Polymorphism of N-acetyltransferase 2 (NAT2) Gene Polymorphism in Shanghai population: Occupational and Non-occupational Bladder Cancer Patient Groups[1]

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Objective Arylamine N-acetyltransferases (NATs) are involved in the detoxification of aromatic amines and hydrazine. In order to explore the possible association of NAT2 polymorphism with bladder cancer risk in benzidine exposed or non-exposed Chinese individuals, healthy subjects, subjects with bladder cancer of a former benzidine exposed cohort in Shanghai dyestuff industry and a group of bladder cancer patients without known occupational exposure to aromatic amines were genotyped for NAT2 gene polymorphism. **Methods** NAT2 genotyping was performed with a set of RFLP procedures at seven major polymorphic loci of gene coding area: G191A, C282T, T341C, C481T, G590A, A803G and G857A. **Results** The wild allele NAT2 *4 was the most prevalent allele (59%) in healthy individuals. The alleles NAT2*6A and NAT2*7B were also frequently observed (21% and 17%, respectively). In contrast to Caucasians, the percentage of slow acetylators was lower (12% in Chinese *vs.* 58% in Caucasians, $P<0.001$). No relevant differences were observed for homogenous rapid, heterogeneous rapid/slow and homogeneous slow acetylation genotypes between the healthy subjects and both groups of bladder cancer patients. **Conclusion** The present work did not support the association of slow acetylating genotypes of NAT2 gene with elevated risk of bladder cancer in Chinese whereas it was documented as an important genetically determined risk factor in Caucasians. Different mechanisms might play a role in individual susceptibility to bladder cancer related with aromatic amine exposure in various races or ethnic groups.

Key words: Benzidine; Occupational exposure; N-Acetyltransferase 2; Polymorphism; Bladder cancer; Dyestuff industry

INTRODUCTION

Arylamine N-acetyltransferases (NATs, EC 2.3.1.5) are involved in the detoxification of aromatic amines and hydrazine[1]. NAT2 polymorphism affects individual's acetylation ability for these substances[2]. Individuals can be phenotyped for the NAT2 status by

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caffeine[3], isoniazid[4], or sulphamethazine[5] as a substrate. NAT2 slow acetylation status shows various frequencies in different races and ethnic groups, ranging from about 10% in Asians to over 90% in Moroccans[6].

NAT2 polymorphism is based on several point mutations in the coding area[7]. A consensus nomenclature for NATs was first published in 1995[8] and updated in 2000[9]. The most actual information on the nomenclature of N-acetyltransferases is available in the World Wide Web (http://www.louisville.edu/medschool/pharmacology/ NAT.html).

There are 28 allelic variants of NAT2 known so far. Using a modification of a PCR-RFLP based procedure developed by Cascorbi *et al*.[10], seven major mutations in the NAT2 gene can be detected simultaneously. The NAT2 polymorphism has been reported to be associated with the susceptibility to various types of cancers[11-13] or other diseases[14] within certain ethnic populations. Point mutations in the coding region may affect the NAT2 expression and catalysis activity[15]. Thus, it is necessary to investigate the frequencies of all major polymorphic loci in the gene and the role they might play in the individual susceptibility in different ethnic populations. In some previously published studies only three of the polymorphic loci of the NAT2 gene, i.e. 481, 590 and 857 were investigated. Many of the substitutions were linked within single alleles, such as 341, 481 and 803 mutations in allele *5B, 282 and 590 in allele *6A, etc. Because there were discrepancies between the genotype and the phenotype of some individuals[16], it would be essential to investigate as many point mutations as possible. In this study, 7 major polymorphic loci of the NAT2 gene were genotyped.

Benzidine, an evident human bladder carcinogen, was introduced for dye synthesis in Shanghai in 1946 and was widely used until it was officially banned for all industrial purposes in China in 1976[17,18]. Workers occupationally exposed to benzidine were regarded as a high-risk group for bladder cancer. We have previously investigated the bladder cancer risk for benzidine-exposed workers in Shanghai dyestuff industry. Our results indicated that the standardized incidence ratio (SIR) for bladder cancer in workers of Shanghai dyestuff industry reached 3500 for the entire cohort and was even higher (up to 7500) for those at highly exposed working positions[19]. The association of susceptibility to bladder cancer with the status of glutathione S-transferases T1, M1, and P1 or the aryl hydrocarbon receptor (*Ahr*) gene in this cohort has been reported elsewhere [17,18,20].

Cartwright *et al*.[21] first reported a strong association of bladder cancer incidence with the slow acetylation phenotype (OR=16.7; *P*=0.00005) in a group of workers in UK exposed to aromatic amines. Later on, a similar study conducted in former benzidine exposed workers in several Chinese cities, including Shanghai, failed to confirm such an association[22]. In the present study, genotyping for the seven major loci (G191A, C282T, T341C, C481T, G590A, A803G and G857A) of the NAT2 gene was conducted in three groups of Shanghai residents to enhance the limited data bases.

MATERIALS AND METHODS

Subjects

All the subjects in this study were ethnic Han Chinese.

The subjects of this study included three groups of Shanghai residents[17]: (1) Healthy individuals in Shanghai (*n*=112). All the subjects in this group were farmers of a rural area in Shanghai suburb. A self-designed questionnaire was used to obtain information relevant for this study from each individual. Subjects reporting diseases such as cancer, cardiovascular disease, mental disorder or any other serious health problems were excluded.

(2) Bladder cancer patients occupationally exposed to benzidine in dyestuff industry in the past (*n*=29): A research cohort with a total number of 700 former benzidine exposed workers in Shanghai dye industry was established in 1984. A follow-up study and regular surveillance had been persisting since then. The number of diagnosed bladder cancer patients decreased from year to year as subjects were getting aged. Twenty-nine patients with histologically confirmed bladder cancer from this cohort were investigated. (3) Non-occupational bladder cancer patients (*n*=32). The subjects were inpatients in a urological division of a local hospital. A possible occupational exposure to benzidine or other aromatic amines was excluded by questionnaire.

DNA Extraction

Genomic DNA was isolated as described elsewhere[23].

NAT2 *Genotyping*

A modification of the PCR-RFLP procedure based on Cascorbi *et al*.[10] was used for NAT2 genotyping at loci 191, 282, 341, 481, 590, 803 and 857. Two sets of primers were used to amplify two segments in the NAT2 gene from genomic DNA. Restriction enzymes *Msp* I, *Fok* I, *Dde* I, *Kpn* I, *Taq* I and *Bam*H I were used to digest PCR products to determine the mutation. Individuals with two variant alleles, except NAT2*13/*13 were classified as slow acetylators. The NAT2*13 allele has a nucleotide substitution at position 282 that does not result in the change of amino acid. Heterozygous genotypes that showed a wild-type allele *4 or *13 and one copy of other variant alleles were classified as intermediate acetylators. Homozygous genotypes (*4/*4 or *13/*13) were classified as rapid acetylators.

Statistics

Chi-square test was used to compare the distribution of NAT2 genotypes in different groups. Hardy-Weinberg equilibrium was used to calculate the expected frequencies of the investigated heterozygous genotypes.

RESULTS

Frequencies of the detected NAT2 genotypes and alleles in the three investigated groups are listed in Tables 1 and 2, respectively. The distribution of the genotypes among healthy subjects was in Hardy-Weinberg equilibrium.

Eleven genotypes were found in healthy subjects in Shanghai, the most frequent (31%) genotype was the homozygous wild type *4/*4. Major genotypes containing variant alleles, *4/*6A and *4/*7B represented 27% and 24% of the individuals, respectively. NAT2*6A/*7B was the most common genotype with two variant alleles, which accounted for 8% of the total subjects. On the basis of trichotomous genotype classification, 14 (12.5%) individuals, 61 (54.5%) and 37 (33.0%) individuals were slow, intermediate rapid, and rapid acetylators, respectively.

Using a dichotomous classification, 87.5% of the individuals were rapid acetylators and 12.5% of the individuals were slow acetylators. Our study revealed a similar level of population frequency of slow acetylation genotypes, compared with a Japanese population (12.5% in Chinese in Shanghai *vs.* 10.1% in Japanese[24], *P*=0.46, and *vs.* 10.9% in Korean[25], *P*=0.65). A significantly higher presentation of the rapid acetylation allele NAT2 *4 was confirmed when compared with a Caucasian population reported by Cascorbi *et al*.[10]

(58.5% in Chinese and 23.4% in Caucasian, $P<0.001$).

TABLE 1

NAT2 Genotypes in Three Subgroups of Shanghai Population

	NAT2 Genotype	Healthy Controls (n=112)	Bladder Cancer Patients Exposed to Benzidine (n=29)	Bladder Cancer Patients Not Overtly Exposed (n=32)
Homozygous Rapid Genotype	*4/*4	35 (31%)	4 (14%)	9 (28%)
	*4/*13	1 (0,9%)	0	0
	*13/*13	1 (0.9%)	2 (6.9%)	2 (6.3%)
Heterozygous (Intermediate) Rapid Genotype	*4/*5B	3 (2.7%)	2 (6.9%)	0
	*4/*5D	1 (0.9%)	0	0
	*4/*6A	30 (27%)	9 (31%)	9 (28%)
	*4/*6B	0	1 (3.4%)	0
	*4/*7B	27 (24%)	7 (24%)	5 (16%)
	*13/*6A	0	0	0
	*13/*7B	0	0	0
	*12B/*7A	0	0	0
	*11/*6A	0	0	1 (3.1%)
Homozygous Slow Genotype	*5B/*5B	0	1 (3.4%)	0
	*5B/*6A	1 (0.9%)	0	0
	*5B/*7B	0	0	1 (3.1%)
	*6A/*6A	3 (2.7%)	2 (6.9%)	2 (6.3%)
	*6A/*7B	9 (8.0%)	1 (3.4%)	3 (9.4%)
	*7B/*7B	1(0.9%)	0	0

TABLE 2

Comparison of NAT2 Genotype Distribution in Chinese and Caucasians (Chi-squared test was used to compare the mutual genotype in the two populations)

Genotype	Population		P Value
	Chinese[a] (n=112)	Caucasians[b] (n=278)	
*4/*4	35 (31.3%)	14 (5.0%)	<0.001
*4/*5B	3 (2.7%)	49 (17.6%)	<0.001
*4/*6A	30 (26.7%)	39 (14.0%)	0.003
*4/*7B	27 (24.1%)	2 (0.7%)	<0.001
*5B/*6A	1 (0.9%)	66 (23.7%)	<0.001
*6A/*6A	3 (2.7%)	29 (10.4%)	0.012
*6A/*7B	9 (8.0%)	50 (18.0%)	0.013

Note. [a]This work. [b]Cascorbi *et al.*, 1996.

TABLE 3

Comparison of Allelic Distribution in Chinese and Caucasians (Chi-squared test was used to compare the mutual alleles of the two populations)

Alleles	Population		*P* Value
	Chinese[a] (*n*=112)	Caucasians[b] (*n*=278)	
*4	132 (58.9%)	130 (23.4%)	<0.001
*5B	4 (1.8%)	214 (38.5%)	<0.001
*6A	46 (20.5%)	173 (31.1%)	0.003
*7B	38 (17.0%)	8 (1.4%)	<0.001

Note. [a]This work. [b]Cascorbi *et al*., 1996.

TABLE 4

Comparison of NAT2 Genotype Distribution in Chinese Population of Shanghai Area and in Other Populations

Populations	NAT2 Genotype			*n*	*P* Value	References
	RR	RS	SS			
Shanghai*	37 (33.0%)	61 (54.5%)	14 (12.5%)	112	Ref.	This Work
Korean**	95 (43.4%)	100 (45.7%)	24 (10.9%)	219	0.190	Lee *et al*., 2000[25]
Japanese**	133 (44.8%)	134 (45.1%)	30 (10.1%)	297	0.099	Koizumi *et al*., 1998[24]
Caucasians*	14 (5.0%)	102 (36.7%)	162 (58.3%)	278	<0.001	Cascorbi *et al*., 1996[10]

Note. *Result of assay at 7 loci. **Result of assay at 3 loci.

In benzidine exposed bladder cancer patients, and non-occupational bladder cancer patients, *4/*4, *4/*6A, *4/*7B, *6A/*7B were found to be the most common alleles. Nevertheless, some genotypes not observed in healthy subjects, such as: *4/*6B, *11/*6A, *5B/*5B and 5B/*7B have been detected among bladder cancer patients. On the other side, the genotypes*4/*13, *4/*5D, *5B/*6A, *7B/*7B observed in healthy individuals were missing in the two different groups of bladder cancer patients.

TABLE 5

Comparison of Homozygous Rapid (RR), Intermediate (RS) and Homozygous Slow (SS) NAT2 Genotypes Among Healthy Individuals and Two Different Groups of Bladder Cancer Patients in Shanghai Area

Groups	Genotypes	*n* (%)	*P*	OR (CI 95%)
Healthy Individuals	RR	37 (33.0%)	Ref.	
In Shanghai	RS	61 (54.5%)	Ref.	
(*n*=112)	SS	14 (12.5%)	Ref.	
Bladder Cancer Patients,	RR	6 (20.7%)	0.198	0.53 (0.20-1.41)
Benzidine Exposed	RS	19 (65.5%)	0.284	1.59 (0.68-3.72)
(*n*=29)	SS	4 (13.8%)	0.853	1.12 (0.34-3.70)
Bladder Cancer Patients,	RR	11 (34.4%)	0.887	1.06 (0.46-2.43)
Overtly Non-exposed	RS	15 (46.8%)	0.448	0.74 (0.34-1.62)
(*n*=32)	SS	6 (18.8%)	0.367	1.62 (0.57-4.61)

Using a trichotomous classification, no significant deviation in the population frequency could be confirmed between the healthy subjects and both groups of bladder cancer patients, though the tendency of lower representation of homozygous rapid genotypes in occupational bladder cancer groups was found, compared with healthy subjects (P=0.20, OR=0.53). The over-presentation of intermediate genotypes was also detected in this group of bladder cancer patients in comparison with normal population (P=0.28, OR=1.59), though the statistical significance was not reached.

DISCUSSION

Few studies have characterized NAT2 genotype distributions in Chinese population in the Chinese mainland. In this study, 7 point mutations in the NAT2 gene were detected and the genotype frequencies observed were compared with other ethnic populations.

Nucleotide substitutions of NAT2 occur at 13 positions, including 111, 190, 191, 282, 341, 434, 481, 499, 590, 759, 803, 845, and 857 (see website for NAT2 nomenclature, Hein *et al*., 2003). All individuals were tested for 7 of these substitutions. The mutation at locus 191 was not detected in this study, suggesting that this nucleotide substitution is uncommon in Chinese. Allele *5 (including 341 mutation) is uncommon in the individuals in this part of China, too. The most prevalent genotypes in the subjects of Shanghai area were the homozygous wild type *4/*4 and the genotypes containing variant alleles *4/*6A and *4/*7B, and genotype with two variant alleles *6A*7B (total coverage is 90%). For all alleles, the most frequent ones were *4**,** *6A and *7B, accounting for 58.9%, 20.5%, and 17.0%, respectively.

When the prevalence of rapid, intermediate, and slow genotypes was compared between Chinese in Shanghai and Caucasian in Germany[10], it revealed that Chinese have a significantly higher portion of homozygous rapid acetylators than Caucasians.

As for allele frequencies, Chinese in Shanghai and Caucasian in Germany displayed a clear difference. In Chinese population, the alleles *4**,** *6A and *7B are the most common ones, whereas in German Caucasian population *5B**,** *6A and *4 are the most frequent. Allele *5B is the most frequent form (39%) in Caucasian population but rare (1.8%) in Chinese. In this study, only one *4/*5D carrier was identified. Allele *5D is also a rare one. In two studies of Agundez *et al*.[26,27], this allele had a frequency of 0.3% in general Spanish population. Several other alleles such as NAT2*12A and NAT2*13 are also rare in human populations. In the present study only one NAT2*13/*13 and one NAT2*4/*13 carrier were recorded, and no NAT2*12A allele was detected in this study. The NAT2 gene spectrum of Chinese in this part of China (middle coastal area of the Chinese mainland) was quite different from the well-documented spectrum of Caucasians[28,29]. In the present study, the slow acetylation genotype carriers only represented 12.5% of the healthy individuals investigated. It was considerably less profound than in Caucasians (50%-65%). This should be taken into account when the impact of polymorphism of NAT2 genotypes on certain diseases such as bladder cancer is concerned.

For the genotyping of NAT2 polymorphism in two groups of bladder cancer patients in Shanghai area, no significant deviation on the frequency of slow acetylation genotypes was confirmed compared to healthy individuals. The slow acetylation genotypes were only found in 13.8% of the histologically confirmed bladder cancer patients in the cohort. This was in line with the findings of Hayes *et al*.[22] who also found no overall increase in bladder cancer risk for individuals with the NAT2 slow acetylation genotypes among 38 bladder cancer cases and 43 controls in several Chinese cities. Five of 38 cancer cases genotyped at

3 loci showed the slow acetylation status (13.2%). These results were in contrast to the situation in Caucasian population where slow acetylation status was proved to be a risk factor for susceptibility to bladder cancer for those exposed to aromatic amine in the past[21]. Among Chinese and other ethnic groups in East Asia area [25,30], rapid and intermediate rapid acetylators are the dominant phenotypes, which were in sharp contrast with those among Caucasians[28,31]. We conclude that the study on individual susceptibility to various cancers should take into account genetic factors, which may vary dramatically among different racial and ethnic populations. Thus, different mechanisms might play a role in individual susceptibility to bladder cancer related with exposure to aromatic amines in various races or ethnic groups[29].

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