Available online at www.sciencedirect.com
ScienceDirect
Toxicology and Applied Pharmacology 227 (2008) 1–7
Toxicology and Applied Pharmacology
www.elsevier.com/locate/ytaap

Risk of carotid atherosclerosis associated with genetic polymorphisms of apolipoprotein E and inflammatory genes among arsenic exposed residents in Taiwan

Yi-Chen Hsieh [a], Fang-I Hsieh [a], Li-Ming Lien [b], Yi-Li Chou [a], Hung-Yi Chiou [a,c,*], Chien-Jen Chen [d]

[a] *School of Public Health, Taipei Medical University, Taipei, Taiwan*
[b] *Department of Neurology, Shin Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan*
[c] *Topnotch Stroke Center, Taipei Medical University, Taipei, Taiwan*
[d] *Graduate Institute of Epidemiology, College of Public Health, National Taiwan University, Taipei, Taiwan*

Received 16 July 2007; revised 6 October 2007; accepted 16 October 2007
Available online 22 October 2007

Abstract

Arsenic had been reported to be associated with carotid atherosclerosis. However, there were few studies to evaluate the association between the susceptible gene of lipid metabolism and inflammation and carotid atherosclerosis among arsenic exposure residents. The aim of the study was to investigate the associations between the genetic polymorphisms of APOE and MCP-1 and the risk of carotid atherosclerosis among residents of Lanyang Basin in Taiwan which was a newly confirmed arsenic-endemic area. In total, 479 residents who had been genotyped of these two genes and examined the severity of carotid atherosclerosis were included in this study. The study subjects with carotid intima media thickness (IMT) $\geq$1.0 mm or with the observable plaque in the extracranial carotid artery were diagnosed as carotid atherosclerosis. A significantly age- and gender-adjusted odds ratio of 2.0 for the development of carotid atherosclerosis was observed in study subjects with ε4 allele of APOE than those without ε4 allele. Compared with study subjects who carried wild genotypes of APOE and MCP-1, those with both risk genotypes of APOE and MCP-1 had 2.5-fold risk of carotid atherosclerosis after adjustment for age and gender, revealing a significant dose–response relationship between number of risk genotypes of these genes and risk of carotid atherosclerosis. Additionally, study subjects with two risk genotypes of APOE and MCP-1 and either had ingested well water contained arsenic level >10 μg/L or had arsenic exposure >0.22 mg/L-year would have strikingly highest risk of 10.3-fold and 15.7-fold, respectively, for the development carotid atherosclerosis, showing significant joint effect of arsenic exposure and risk genotypes of APOE and MCP-1.

Keywords: Atherosclerosis; Arsenic; APOE; MCP-1; Polymorphism

Introduction

Arsenic is a metalloid element and widely distributed in the environment. Drinking arsenic-contaminated groundwater is the main way of human exposure to arsenic (Sheehy and Jones, 1993). Epidemiological evidence has shown that long-term chronic arsenic exposure in drinking water is associated with an increased risk of peripheral arterial disease (Tseng et al., 1996), ischemic heart disease (Chen et al., 1996) and cerebral infarction (Chiou et al., 1997a). A recent report also indicated a dose–response relationship between long-term exposure to inorganic arsenic from groundwater and carotid atherosclerosis in Taiwan (Wang et al., 2002). Accumulating researches demonstrated that arsenic induces pathophysiological events relevant with atherogenic potential including increased oxidative stress (Barchowsky et al., 1996, 1999; Del Razo et al., 2001; Kitchin and Ahmad, 2003). The generation of reactive oxidants is a general manifestation of an inflammatory reaction, an important modifying factor of atherosclerosis progression, which involved low-density lipoprotein (LDL) deposition and oxidation, the interactions of migratory leukocytes with resident vascular endothelial cells, smooth muscle cells and fibroblasts (Ross, 1999).

* Corresponding author. School of Public Health, Taipei Medical University, 250 Wusing St., Taipei 110, Taiwan. Fax: +886 2 23779188.
E-mail address: hychiou@tmu.edu.tw (H.-Y. Chiou).

0041-008X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.taap.2007.10.013

Several reports have indicated that chemokines have been detected in atherosclerotic plaques and they mediate not only migration, but also maturation and activation of leukocytes and other inflammatory cells present in the lesions (Reape and Groot, 1999). It is implied that chemokines play an important role in the development and progression of atherosclerosis, particularly monocyte chemoattractant protein-1 (MCP-1), since various murine models of atherosclerosis revealed that deletion of MCP-1 leads to a decrease in the extend of atherosclerotic plaque size (Boring et al., 1998; Dawson et al., 1999; Gu et al., 1998). Moreover, the expression of MCP-1 gene is increased in human subjects with increased arsenic exposure in our previous study (Wu et al., 2003). In addition, an association between MCP-1 plasma protein level and blood arsenic concentration was also observed in 65 study subjects with different arsenic exposure level through drinking well water (Wu et al., 2003). Recently, a functional single nucleotide (A/G) polymorphism at position −2518 in the promoter region of the MCP-1 gene is identified, and several studies have confirmed the finding that G allele increased the MCP-1 level in serum or in culture (Fenoglio et al., 2004; Muhlbauer et al., 2003; Rovin et al., 1999).

In addition, arsenic exposure, through drinking water, was found to increase atheroma formation in apolipoprotein E (APOE)−/− mice in parallel with increasing levels of arsenic in vessel wall (Simeonova et al., 2003). APOE, one of several lipoprotein transfer genes (Nakashima et al., 1994; Palinski et al., 1994), encoded by APOE gene serves as a ligand for LDL receptor and LDL receptor-related protein (LRP) and plays an important role in removing chylomicron and very low density lipoprotein (VLDL) remnants from plasma (Davignon et al., 1999). APOE polymorphism which has three isoforms encoded by distinct alleles (ε2, ε3 and ε4) modulates lipid metabolism (Mahley, 1988). These isoforms differ in affinity for the LDL receptor, which results in different lipoprotein concentrations. The ε4 allele is known to increase LDL cholesterol and triglycerol concentrations (Eto et al., 1986) and seems to be a risk factor for the development of atherosclerosis and coronary artery disease (CAD) (Wilson et al., 1996).

It is known that participation of single genes in a pathogenesis of atherosclerosis is relatively small; however, coexistence of several polymorphic variants of candidate genes may have higher impact on atherosclerosis. The aim of the present study was to investigate the joint effects of arsenic exposure through drinking water and genetic polymorphisms of APOE and MCP-1 on the risk of carotid atherosclerosis.

Methods

Study area and population. Study subjects were recruited from the Lanyang Basin of Ilan County in northeastern Taiwan. The design of the study has previously been described (Chiou et al., 1997a). Briefly, a total of 8088 residents aged ≥40 years from 18 villages in four townships were interviewed and included as the study cohort during the years of 1991–1994. In the year of 1996, we had completed home visit follow-up interview and had sent invitation letters to 5146 subjects out of the original cohort for attending a health examination held in 1997–1998. Finally, 1318 cohort members who agreed to participate in this study finished the health examination including the ultrasonographic assessment of external cranial carotid artery (ECCA) being conducted in Lotung Poh-Ai hospital. For the present study, a random sample of 479 subjects inclusive of 235 cases and 244 controls who had been genotyped of APOE and MCP-1 were selected without any matched factors. These study subjects were not substantially different from the original cohort by age, gender and educational level. This study was approved by the institutional review board for human subjects of Taipei Medical University, Taipei, Taiwan, and each subject provided written informed consent.

Questionnaire interview and arsenic measurement in the well water. A standardized questionnaire interview was conducted by well-trained public health nurses. Information of the interview included history of well water consumption, residential history, sociodemographic characteristics, levels of cigarette smoking and alcohol consumption, physical activities, as well as personal and familial history of hypertension, diabetes mellitus, cerebrovascular disease, heart disease and cancer of various organs. Well water samples were collected during the home interview, immediately acidified with hydrochloric acid and then stored at −20 °C until the subsequent assay. Hydride generation combined with flame atomic absorption spectrometry was used to determine the arsenic concentration in these samples (Kuo, 1968). The standard procedure of quality control and quality assurance was executed in this study. The quantitative determination was performed by the standard calibration curve using standard arsenic solution disposed by 0.12 N hydrochloric acid. The standard calibration curves were generated between experiments, which started in the morning and in the noon, respectively, at the same day. The correlation coefficients of the standard calibration curve which was the index of precision were between 0.996 and 0.999 during entire experimental time. Recovery rate which was the index of accuracy ranged from 95% to 106%. The arsenic concentration in well water was found to range from undetectable (<0.15 μg/L) to 3.59 mg/L. The 25%, median, 75% and mean of arsenic concentration of study subjects was 43.0 μg/L, 87.2 μg/L, 182.2 μg/L and 228.5 μg/L, respectively. The arsenic exposure level of each study subjects from drinking well water was derived from the arsenic concentration in well water of the household. In order to reflect the overall exposure to ingested arsenic through well water for each study subjects, cumulative arsenic exposure from drinking well water was also applied. The cumulative arsenic exposure from drinking well water was calculated as the sum of products derived by multiplying the arsenic concentration in well water (in milligrams per liter) by the year of drinking well water (in years) during successive periods of living in different villages.

Evaluation of carotid atherosclerosis. All ultrasound examinations were performed with the use of Hewlett-Packard SONO 1000 ultrasound system, equipped with 7.5 MHz real-time B-mode scanner and a 5.6-MHz pulsed-Doppler mode scanner. The duplex scanning and operation on study subjects were described in a previous study (Wang et al., 2002, 2007; Wu et al., 2006). Indications of carotid atherosclerosis were evaluated mainly based on three indices: the intima media thickness (IMT), the plaque score and the maximal level of stenosis of ECCA. IMT was measured in the far wall of the common carotid artery (CCA) at the most stenotic location between 0 and 2 cm proximal to the carotid bifurcation. The plaque score, also used as an index of atherosclerosis, was counted if any plaque was observed in the areas of the external carotid artery (ECA), internal carotid artery (ICA), carotid bulb or CCA. All measurements were bilateral and performed by one neurologist who was blinded from the patients' clinical details. Patient subjects were diagnosed based on a mean carotid IMT of ≥1.0 mm and either plaque occurrence in at least two locations on one side or the presence of stenosis of >50% in the left or right CCA.

Blood biochemical markers and genotype determination. Blood biochemical markers, including total cholesterol, triglyceride (TG), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol and fasting glucose were assessed and performed using a standard automatic analyzer. Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg or having taken antihypertensive medication. Subjects were considered as diabetes if they had ever been diagnosed by a physician or had a fasting blood sugar level of ≥126 mg/dL. Meanwhile, fasting blood samples were collected from study subjects during the health examination, centrifuged to separate the buffy coat and plasma and stored at −70 °C until the subsequent analysis. DNA was extracted from the buffy coat using the Viogene Blood and Tissue Genomic DNA Miniprep System kit (Viogene Inc., Taipei, Taiwan). Samples were stored at −20 °C until genotyping.

MCP-1 and APOE genotyping were performed on DNA samples by laboratory personnel blinded to clinical information.

The MCP-1 genotype was determined with a PCR-RFLP assay. Amplification with the primers forward primer 5′-CCG AGA TGT TCC CAG CAC AG and reverse primer 5′-CTG CTT TGC TTG TGC CTC TT generated a 930-bp product. Digestion with *Pvu*II yields 708 and 222 bp fragments when G is at position −2518. The products were separated in 3% agarose gel, stained with ethidium bromide.

The APOE genotypes were determined using the following primers: 5′-TCGGAACTGGAGGAACAACT-3′, 5′-CTGCCCATCTCCTCCATC-3′, 5′-ACAGAATTCGCCCCGGCCTGGTACAC-3′ and 5′-TAAGCTTGGCACG-GCTGTCCAAGGA-3′. The amplified DNA was 244 bp and cleaved with *Hha*I followed by 10% polyacrylamide.

Statistical analysis. Descriptive analyses were performed for risk factor variables including age, gender, body mass index (BMI), cigarette smoking, alcohol consumption, hypertension, diabetes mellitus, blood biochemical markers, arsenic concentration in well water and genotypes of APOE and MCP-1. To evaluate the associations between these risk factors and the prevalence of carotid atherosclerosis, a multiple logistic regression analysis was performed to estimate the odds ratio (OR) and 95% confidence interval (95% CI) after adjustment for age and gender. The statistical significance of each multivariate adjusted odds ratio was examined by significance testing of the regression coefficient based on the maximum likelihood method. Trend test were derived from logistic regression models, showing dose-dependent effect of risk factors. According to our initial results from univariate analysis, several variables such as age, gender, cigarette smoking, hypertension, diabetes mellitus, total cholesterol, triglyceride and arsenic concentration in well water were included in the subsequent analyses as shown in models I and II to control for potential confounding effect caused by these variables. Statistical analysis was carried out using SAS Version 8.0 software (SAS Institute, Cary, NC, USA). Statistical significance was set at $P<0.05$.

Results

Baseline characteristics of study subjects were shown in Table 1. Age, gender and hypertension were risk factors with the strongest effects on carotid atherosclerosis in this study population. Study subjects with abnormal level of blood lipid profiles had slightly increased risk of carotid atherosclerosis compared with those who have normal value of these lipid profiles. In contrast, study subjects with current cigarette smoking had a non-significantly increased risk of carotid atherosclerosis after adjustment for age and gender. The significant age- and gender-adjusted odds ratio of 2.4-fold higher risk of carotid atherosclerosis was observed in the arsenic exposed group with an arsenic concentration in the well water ≥50 μg/L. In addition, a non-significantly increased odds ratio of 1.5 of carotid atherosclerosis risk was also found in middle level exposed group of arsenic of 10.1–50.0 μg/L. However, a significant dose–response relationship between arsenic exposure and risk of carotid atherosclerosis was observed. Since the average duration of drinking well water was 42 years, we divided cumulative arsenic exposure (mg/L-year) in three groups to evaluate their associations with risk of carotid atherosclerosis. Compared with reference group (≤0.2 mg/L-year), a significant age- and gender-adjusted 1.9-fold risk for the development of carotid atherosclerosis was found among cumulative arsenic exposure >1.0 mg/L-year. In addition, an increased odds ratio of 1.1 for risk of carotid atherosclerosis was also observed among study subjects with cumulative arsenic exposure between 0.3 and 1.0 mg/L-year. Table 2 indicated that a significantly higher age- and gender-adjusted odds ratio of 2.0 for the development of carotid atherosclerosis was observed among study subjects carried ε4 allele of APOE gene. However, study subjects with A/G or G/G genotypes of MCP-1 had a non-significantly increased risk of carotid atherosclerosis after adjustment for age and gender. In gene–gene interaction analysis

Table 1
Risk of carotid atherosclerosis by characteristics of study subjects

	Controls, *n* (%)	Cases, *n* (%)	OR [a] (95% CI)	OR [b] (95% CI)
Age (years)				
≤55	69 (28.3)	27 (11.5)	1.0	1.0
56–65	114 (46.7)	87 (37.0)	2.0 (1.2–3.3) *	1.8 (1.0–3.0)
≥66	61 (25.0)	121 (51.5)	5.1 (3.0–8.7) ***	4.5 (2.6–7.8) ***
Gender				
Female	148 (60.7)	107 (45.5)	1.0	1.0
Male	96 (39.3)	128 (54.5)	1.8 (1.3–2.7) **	1.5 (1.0–2.1) †
Body mass index (kg/m^2)				
Normal	144 (60.0)	147 (63.4)	1.0	1.0
Abnormal [c]	96 (40.0)	85 (36.6)	0.9 (0.6–1.3)	1.1 (0.7–1.6)
Cigarette smoking				
No	171 (70.1)	133 (56.6)	1.0	1.0
Yes	73 (29.9)	102 (43.4)	1.8 (1.2–2.6) **	1.1 (0.6–2.0)
Alcohol drinking				
No	196 (80.3)	170 (72.3)	1.0	1.0
Yes	48 (19.7)	65 (27.7)	1.6 (1.0–2.4) *	1.2 (0.7–2.0)
Hypertension				
No	193 (79.4)	148 (63.5)	1.0	1.0
Yes	50 (20.6)	85 (36.5)	2.2 (1.5–3.3) ***	2.2 (1.4–3.4) ***
Diabetes mellitus				
No	209 (86.0)	206 (88.0)	1.0	1.0
Yes	34 (14.0)	38 (12.0)	0.8 (0.5–1.4)	0.9 (0.5–1.5)
Cholesterol (mg/dL)				
<200	117 (48.2)	112 (47.9)	1.0	1.0
≥200	126 (51.9)	122 (52.1)	1.0 (0.7–1.4)	1.1 (0.7–1.6)
Triglyceride (mg/dL)				
<150	191 (78.9)	170 (73.0)	1.0	1.0
≥150	51 (21.1)	63 (27.0)	1.4 (0.9–2.1)	1.3 (0.9–2.1)
Arsenic concentration in well water (μg/L)				
≤10	31 (12.7)	17 (7.2)	1.0	1.0
10.1–50.0	38 (15.6)	23 (9.8)	1.1 (0.5–2.4)	1.5 (0.7–3.5)
≥50.1	175 (71.7)	195 (83.0)	2.0 (1.1–3.8) *	2.4 (1.2–4.6) *
			P_{trend}=0.0054 **	P_{trend}=0.0049 **
Cumulative arsenic exposure (mg/L/year)				
≤0.2	51 (15.7)	32 (11.5)	1.0	1.0
0.3–1	50 (15.4)	25 (9.0)	0.8 (0.4–1.6)	1.1 (0.5–2.1)
≥1.1	224 (68.9)	222 (79.6)	1.6 (1.0–2.6) †	1.9 (1.1–3.1) *
			P_{trend}=0.0117 *	P_{trend}=0.0047 **

[a] Crude odds ratio.
[b] Age- and gender-adjusted odds ratio.
[c] Men with BMI ≥25 or women with BMI ≥24.
* 0.01<*P*<0.05.
** 0.001<*P*<0.01.
*** *P*<0.001.
† 0.05<*P*<0.1.

Table 2
Risk of carotid atherosclerosis by the genotypes of MCP-1 and APOE among study subjects

	Controls, *n* (%)	Cases, *n* (%)	OR [a] (95% CI)	OR [b] (95% CI)
MCP-1				
A/A	67 (27.5)	47 (20.0)	1.0	1.0
A/G or G/G	177 (72.5)	188 (80.0)	1.5 (1.0–2.3)†	1.3 (0.8–2.1)
APOE				
Others [c]	208 (85.3)	182 (77.5)	1.0	1.0
ε4 allele	36 (14.8)	53 (22.6)	1.7 (1.1–2.7)*	2.0 (1.2–3.2)**
MCP-1 and APOE [d]				
0	58 (23.8)	38 (16.2)	1.0	1.0
1	159 (65.2)	153 (65.1)	1.5 (0.9–2.3)	1.3 (0.8–2.2)
2	27 (11.1)	44 (18.7)	2.5 (1.3–4.7)**	2.5 (1.3–4.8)**
			P_{trend}=0.0049**	P_{trend}=0.0096**

[a] Crude odds ratio.
[b] Age- and gender-adjusted odds ratio.
[c] Including allele/allele of ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3 and ε3/ε4.
[d] 0=with non-risk genetic polymorphism of two genes; 1=with at least one risk genetic polymorphism of two genes; 2=with two risk genetic polymorphisms of two genes.
* 0.01<*P*<0.05.
** 0.001<*P*<0.01.
† 0.05<*P*<0.1.

compared with the study subjects with wild-type APOE and MCP-1 as reference group, those who carried two risk genotypes of MCP-1 and APOE had significant age- and gender-adjusted odds ratio of 2.5 for the development of carotid atherosclerosis. It also demonstrated a remarkable dose–response relationship in a trend test.

As illustrated in Table 3, a highly significant dose–response relationship between risk genotype numbers of APOE and MCP-1 and risk of carotid atherosclerosis was observed in both two arsenic exposed groups. A significantly highest risk of 10.3-fold of risk for the development of carotid atherosclerosis was observed in those who drank water contained arsenic concentration >10 μg/L and also carried two risk genotypes of APOE and MCP-1, showing prominent joint effect between arsenic exposure and these two genes. Moreover, a non-significantly increased 5.1-fold risk of carotid atherosclerosis was also found among study subjects with arsenic exposure through well water ≤10 μg/L. Moreover, compared with the study subjects with cumulative arsenic exposure ≤0.2 mg/L-year and wild-type APOE and MCP-1 as reference group, those whose cumulative arsenic exposure >0.2 mg/L-year and carried two risk genotypes of MCP-1 and APOE had significant age- and gender-adjusted odds ratio of 15.7 for the development of carotid atherosclerosis. A remarkable joint effect between cumulative arsenic exposure and these two genes was also found.

Multiple logistic regression analyses were shown in Table 4. Significant multivariate adjusted risk factors for the development of carotid atherosclerosis were observed among study subjects with higher arsenic exposed group, hypertension and ε4 alleles of APOE, respectively, after adjustment for age, gender, cigarette smoking, diabetes mellitus, cholesterol and triglyceride in model I. As shown in model II, significant multivariate adjusted gene–gene interaction of risk of genotypes of APOE and MCP-1 on the risk of carotid atherosclerosis was found after

Table 3
Joint effect on the risk of carotid atherosclerosis between arsenic concentration in well water and cumulative arsenic exposure and risk genotype number of APOE and MCP-1

	APOE and MCP-1 [a]	Controls, *n* (%)	Cases, *n* (%)	OR [b] (95% CI)	OR [c] (95% CI)
Arsenic concentration in well water (μg/L)					
≤10	0	5 (2.1)	1 (0.4)	1.0	1.0
	1	24 (9.8)	13 (5.5)	2.7 (0.3–25.7)	2.7 (0.3–28.1)
	2	2 (0.8)	3 (1.3)	7.5 (0.5–122.7)	5.1 (0.3–89.5)
>10	0	53 (21.7)	37 (15.7)	3.5 (0.4–31.1)	4.2 (0.4–41.5)
	1	135 (55.3)	140 (59.6)	5.2 (0.6–45.0)	5.8 (0.6–54.7)
	2	25 (10.3)	41 (17.5)	8.2 (0.9–74.3)	10.3 (1.0–102.5)*
				P_{trend}=0.0014**	P_{trend}=0.0006***
Cumulative arsenic exposure (mg/L/year)					
≤0.2	0	7 (2.9)	1 (0.4)	1.0	1.0
	1	25 (10.3)	22 (9.4)	7.0 (0.8–60.8)†	6.4 (0.7–59.0)†
	2	2 (0.9)	3 (1.3)	12.0 (0.8–186.4)†	10.1 (0.6–173.1)
>0.2	0	50 (20.6)	37 (15.7)	5.9 (0.7–49.4)	7.0 (0.8–62.1)†
	1	134 (55.1)	131 (55.7)	7.8 (1.0–63.4)†	8.6 (1.0–73.4)*
	2	25 (10.3)	41 (17.5)	13.1 (1.5–111.2)*	15.7 (1.7–141.2)*
				P_{trend}=0.0261*	P_{trend}=0.0078**

[a] 0=with non-risk genetic polymorphism of two genes; 1=with at least one risk genetic polymorphism of two genes; 2=with two risk genetic polymorphisms of two genes.
[b] Crude odds ratio.
[c] Age- and gender-adjusted odds ratio.
* 0.01<*P*<0.05.
** 0.001<*P*<0.01.
*** *P*<0.001.
† 0.05<*P*<0.1.

Table 4
Four models of multiple logistic regression analysis of risk factors on risk of carotid atherosclerosis

	Model I	Model II	Model III	Model IV
	OR[a] (95% CI)	OR[a] (95% CI)	OR[a] (95% CI)	OR[a] (95% CI)
Age (years)				
≤55	1.0	1.0	1.0	1.0
56–65	1.9 (1.1–3.3)*	1.8 (1.0–3.2)*	1.9 (1.1–3.4)*	1.9 (1.1–3.3)*
≥66	4.4 (2.4–7.8)***	4.3 (2.4–7.7)***	4.6 (2.5–8.2)***	4.5 (2.5–8.0)***
Arsenic concentration in well water (μg/L)				
≤10	1.0	1.0		
10.1–50.0	1.8 (1.0–3.2)*	1.8 (1.0–3.2)*		
≥50.1	1.9 (1.1–3.1)	1.9 (1.1–3.1)*		
Cumulative arsenic exposure (mg/L-year)				
≤0.2			1.0	1.0
0.3–1			1.2 (0.5–2.6)	1.2 (0.5–2.6)
≥1.1			1.7 (0.9–3.1)†	1.7 (0.9–3.1)†
Hypertension				
No	1.0	1.0	1.0	1.0
Yes	2.1 (1.3–3.3)**	2.1 (1.3–3.3)**	2.1 (1.4–3.3)***	2.2 (1.4–3.4)***
MCP-1				
A/A	1.0		1.0	
A/G or G/G	1.3 (0.8–2.1)		1.3 (0.8–2.1)	
APOE				
Others	1.0		1.0	
ε4 allele	1.9 (1.1–3.1)*		1.9 (1.1–3.2)*	
APOE and MCP-1				
0		1.0		1.0
1		1.3 (0.8–2.2)		1.3 (0.8–2.2)
2		2.4 (1.2–4.8)*		2.5 (1.2–4.9)**

[a] Multivariate adjusted odds ratio (adjustment for gender, cigarette smoking, diabetes mellitus, cholesterol and triglyceride).
* $0.01<P<0.05$.
** $0.001<P<0.01$.
*** $P<0.001$.
† $0.05<P<0.1$.

adjustment for major risk factors such as age, hypertension and arsenic exposure etc. Furthermore, in the models III and IV, after adjustment for the above risk factors, a marginal significant multivariate adjusted risk of 1.7-fold for the development of carotid atherosclerosis was found among study subjects whose cumulative arsenic exposure >1 mg/L-year. The study subjects with ε4 alleles of APOE in model III and those carried two risk genotypes of MCP-1 and APOE in model IV had a significant 1.9-fold and 2.5-fold risk for the development of carotid atherosclerosis, respectively.

Discussions

It has been well documented that the atherogenic effects of ingesting inorganic arsenic was through drinking water. In Taiwan, several studies have reported significant dose–response relationships between long-term exposure to arsenic in drinking water and the risk for the development of atherosclerotic vascular diseases including ischemic heart disease, cerebrovascular disease and peripheral vascular disease (Chen et al., 1996; Chiou et al., 1997a; Tseng et al., 1996).

However, only a few studies reported the association between arsenic exposure and carotid atherosclerosis. Based on our current and previous studies (Wang et al., 2007; Wu et al., 2006), though with different sample size, a consistent significantly increased risk for the development of carotid atherosclerosis was observed among arsenic exposure study subjects, providing a strong evidence of atherogenic effect of ingested arsenic through drinking well water. A relatively lower arsenic exposure among study subjects was found in this study compared with residents in BFD (black foot disease) area who had higher arsenic exposure (≥20 mg/L-year) (Wang et al., 2002). A borderline significant risk of 1.7-fold risk for the development of carotid atherosclerosis observed in this study would be expected based on our study subjects with lower cumulative arsenic exposure (>1 mg/L-year).

Our findings showed that age and gender were both significantly related with atherosclerosis risk. The results were the same with previous studies (Sun et al., 2002), which implies that they are both major risk factors for carotid atherosclerosis. It was also found that hypertension was significantly associated with the risk of development of carotid atherosclerosis and was concordant

with previous study which pointed that hypertension alone is a high-risk status for early atherosclerosis (Furumoto et al., 2002).

Many experimental data and epidemiological evidence have recently suggested several possible mechanisms of arsenic-induced atherosclerosis (Kitchin and Ahmad, 2003; Simeonova and Luster, 2004). Accumulating evidence indicated that arsenic could induce oxidative stress which increases the level of accumulation of oxidized lipoproteins and then result in the dysfunction of endothelial cells. Endothelial dysfunction is thought to be an early event in atherosclerosis progression (Libby et al., 2002), resulting in inflammatory cell infiltration and platelet thrombus formation (Simeonova and Luster, 2004). In the early stage of atherosclerotic lesions, MCP-1 is thought to play a crucial primarily role in monocyte recruitment into the subendothelial lesions (Harrington, 2000). The MCP-1-2518 G allele located in the regulatory region was associated with higher MCP-1 levels in both human and cell line researches (Rovin et al., 1999; Tabara et al., 2003). In addition, our data indicated that subjects with A/G or G/G genotypes of MCP-1 had a borderline increased risk of carotid atherosclerosis. It is implied that G allele carriers may be associated with risk of carotid atherosclerosis.

APOE, a critical role in the formation of very low density lipoprotein (VLDL) and chylomicrons, helps to stabilize and solubilize lipoproteins as they circulate in the blood. In general, the role of apolipoproteins in lipid metabolism includes maintaining the structural integrity of lipoproteins, serving as cofactors in enzymatic reactions and acting as ligand for lipoprotein receptors. The variants of APOE gene influence lipoprotein metabolism and the plasma concentration of total cholesterol and LDL cholesterol. The ε2 allele is associated with lower levels of total cholesterol, LDL cholesterol and elevated level of TG and APOE compared with the ε3 allele. Conversely, the ε4 allele is related to higher levels of total and LDL cholesterol and lower levels of APOE (Lehtinen et al., 1995). Our data consistently showed that no matter what arsenic concentration level in the well water is, ε2 carriers have lower LDL cholesterol while ε4 carriers have higher LDL cholesterol (116.1±25.2 mg/dL vs. 132.9± 143.5 mg/dL, $P=0.0004$). It is known that high-level LDL cholesterol was prone to form oxidized LDL which can initiate a series of inflammation process in the blood vessels and lead to develop atherosclerosis. This may be the reason why the study subjects with ε4 allele of APOE gene have 2.0-fold risk for the development of carotid atherosclerosis.

Our data further demonstrated that those with both variant genotypes of APOE and MCP-1 had significant age- and gender-adjusted odds ratios of 2.5 compared with wild genotypes of APOE and MCP-1 as a reference group. The risk of carotid atherosclerosis increased as the number of risk genotypes of these two genes increment. Participation of single genes in pathogenesis of atherosclerosis is relatively small, but coexistence of two risk genotypes of study genes may have higher impact on atherosclerosis formation. Our study showed that a non-significant increased risk of 5.1-fold for the development of carotid atherosclerosis was observed among low arsenic exposure group who drank well water contained arsenic concentration ≤10 μg/L. However, the highest risk of carotid atherosclerosis was observed in study subjects with exposure to arsenic concentration >10 μg/L and carried two risk genotypes of APOE and MCP-1. It is suggested that significant joint effect between environmental exposure and individual susceptible genes of atherosclerosis. Furthermore, after adjustment for several traditional risk factors including age, gender, cigarette smoking, hypertension, diabetes mellitus, cholesterol, triglyceride and arsenic exposure, significant multivariate adjusted odds ratio of 2.4 was observed among those with risk genotypes of APOE and MCP-1 in model II.

There were some potential limitations of this study. First, the family history of stroke or cardiovascular disease was not included in our analysis, which might reduce the precision of our results. However, we had collected information of almost risk factors of carotid atherosclerosis including age, gender, BMI, cigarette smoking, alcohol consumption, hypertension, diabetes mellitus, total cholesterol, triglyceride, LDL and HDL. They had been included in the subsequent multivariate models to control for their confounding effect on the estimation of joint effect on risk of carotid atherosclerosis between risk genotypes of APOE and MCP-1 and arsenic exposure. Second, the plasma levels of APOE and MCP-1 were not determined for subjects, which might have contributed to some of the unexplained variation in this study. Third, urinary arsenic concentration might be a valuable indicator to estimate the risk for the development of carotid atherosclerosis (Chiou et al., 1997b). However, we only have data of 310 study subjects. Due to limited data and risk of 0.8-fold for the development of carotid atherosclerosis in study subjects with urinary arsenic concentration greater than 56.5 μg/L (median value), we consider that arsenic concentration in well water should be a better indicator than urinary arsenic concentration on estimation risk of carotid atherosclerosis in this study. In addition, we could not use arsenic dose per body weight as indicator to estimate risk of carotid atherosclerosis since we did not have body weight of each study subjects. In summary, this study is the first report to show a significant dose–response relationship between number of genetic polymorphisms of APOE and MCP-1 and higher arsenic exposure through consuming water on the risk of carotid atherosclerosis. Contemporaneous analysis of more than one candidate genes involved in the mechanism of atherosclerosis formation may provide more plausible results and may be helpful in understanding the genetic basis of atherosclerosis. This study also showed that study subjects who carried two risk genotypes of APOE and MCP-1 and had ingested well water contained arsenic level >10 μg/L would have striking highest risk of 10.3-fold for the development carotid atherosclerosis, showing significant joint effect of arsenic exposure and risk genotypes of APOE and MCP-1.

Conflict of interest statement

The author(s) declare that they have no competing interests.

Acknowledgments

This study was supported by grants from the National Science Council of Taiwan (NSC90-2320-B-038-044; NSC92-2321-B-038-005; NSC93-2321-B-038-008), Topnotch Stroke

Research Center Grant, Ministry of Education; Center of Excellence for Clinical Trial and Research in Storke and TBI (DOH-TD-B-1111-002).

References

Barchowsky, A., Dudek, E.J., Treadwell, M.D., Wetterhahn, K.E., 1996. Arsenic induces oxidant stress and NF-KB activation in cultured aortic endothelial cells. Free Radic. Biol. Med. 21, 783–790.

Barchowsky, A., Klei, L.R., Dudek, E.J., Swartz, H.M., James, P.E., 1999. Stimulation of reactive oxygen, but not reactive nitrogen species, in vascular endothelial cells exposed to low levels of arsenite. Free Radic. Biol. Med. 27, 1405–1412.

Boring, L., Gosling, J., Cleary, M., Charo, I.F., 1998. Decreased lesion formation in CCR2−/− mice reveals a role for chemokines in the initiation of atherosclerosis. Nature 394, 894–897.

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

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

Chiou, H.Y., Hsueh, Y.M., Hsieh, L.L., Hsu, L.I., Hsu, Y.H., Hsieh, F.I., Wei, M.L., Chen, H.C., Yang, H.T., Leu, L.C., Chu, T.H., Chen, W.C., Yang, M.H., Chen, C.J., 1997b. Arsenic methylation capacity, body retention, and null genotypes of glutathione S-transferase M1 and T1 among current arsenic-exposed residents in Taiwan. Mutat. Res. 386, 197–207.

Davignon, J., Cohn, J.S., Mabile, L., Bernier, L., 1999. Apolipoprotein E and atherosclerosis: insight from animal and human studies. Clin. Chim. Acta 286, 115–143.

Dawson, C., Kuziel, A., Osahar, A., Maeda, N., 1999. Absence of CC chemokine receptor-2 reduces atherosclerosis in apolipoprotein E-deficient mice. Atherosclerosis 143, 205–211.

Del Razo, L.M., Quintanilla-Vega, B., Brambila-Colombres, E., Calderon-Aranda, E.S., Manno, M., Albores, A., 2001. Stress proteins induced by arsenic. Toxicol. Appl. Pharmacol. 177, 132–148.

Eto, M., Watanabe, K., Ishii, K., 1986. Reciprocal effects of apolipoprotein E alleles (epsilon 2 and epsilon 4) on plasma lipid levels in normolipidemic subjects. Clin. Genet. 29, 477–484.

Fenoglio, C., Galimberti, D., Lovati, C., Guidi, I., Gatti, A., Fogliarino, S., Tiriticco, M., Mariani, C., Forloni, G., Pettenati, C., Baron, P., Conti, G., Bresolin, N., Scarpini, E., 2004. MCP-1 in Alzheimer's disease patients: A-2518G polymorphism and serum levels. Neurobiol. Aging 25, 1169–1173.

Furumoto, T., Saito, N., Dong, J., Mikami, T., Fujii, S., Kitabatake, A., 2002. Association of cardiovascular risk factors and endothelial dysfunction in japanese hypertensive patients: implications for early atherosclerosis. Hypertens. Res. 25, 475–480.

Gu, L., Okada, Y., Clinton, S.K., Gerard, C., Sukhova, G.K., Libby, P., Rollins, B.J., 1998. Absence of monocyte chemoattractant protein-1 reduces atherosclerosis in low density lipoprotein receptor-deficient mice. Mol. Cell 2, 275–281.

Harrington, J.R., 2000. The role of MCP-1 in atherosclerosis. Stem Cells 18, 65–66.

Kitchin, K.T., Ahmad, S., 2003. Oxidative stress as a possible mode of action for arsenic carcinogenesis. Toxicol. Lett. 137, 3–13.

Kuo, T.L., 1968. Arsenic content of arsenic well water in endemic area of chronic arsenic poisoning. Rep. Inst. Pathol. Natl. Taiwan Univ. 19, 7–13.

Lehtinen, S., Lehtimaki, T., Sisto, T., Salenius, J.P., Nikkila, M., Jokela, H., Koivula, T., Ebeling, F., Ehnholm, C., 1995. Apolipoprotein E polymorphism, serum lipids, myocardial infarction and severity of angiographically verified coronary artery disease in men and women. Atherosclerosis 114, 83–91.

Libby, P., Ridker, P.M., Maseri, A., 2002. Inflammation and atherosclerosis. Circulation 105, 1135–1143.

Mahley, R.W., 1988. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. Science 240, 622–630.

Muhlbauer, M., Bosserhoff, A., Hartmann, A., Thasler, W., Weiss, T., Herfarth, H., Lock, G., Scholmerich, J., Hellerbrand, C., 2003. A novel MCP-1 gene polymorphism is associated with hepatic MCP-1 expression and severity of HCV-related liver disease. Gastroenterology 125, 1085–1093.

Nakashima, Y., Plump, A.S., Raines, E.W., Breslow, J.L., Ross, R., 1994. ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. Arterioscler. Thromb. 14, 133–140.

Palinski, W., Ord, V.A., Plump, A.S., Breslow, J.L., Steinberg, D., Witztum, J.L., 1994. ApoE-deficient mice are a model of lipoprotein oxidation in atherogenesis. Demonstration of oxidation-specific epitopes in lesions and high titers of autoantibodies to malondialdehyde-lysine in serum. Arterioscler. Thromb. 14, 605–616.

Reape, T.J., Groot, P.H.E., 1999. Chemokines and atherosclerosis. Atherosclerosis 147, 213–225.

Ross, R., 1999. Atherosclerosis—an inflammatory disease. New Engl. J. Med. 340, 115–126.

Rovin, B.H., Lu, L., Saxena, R., 1999. A novel polymorphism in the MCP-1 gene regulatory region that influences MCP-1 expression. Biochem. Biophys. Res. Commun. 259, 344–348.

Sheehy, J.W., Jones, J.H., 1993. Assessment of arsenic exposures and controls in gallium arsenide production. Am. Ind. Hyg. Assoc. J. 54, 61–69.

Simeonova, P.P., Luster, M.I., 2004. Arsenic and atherosclerosis. Toxicol. Appl. Pharmacol. 198, 444–449.

Simeonova, P.P., Hulderman, T., Harki, D., Luster, M.I., 2003. Arsenic exposure accelerates atherogenesis in apolipoprotein E(−/−) mice. Environ. Health Perspect. 111, 1744–1748.

Sun, Y., Lin, C.H., Lu, C.J., Ping, K., Chen, R.C., 2002. Carotid atherosclerosis, intima media thickness and risk factors—an analysis of 1781 asymptomatic subjects in Taiwan. Atherosclerosis 164, 89–94.

Tabara, Y., Kohara, K., Yamamoto, Y., Igase, M., Nakura, J., Kondo, I., Miki, T., 2003. Polymorphism of the monocyte chemoattractant protein (MCP-1) gene is associated with the plasma level of MCP-1 but not with carotid intima-media thickness. Hypertens. Res. 26, 677–683.

Tseng, C.H., Chong, C.K., Chen, C.J., Tai, T.Y., 1996. Dose–response relationship between peripheral vascular disease and ingested inorganic arsenic among residents in blackfoot disease endemic villages in Taiwan. Atherosclerosis 120, 125–133.

Wang, C.H., Jeng, J.S., Yip, P.K., Chen, C.L., Hsu, L.I., Hsueh, Y.M., Chiou, H.Y., Wu, M.M., Chen, C.J., 2002. Biological gradient between long-term arsenic exposure and carotid atherosclerosis. Circulation 105, 1804–1809.

Wang, Y.H., Wu, M.M., Hong, C.T., Lien, L.M., Hsieh, Y.C., Tseng, H.P., Chang, S.F., Su, C.L., Chiou, H.Y., Chen, C.J., 2007. Effects of arsenic exposure and genetic polymorphisms of p53, glutathione S-transferase M1, T1, and P1 on the risk of carotid atherosclerosis in Taiwan. Atherosclerosis 192, 305–312.

Wilson, P.W.F., Schaefer, E.J., Larson, M.G., Ordovas, J.M., 1996. Apolipoprotein E alleles and risk of coronary disease: a meta-analysis. Arterioscler. Thromb. Vasc. Biol. 16, 1250–1255.

Wu, M.M., Chiou, H.Y., Ho, I.C., Chen, C.J., Lee, T.C., 2003. Gene expression of inflammatory molecules in circulating lymphocytes from arsenic-exposed human subjects. Environ. Health Perspect. 111, 1429–1438.

Wu, M.M., Chiou, H.Y., Hsueh, Y.M., Hong, C.T., Su, C.L., Chang, S.F., Huang, W.L., Wang, H.T., Wang, Y.H., Hsieh, Y.C., Chen, C.J., 2006. Effect of plasma homocysteine level and urinary monomethylarsonic acid on the risk of arsenic-associated carotid atherosclerosis. Toxicol. Appl. Pharmacol. 216, 168–175.