

Nonassociation of aryl hydrocarbon receptor genotypes with susceptibility to bladder cancer in Shanghai population¹

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ABSTRACT

AIM: To assess two polymorphic forms of aryl hydrocarbon receptor (AHR) gene, G₁₇₂₁A (R₃₅₄K), and G₁₇₆₈A (V₅₇₀I) in Chinese population and to explore the possible association of human AHR gene polymorphism with elevated incidence of bladder cancer among Chinese Han subjects in east of China. **METHODS:** An allele-specific PCR-based procedure for AHR gene polymorphism genotyping was developed by this work. Genotyping on three groups of subjects in Shanghai area had been performed; a bladder cancer group with the occupational exposure to benzidine, a non-occupational bladder cancer patient group whose members lack an obvious aromatic amine exposure record, and a normal population in the same city as controls. **RESULTS:** A significant difference ($P < 0.01$) in frequency distribution at locus G₁₇₂₁A between normal population in Shanghai and a Caucasian population reported by other authors was observed. No mutant allele (A₁₇₆₈) at locus G₁₇₆₈A had ever been detected in our study. The observed frequencies were similar between both genders in the normal population ($P = 0.54$), and there were no significant difference confirmed between the case group and the control group. **CONCLUSION:** The locus G₁₇₆₈ of human AHR gene seems to be monomorphic among Chinese in this area. The significant distribution difference at locus G₁₇₂₁A of human AHR gene between Chinese Han and Caucasian was confirmed. This study

did not support the association of AHR G₁₇₂₁A polymorphism with higher risk to bladder cancer among the residents in this area, either in a group of occupationally benzidine-exposed individuals or among the persons who never have an obvious aromatic amine exposure record.

INTRODUCTION

Benzidine, as an evident human bladder carcinogen, has been listed in the Group I of chemical carcinogens by the International Agency of Research on Cancer (IARC, 1987)⁽¹⁾. Therefore, a population exposed to benzidine is regarded as a high-risk group for bladder cancer⁽²⁾. The aromatic amines are mainly activated by CYP1A1/2 in human body, the activated metabolites can be further detoxicated by conjugating with GSH and other small endogenous molecules, which made them more polar and hydrophilic, and less toxic to the organisms in most cases; on the other hand, they can also covalently bind with DNA and other biomacromolecules, thus initiate the process of chemical carcinogenesis⁽³⁾. Many of these metabolites serve as the ligands of AHR system. The ligand-receptor binding triggers the induction process of xenobiotic metabolic enzymes, including CYP1A1, CYP1A2, CYP1B1, glutathione transferase Ya (GST-Ya), aldehyde dehydrogenase 3 (ALDH3), NAD(P)H:quinone oxidoreductase (NQO1), UDP-glucuronosyltransferase-6 (UGT-6). The mutant phenotype of AHR is considered as a risk factor for laryngeal carcinoma, cancer of oral cavity and bronchogenic carcinoma⁽⁴⁾. Furthermore, AHR also involves in many other critical life processes (eg, cell differentiation and cell division) by signal transduction mechanism⁽⁵⁾. The human AHR gene is located at chromosome 7p15⁽⁶⁾. It consists of 11 exons and 10 introns; its cDNA was cloned in 1993⁽⁷⁾, and the report about full length of AHR gene sequence has not been seen so far.

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Studies have shown that the inducibility of CYP1A1 alters in mutant CYP1A1 gene, and that some mutant alleles were considered as risk factors for lung cancer and some other diseases in some oriental populations^(8,9). The high inducibility form of CYP1A2 was also correlated with elevated incidence of bladder cancer⁽¹⁰⁾. Meanwhile, studies in mice have shown that the polymorphism of AHR, which exists in some mouse strains, changed the cyplal activity significantly⁽¹¹⁾. Smart *et al* reported two polymorphic forms in AHR exon 10, G₁₇₂₁A (R₅₅₄K), and G₁₇₆₈A (V₅₇₀I)⁽¹²⁾. Both polymorphisms exist in a region that shows high transcription activity in mouse AHR⁽¹³⁾. The G₁₇₂₁A polymorphism has been detected previously in Japanese population. Data showed no difference in the inducibility of aromatic hydrocarbon hydrolase (AHH) activity, the genotype distribution did not show significant difference between the cases and the controls. These suggested that the mutant allele did not serve as a risk factor for lung cancer, at least, in Japanese population⁽⁸⁾. While Smart *et al* found that the presence of the variant allele at locus G₁₇₂₁A was connected with increasing mean ethoxyresorufin-O-deethylase (EROD) activity in Caucasian population⁽¹²⁾.

To explore the possible association of human AHR gene polymorphism with bladder cancer risk, this work investigated the AHR gene polymorphism at G₁₇₂₁A and G₁₇₆₈A loci in three groups of subjects.

MATERIALS AND METHODS

Populations All the subjects in the three groups of this study are Chinese Han. (1) Control group ($n = 183$); control subjects were selected from healthy individuals among Shanghai residents. At the time of sample collecting, none of the subjects had been diagnosed to suffer from any kind of cancers, cardiovascular disease, mental disorder, or any other serious diseases. (2) Benzidine-exposed bladder cancer group ($n = 29$); benzidine was firstly introduced to dye synthesis in Shanghai in 1946 and had been widely used in dye factories for 30 a before it was finally forbidden for any industrial purpose in China in 1976. A cohort of 700 former benzidine-exposed workers in Shanghai dye industry was established in 1984. The follow-up studies and regular surveillance have been persisted since then. By the end of 1999, 215 death cases (some of the cases died before the research cohort was established) in the

group were registered, 91 of them have been diagnosed as bladder cancer (with bladder cancer at the top of incidence rate), among which 29 bladder cancer patients survived were included in this study. (3) Non-occupational bladder cancer patients ($n = 32$); The subjects in this group were inpatients in the urological division of a local hospital at the time of sample collection. Any possible occupational exposure history to benzidine and other major aromatic amine was excluded by questionnaire.

Blood sample collection and DNA extraction

Edetic acid was used as blood anticoagulant. Genomic DNA was isolated from peripheral leukocytes by proteinase K digestion and phenol/chloroform extraction⁽¹⁴⁾.

AHR genotyping at the loci of G₁₇₂₁A and G₁₇₆₈A AHR genotyping was performed by a newly developed allele-specific PCR (AS-PCR). Two primers, differing by only 1 base in the 3' end, according to the polymorphic site, were separately used, together with a common upstream primer in the AS-PCR (as shown in the primer sequences); the two resulting PCR products were subjected to agarose gel electrophoresis in parallel lanes to identify the genotypes. Primers were designed according to the cDNA sequence of exon 10 of AHR gene⁽⁷⁾. Forward primer for detecting G₁₇₂₁A polymorphism was 5'-ACTCTCTCAATCCTAGTTC-3' and the corresponding reverse primer was 5'-TTTCATTCTGCATGTGTC-3' or 5'-TTTCATTCTGCATGTGT-3'. The PCR product was 333 bp. coamplification of a 493-bp segment of human actin gene was performed as an internal control, with a pair of primers 5'-GGGCACGAAGGTCATCATT-3' and 5'-GGCCCCTCCATCGTCCACCG-3'. Forward primer for detecting G₁₇₆₈A polymorphism was 5'-ACTCTCTCAATCCTAGTTC-3' and the corresponding reverse primer was 5'-GTCAATGTCTCTGAAGTCAAT-3' or 5'-GTCAATGTCTCTGAAGTCAAC-3'. The PCR product was 383 bp, a 268-bp fragment of human β -globin gene was coamplified as an internal control with a pair of primers; 5'-CAACTTCATCCACGTTACC-3' and 5'-GAAGAGCCAAGGACAGGTAC-3'. All primers used in this study were synthesized by Gibco-BRL Life Technologies (Grand Island, NY, USA).

Two polymorphic loci were genotyped separately. All PCR reactions were carried out in a 30 μ L of 10 \times PCR buffer (3 μ L), dNTP 1.50 mmol \cdot L⁻¹, 0.5 mmol \cdot L⁻¹ of each primer, 1.00 units of *Taq* DNA polymerase

(Sangon, shanghai) and approximately 0.2 µg of genomic DNA, overloaded with a drop of mineral oil.

The touchdown PCR procedure was chosen for our purpose. The program began with denaturation at 94 °C for 5 min, and the first 5 cycles were programed as following: denaturation at 94 °C for 40 s, annealing at 62 °C for 40 s, and elongation at 72 °C for 40 s. For the following cycles, parameters remained the same but the annealing temperature decreased 2 °C per 5 cycles until it touched 56 °C. The last 15 cycles were programmed as: denaturation at 94 °C for 40 s, annealing at 50 °C for 40 s, and elongation at 72 °C for 40 s. The program ended with elongation at 72 °C for 10 min. All the PCR runs had negative (no DNA) controls. All amplications were performed in a Thermal Cycler (PTC-100, MJ Research, Inc, Watertown, Massachusetts, USA). PCR products were subjected to electrophoresis on a 2 % agarose gel containing ethidium bromide and visualised by UV illumination.

Statistical analysis χ^2 -test was used to test the genotype distribution differences between different groups. Odd ratios (OR) and the 95 % confidential internals (CI) were calculated on the basis of genotype to assess the relative risks. According to Smart *et al*, the G/G genotype codes for the lower-active form, and the G/A and A/A code for the higher-active ones in the case of G₁₇₂₁ locus. Thus, the risk of combined G/A and A/A genotypes versus G/G genotype was assumed^[12].

RESULTS

Polymorphism genotyping at locus G₁₇₆₈A and G₁₇₂₁A in a normal population in Shanghai

Present study did not detect any mutant allele (A₁₇₆₈) in all 183 subjects assayed, even in heterozygous state. This finding excluded the possible association of polymorphism at this locus with most kind of environmental chemical-related human health risk in the population studied. So the genotyping of G₁₇₆₈A of AHR gene was not included in our further study on case group.

The distribution of genotypes at locus G₁₇₂₁ was in agreement with Hardy-Weinberg equilibrium in three target populations, which indicated that the groups chosen for this study were random and representative (data not shown).

The comparison of genotype distribution of this polymorphism in the normal population in Shanghai with

that of other ethnic groups reported by other authors was performed. A statistically significant difference between the Chinese in Shanghai and the Caucasian was proved in the comparison ($P < 0.01$, Tab 1).

Tab 1. The comparison of genotype distribution at AHR G₁₇₂₁A locus in different ethnic groups.

Population	n	G/G	G/A	A/A	P value
Chinese Han in Shanghai	183	81(44 %)	75(41 %)	27(15 %)	1
Japanese ⁽⁸⁾	277	94(34 %)	129(47 %)	54(19 %)	0.07
Caucasians ⁽¹²⁾	105	83(79 %)	21(20 %)	1(1 %)	<0.01
African-Americans ⁽¹²⁾	110	36(33 %)	57(52 %)	17(15 %)	0.13

Some reports elucidated that the inducibility of CYP1A1/2 activity in female was lower than that in male^(15,16). It would be the case that the G₁₇₂₁A polymorphism might be due to the gender difference. So the polymorphism frequency was stratified according to the gender. There was no significant difference in the genotype distribution between the male and female (Tab 2).

Tab 2. The distribution of G₁₇₂₁A polymorphism between genders in a normal population in Shanghai.

	n	G/G	G/A	A/A	P value
Males	91	43(47 %)	37(41 %)	11(12 %)	1
Females	92	38(41 %)	38(41 %)	16(17 %)	0.54

Polymorphism genotyping at locus G₁₇₂₁A in a group of benzidine exposure-related bladder cancer patients and a group of non-occupation-related bladder cancer patients

According to Smart *et al*, it was assumed that both heterozygous and homozygous mutant alleles, ie, G/A or A/A, at locus G₁₇₂₁A of AHR gene was related with elevated risk for bladder cancer. So the OR and CI were calculated on the basis of G/A plus A/A vs G/G alone.

The genotype frequencies were similar among three groups assayed. It would be a good reason to postulate that the human G₁₇₂₁A polymorphism locus of AHR gene would not be a risk factor either for occupational benzidine exposure-related, or for non-occupation-related bladder cancer (Tab 3).

Tab 3. The distributions of G₁₇₂₁A polymorphism in two groups of bladder cancer patients and a normal population in Shanghai.

	n	G/G	G/A	A/A	P value	OR	95 % CI
Normal population	183	81(44 %)	75(41 %)	27(15 %)	1	1	1
Bladder cancer patients	61	29(47 %)	23(38 %)	9(15 %)	0.66	0.88	0.49 - 1.57
Occupational benzidine exposure-related	29	13(45 %)	11(38 %)	5(17 %)	0.95	0.98	0.44 - 2.15
Non-occupational exposure-related	32	16(50 %)	12(37 %)	4(13 %)	0.55	0.79	0.37 - 1.68

DISCUSSION

Recently, studies on the susceptibility to occupational diseases mass densely on the polymorphism of certain xenobiotic metabolic enzymes (XME). The signal transduction pathways can modulate the activity and expression of XME, so it is necessary to investigate on possible correlation between the polymorphism of some signal mechanisms and the susceptibility to certain diseases.

In this study, a suitable method for genotyping polymorphisms at the loci G₁₇₂₁A and G₁₇₆₈A of AHR gene had been developed. The allele specific PCR (AS-PCR), together with the touchdown temperature program, proved to be convenient for our purpose. The population frequencies of two polymorphic forms of human AHR gene were investigated in Chinese population. The data revealed a significant difference in distribution frequency of G₁₇₂₁A allele between Chinese Han population stationed in Shanghai area and Caucasian population. Smart *et al* detected G₁₇₆₈A polymorphism in African-American and Caucasian populations^[12], but no mutant allele ever was detected in the present study, so it is postulated that human AHR gene is monomorphic at locus G₁₇₆₈ in southeastern Chinese sub-population (Polymorphism, as indicated by some authors, means that different allelic forms exist in a considerable part (> 1 %) of the population^[17]). Therefore there is no reason to expect any implication on CYP1A1 activity in terms of G₁₇₆₈A polymorphism in the population studied. These data might also offer clues to explore the ethnic differences in susceptibilities to certain kinds of xenobiotic-related occupational diseases.

The incidence of many diseases are significantly different between different sexes, which may partially due to the different levels of activities of some XME. Studies have confirmed that CYP1A1 and CYP1A2 activities are lower in women than in men^[15,16]. Because both of these two enzymes are under the regulation of AHR system, we want to investigate

whether or not the difference partially results from the different distribution of A₁₇₂₁G polymorphism of AHR gene in men and women. Data from this study showed similar genotype frequencies, these data may be helpful for further studies on the relationship between the A₁₇₂₁G polymorphism and some sex-related diseases.

A few epidemiological studies have been published on the association of polymorphism of human AHR gene with the susceptibility to some diseases. Kawajiri *et al* reported that there were no correlation between AHR G₁₇₂₁A polymorphism and higher risk of lung cancer in Japanese population^(B). In this study, we found that the distributions of G₁₇₂₁A polymorphisms were similar in the case group and in the control group, the comparison of data from the two subgroups of the cancer patients showed no significant difference, either. Therefore, it is postulated that this polymorphism might not be a risk factor for bladder cancer.

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上海地区人群中的芳香烃受体基因多态性与膀胱癌易感性无关¹

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关键词 芳香烃受体; 多态现象(遗传学); 膀胱肿瘤; 联苯胺类

目的: 研究芳香烃受体(AHR)基因两个多态性位点: G₁₇₂₁A (R₅₅₄K) 和 G₁₇₆₈A (V₅₇₀I), 在中国人群中的分布频率及与膀胱癌易感性的关系. **方法:** 我们建立了基于等位基因特异性 PCR (Allele-specific PCR, AS-PCR) 的基因型鉴定方法, 并对三组人群进行了基因型鉴定; 一个作为对照的本地正常人群, 一个有膀胱癌致癌剂联苯胺职业暴露史的膀胱癌病人组, 和一个无明显芳香胺类物质接触史的膀胱癌病人组. **结果:** 我们在本地区正常人群中没有发现 G₁₇₆₈ 位点的多态性; 在对照人群中, G₁₇₂₁ 多态性位点的基因型分布频率与已报道的一个高加索人群有显著差异 ($P < 0.01$); 该多态性位点在不同性别中的分布差异不显著 ($P = 0.54$). 在所研究的三个人群中, 其基因型分布频率相近. **结论:** AHR 基因 G₁₇₆₈ 位点在上海地区的人群中表现为野生型单态性; G₁₇₂₁A 多态性基因型分布在该人群和高加索人群中有显著差异; 在该地区人群中, G₁₇₂₁ 位点的多态性并不是构成致膀胱癌的危险因子.

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