterization of mitochondrial manganese superoxide dismutase (MnSOD) from *Capsicum annuum* L. *Mol. Cells.* 9:625–630.

Lai, M. S., Hsueh, Y. M., Chen, C. J., Shyu, M. P., Chen, S. Y., Kuo, T. L., Wu, M. M., and Tai, T. Y. 1994. Ingested inorganic arsenic and prevalence of diabetes mellitus. *Am. J. Epidemiol.* 139:484–492.

Lee, M. Y., Jung, B. I., Chung, S. M., Bae, O. N., Lee, J. Y., Park, J. D., Yang, J. S., Lee, H., and Chung, J. H. 2003. Arsenic-induced dysfunction in relaxation of blood vessels. *Environ. Health Perspect.* 111:513–517.

Lee, W. H., Hwang, T. H., Oh, G. T., Kwon, S. U., Choi, Y. H., and Park, J. E. 2001. Genetic factors associated with endothelial dysfunction affect the early onset of coronary artery disease in Korean males. *Vasc. Med.* 6:103–108.

Li, A., Prasad, A., Mincemoyer, R., Satorius, C., Epstein, N., Finkel, T., and Quyyumi, A. A. 1999. Relationship of the C242T P22 phox gene polymorphism to angiographic coronary artery disease and endothelial function. *Am. J. Med. Genet.* 86:57–61.

Lin, P., Hsueh, Y. M., Ko, J. L., Liang, Y. F., Tsai, K. J., and Chen, C. Y. 2003. Analysis of NQO1, GSTP1, and MnSOD genetic polymorphisms on lung cancer risk in Taiwan. *Lung Cancer* 40:123–129.

Lin, T. H., Huang, Y. L., and Wang, M. Y. 1998. Arsenic species in drinking water, hair, fingernails, and urine of patients with blackfoot disease. *J Toxicol. Environ. Health A.* 53:85–93.

Lynn, S., Gurr, J. R., Lai, H. T., and Jan, K. Y. 2000. NADH Oxidase activation is involved in arsenite-induced oxidative DNA damage in human vascular smooth muscle cells. *Circ. Res.* 86:514–519.

Mitrunen, K., Sillanpaa, P., Kataja, V., Eskelinen, M., Kosma, V. M., Benhamou, S., Uusitupa, M., and Hirvonen, A. 2001. Association between manganese superoxide dismutase (MnSOD) gene polymorphism and breast cancer risk. *Carcinogenesis* 22:827–829.

Miyamoto, Y., Saito, Y., Kajiyama, N., Yoshimura, M., Shimasaki, Y., Nakayama, M., Kamitani, S., Harada, M., Ishikawa, M., Kuwahara, K., Ogawa, E., Hamanaka, I., Takahashi, N., Kaneshige, T., Teraoka, H., Akamizu, T., Azuma, N., Yoshimasa, Y., Yoshimasa, T., Itoh, H., Masuda, I., Yasue, H., and Nakao, K. 1998. Endothelial nitric oxide synthase gene is positively associated with essential hypertension. *Hypertension* 32:3–8.

Mohazzab, K. M., and Wolin, M. S. 1994. Sites of superoxide anion production detected by lucigenin in calf pulmonary artery smooth muscle. *Am. J. Physiol.* 267:L815–L822.

Moncada, S., Palmer, R. M., and Higgs, E. A. 1991. Nitric oxide: Physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.* 43:109–142.

Morawietz, H., Weber, M., Rueckschloss, U., Lauer, N., Hacker, A., and Kojda, G. 2001. Upregulation of vascular NAD(P)H oxidase subunit Gp91phox and impairment of the nitric oxide signal transduction pathway in hypertension. *Biochem. Biophys. Res. Commun.* 285:1130–1135.

Mylona, P. V., Polidoros, A. N., and Scandalios, J. G. 1998. Modulation of antioxidant responses by arsenic in maize. *Free Radical Biol. Med.* 25:576–585.

Nordstrom, D. K. 2002. Public health—Worldwide occurrences of arsenic in ground water. *Science* 296:2143–2145.

Oberley, T. D., and Oberley, L. W. 1997. Antioxidant enzyme levels in cancer. *Histol. Histopathol.* 12:525–535.

Pi, J., Horiguchi, S., Sun, Y., Nikaido, M., Shimojo, N., Hayashi, T., Yamauchi, H., Itoh, K., Yamamoto, M., Sun, G., Waalkes, M. P., and Kumagai, Y. 2003. A potential mechanism for the impairment of nitric oxide formation caused by prolonged oral exposure to arsenate in rabbits. *Free Radical Biol. Med.* 35:102–113.

Rahman, M., Tondel, M., Ahmad, S. A., Chowdhury, I. A., Faruquee, M. H., and Axelson, O. 1999. Hypertension and arsenic exposure in Bangladesh. *Hypertension* 33:74–78.

Rose, G. A., Blackburn, H., Gillum , R. F., and Prineas , R. J. 1982. *Cardiovascular survey methods.* 2nd ed. Geneva, Switzerland: World Health Organization.

Schachinger, V., Britten, M. B., Dimmeler, S., and Zeiher, A. M. 2001. NADH/NADPH oxidase P22phox gene polymorphism is associated with improved coronary endothelial vasodilator function. *Eur. Heart J.* 22:96–101.

Smith, K. R., Klei, L. R., and Barchowsky, A. 2001. Arsenite stimulates plasma membrane NADPH oxidase in vascular endothelial cells. *Am. J. Physiol. Lung Cell Mol. Physiol.* 280:L442–L449.

Tsai, S. M., Wang, T. N., and Ko, Y. C. 1998. Cancer mortality trends in a blackfoot disease endemic community of taiwan following water source replacement. *J. Toxicol. Environ. Health A.* 55:389–404.

Tseng, C. J., Liu, H. Y., Lin, H. C., Ger, L. P., Tung, C. S., and Yen, M. H. 1996. Cardiovascular effects of nitric oxide in the brain stem nuclei of rats. *Hypertension* 27:36–42.

Tseng, W. P. 1989. Blackfoot disease in Taiwan: a 30-year follow-up study. *Angiology* 40:547–558.

Wang, T. S., Hsu, T. Y., Chung, C. H., Wang, A. S., Bau, D. T., and Jan, K. Y. 2001. Arsenite induces oxidative DNA adducts and DNA–protein cross-links in mammalian cells. *Free Radical. Biol. Med.* 31:321–330.

Wang, X. L., Sim, A. S., Wang, M. X., Murrell, G. A., Trudinger, B., and Wang, J. 2000. Genotype dependent and cigarette specific effects on endothelial nitric oxide synthase gene expression and enzyme activity. *FEBS Lett.* 471:45–50.

Wu, H. Y., Chen, K. P., Tseng, W. P., and Hsu, C. L. 1961. Epidemiologic studies on blackfoot disease: I. Prevalence and incidence of the disease by age, sex, occupation and geographical distribution. *Mem. Coll. Med. Natl. Taiwan Univ.* 7:33–50.

Wu, M. M., Kuo, T. L., Hwang, Y. H., and Chen, C. J. 1989. Dose-response relation between arsenic concentration in well water and mortality from cancers and vascular diseases. *Am. J. Epidemiol.* 130:1123–1132.