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Adiponectin gene polymorphisms and obesity increase the susceptibility to arsenic-related renal cell carcinoma

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ABSTRACT

Our recent study found that high urinary total arsenic levels were associated with renal cell carcinoma (RCC). Recent studies demonstrated that low circulating adiponectin was related to RCC. The aim of the present study was to explore the relationship between adiponectin gene (*ADIPOQ*) polymorphisms and RCC and investigate whether individuals with an *ADIPOQ* risk genotype, obesity, and high urinary total arsenic levels have a modified odds ratio (OR) of RCC. A total of 389 RCC patients and 389 age- and sex-matched controls were recruited between November 2006 and December 2012 in Taiwan. Image-guided biopsy or surgical resection of renal tumors was performed to pathologically verify RCC. Genomic DNA was used to examine the genotypes of the *ADIPOQ* rs182052, *ADIPOQ* rs2241766, *ADIPOQ* rs1501299, and *ADIPOQ* rs1063539 SNPs by PCR-RFLP. HPLC-HG-AAS was used to measure the concentrations of urinary arsenic species. Participants with the *ADIPOQ* rs182052 G/A + A/A genotype had a significantly higher OR of RCC compared with those with the *ADIPOQ* rs182052 G/G genotype. The OR (95% confidence interval [CI]) was 1.70 (1.23–2.36). The OR of RCC for the combined effect of high urinary total arsenic levels and obesity, which was dose-dependent, in individuals with the *ADIPOQ* rs182052 G/A + A/A genotype was 9.33 (3.85–22.62). The present study found significant combined effects of obesity and the *ADIPOQ* rs182052 G/A + A/A genotype on the arsenic-related risk of RCC in a population with low arsenic exposure. Arsenic exposure, obesity, and the *ADIPOQ* rs182052 polymorphism could be predictors of a higher OR of RCC.

1. Introduction

The incidence of renal cell carcinoma (RCC) in men and women has increased annually in Taiwan. A total of 644 males and 310 females were diagnosed with RCC in 2011, accounting for 1.03% of all cancer patients (Ministry of Health and Welfare, 2011). Both genetic susceptibility and environmental factors contribute to the pathogenesis of RCC (Chow and Devesa, 2008). Our previous study found that people with high urinary total arsenic levels and low estimated glomerular filtration (eGFR) rates had a higher odds ratio (OR) of RCC compared with those with low urinary total arsenic levels and high eGFR rates in an area with low arsenic exposure (Huang et al., 2011). However, a large cohort study reported a strong association between obesity and the risk of RCC (Adams et al., 2008). Other studies found that a greater body mass index (BMI) was significantly associated with a higher risk of RCC (Bergstrom et al., 2001; Renehan et al., 2008) and unfavorable RCC prognosis (Calle et al., 2003). Although our recent study found that obesity had a marginally significant positive association with RCC (Yang et al., 2016), the mechanism by which obesity triggers RCC needs to be explored further.

Adiponectin is a protein that is produced and secreted by adipocytes. It plays a key role in glucose metabolism, fatty acid oxidation,

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and insulin sensitivity (Ahima, 2006). Serum adiponectin concentrations are low in obese people (Arita et al., 2012). Plasma adiponectin levels decrease as visceral adipose tissue accumulates, which induces insulin resistance (Matsuzawa et al., 2004). A recent study reported that low plasma adiponectin levels were a diagnostic and prognostic biomarker of RCC (Choi et al., 2016). Another study found that low plasma adiponectin levels were related to a higher incidence of clear-cell RCC (Wang et al., 2016). An animal study found an inverse relationship between urinary total arsenic levels and serum adiponectin levels, suggesting that arsenic exposure may disturb the metabolic lipid profile (Song et al., 2017). Arsenic exposure and obesity can reduce adiponectin levels. Whether obesity interacts with arsenic exposure to increase the risk of RCC requires further investigation.

The adiponectin protein is encoded by the adiponectin gene (*ADIPOQ*), which is located on chromosome 3q27 and comprises three exons and two introns (17 kb) (Ling et al., 2009). A previous meta-analysis suggested that the *ADIPOQ* rs2241766 (T45G) GG genotype might be associated with obesity in Chinese adults (Wu et al., 2014). Another meta-analysis found that the G allele of the *ADIPOQ* rs2241766 (T45G) single-nucleotide polymorphism (SNP) significantly decreased the risk of cancer, with an OR (95% confidence interval [CI]) of 0.65 (0.50–0.85) (Zhou et al., 2013). A recent study reported that the *ADIPOQ* rs182052 variant A allele was significantly associated with a higher risk of clear-cell RCC in Chinese adults, with an OR (95% CI) of 1.36 (1.07–1.74) (Zhang et al., 2015). Few studies have investigated associations between obesity, the *ADIPQ* gene, and RCC. The aim of the present study was to explore the impact and joint effects of obesity, arsenic exposure, and *ADIPOQ* gene polymorphisms on the risk of RCC.

2. Materials and methods

2.1. Study participants

We recruited 389 patients who were diagnosed with pathologically proven RCC and 389 age- and gender-matched controls with no evidence of RCC or any other malignancy from National Taiwan University Hospital, Taipei Municipal Wan Fang Hospital, and Taipei Medical University Hospital between November 2006 and December 2012 (Huang et al., 2016). Image-guided biopsy or surgical resection of renal tumors was performed to pathologically verify RCC. Pathological staging was evaluated based on tumor-node-metastasis (TNM) classification by the American Joint Committee on Cancer (American Joint Committee on Cancer, 2002). Tumor grading was based on the Fuhrman grading system (Fuhrman et al., 1982). The present study was approved by the Research Ethics Committee of National Taiwan University Hospital. All of the participants signed written informed consent forms before sample and data collection. The present study complied with the World Medical Association Declaration of Helsinki. All of the participants who lived in Taipei and the surrounding areas drank tap water from the Taipei Water Department of the Taipei city government, which had arsenic levels that were less than the 10 μg/L arsenic standard according to WHO drinking water standards (WHO, 2011). Although Taipei has no arsenic-related factory, in addition to exposure from drinking water, arsenic or arsenic species have been detected in seafood (Chen et al., 2010), agricultural rice (Hsu et al., 2012), edible oil (Chu and Jiang, 2011), and cereals (Tsai and Jiang, 2011) in Taiwan. Therefore, the exact source of the participant's exposure to arsenic in the present study was unknown.

2.2. Questionnaire interview and biological specimen collection

Standardized personal interviews, based on a structured questionnaire, were conducted by well-trained personnel during subject recruitment. The questionnaire included demographic and socioeconomic characteristics, cigarette smoking habits, and histories of hypertension and diabetes (Huang et al., 2011). Body mass index was calculated as weight (kilograms) divided by height (meters squared). Blood samples and daytime midstream urine samples were collected after diagnosis but before surgery or treatment. Urine samples were stored at −20 °C until the analysis of urinary arsenic species. Urinary creatinine levels were used to adjust urinary total arsenic levels for variations in hydration status (Barr et al., 2005). Spot-collected urine samples can be reliably used to determine arsenic excretion levels over 24 h (Calderon et al., 1999).

2.3. Determination of urinary arsenic species

The frozen urine samples were thawed at room temperature. The samples were then dispersed by means of ultrasonic waves and filtered through a Sep-Pak C18 column (Mallinckrodt Baker, Phillipsburg, NJ, USA). Arsenic species were analyzed in 200-μL aliquot samples using high-performance liquid chromatography (HPLC; Waters 501, Waters, Milford, MA, USA) with columns that were obtained from Phenomenex (Nucleosil, Torrance, CA, USA). The concentrations of arsenite (As^{III}), arsenate (As^{V}), monomethylarsonic acid (MMA^{V}), and dimethylarsenic acid (DMA^{V}) were quantified by hydride generator-atomic absorption spectrometry (Hsueh et al., 1997). This method is used for the determination of arsenic species and is not influenced by the ingestion of shellfish, fish, or any other seafood (Hsueh et al., 2002). The recovery rates of the four arsenic species were calculated as the following: [(sample spiked standard solution concentration) − sample concentration] / standard solution concentration × 100. The detection limits for As^{III}, As^{V}, MMA^{V}, and DMA^{V} were 0.02, 0.06, 0.07, and 0.10 μg/L, with recovery rates that ranged from 93.8% to 102.2%. The standard reference material, SRM 2670 (National Institute of Standards and Technology; Gaithersburg, MD, USA), contained 480 ± 100 μg/L inorganic arsenic and was used as a quality standard for comparisons with the urine samples. The concentration (mean ± standard deviation [SD]) of SRM 2670 that was determined by our system was 507 ± 17 μg/L ($n = 4$).

2.4. Genotype determination

Genomic DNA was extracted by proteinase K digestion, followed by phenol and chloroform extraction. We selected appropriate single nucleotides polymorphisms (SNPs) according to the frequency of Han Chinese in Beijing, China, and Asian from the dbSNP Short Genetic Variations which was provided by the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/projects/SNP/). The genotyping of *ADIPOQ* rs182052, *ADIPOQ* rs2241766, *ADIPOQ* rs1501299, and *ADIPOQ* rs1063539 was performed using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) (Aminimoghaddam et al., 2015; Ramya et al., 2013; Zhang et al., 2015). The following primers were used to amplify 302, 372, 153, and 481 bp PCR products for *ADIPOQ* rs182052, *ADIPOQ* rs2241766, *ADIPOQ* rs1501299, and *ADIPOQ* rs1063539, respectively: 5′-GGATC ACCCAGGCTCTCC-3′ (forward) and 5′-GAGGCAATGTATTCCCTTA TCC-3′ (reverse) for *DIPOQ* rs182052; 5′-GAAGTAGACTCTGCTGAGA TGG-3′ (forward) and 5′-TATCAGTGTAGGAGGTCTGTGATG-3′ (reverse) for *ADIPOQ* rs2241766; 5′-CCAGTAGTCCTGATACCTTAG-3′ (forward) and 5′-ATCAAGTTCAGGAGAGGATG-3′ (reverse) for *ADIPOQ* rs1501299; and 5′-TGGCTATGCTCACAGTCT-3′ (forward) and 5′-TCC TTCCTTCCTTCCTCC-3′ (reverse) for *ADIPOQ* rs1063539. All of the PCR products were obtained in a total volume of 30 μL that contained 80 ng DNA sample, 10× Taq buffer, 2.5 mM dNTP, 2 μM of each primer, and 2 U Taq polymerase. After initial denaturation for 5 min at 94 °C, 35 cycles were performed at 94 °C for 30 s (denaturation), at 58 °C for 30 s (annealing), and at 72 °C for 30 s (extension), followed by a final step at 72 °C for 5 min for *ADIPOQ* rs182052. For *ADIPOQ* rs2241766, *ADIPOQ* rs1501299, and *ADIPOQ* rs1063539, the annealing temperatures were 60 °C, 56 °C and 57 °C, respectively. The PCR products were digested with *MwoI* for *ADIPOQ* rs182052, *SmaI* for *ADIPOQ*