Toxicology and Applied Pharmacology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

Renin–angiotensin–aldosterone system related gene polymorphisms and urinary total arsenic is related to chronic kidney disease

Wei-Jen Chen [a], Ya-Li Huang [b], Horng-Sheng Shiue [c], Tzen-Wen Chen [d,e], Yuh-Feng Lin [f,g], Chao-Yuan Huang [h], Ying-Chin Lin [i,j], Bor-Cheng Han [a], Yu-Mei Hsueh [a,b,*]

[a] School of Public Health, College of Public Health and Nutrition, Taipei Medical University, Taipei, Taiwan
[b] Department of Public Health, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan
[c] Department of Chinese Medicine, Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Taoyuan, Taiwan
[d] Division of Nephrology, Department of Internal Medicine, Taipei Medical University Hospital, Taipei, Taiwan
[e] Department of Internal Medicine, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan
[f] Graduate Institute of Clinical Medicine, School of Medicine, Taipei Medical University, Taipei, Taiwan
[g] Division of Nephrology, Department of Internal Medicine, Shuang Ho Hospital, New Taipei, Taiwan
[h] Department of Urology, National Taiwan University Hospital, College of Medicine National Taiwan University, Taipei, Taiwan
[i] Department of Family Medicine, Shung Ho Hospital, Taipei Medical University, New Taipei, Taiwan
[j] Department of Health Examination, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan

ARTICLE INFO

Article history:
Received 9 March 2014
Revised 14 May 2014
Accepted 20 May 2014
Available online xxxx

Keywords:
Arsenic
Renin–angiotensin–aldosterone system
Angiotensinogen (*AGT*)
Aldosterone synthase (*CYP11B2*)
Gene polymorphisms
Chronic kidney disease

ABSTRACT

A recent study demonstrated that an increased risk of chronic kidney disease (CKD) was associated with high urinary total arsenic levels. However, whether genomic instability is related to CKD remains unclear. An association between CKD and genetic polymorphisms of regulation enzymes of the renin–angiotensin–aldosterone system (RAAS), such as angiotensin-converting enzyme (*ACE*), angiotensinogen (*AGT*), angiotensin II type I receptor (*AT1R*), and aldosterone synthase (*CYP11B2*) has not been shown. The aim of the present study was to investigate the relationship between arsenic, genetic polymorphisms of RAAS enzymes and CKD. A total of 233 patients and 449 age- and gender-matched controls were recruited from the Taipei Medical University Hospital, Taipei Municipal Wan Fang Hospital and the Shin Kong Wu Ho-Su Memorial Hospital. Concentrations of urinary arsenic were determined by a high-performance liquid chromatography-linked hydride generator, and atomic absorption spectrometry. Polymorphisms of *ACE*(I/D), *AGT*(A[−20]C), (T174M), (M235T), *AT1R*(A1166C) and *CYP11B2*(C[−344]T) were examined by polymerase chain reaction and restriction fragment length polymorphism. Subjects carrying the *CYP11B2* TT genotype had a higher odds ratio (OR), 1.39 (0.96–2.01), of CKD; while those with the *AGT*(A[−20]C) CC genotype had an inverse OR of CKD (0.20 (0.05–0.81)), and a high-risk genotype was defined as A/A + A/C for *AGT*(A[−20C]) and T/T for *CYP11B2*(C[−344]T). The trend test showed a higher OR for CKD in patients who had either high urinary total arsenic levels or carried the high-risk genotype, or both, compared to patients with low urinary total arsenic levels, who carried the low-risk genotype, and could also be affected by the hypertension or diabetes status.

Introduction

Chronic kidney disease (CKD) is a major worldwide public health issue, which can progress into end-stage renal disease (ESRD) (Iseki et al., 2003) or other cardiovascular complications (Weiner et al., 2004), thus increasing the risk of mortality. In Taiwan, the national prevalence of CKD is 11.93%, but only 3.54% of CKD patients were actually aware of their disorder (Wen et al., 2008). CKD is a multifactorial disorder, with the major risk factors being diabetes mellitus and hypertension (Yamagata et al., 2007) and habitual analgesic usage (Kuo et al., 2010); however, genetic (Maeda, 2008) and environmental (Soderland et al., 2010) effects including gene-environment interactions cannot be ignored.

Arsenic is one of the most significant environmental hazards; arsenic has been proven to be associated with various cancers (Chung et al., 2013) and chronic diseases, including diabetes (Kim and Lee, 2011), hypertension (Jones et al., 2011), carotid atherosclerosis (Wu et al., 2006) and microvascular diseases (Chiou et al., 2005). Absorption of arsenate (As^V) by humans, from drinking water or food consumption, is subsequently reduced to arsenite (As^{III}), and undergoes methylation to the low toxic monomethylarsonic acid (MMA^V) and dimethylarsinic acid (DMA^V) in the liver, followed by excretion through urine by the kidney (Vahter, 2002). Several ecological studies have indicated that arsenic

* Corresponding author at: Department of Public Health, School of Medicine, College of Medicine, Taipei Medical University, No. 250 Wu-Hsing Street, Taipei 110, Taiwan. fax: +886 2 27384831.
E-mail address: ymhsueh@tmu.edu.tw (Y.-M. Hsueh).

http://dx.doi.org/10.1016/j.taap.2014.05.011
0041-008X/

exposure from drinking water is associated with kidney diseases (Chiou et al., 2005; Meliker et al., 2007). A recent cross-sectional study in Taiwan examined that urinary total arsenic was associated with renal dysfunction (Chen et al., 2011a). Our previous study reported that urinary total arsenic, which is the sum of inorganic arsenic and its metabolites, was significantly associated with CKD (eGFR less than 60 mL/min/1.73 m^2 consistent for 3 months) in a dose–response relationship (Hsueh et al., 2009).

The renin–angiotensin–aldosterone system (RAAS) mainly consists of renin, angiotensin converting enzyme (*ACE*), angiotensinogen (*AGT*), angiotensin II type I receptor (*AT1R*), aldosterone and other enzymes, which play an important role in mediating diverse physiological functions, including vasoconstriction, sodium homeostasis, fluid balance, aldosterone secretion, inflammation, fibrosis and oxidative stress (Paul et al., 2006). If the RAAS becomes overactive, there is an excess of angiotensin II excretion, along with aldosterone which results in the increase of glomerular pressure, thereby enabling glomerular arteriosclerosis, resulting in renal function decline (Brewster and Perazella, 2004; Siragy and Carey, 2010). In addition, angiotensin II can also activate oxygen species related enzymes, increase oxidative stress and pro-inflammatory factors, eventually leading to hypertension and renal glomerular sclerosis and injury (Nistala et al., 2009). Various factors are involved in the deregulation of RAAS, and polymorphisms of RAAS genes are the most common basis for increasing RAAS activity (Anbazhagan et al., 2009). RAAS genes have also been reported as candidate genes for diabetic nephropathy (Maeda, 2008). Regarding the insertion/deletion polymorphism in *ACE*, participants with the D/D genotype had significantly higher plasma angiotensin II levels than those with the I/I or I/D genotypes (San Jose et al., 2009); participants with the *AGT*(Met235Thr) Met/Thr and Thr/Thr genotypes were associated with increased angiotensinogen levels (Sethi et al., 2003); and participants carrying the aldosterone synthase *CYP11B2*(C[−344]T) TT genotype had significantly higher plasma aldosterone levels than those with the CC and CT genotypes (Ko et al., 2008). However, the association between RAAS gene polymorphisms and CKD remains controversial and inconclusive. Fabris et al. (2005) suggested that *AGT*(Met235Thr) Thr/Thr genotypes were associated with renal failure in Italian hypertensive patients, but these genotypes were not related to the diabetic nephropathy patients in Taiwan (Tien et al., 2009). Nevertheless, studies on RAAS gene polymorphisms and the link with CKD in Taiwan are scarce.

Although previous studies have confirmed the significance of arsenic-induced renal dysfunction, whether gene susceptibility affects arsenic-related CKD remains to be determined. Therefore, we conducted a hospital-based case–control study to investigate the combined effects of the gene polymorphisms of *ACE*, *AGT*, *AT1R*, and *CYP11B2*, and arsenic, on CKD, in an area without obvious arsenic exposure. In addition, we explored the influence of hypertension or diabetes on the combined effects of gene and environment on CKD.

Materials and methods

Study participants. We performed a hospital-based case–control study including 233 CKD cases and 449 age- and sex-matched controls recruited from the Taipei Medical University Hospital, Taipei Municipal Wan Fang Hospital and the Shin Kong Wu Ho-Su Memorial Hospital between July 2007 and September 2011. All study participants lived in Taipei City or New Taipei City. The potable tap water provided by the Taipei Water Department of the Taipei City Government had arsenic levels less than the standard 10 μg/L, which is the same standard value set by the United States Environmental Protection Agency (USEPA, 2001) and WHO guidelines (WHO, 2011) for arsenic in drinking water (10 μg/L). Participants provided written informed consent before the questionnaire interview and biospecimen collection. The Research Ethics Committee of the Taipei Medical University approved the study, and complied with the World Medical Association Declaration of Helsinki.

All CKD outpatients were diagnosed by biochemical criteria such as blood urea nitrogen, proteinuria and serum creatinine, at the Department of Internal Medicine/Nephrology. CKD was defined by using the equation from the Modification of Diet in Renal Disease Study (Levey et al., 1999) to eGFR as $186.3 \times (\text{serum creatinine})^{-1.154} \times \text{age}^{-0.203}$ ($\times 0.742$ if female) and characterized five stages of CKD, according to the Kidney Disease Outcomes Quality Initiative from the United States National Kidney Foundation (KDOQI, 2002). Subjects who had an eGFR of less than 60 mL/min/1.73 m^2 that continued for 3 months were defined as having stages 3–5 CKD. Matched controls with no evidence of CKD were recruited from receiving adult health examinations and senior citizen health examinations at the Department of Family Medicine.

Questionnaire interview and biospecimen collection. Well trained interviewers carried out standardized personal interviews based on a structured questionnaire. Collected information included demographics and socioeconomic characteristics, lifestyle factors such as cigarette smoking, alcohol, tea and coffee consumption, analgesic usage (if participants took any kind of analgesic more than seven times in one week, or continuous medication for a month, defined as "yes, habitual") and personal histories of hypertension and diabetes (based on participants' reports of ever having been diagnosed by physicians, or ever using antihypertension or diabetes drugs, defined as having a history of hypertension or diabetes).

Spot urine samples were collected and stored in 50 mL acid-washed tubes at the time of recruitment, and immediately transferred to a −20 °C freezer until required for arsenic species analyses. Concurrently, we used ethylene-diamine-tetraacetic-acid (EDTA) vacuumed syringes to collect 5–8 mL peripheral blood samples and separated buffy coat was frozen at −80 °C for DNA extraction and measurement of gene polymorphisms.

Genotyping of SNPs in ACE, AGT, AT1R and CYP11B2. Genomic DNA was extracted from buffy coats, followed by storage at −80 °C until genotype measurements were taken. Genotyping was carried out by the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) (Lindpaintner et al., 1995; Kupari et al., 1998; Andersson et al., 1999; Niu et al., 1999; Padro-Miquel et al., 2009). All PCR products were obtained in a total volume of 30 μL, consisting of an 40 ng DNA sample, 10× Taq buffer (Tris–HCl, PCR enhancers, $(NH_4)_2SO_4$, and $MgCl_2$) 2.5 mM dNTP, 2 μM of each primer and 2 U Taq polymerase (Prime Taq™, Genet Bio, Korea). Amplified products were visualized by electrophoresis using a 2% agarose gel. After digestion by restriction enzymes, products were re-analyzed by electrophoresis on 3% agarose gels. Ten percentages of random samples were tested twice for quality control, and reported with a concordance of 100% (reproducibility). Furthermore, direct sequencing using an ABI PRISM Model 3730 automated sequencer (Applied Biosystems, CA, USA) for different genotypes in each gene were confirmed. Detailed sequences of individual primers, annealing temperatures, restricted enzyme and fragment sizes of *SNPs* are presented in Table 1.

Determination of urinary arsenic species levels. Stored urine samples were retrieved, and thawed at room temperature, ultrasonically mixed, and filtered through a Sep-Pak C_{18} column (Mallinckrodt Baker, Phillipsburg, NJ, USA), 200 μL urine was analyzed using high-performance liquid chromatography (Merck Hitachi, Tokyo, Japan), equipped with a hydride generator and atomic absorption spectrometer (PerkinElmer, Waltham, MA, USA) (HPLC–HG-AAS) to analyze the urinary arsenic species concentrations. The detailed method for the analysis has been reported in a previous study (Hsueh et al., 1998). The correlation coefficient of the calibration curve was greater than 0.995 and the average recovery rates for As^{III}, DMA^{V}, MMA^{V}, and As^{V} were between 93.8 to 102.2%, with detection limits of 0.02, 0.08, 0.05 and 0.07 μg/L, with quantization limits of 0.07, 0.27, 0.17 and 0.23 μg/L,

Please cite this article as: Chen, W.-J., et al., Renin–angiotensin–aldosterone system related gene polymorphisms and urinary total arsenic is related to chronic kidney disease, Toxicol. Appl. Pharmacol. (2014), http://dx.doi.org/10.1016/j.taap.2014.05.011