Relationship between bladder cancer and slow N-acetylators phenotyped with caffeine as a probe drug in Chinese population

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Aim Acetylator status of 203 healthy controls and 67 patients with bladder cancer was observed. **Methods** All the subjects were N acetylate-phenotyped with caffeine as a metabolic probe drug. Urine samples were collected 2 hours after a cup of 140 mg-caffeine-spiked coffee was taken under fasting condition in the morning. The caffeine metabolites, 5-acetylamino-6-formylamino-1-methyluracil (AFMU) and 1-methylxanthine (1X) were analysed by High Performance Liquid Chromatography (HPLC). The frequence histogram and probit plot were constructed to select the anti-mode which was used to assess slow and fast acetylator status both in healthy controls and patients with bladder cancer. **Results** The peak hight ratios (PHR) of AFMU/1X were from 0.06 to 6.5 for healthy voluteers and 0.1 to 6.31 for patients with bladder cancer. Of the 203 healthy controls involved in this study 73.7% (149/203) had the AFMU/1X >1.10, and were classified as fast acetylators, and 26.3% (54/203) as slow acetylators, while 53.7% (36/67) were fast acetylators and 46.3% (31/67) were slow acetylators in patients with bladder cancer. The odds ratio was 2.376, and the gene freqency for healthy controls and for patients with urinary bladder cancer was 0.51 and 0.68 respectively. **Conclusion** The acetylator status of Chinese population is polymorphic and completely in concordance with that determined by dapsone, isoniazid or sulfamethazine methords as previously reported. Slow acetylators might be susceptible to urinary bladder cancer.

Key words N-acetylation; polymorphism; bladder cancer; caffeine

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N-acetylation was proposed as a detoxification pathway with respect to arylamine bladder carcinogens. Although pharmacoepidemilolgical studies on patients with arylamine induced bladder cancer showed that slow acetylator phenotype plays a crucial role as genetic predisposition to development of this occupational type of diseases most of studies were conducted in Europe, North America or other non-Asia countries[1~3], where slow acetylators account for 40~60%, as compared with 10~20% of Chinese origins. To find (1) the interethnic and interreginal differences in the incidence of bladder cancer in Chinese population, (2) to find the susceptible factors and important implications for its prevention and management, and (3) to answere questtion whether slow acetylator phenotype might also

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predispose individual to non-occupational or spontaneous urinary bladder cancer, 203 healthy volunteers and 67 patients with urinary bladder cancer of Chinese origin were N-acetylate-phenotyped, using caffeine as metabolic probe drug.

1 Materials and Methods

1.1 Subjects The 67 patients with bladder cancer, including 51 males and 16 femals, with mean age of 48±7 years, and mean weight of 64 ±13 kg, were admitted to the department of urology. Affiliated Hospital of Shangdong Medical University. Among all patients involved, 56 were before surgery and 11 after surgery and all were diagnosed by CT or bladder cystoscope. Those with cardiac, renal or hepatic desease were excluded.

The controls comprised 203 healthy students and hospital staff members(male 129, female 74, mean age 19±3 years, mean weight 63±9 kg) in whom 200 were of Han nationality and each of the other three was of Hui Manchu and Korean respectively.

All the subjects were required orally or in written form before text absolutely to abstain from tea, coko, chocolate, tobacco, alcohol and caffeine-containing beverage or food which might influence caffeine metablism in liver or interfere with the acetylate and oxidative procedures.

1.2 Chemicals and Instrument 1-Methylxanthine (1X, AR) was from Aldrich (Steinheim, Germany), 5-acetylamino-6-formylamino-3-methyluricil (AFMU) was a kind gift of Nestle (Vervey, Switzerland), N-acetyl-p-aminophenol (As internal standard, IS) was from Fluka (Buches, Switzerland) Coffee (940425) was from Nestle' Dongguan Ltd (Dongguan, Guangdong). Chloroform and Ammonium Sulfate (950504) were from Laiyang Chemical Ltd (Laiyang, Shandong). Acetonitrile (950310) and methanol (930418), all of HPLC grade, were from Beijing Chemical Ltd (Beijing).

HPLC analysis was performed using Waters 510 solvent delivery system, 440 ultraviolet spectrophotometer, ubondapack C_{18} stainless steel column (6mmx30mm) and V6k sampler. Chromatograms were recorded and integrated by waters 730 data station.

Penotyping Acetylate status was phenotyped using coffeine as a probe drug. An aliquot of urine sample was collected 2 hours after a cup of 140 mg-caffeine-spiked coffee and was stored at −20 ℃ untill analysis.

0.2 ml of defrozened urine sample was mixed with 120 mg Ammonium Sulfate and 6 ml of Chloroform in a 10 ml plugged centrifuging tube, vigorously shaked, and centrifugalized. Water phase was removed, and organic phase was transfered, dehydrated with anhydrous Sodium Sulfate and evaporated to dryness under gental Nitrogen straem at 60 ℃ water bath.

Residual was dissolved in 0.5 ml of mobile phase, and shaked for 60 sec. A volume of 10μl with paracetamol as internal standard was injected and eluted with 0.05 HAC/ Acetonitrile (90∶10 V∶V) at a flow rate of 0.5 ml/min and a pressure of 3273-12410 KPa. Detection was performed at 280 nm. Under these chromatographic conditions, AFMU, 1X and internal standard were eluted after about 7.1, 8.8 and 10.7 min respectively.

The peak hight ratio (PHR) of AFMU and 1X was calculated and the distribution histogram and probit plot of patients with bladder cancer and healthy controls were constructed to sellect antimode and to determine N-acetylator status.

2 Results

The Distribution histogram and probit plot of urinary AFMU/1X ratio in 203 healthy sub-

jects showed apparent bimodal and non-linear character (Fig 1 A, B)indicating that the acetylator distributionm in Chinese population was polymorphic. AFMU/1X was from 0.06 to 6.5 With the antimode of 1.10 73.4% (46/203) of the healthy subjecls were fast actylators and 26.6% were slow scetylators (Tab 1), which coincided with the previous research in Chinese population[5]。

AFMU/1X for patients with bladder cancer was from 0.10 to 6.31. Using the same antimode of 1.10 (Fig 1 C, D), the fast and slow acetylators of the patients accounted for 53.7% (36/67) and 46.4(31/67) respectively.

The odds ratio, according to comparative analysis of two groups, was 2.376 (95% confidence interval: 1.351~4.177) with a significan difference of $P<0.001$.

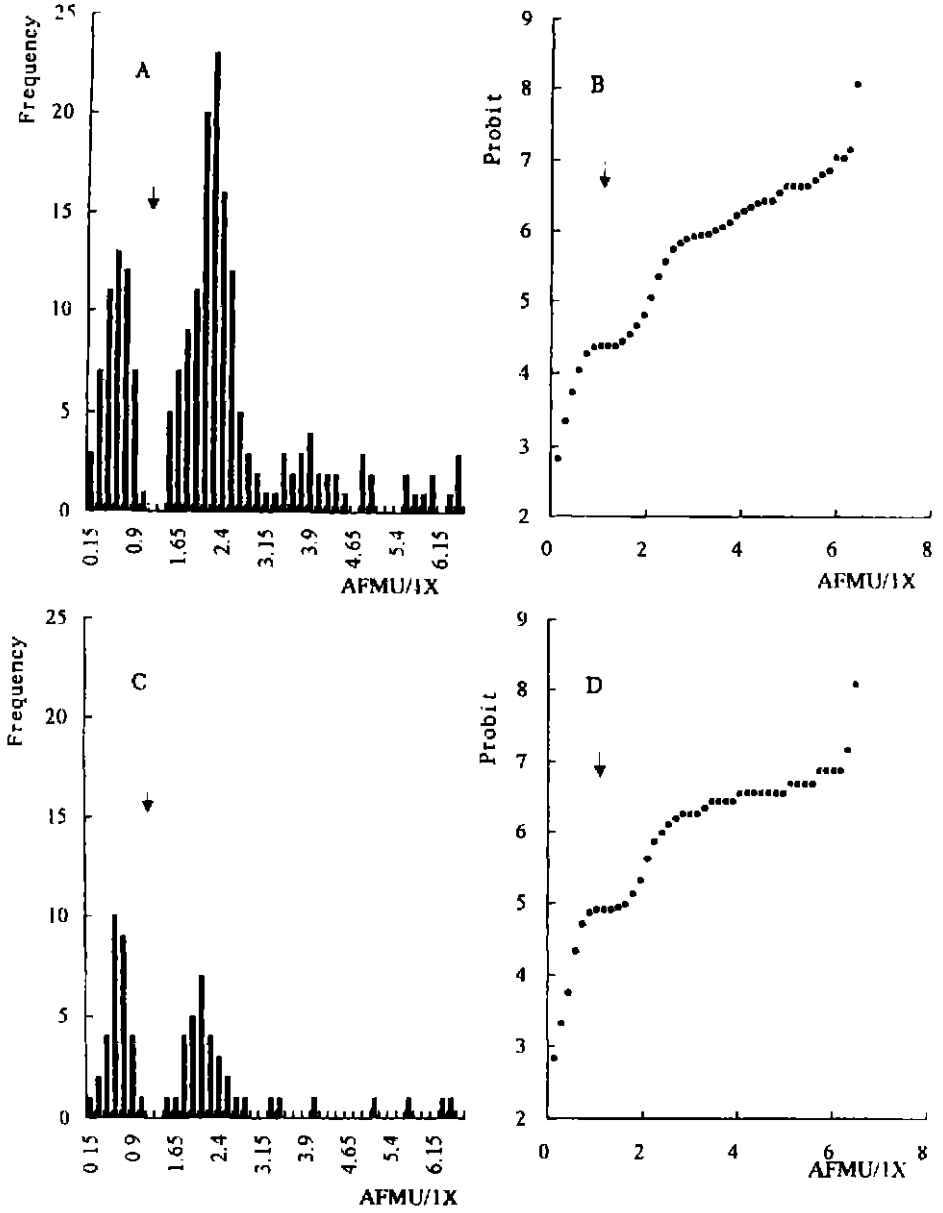

Fig1 Frequency distribution histogram and probit plot of caffeine metabolic ratio in 203 health volunteers (A, B) and 67 patients with bladder cancer (C, D). Arrows indicate apparent antimode. Column division of 0.15 was selected as horizental coordinate.

Tab 1 Statistical analysis for patients with bladder cancer and healthy controls

Group	Subjects	SA	%	GF	OR	95% CI	$\chi^2(P)$
Controls	203	54	26.3	0.5218			
Patients	67	31	46.3	0.6804	2.376	1.3513~4.1776	9.0334(<0.01)

SA: slow actylator, GF: Gene frequency, OR: Odds ratio, CI: Confidence interval

3 Discussion

The metabolism of amine, arylamine or hydrodazine is catalysed by N-acetylatransferase which is controled by an autosomal single gene, and appears to be of polymorphism[6]. The activity of N-acetyltransferase is relatively stable and not influenced by postnatal factors as age, sex and other diseases. Therefore its determination could be used for the study of genetic phenotype. Many drug induced diseases and adverse drug reactions are supposed to be related with human acetylator status. The adverse dapsone reactions of AIDs patients in slow acetylators are more serious and frequent that those in fast N-acetylators[7], while fast acetylators are more closely associated with increased risk of isoniazid induced liver toxicity[1]. Similar relationship between slow acetylators and procainimide induced system it lupus erythematosus has also been proposed[8]. The links between diabetics, Graves' disease and Down's diseases and acetylate status has been also well established[9]. The result of this study shows slow acetylators in healthy controls were 26.3%, whereas in patients with bladder cancer were 46.3%. So it was concluded that slow acetylator may be a risk factor of the development of bladder cancer.

It is generally considered that β-naphthylamine, bezidine and 4-aminobiphenyl are most important bladder cancer carcinogens. Those who have contacted with arylamine, such as dye, rubber tar and pesticide for a long term are most likely to be at risk of bladder cancer. Arylamine is metabolized by N-oxidation, glucuronidated in liver, secreted into bladder, and finally hydrolyzed under mild acidic condition (pH 5~6) to yield active arylnitrenium ions which covalently bound with urothelial DNA and result in urinary bladder cancer[1]. N-acetylation of arylamine is mainly considered as a detoxification step, whil N-oxidation an active step of these bladder carcinogenes. When metabolic processes of human bodies are at certain conditions as N-acetylation reduced, and / or N-oxidation enhenced in slow acelylators, or congenitally lack of N-acetyl transferase on the gastrointestinal and bladder mucous membrane, and either because its amount is reduced or its activity disappears, more exotic arylamine carcinogens accumulate in bladder or remain in its for a longer period of time, then bladder cancer would occur. It is well established that the bladder cancer more frequently occurs in Europe than in Asia countries, Besides the influence of industrial pollution, more slow acelylators in European population may also be one of other factors.

Odds ratio (OR) is a criterion indicating the relationship between two groups of objects. The higher the value is, the closer the relationship between these two groups is. It is generally considered that when OR is over 1.5, the close relationship will exist. In present study the OR is 2.376, showing a strong connection, but it is lower than 8.8 as reported in a study of Chinese population[9], Why the difference exists between studies of same

population needs to be further investigated. The genetic frequencies of controls and of patients with bladder cancer are 0.51 and 0.65 respectively, and X^2 is 9.0334, indicating the significant relationship between these two group.

Many studies were carried out at different laboratories in this field recent years, but the results on the extent of relationship between acetylator status and bladder cancers were different. In 1979, N-acetylation was proposed as a detoxification pathway with respect to arylamine bladder carcinogens[10]。Four subsequent studies in subjects occupationally exposed to arylamine carcinogens showed, increased risks, ranging from 2 to 17 fold, for bladder cancer associating with the slow acetylator phenotype [11~14], while the other studies were 9, 4 and 2 fold respectively[15, 16]. However the recent reports seemed to be contrary to the previous results. Richard B, *et al* showed no interaction between genotype and bezidine exposure, and provided the evidence that the NAT2-related slow N acetylation polymorphism was not associated with an increased risk of bladder cancer in worker exposed to benzidine [17]. The results of the research of Orzechowska JK *et al* also hold that there was no relationship between acetylator phenotype and bladder cancer[12, 18]. The differences might be due to different determining procedures, the influence of other medications, different probe drugs or ethnic origin and different disease conditions. Therefore, a further study of larger amount of samples on the possible association between polymorphism of N acetylation and susceptibility to bladder carcinogens and on the determination of polymorphic genotype of N-acetyltransferase are required, so that the effect of gene and environment on the occurrence and development of bladder cancer could be established.

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咖啡因代谢探针研究乙酰化代谢表型与膀胱癌关系

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目的　以咖啡因为代谢探针，研究膀胱癌发生发展的N-乙酰化代谢表型分布的统计学相关性。方法　根据人尿液中咖啡因代谢物5—乙酰氨基-6-甲酰氨基-3-甲基尿嘧啶（AFMU）和甲磺嘌呤1X）的峰高比值绘制概率分布直方图和概率单位图，寻找区分快慢乙酰化代谢表型的截点，确定人的乙酰化代谢表型分布。结果　健康志愿者和膀胱癌患者概率分布直方图和概率单位图呈明显多态性，截点为1.10，即大于1.10为快乙酰化代谢表型，小于1.10为慢乙酰化代谢表型。健康志愿者中快、慢人乙酰化表型分别为149例（73.7%）和54例（26.3%）。膀胱癌患者分别为36例（53.7%）和31例（46.3），两者存在显著统计学差异（$P<0.01$）。志愿者和膀胱癌患者基因频率分别为0.51和0.68，优势比为2.376（95%可信区间为1.3513和4.1776）。结论　中国人乙酰化代谢表型分布呈多型性。慢乙酰化代谢表型个体可能为膀胱癌多发和易感人群。

关键词　乙酰化代谢多态性　膀胱癌　咖啡因　表型