occupationally exposed to BPA (20). Thus, our present values are similar to those obtained in other studies in Japan but not as high as those in Korean subjects.

In addition to BPA concentrations in spot urine, daily urinary BPA excretion rates based on 24-hour urine sampling has been measured (10, 16). Of interest, the rates of daily urinary BPA excretion are in direct accord with estimated values for daily BPA intake. The following values are also consistent with the daily BPA intake observed here (Table 1). The daily urinary excretion rates of BPA based on 24-hour urine samples collected from 36 male university students (mean age, 24.7 years) in 2003 ranged from <0.003 to 0.23 (median, 0.02) μg/kg body weight/day (10). Another study in which values were determined from whole-day urine samples from 11 male and 11 female volunteers showed daily urinary BPA excretion rates ranging from 0.48 to 4.5 (average, 1.68) μg/day (16). Furthermore, the observed range of estimated daily BPA intake (Table 1) was much lower than the tolerable daily intake of 50 μg/kg body weight/day established by the European Food Safety Authority in 2007 (21).

A change in BPA exposure over time has also been reported. Matsumoto et al. (22) measured BPA concentration in urine samples collected from university students in Japan and showed that the median of total BPA concentrations in 1992 was 2.2-fold higher than that in 1999. Furthermore, daily dietary BPA intake rates determined by measuring BPA concentrations in hospital-meal samples in Japan were in the range of 0.15–1.34 (average, 0.64) μg/day in 2000 but were only in the range of 0.06–0.68 (average, 0.20) μg/day in 2001 (23). Given the frequent detection of BPA in canned foods and retort foods (24), these reductions may have partly resulted from changes in the protective coating of epoxy on food and beverage cans (3). This finding may be useful in interpreting the meaning of urinary BPA levels in respective studies.

In addition to our exposure survey, we also explored whether urinary BPA concentration is associated with endometriosis in infertile Japanese women. Analysis of these data showed the absence of association. Although the pathogenesis of endometriosis is poorly understood, epidemiological evidence has suggested its association with several estrogen-dependent factors, namely early menarche, shorter menstrual cycle length, and lower parity (25–27). Based on this, endometriosis probably represents a sensitive detector of the effect of xenoestrogens in humans. However, two recent human experimental studies showing that orally administered BPA is quickly recovered in urine suggest that accurate exposure assessment based on urinary excretion is problematic, because it likely reflects recent rather than cumulative or long-term exposure (16, 17). The probable importance of chronic over recent exposure may partly explain our finding of the lack of association between BPA exposure and endometriosis, and future investigation should focus on the measurement of cumulative exposure to BPA.

Several other suggestions to improve studies of the association between BPA exposure and endometriosis can be made. In addition to accounting for cumulative exposure, investigation would be facilitated by a better study design, such as prospective cohort rather than cross-sectional studies. Measurement of free urinary BPA would be informative, providing its accuracy is confirmed, because BPA glucuronide shows no significant estrogenic activity, *in vitro* at least (2). Furthermore, a previous prospective cohort study showing that the risk of endometriosis is associated with exposure to diethylstilbestrol *in utero* indicates that early-life exposure to BPA, including fetal exposure, might also be critical to the development of endometriosis (28). Additionally, measurement of endogenous estrogen level would allow evaluation of the interaction between serum endogenous estrogens and BPA; this was not possible in the present study because individual endogenous estrogen level in individual premenopausal women periodically fluctuates in accordance with their menstrual cycle.

At a more basic concentration, studies of the effect of BPA on animals have also been inconclusive. BPA showed estrogenic activity generally 10^{-5}–10^{-3} times that of 17β-estradiol in a MCF-7 cell assay in a human breast cancer cell line (2). Furthermore, oral administration of BPA at 200 mg/kg body weight/day to a strain of immature rats resulted in an increase in uterine weight (2). In contrast, BPA showed no clear endocrine disrupting effect on rodents at estimated human exposure doses (5). Moreover, experimental results for BPA have been shown to depend on a number of factors, namely measurement endpoint, cell line, animal species and strain, and conjugate form and dose (2).

The strength of our study includes the use of a reliable biomarker measurement method and detailed information on subjects. In particular, we employed the most reliable analytical method currently available, namely, enzymatic deconjugation of both BPA sulfate and glucuronide and isotope-dilution LC-MS/MS measurement. Moreover, to monitor and control sample contamination, method blank tests were conducted in parallel with unknown sample analysis for all measurements, in addition to the rinsing of glassware and plastic tubing with methanol or acetonitrile before each test.

Two limitations of our study warrant mention. First, our subjects were restricted to urban residents of reproductive age, as well as other characteristics; thus, the generalizability of our results may also be limited. Second, intraindividual variation in BPA exposure and uncertainty in laboratory analysis may have contributed to urinary BPA concentration measurement errors. One study in which between-day variation in daily urinary BPA excretion was examined suggested that the magnitude of intraindividual variation is comparable to that in interindividual variation (10). If present, however, such errors would at least not tend to bias our results toward one side; thus, the median or geometric mean urinary BPA concentration would have been properly estimated. In contrast, interindividual variation, such as geometric standard deviation, might have been overestimated and thus remain to be corrected.

In conclusion, we report urinary concentrations of BPA in Japanese women who consulted a physician for infertility. Values were derived from the largest subject sample size studied in Japan to date and were obtained using the most reliable analytical method. Results showed that urinary BPA concentrations in these women were not higher than those in other populations. Moreover, this cross-sectional study revealed no association between urinary BPA concentration and endometriosis. Further study is required to confirm this result.

Appendix: Urinary BPA analysis

In the present study, we measured urinary bisphenol A (BPA) concentration. Here, we detail the methods used to measure urinary BPA concentration.

We analyzed urine samples without information on the participant's endometriosis status. Urinary BPA was separated, detected and measured using enzymatic deconjugation, offline solid-phase extraction, and high-performance liquid chromatography isotope-dilution tandem mass spectrometry (isotope-dilution LC-MS/MS). In addition to urinary BPA glucuronide, the recently identified compound urinary BPA sulfate has also been included in urinary BPA analysis in recent years (6–8, 29, 30). To deconjugate these BPAs into their free forms, we used β-glucuronidase/sulfatase (from *Helix pomatia* H-1, 492,000 units/g solid, Sigma Aldrich, St. Louis, MO, USA), a hydrolytic enzyme with sulfatase activity, as described in most recent studies (7, 29).

We purchased the native BPA standard (99.8%, for environmental analysis) and isotopically labeled standard (BPA-d_{16}, 99.9%, for environmental analysis) from Kanto Chemical Co., Inc. (Tokyo, Japan). Methanol (≥99.8%, residual-pesticide analysis grade 5000) and acetonitrile (≥99.8%, HPLC grade) were purchased from Sigma Aldrich Japan (Tokyo, Japan). Ammonium acetate (≥97.0%), sodium acetate trihydrate (≥99.0%), formic acid (≥98.0%), and acetic acid (≥99.7%) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). We used ultrapure water (Milli-Q Gradient A10, Millipore, Bedford, MA). To prepare 0.1 M acetate buffer, we diluted sodium acetate trihydrate (0.5 mmol) and acetic acid (0.5 mmol) with water to 10 ml. Standard substances were measured by weight and then dissolved in acetonitrile. They were serially diluted with acetonitrile:water=2:3 (v/v) to their respective target concentrations. Except for unused vials, spatula and glassware were previously rinsed with methanol three times and dried.

Frozen urine was thawed at room temperature. A 0.2-mL aliquot was decanted into a 2-mL silanized glass vial. We dissolved β-glucuronidase/sulfatase (0.2 mg) in 0.1 M acetate buffer solution (0.1 mL). This hydrolytic enzyme solution and 20 μL of the surrogate solution (50 μg/L) were added to the vial. This sample was mixed well and then incubated at 37°C overnight, as described in recent studies (7, 29).

A solid-phase extraction (SPE) cartridge (FOCUS Versa-Plate Tube, 10 mg/1.8 mL, Varian, Lake Forest, CA, USA) was preconditioned with 1-mL methanol followed by 1-mL water. After dilution with 1 M ammonium acetate aqueous solution (1 mL), the urine sample was vortex-mixed, and then loaded onto this SPE column. The SPE column was then washed with 1-mL water followed by acetonitrile:water=1:4 (v/v; 1 mL). Analytes were finally eluted from the SPE column using 1-mL solvent (methanol:acetonitorile:water:formic acid=6:3:1:0.01, v/v/v/v). A plastic tube (1.1 mL) into which the SPE eluate was collected was previously washed with acetonitrile. The eluate was dried out using a vacuum centrifuge (100 min) and the residue was reconstituted with acetonitrile:water=2:3 (v/v; 200 μL). The resulting solution was decanted into a silanized-glass insert.

We then measured BPA concentration in the extracted urine sample using a tandem quadrupole mass spectrometer (API 2000, Applied Biosystems, Foster City, CA, USA) connected to a high-performance liquid-chromatography (HPLC) system (LC-10 AD_{VP} pomp, SIL-10 AD_{VP} autosampler, CTO-10AC_{VP} column oven, and SCL-10A_{VP} system controller, Shimadzu, Kyoto, Japan) equipped with a C_{18} column (S-3 μm, 8 nm, 2.0 mm i.d.×100 mm, YMC-pack Pro RS, YMC Co., Ltd., Kyoto, Japan). We used 0.1-mL acetic acid in 1-L water (solvent A) and 0.1-mL acetic acid in 1-L acetonitrile (HPLC grade) (solvent B) as HPLC mobile phases with a constant flow rate (0.2 mL/min). A 20-μl aliquot of the sample was injected into the HPLC column using an auto sampler. The sample vial was cooled at 4.0°C. The percentage of solvent B was elevated from 40% (0 min) to 100% (5 min) for separation, kept at 100% (5–8 min) for flushing, and then kept at 40% (8.01–20 min) for re-equilibrium. The temperature of the HPLC column was kept constant (40.0°C). We used electrospray ionization and multiple-reaction monitoring to produce the combinations of the precursor and product ion of m/z 227.1 and 132.9 for BPA, and m/z 241.2 and 141.8 for BPA-d_{16}, respectively. The ion source temperature and collision energy were 500°C and −30 volts, respectively. MS/MS parameters on API 2000 were automatically optimized using a personal computer-based instrument software program (Analyst version 1.4, Applied Biosystems, Foster, CA, USA), which was also used to acquire and process the data obtained. To obtain a calibration curve (0.6–20 μg/L), each calibration point was weighted by the reciprocal of concentration (1/X), and the origin was ignored. In each analytical batch, one quality control material, all portions of which were obtained from a single sample of pooled urine (2.4 μg/L), and five method blanks were also analyzed along with unknown samples to ensure the accuracy of analysis. Intraday and interday reproducibilities (CV=8.8% and 19%, n=5, respectively) were previously checked by repeated measurements. In the batches where no method blank was observed, the standard deviation derived from six repeated measurements of a low-concentration standard solution (1 μg/L) was employed as the standard deviation at concentration zero (S_0). In the batch where method blanks were observed, S_0 was calculated from the standard deviation of the method blanks. We defined $3S_0$ as the analytical limits of detection (LODs), which were 0.30–0.55 μg/L. Mean total surrogate recovery was 72% in one batch (n=5). Any loss of BPA through analysis was automatically corrected on the basis of individual surrogate recovery. The mean of the method blank values was subtracted from the BPA measurement value in each batch.

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[Review]

Molecular Epidemiology and Urothelial Cancer

Hiromasa TSUKINO[1], Hisamitsu OMORI[2], Kiyotaka KOHSHI[3] Yuko YAMANO[4] and Takahiko KATOH[5]

[1] *Department of Urology, Faculty of Medicine, University of Miyazaki, Miyazaki 889-1692, Japan*

[2] *Japanese Red Cross Kumamoto Health Care Center, Kumamoto 862-8528, Japan*

[3] *Division of Hyperbaric Medicine and Department of Neurosurgery, University of Occupational and Environmental Health, Kitakyushu 807-8555, Japan*

[4] *Department of Hygiene and Preventive Medicine, Showa University, School of Medicine, Tokyo 142-8555, Japan*

[5] *Department of Public Health, Graduate School of Medical Sciences, Kumamoto University, Kumamoto 860-8556, Japan*

Abstract : Tobacco smoking is the main cause of human urothelial cancer. It has been suggested that genetic susceptibility may contribute to the risk, because only a small portion of smokers develops urothelial cancer. Tobacco smoke contains many carcinogens which are activated or detoxified by phase-I or phase-II enzymes. The concentration of the ultimate carcinogen, which will react with DNA, is determined by the rate of activation and detoxification. Individuals with an increased rate of activation or a decreased rate of detoxification have a slightly higher level of bulky carcinogen-DNA adduct in the urothelial mucosa. Thus metabolic polymorphisms have been recognized as important determinants of carcinogen susceptibility, and recent efforts have shown that inter-individual differences in specific cytochrome P450 enzymes (CYPs), N-acetyltransferases (NAT), glutathione S-transferases (GST) and sulfotransferases (SULT) are often disproportionately represented in epidemiological studies between urothelial cancer cases and controls. It has been revealed that GSTM1 null genotype or NAT2 slow acetylator genotype may be associated with a small increase in urothelial cancer risk. Associations between other polymorphisms of metabolic enzymes and urothelial cancer are not well-known or are inconsistent. To reveal these associations, further well-designed and large-scale studies are needed.

Key words : molecular epidemiology, bladder cancer, cytochrome P450, glutathione S-transferase, N-acetyltransferase.

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All correspondence abbressed to: Takahiko Katoh, Department of Public Health, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan. 1-1-1 Honjou, Kumamoto City, Kumamoto 860-8556 Japan.

Tel: 096-373-5112, Fax: 096-373-5113, E-mail: katoht@gpo.kumamoto-u.ac.jp

Introduction

Many diseases are affected by both host factors and the external environment. In the case of urothelial cancer, the process of urothelial carcinogenesis is characterized by a diversity of risk factors. However, transitional cell urothelial cancer is the malignancy most causally linked to the process of chemical carcinogenesis. Extensive epidemiological studies have reported that 60−70% of bladder cancer cases can be attributed to exposure to certain chemical carcinogens [1, 2], the main sources of which are smoking and hazardous occupational exposure [2]. Tobacco smoke in particular is estimated to be responsible for 40−50% and 30% of all bladder cancer cases among males and females, respectively [1, 3, 4], whereas occupational exposure to chemicals, the oldest known causal factor in the development of bladder cancer, is now considered to be responsible for 19% of male and 6% of female cases [1, 3, 5]. There are 55 carcinogens in cigarette smoke that have been evaluated by the International Agency for Research on Cancer (IARC) and for which there is "sufficient evidence for carcinogenicity" in either laboratory animals or humans [6]. Polycyclic aromatic hydrocarbons (PAHs), aromatic amines, heterocyclic aromatic amines and *N*-nitroso compounds in tobacco smoke are thought to be urothelial carcinogens. However, many such compounds are not themselves carcinogenic but require activation by xenobiotic metabolizing enzymes. Many compounds are converted to reactive electrophilic metabolites by the oxidative (phase-I) enzymes, which are mainly cytochrome P450 enzymes (CYPs). Phase-II conjugating enzymes, such as glutathione *S*-transferases (GST), UDP-glucuronosyltransferases, sulfotransferases (SULT) and *N*-acetyltransferases (NAT), usually act as inactivation enzymes. Thus, the concerted action of these enzymes may be crucial in determining the final biological effects of a xenobiotic chemical. A number of genes that encode carcinogen-metabolizing enzymes are presently known. Individual variation in enzymes activating or detoxifying carcinogens and other xenobiotics have subsequently been related to discovered genetic polymorphisms in these genes. Epidemiological studies may contribute to our understanding of and may quantify the impact of xenobiotic metabolism on carcinogenesis in humans. This type of analysis is especially suitable for those enzymes that are polymorphically expressed on a heritable basis, enabling a comparison of cancer incidence among subjects with genetically deficient or extremely active metabolism to incidence among those with normal activity. Many molecular epidemiological studies showing an association between enzymatic polymorphisms and urothelial cancer susceptibility have been reported. Some enzymatic polymorphisms were associated with urothelial cancer susceptibility, but others were not associated with urothelial cancer susceptibility. There are reports with some significant association between enzymatic polymorphisms and urothelial cancer susceptibility related to cigarette smoking.

CYPs polymorphisms

1. *CYP1A1*

CYP1A1 is expressed in the lung, larynx, kidney, placenta, lymphocytes and fetal liver [7−9]. Substrates for and inducers of CYP1A1 include PAHs such as benzo[a]pyrene. PAHs

have been known as urothelial carcinogens from epidemiological and animal studies [10]. The CYP1A1-dependent phenotype has been determined through assay of the aryl hydrocarbon hydroxylase (AHH) metabolism of benzo[a]pyrene in human-derived tissues, usually peripheral blood lymphocytes [11]. Korsggard *et al.* [12] reported an association between AHH inducibility and urothelial cancer, and suggested that the role of AHH in urothelial carcinogenesis seemed to be less explicit (Table 1). Two genetic polymorphisms of the *CYP1A1* gene are thought to be associated with the large inter-individual differences in AHH enzyme activity. The *CYP1A1* Ile462Val polymorphism is a result of A (*CYP1A1*1A*) to G (*CYP1A1*2C*) substitution in exon 7, causing an amino acid change in the heme-binding region [13]. It has been reported that the Val/Val genotype resulted in a reduced catalytic enzyme activity [14]. The frequency of *CYP1A1* Val/Val genotype was reported to be 4.7－5.0% in Japanese populations and very rare in European populations [15]. The other *CYP1A1* polymorphism is a T (*CYP1A1**1A) to C (*CYP1A1**2A) transition 1197 bp downstream of exon 7, the MspI variant allele [16]. The MspI polymorphism can be classified into 3 genotypes: predominant homozygous alleles (genotype A), heterozygote (genotype B) and homozygous rare alleles (genotype C) [17]. The genotype C is closely related to high inducible CYP1A1 phenotypic activity [14]. Individuals with genotype C are most common among Chinese and Japanese (10%), and least common among Caucasians (0－4%), with African-Americans and Koreans (5－6%) [18]. The MspI polymorphism is closely linked to the *CYP1A1* Ile462Val polymorphism not only in a Japanese population but also in Northern Europeans.

There are two reports that showed a negative association between CYP1A1 genetic polymorphisms and urothelial cancer (Table 1) [19, 20]. Katoh *et al.* [19] reported that the frequency distribution of the *CYP1A1* Ile462Val genotypes in urothelial cancer patients showed no significant difference from that in healthy controls among Japanese populations. Brockmoller *et al.* [20] reported that polymorphisms in *CYP1A1* Ile462Val or MspI had no statistically significant impact in a German population. As the *CYP1A1* Val/Val genotype and genotype C are common among Asian populations but very rare among Europeans, the association between *CYP1A1* polymorphisms and urothelial cancer is interesting in Asian populations [21].

2. *CYP1A2*

CYP1A2 is involved in the metabolism of arylamines including 4-aminobiphenyl (ABP), nitrosamines and aromatic hydrocarbons, and the dehalogenation of chlorinated hydrocarbons. In humans, CYP1A2 has been detected only in the liver. A wide variation across racial/ethnic groups is one factor that may influence the results on the phenotypic distribution of slow, intermediate and rapid metabolizers of CYP1A2, as well as on the inducibility of this gene. Overall, slow CYP1A2 metabolizers are represented in about 10% of Caucasians, while their frequency in Japanese people seems to be much lower [22]. Evidence linking elevated CYP1A2 activity to increased bladder cancer risk has been reported [23, 24]. Lee *et al.* [24] reported the capacity for 3-demethylation of theophylline, as a reflection of CYP1A2 activity, was significantly associated with increased risk of non-occupational urinary bladder cancer (P=0.006). Moreover, molecular dosimetry studies indicate that the slow NAT2/rapid

Table 1. Previous reports of association between CYPs polymorphisms and urothelial cancer

Enzyme	Author, year of publication (reference number)	Population	Number of cases[1]	Number of controls	Phenotyping and genotyping (drug used for phenotyping or mutant allele)	Crude OR or result-urothelial cancer OR (95%CI)
CYP1A1	Korsgaard *et al*, 1984(12)	Swedish	76[2]	92	Phenotyping (3-methylcholanthrene)	1.42 (0.40−4.99) (renal pelvis and ureter) 1.38 (0.46−4.15) (bladder) (high/low+intermediate AHH ratio)
	Katoh *et al*, 1995(19)	Japanese	83[3]	101	Genotyping (*CYP1A1*2C*)	0.86 (0.47−1.54) (WM+MM/WW) 0.72 (0.17−3.11) (MM/WM+WW)
	Brockmoller *et al*, 1996(20)	German	374	373	Genotyping (*CYP1A1*2A,*2C*)	0.92 (0.61−1.41) (CYP1A1*2A WM+MM/WW) 0.67 (0.33−1.39) (CYP1A1*2C WM+MM/WW)
	Bringuier *et al*, 1998(21)	Australian	105[4]	0	Genotyping (*CYP1A1*2C*)	No association between p53 mutation and CYP1A1 polymorphism among cancer cases
CYP1A2	Lee *et al*, 1994(24)	Korean	100	84	Phenotyping (Theophylline)	A significant association between high CYP1A2 activity and bladder cancer risk(P=0.006)
CYP2A6	Tsukino *et al*, 2002(42)	Japanese	137[5]	92	Genotyping (*CYP2A6* deletion)	0.90 (0.26−3.14) (MM/WM+WW)
CYP2C19	Kaisary *et al*, 1987(44)	British	98	110	Phenotyping (Mephenytoin)	Not significant, but a weak association between non-aggressive bladder cancer and high CYP2C19 activity(P=0.04).
	Brockmoller *et al*, 1996(20)	German	374	373	Genotyping (*CYP2C19*2A*)	2.7 (0.9−7.7) (WM+WW/MM)
CYP2D6	Cartwright *et al*, 1984(49)	British	122	94	Phenotyping (Debrisoquine)	1.30 (0.09−18.28) (EM/PM) 0 WM or MM, P=0.04 among cases with past benzidine exposure (EM/PM)
	Kaisary *et al*, 1987(44)	British	98	110	Phenotyping (Debrisoquine)	Significant higher frequency of EM in aggressive cases(P=0.006), but not in non-aggressive cases
	Horai *et al*, 1989(50)	Japanese	51	203	Phenotyping (Metoprolol)	No significant association of WW status and cancer(P=1.0) No significantly different CYP2D6 frequency by gender, age, or tumor grade (EM/PM)
	Benites *et al*, 1990(51)	Spanish	125	556	Phenotyping (Debrisoquine)	2.6 (0.80−8.70) (EM/PM)
	Wolf *et al*, 1992(56)	British	184	720	Genotyping (*CYP2D6*4A*)	0.99 (0.45−2.19) (WW+WM/MM) 0.60 (0.43−0.83) (WW/WM+MM)
	Lee *et al*, 1994(24)	Korean	100	84	Phenotyping (Metoprolol/ α-hydroxymetoprolol)	0.59 (0.05−6.50) (EM/PM)
	Spurr *et al*, 1995(54)	British	126	132	Genotyping (*CYP2D6*4A*)	0.61 (0.22−1.68) (WW+WM/MM)
	Brockmoller *et al*, 1996(20)	German	374	373	Genotyping (*CYP2D6*2A*)	1.05 (0.57−1.95) (WW+WM/MM) 0.98 (0.74−1.31) (WW/WM+MM)
	Anwar *et al*, 1996(55)	Egyptian	22	21	Genotyping (*CYP2D6*3A, CYP2D6*4A, CYP2D6*5*)	2.36 (0.68−9.90) (WW+WM/MM)
	Chinegwundoh *et al*, 1996(57)	British	126	0	Genotyping (*CYP2D6*4A*)	No significantly different CYP2D6 frequency in smokers compared to nonsmokers(P=0.92).
CYP2E1	Anwar *et al*, 1996(55)	Egyptian	22	21	Genotyping (*CYP2E1*5B*)	Not significant(P=0.48)(0 MM or WM genotype among cases)
	Brockmoller *et al*, 1996(20)	German	374	373	Genotyping (*CYP2E1*1B,*5A,B*)	0.76 (0.54−1.08) (CYP2E1*1B WM+MM/WW) 1.16 (0.73−1.82) (CYP2E1*5A WM+MM/WW) 0.54 (0.27−1.08) (CYP2E1*5B WM+MM/WW)
	Farker *et al*, 1998(61)	German	273[6]	298	Genotyping (*CYP2E1*5A,B*)	1.14 (0.71−1.83) (CYP2E1*5A WM+MM/WW) 1.35 (0.65−2.78) (CYP2E1*5B WM+MM/WW)
	Farker *et al*, 2000(62)	German	158[6]	150	Genotyping (*CYP2E1*2*)	No variant allele among case and control patients
	Tsukino *et al*, 2002(42)	Japanese	137[5]	92	Genotyping(*CYP2E1*5B*)	0.67 (0.43−1.05) (WM+MM/WW)

[1]Cases were bladder cancer patients, [2]Cases were 46 bladder cancer and 30 renal pelvis cancer patients, [3]Cases were 65 bladder cancer,12 renal previs cancer and 6 ureter cancer patients, [4]Cases were renal pelvic cancer patients, [5]Cases were 95 bladder,14 renal pelvis, 16 ureter and 12 overlapping cancer patients, [6]Cases were renal and urothelial cancer patients

CYP1A2 individual has the highest level of ABP-hemoglobin adducts and, conversely, the lowest level of ABP-hemoglobin adducts, as observed in individuals who are rapid NAT2/slow CYP1A2 [23, 25]. Thus, those individuals who are rapid for CYP1A2 and slow for hepatic NAT2 might be at a higher risk for arylamine-induced urothelial cancer, compared with those who are slow for CYP1A2 and rapid for NAT2 [26]. Recently, several polymorphisms of *CYP1A2* have been reported [27−30], and two variant alleles which affect CYP1A2 activity were reported. One variant allele was a point mutation from guanine (*CYP1A2*1A*) to adenine (*CYP1A2*1C*) at position −2964 in the gene, which caused a significant decrease in CYP1A2 activity [28]. Another variant allele, a C to A transversion (*CYP1A2*1F*) in intron 1 at position 734 downstream of the first transcribed nucleotide, has recently been associated with increased CYP1A2 inducibility [29]. The outcome of these polymorphisms on susceptibility to urothelial cancer is not known.

3. *CYP2A6*

CYP2A6 is a constitutive cytochrome P450 which is expressed in human liver at variable levels [31]. This enzyme may also be expressed in other tissues, including nasal tissue, but not in the kidney, duodenum, lung, alveolar macrophages, peripheral lymphocytes, placenta or uterine endometrium [32]. CYP2A6 catalyzes the metabolic activation of several pre-carcinogens, including several nitrosamines, aflatoxin B1 and 1,3-butadiene. In addition, CYP2A6 is involved in the metabolism of nicotine, the primary compound in tobacco that establishes and maintains tobacco dependence [33, 34]. Individual variation in the activity of this enzyme has been suggested to be linked to differential smoking behaviours [35]. A large inter-individual difference in CYP2A6-mediated coumarin 7-hydroxylase activity [36, 37] suggested the existence of a genetic polymorphism of this enzyme. Several variants of the *CYP2A6* gene have been identified, and recently, several deletion-type alleles of the *CYP2A6* (*CYP2A6*4*) have been identified [38-41]. The homozygous deletion *CYP2A6* frequency of 3.2−4.0% was found only in Oriental populations [42]. The homozygous deletion *CYP2A6* genotype causes complete lack of enzymatic activity. Seventy to eighty percent of nicotine is converted to cotinine, mainly by CYP2A6, and the proportion of urinary cotinine excretion in the individuals with the homozygous deletion of *CYP2A6* was about one-seventh compared to the *CYP2A6* wild types [41]. It has been hypothesized that a lack of CYP2A6 activity might decrease the production of genotoxic metabolites of these nitrosamines and potentially reduces the risk of tobacco-smoking related cancer by this mechanism. Our study revealed the frequency of the homozygous deletion of *CYP2A6* genotype was 2.9% (4/137) in the urothelial patients, compared with 3.2% (7/210) in the controls (OR=0.84, 95% CI=0.24−2.96) [42]. However, the sample size of our study was small, and future research is needed to establish a significant relationship.

4. *CYP2C19*

The human CYP2Cs metabolizes approximately 20% of clinically used drugs, and there are four members in the subfamily: CYP2C8, CYP2C9, CYP2C19 and CYP2C18. The most well-known of CYP2Cs' polymorphism is in CYP2C19. Several defective CYP2C19 alleles are the

basis for the (S)-mephenytoin 4'-hydroxylase polymorphism in human populations. Poor metabolizers (PMs) of CYP2C19 represent approximately 3−5% of Caucasians, a similar percentage of African-Americans and 18−23% of Asians [43]. Kaisary *et al.* [44] reported an association between bladder cancer and CYP2C19 phenotype by using measurement of S-mephenytoin hydroxylation. CYP2C19 phenotype was not associated with increased risk of bladder cancer, but a weak association was found between non-aggressive bladder cancer and high CYP2C19 activity. The most common variant allele, *CYP2C19*2A*, has an aberrant splice site in exon 5 [45]. There is one report about a negative association between *CYP2C19*2A* polymorphism and bladder cancer risk [20]. Despite the wide range of substrate specificity and abundance in the liver, the CYP2C enzymes do not seem to have a significant role in carcinogen metabolism. The possibility should not, however, be ruled out.

5. *CYP2D6*

CYP2D6 is expressed in the liver, gut and brain neurons [46]. CYP2D6 metabolizes several important clinically used drugs [47], but there is little evidence of it having a role in carcinogen activation. CYP2D6 is suspected to be involved in the activation of tobacco-specific nitrosamines, such as 4-(metylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) [48]. With debrisoquine as a substrate, a high inter-individual variability in CYP2D6 activity has been observed *in vivo*. Inter-individual differences in the metabolic capacity of the CYP2D6 may be expected to be a key factor in susceptibility to developing urothelial cancer where environmental chemicals are implicated. A genetic polymorphism at CYP2D6 debrisoquine hydroxylase gene locus affects 5−10% of the Caucasian population and < 1% of the Chinese or Japanese population, and is responsible for the compromised metabolism (poor metabolizer phenotype). There are some reports that investigated the association between CYP2D6 phenotype and urothelial cancer (Table 1). Three papers reported that there was no difference in the distribution of oxidative polymorphism of debrisoquine [24, 49, 50]. Kaisary *et al.* [44] detected a higher oxidative rate of debrisoquine in patients with aggressive urothelioma than in those with a less undifferentiated disease. Benitez *et al.* [51] reported that the distribution of frequencies of metabolic ratio values tend to have lower values in the patients ($P<0.05$), and patients with a high occupational risk for urothelioma had lower metabolic ratio values ($P=0.03$).

The *CYP2D6* gene is located on chromosome 22q13.1. A number of alleles have now been characterized at the *CYP2D6* locus. Inactivating mutations at the *CYP2D6* gene are *CYP2D6*3* (deletion of A2549 in exon 5), *CYP2D6*4* (G1864A at splicing site), *CYP2D6*5* (complete deletion of the wild-type allele *CYP2D6*1*), *CYP2D6*6A* (deletion of T1707 in exon 3) and *CYP2D6*11, *12, *13, *14, *15, *16.* The *CYP2D6*3*, *CYP2D6*4* and *CYP2D6*5* alleles account for the majority (greater than 90%) of the poor metabolizer phenotype [52]. The phenotype-genotype concordance, which predicts the metabolic phenotype by genetic analysis, was found to be between 93.4% and 100% [53]. Three reports showed a negative association between CYP2D6 genetic polymorphism and urothelial cancer [20, 54, 55], but one reported that a significant increase in the proportion of poor metabolizers or heterozygotes was seen in

urothelial cancer patients [56]. Chinegwundoh *et al.* [57] found that there was a trend for those heterozygous at the *CYP2D6* locus and with a history of smoking to develop more aggressive diseases, but this trend did not reach statistical significance.

Among control groups, the frequency of the CYP2D6 poor metabolizers has been assumed in some ethnic groups (Table 1). The frequency of the CYP2D6 poor metabolizers are 0.5－1.2% in Asian populations and 2.1－6.1% in European populations. The meta-analysis of two studies in Asian populations (151 cases, 287 controls) and six studies in European populations (1057 cases, 1875 controls) was conducted [20, 24, 49－51, 54, 56, 57]. The summary odds ratio(OR) for the CYP2D6 poor metabolizers was 1.91 (95%Confidence interval(CI)=0.28－13.30) in Asians and 1.06 (95%CI=0.76－1.49) in Europeans. To reveal these associations relating to tobacco smoking, future research is needed.

6. *CYP2E1*

CYP2E1 is an ethanol inducible enzyme, known to be involved in the metabolic activation of several organic compounds with low molecular weight, including *N*-nitrosamines found in tobacco smoke. The CYP2E1 is expressed at high levels in the liver and at lower levels in several extra-hepatic tissues. Wide inter-individual variation in the expression of the *CYP2E1* gene has been reported in humans, which is possibly attributable to gene-environment interaction. Significant inter-ethnic differences exist in *CYP2E1* polymorphism, but there is no clear evidence that any of these polymorphisms are related to altered function *in vivo*. All polymorphisms reported in the literature are located in the non-coding regions of *CYP2E1*, while the coding regions of *CYP2E1* seem to be well conserved among different ethnic groups and species [58]. Restricton fragment length polymorphisms (RFLPs) have been detected for TaqI (intron 7; *CYP2E1*1B*)[58], RsaI (intron 5)[60], DraI (intron 6; *CYP2E1*6*) [59]. Interestingly, PstI and RsaI RFLPs identify two further variant sequences in the 5' untranslated region (*CYP2E1*5A* and *CYP2E1*5B*), and, furthermore, the RsaI RFLP has been associated with the alteration in the transcriptional activation of the gene. There are some reports that investigated the association between *CYP2E1* polymorphisms and urothelial cancer risk, but none of them reported significant associations (Table 1) [20, 42, 55, 61, 62].

GSTs polymorphism

The GSTs are a family of enzymes, which catalyze the conjugation of a wide variety of xenobiotics, including environmental carcinogens, with glutathione. Although the vast majority of GST conjugates represents detoxification products, several instances exist where GST activity does not result in the detoxification, but rather activation. In humans, there are four main classes of α (glutathione S-transferase-α), μ (glutathione S-transferase-μ), π (glutathione S-transferase-π), θ (glutathione S-transferase-θ), each of which contains 1 or more of the homodimeric or heterodimeric isoforms, glutathione S-transferase-α 1-1 (GSTA1-1), GSTA1-2 and so forth [63, 64]. Genetic polymorphisms have been reported for *GSTM1*, *GSTT1* and *GSTP1*, resulting in either decreased or altered enzyme activity. Because

of their detoxification role, these polymorphisms may play an important role in urothelial cancer susceptibility.

1. *GSTM1*

Five μ class genes (M1－M5) situated in tandem on chromosome 1p13 have been identified. *GSTM1* is expressed in human liver, stomach, brain and other tissues, while GSTM2－M5 subunits have been detected in extrahepatic tissues. *GSTM1* enzyme has received considerable attention in relation to urothelial cancer because of its role in the detoxification of benzo[a]pyrene and other polycyclic aromatic hydrocarbons found in tobacco smoke. Three polymorphisms of the *GSTM1* gene, namely *GSTM1*0*, *GSTM1*A* and *GSTM1*B*, have been identified. *GSTM1*0* is a deleted allele, and the homozygotes allele (*GSTM1* null genotype) express no *GSTM1* protein [65]. *GSTM1*A* and *GSTM1*B* differ by only a single base in exon 7, however, there is no evidence of a functional difference between *GSTM1*A* and *GSTM1*B*, and the two are typically categorized together as a single functional phenotype. Most studies of *GSTM1* polymorphism and cancer have compared the homozygous deletion genotype with the genotypes containing at least one functional allele. The genotype with the homozygous deletion of the *GSTM1* gene is called "*GSTM1*-null", whereas the genotype having at least one copy of the gene is called "*GSTM1*-positive". *GSTM1* null genotype was shown to occur in approximately 50% of the population of various ethnic origins among controls (Table 2).

Many studies investigated the association between *GSTM1* polymorphism and urothelial cancer (Table 2) [20, 21, 55, 66－88]. Most studies recognized an increased risk associated with a lack of *GSTM1* activity. Recently, two meta-analyses of published studies have been reported [89, 90]. Johns *et al.* [89] reported that a meta-analysis of 15 case-control studies had been carried out using a random effects model, and the OR for risk of bladder cancer risk associated with *GSTM1* deficiency was 1.53 (95%CI=1.28－1.84). Engel *et al.* [90] performed meta- and pooled analyses of published and unpublished, case-control, genotype-based studies that examined associations between *GSTM1* polymorphism and bladder cancer (17 studies, 2149 cases, 3646 controls). They excluded studies conducted in populations with a high prevalence of exposure to known bladder cancer risk factors other than tobacco smoke. Using a random effects model in the meta-analysis, they obtained a summary OR of 1.44 (95%CI= 1.23－1.68) for *GSTM1* null status with all studies included. Studies conducted in Asia generated a summary OR of 1.73 (95% CI=1.66－1.81); in Europe, a summary OR of 1.39 (95% CI= 1.09－1.77); and in the USA, a summary OR of 1.44 (95% CI=1.38－1.50). Pooled analyses using original data sets from 10 studies (1496 cases, 1444 controls) and adjusted for age, sex and race produced similar results. There was no evidence of multiplicative interaction between the *GSTM1* null genotype and ever smoking in relation to bladder cancer, although there was a suggestion of addictive interaction (addictive interaction=0.45, 95%CI=0.03－ 0.93).

2. *GSTT1*

To our knowledge, there are 14 reports investigating the associations between *GSTT1*

polymorphism and urothelial cancer (Table 2) [20, 21, 75−81, 83−85, 87, 88]. Among control groups, the frequency of the null genotype has been assumed in some ethnic groups. The frequency of null genotype is highest among Asian populations (46−52%) (Table 2). Among European populations, the frequency was measured to range from 11 to 22% (Table 2).

We reviewed 12 case-control studies that investigated associations between *GSTT1* polymorphisms and urothelial cancer (Table 2) [20, 75−80, 83−85, 87, 88]. Ten case-control studies reported no associations between *GSTT1* null genotype and urothelial cancer risks [20, 75, 77, 79, 80, 83−85, 87, 88]. However, two of those reported that the risk of bladder cancer with *GSTT1* null genotype was significantly higher among non-smokers [20, 75]. Two studies reported significant associations between *GSTT1* null genotype and urothelial cancer risk [76, 78]. Furthermore, individuals with the null genotype for both *GSTM1* and *GSTT1* were at a significantly higher risk for developing bladder cancer than individuals with both genes present.

A meta-analysis of three studies in Asian populations (489 cases, 530 controls) and seven studies in European populations (1034 cases, 1094 controls) was conducted (20, 75, 77, 79, 80, 81, 83−85, 88). The summary OR for the *GSTT1* null type was 1.06 (95%CI=0.83−1.35) in Asians and 0.82 (95%CI=0.65−1.03) in Europeans. These results suggested that *GSTT1* null type might be a reduced risk for urothelial cancer in Europeans, but not in Asians.

3. *GSTP1*

There are five case-control studies that investigated the association between *GSTP1* polymorphisms and urothelial cancer [83, 85, 87, 91, 92]. Three of these [83, 87, 91] reported that *GSTP1*a/b or b/b genotypes might be associated with an increase in bladder cancer risk among British, Italian and Turkish populations. Furthermore, the risk for *GSTP1*a/b or b/b genotypes with bladder cancer was elevated in individuals with the combination of cigarette smoking and *GSTM1* null genotype [87]. Two papers reported that no significant increase in the frequency of the *GSTP1*b allele was found in tumor patients among Japanese and Germans [85, 92].

NATs polymorphism

Although N-hydroxy arylamines can react with DNA at acidic urinary pH, further activation by NAT1 in the urothelial has been suggested as a final activation step leading to DNA adducts, mutations and neoplasia [93]. This metabolic pathway led the hypothesis that the NAT2 slow activity type and NAT1 high activity type were risk factors for urothelial cancer among smoking groups. A number of reports supported this hypothesis, but some did not support it.

1. *NAT1*

A significant association between urothelial cancer and smokers possessing the *NAT1*10* allele was reported in two studies [94, 95], but not in two others (Table 3) [96, 97]. In the two former studies, the association was highest among smokers who possessed both *NAT1*10*

Table 2. Previous reports of association between GSTs polymorphisms and urothelial cancer

Genotype	Author, year of publication (reference number)	Population	Number of cases[1]	Number of controls	Analysis methods	Frequencies of variant genotype cases/controls(%)	Crude OR or results OR (95%CI)
GSTM1 deletion polymorphism	Zhong *et al*, 1993(66)	British	97	225	Southern blotting	40/42	0.9 (0.6−1.5)
	Daly *et al*, 1993(67)	British	53	52	PCR	85/60	3.8 (1.5−9.5)
	Lafuente *et al*, 1993(68)	Spanish	75	75	ELISA	67/45	2.4 (1.2−4.7)
	Bell *et al*, 1993(69)	American	229	211	PCR	60/47	1.7 (1.2−2.5)
	Lin *et al*, 1994(70)	American	114	1104	PCR	57/49	1.4 (0.9−2.0)
	Brockmoller *et al*, 1994(71)	German	296	400	ELISA, PCR	59/51	1.4 (1.0−1.9)
	Rothman *et al*, 1996(72)	Chinese	38	43	PCR	61/61	1.0 (0.4−2.5)
	Lafuente *et al*, 1996(73)	Egyptian	80	70	ELISA	59/51	1.3 (0.7−2.6)
	Brockmoller *et al*, 1996(20)	German	374	373	PCR	58/52	1.3 (1.0−1.8)
	Okkels *et al*, 1996(74)	Danish	234	202	PCR	57/50	1.3 (0.9−2.0)
	Kempkes *et al*, 1996(75)	German	113	170	PCR	68/54	1.8 (1.1−3.0)
	Anwar *et al*, 1996(55)	Egypitan	22	21	PCR	86/48	7.0 (1.7−28.8)
	Abdel-Rahman *et al*, 1998 (76)	Egyptian	37	34	PCR	70/44	3.0 (1.1−7.9)
	Bringuier *et al*, 1998(21)	Australian	62[2]	0	PCR	39/*[3]	No association between p53 mutation and GSTM1 polymorphism among cancer cases
	Katoh *et al*, 1998(77)	Japanese	145[4]	145	PCR	57/45	1.6 (1.0 2.5)
	Salagovic *et al*, 1999(78)	Slovakian	76	248	PCR	53/50	1.1 (0.7−1.9)
	Georgiou *et al*, 2000(79)	Greek	89	147	PCR	63/38	2.8 (1.6−4.7)
	Kim *et al*, 2000(80)	Korean	112	220	PCR	70/56	1.8 (1.1−2.9)
	Martone *et al*, 2000(81)	Italian	45	0	PCR	56/*[3]	No association between p53 mutation and GSTM1 polymorphism among cancer cases
	Mungan *et al*, 2000(82)	Dutch	61	61	PCR	62/43	2.1 (1.1−4.3)
	Peluso *et al*, 2000(83)	Italian	130	54	PCR	47/54	0.8 (0.4−1.4)
	Schnakenberg *et al*, 2000 (84)	German	157	223	PCR	59/58	1.1 (0.7−1.6)
	Steinhoff *et al*, 2000(85)	German	135	127	PCR	59/45	1.8 (1.1−2.9)
	Aktas *et al*, 2001(86)	Turkish	103	202	PCR	54/35	2.2 (1.4−3.6)
	Toruner *et al*, 2001(87)	Turkish	121	121	PCR	62/45	2.0 (1.2−3.3)
	Lee *et al*, 2002(88)	Korean	232	165	PCR	64/52	1.6 (1.1−2.5)

Table 2. Previous reports of association between GSTs polymorphisms and urothelial cancer(continued)

Genotype	Author, year of publication (reference number)	Population	Number of cases[1]	Number of controls	Analysis methods	Frequencies of variant genotype cases/controls(%)	Crude OR or results OR (95%CI)
GSTT1 deletion polymorphism	Kempkes *et al*, 1996(75)	German	113	170	PCR	18/18	1.0 (0.5−1.8)
	Brockmoller *et al*, 1996(20)	German	374	373	PCR	18/21	0.8 (0.6−1.2)
	Katoh *et al*, 1998(77)	Japanese	145[4]	145	PCR	41/48	0.8 (0.5−1.2)
	Abdel-Rahman *et al*, 1998 (76)	Egyptian	37	34	PCR	46/15	4.9 (1.6−14.9)
	Bringuier *et al*, 1998(21)	Australian	82[2]	0	PCR	5/*[3]	No association between p53 mutation and GSTT1 polymorphism among cancer cases
	Salagovic *et al*, 1999(78)	Slovakian	76	248	PCR	28/17	1.9 (1.0−3.4)
	Georgiou *et al*, 2000(79)	Greek	89	147	PCR	6/11	0.5 (0.2−1.4)
	Kim *et al*, 2000(80)	Korean	112	220	PCR	42/46	0.9 (0.5−1.3)
	Martone *et al*, 2000(81)	Italian	44	0	PCR	9/*[3]	No association between p53 mutation and GSTT1 polymorphism among cancer cases
	Peluso *et al*, 2000(83)	Italian	122	54	PCR	11/11	1.0 (0.4−2.9)
	Schnakenberg *et al*, 2000 (84)	German	157	223	PCR	18/22	0.8 (0.5−1.3)
	Steinhoff *et al*, 2000(85)	German	135	127	PCR	15/13	1.1 (0.6−2.3)
	Toruner *et al*, 2001(87)	Turkish	121	121	PCR	20/17	1.2 (0.6−2.2)
	Lee *et al*, 2002(88)	Korean	232	165	PCR	58/52	1.3 (0.9−2.0)
GSTP1 Ile-Val polymorphism[6] (exon 5)	Harries *et al*, 1997(91)	British	71	155	PCR	65/49	1.9 (1.1−3.4)
	Katoh *et al*, 1999(92)	Japanese	106[5]	122	PCR	29/24	1.3 (0.7−2.4)
	Martone *et al*, 2000(81)	Italian	45	0	PCR	60/*[3]	The frequency of GSTP1 rapid genotype with p53 mutation was 3.5-fold higher than that of GSTP1 slow genotype with p53 mutation (P=0.03)
	Peluso *et al*, 2000(83)	Italian	123	54	PCR	59/41	2.1 (1.1−4.1)
	Steinhoff *et al*, 2000(85)	German	135	127	PCR	50/45	1.2 (0.8−2.0)
	Toruner *et al*, 2001(87)	Turkish	121	121	PCR	45/31	1.8 (1.0−2.9)

[1]Cases were bladder cancer patients, [2]Cases were renal pelvis cancer patients, [3]There were no controls, [4]Cases were 112 bladder cancer, 12 ureter cancer, 10 renal pelvis cancer and 11 overlapping cancer patients, [5]Cases were 73 bladder cancer, 9 ureter cancer, 13 renal pelvis cancer and 11 overlapping cancer patients, [6]Variant genotypes were GSTP1A/G or G/G, respectively

Table 3. Previous reports of association between NAT1 polymorphisms and urothelial cancer

Author, year of publication (reference number)	Population	Number of cases	Number of controls	Phenotyping and genotyping (drug used for phenotyping or mutant allele)	Crude OR or results OR (95%CI)
Okkels *et al*, 1997(96)	Danish	254[1]	242	Genotyping (*NAT1*3,10,11*)	0.99 (0.98−1.01) (rapid vs slow acetylators) 3.76 (1.07−13.31) (NAT2 slow/NAT1 rapid vs NAT2 rapid/NAT1 slow)
Bringuier *et al*, 1998(21)	Australian	105[2]	0	Genotyping (*NAT1*10,11*)	No association between p53 mutation and NAT1 polymorphism among cancer cases.
Taylor *et al*, 1998(95)	American	230[1]	203	Genotyping (*NAT1*3,10,11*)	3.8 (2.1−7.1) (NAT1*10(+)/smoker vs NAT1*10(−)/nonsmoker) 5.7 (1.9−17.7) (NAT1*10(+)/NAT2 slow/smoker vs NAT1*10(−)/NAT2 rapid/nonsmoker)
Hsieh *et al*, 1999(97)	Taiwanese	74[1]	184	Genotyping (*NAT1*3,10,11*)	2.34 (1.03−5.31) (NAT1*10/NAT2 slow ever smoker vs never smoker)
Katoh *et al*,1999(94)	Japanese	116[3]	122	Genotyping (*NAT1*3,10*)	2.09 (1.02−4.35 ((NAT1*10/*10 vs *4/*4) 7.28 (2.13−32.06) (NAT2 slow/NAT1*10 vs NAT2 rapid/-NAT1*4)
Cascorbi *et al*, 2001(98)	German	425[1]	343	Genotyping (*NAT1*3,10,11,14,15,17,22*)	0.53 (0.20−1.40) (NAT1*10/*10 vs *4/*4) 2.09 (1.36−3.22) (NAT2 slow/NAT 1 *4 vs NAT2 rapid/NAT 1 *10)

[1]Cases were bladder cancer patients, [2]Cases were renal pelvic cancer patients, [3]Cases were 96 bladder cancer, 7 renal pelvis and 13 ureter cancer patients

allele and were slow *NAT2* acetylators. In the two latter studies, significant differences were found by a combination of *NAT1*10* allele and slow *NAT2* acetylator and smoking. They suggested that higher levels of NAT1-catalyzed activation (*O*-acetylation) within the urothelial increase risk. On the other hand, Cascorbi *et al.* [98] reported that genotypes including *NAT1*10* were significantly less frequent among the cases (P=0.013). Furthermore, the bladder cancer risk for *NAT2* slow acetylators combined with *NAT1*4* was increased 2.3 times compared with *NAT2* rapid acetylators with *NAT*10* genotypes (P=0.0001). Perhaps *NAT1*10* allele might affect urothelial cancer development, but few studies and small sample size might cause the discrepancy in the results.

2. *NAT2*

Slow NAT2 acetylator status as a risk factor for urothelial cancer was first proposed in the late 1970s and early 1980s [99, 100]. Since then, a large number of studies have appeared in the literature confirming or refuting an association between NAT2 status and urothelial cancer risk (Table 4) [20, 24, 80, 81, 83, 84, 92, 97, 99−126]. Recently, some meta-analyses of published studies have been reported. Green *et al.* [127] (21 studies, 2700 cases, 3426 controls) and Johns *et al.* [128] (21 studies, 2462 cases, 3450 controls) suggested that NAT2 slow acetylator may be associated with a small increase in bladder cancer risk, and these effects may be greater in smokers than in non-smokers. Marcus *et al.* [129] (22 studies, 2496 cases, 3340 controls) reported that slow acetylators had an approximately 40% increase in risk compared with rapid acetylators (OR=1.4, 95% CI=1.2−1.6). However, studies conducted in Asia generated a summary OR of 2.1 (95% CI=1.2−3.8); in Europe, a summary OR of 1.4 (95% CI=1.2−1.6); and in the USA, a summary OR of 0.9 (95% CI=0.7−1.3). In addition, a case series meta-analysis using data from 16 bladder cancer studies conducted in the general population (n=1999 cases) has been published [130]. The case-series design can be used to assess multiplicative gene-environment interaction without inclusion of control subjects. There was a weak interaction between smoking and NAT2 slow acetylation (OR=1.3, 95% CI=1.0−1.6) that, again, was stronger when analyses were restricted to studies conducted in Europe (OR=1.5, 95% CI=1.1−1.9). The meta-analyses mentioned above were based on both phenotype and genotype. Vineis *et al.* [131] reported a pooled analysis of *NAT2* genotype-based studies in Caucasian populations (6 studies, 1530 cases, 731 controls), and a significant association between *NAT2* and bladder cancer (OR=1.42, 95% CI=1.14−1.77). The risk of cancer was elevated in smokers and occupationally exposed subjects, with the highest risk among slow acetylators. They suggested that *NAT2* was not a risk factor but modulated the effect of carcinogens contained in tobacco smoke (probably arylamines) or associated with occupational exposures.

Sulfotransferases (SULT)

SULT catalyze both the bioactivation and detoxification of a wide range of promutagens and procarcinogens. SULT1A1 appears to be an important phenol SULT because of its abundance and distribution in many tissues and wide substrate specificity. The *SULT1A1* gene

Table 4. Previous reports of association between NAT2 polymorphisms and urothelial cancer

Author, year of publication (reference number)	Population	Number of cases[1]	Number of controls	Phenotyping and genotyping (drug used for phenotyping or mutant allele)	Frequencies of NAT2 slow cases/controls(%)	Crude OR or results OR (95%CI)
Lower *et al*, 1979(99)	Swedish	115	118	Phenotyping (Sulphamethazine)	70/67	1.1 (0.7–2.0)
Lower *et al*, 1979(99)	Danish	71	74	Phenotyping (Sulphamethazine)	65/51	1.8 (0.9–3.4)
Lower *et al*, 1979(99)	American	34	41	Phenotyping (Sulphamethazine)	65/51	1.5 (0.6–3.8)
Wolf *et al*, 1980(100)	Danish	71	74	Phenotyping (Sulphamethazine)	65/51	1.7 (0.9–3.4)
Cartwright *et al*, 1982(101)	British	111	95	Phenotyping (Dapsone)	67/57	1.5 (0.9–2.7)
Evans *et al*, 1983(102)	British	100	852	Phenotyping (Sulphamethazine)	66/60	1.3 (0.8–2.0v
Miller *et al*, 1983(103)	American	26	26	Phenotyping (Sulphamethazine)	46/69	0.4 (0.1–1.2)
Cartwright *et al*, 1984(104)	Portuguese	47	35	Phenotyping (not stated)	30/29	1.1 (0.4–2.8)
Hanssen *et al*, 1985(105)	German	105	42	Phenotyping (Sulphamethazine)	62/43	2.2 (1.1–4.5)
Ladero *et al*, 1985(106)	Spanish	130	157	Phenotyping (Dapsone)	64/57	1.3 (0.8–2.1)
Mommsen *et al*, 1985(107)	Danish	228	100	Phenotyping (Sulphamethazine)	51/46	1.5 (0.9–2.4)
Karakaya *et al*, 1986(108)	Turkish	23	109	Phenotyping (Sulphamethazine)	39/62	0.4 (0.2–0.9)
Kaisary *et al*, 1987(109)	British	98	110	Phenotyping (Dapsone)	60/49	1.6 (0.9–2.7v
Bicho *et al*, 1988(110)	Portuguese	49	84	Phenotyping (Sulphamethazine)	43/42	1.1 (0.5–2.1)
Horai *et al*, 1989(111)	Japanese	51	203	Phenotyping (Dapsone)	6/6	0.9 (0.3–3.3)
Hanke *et al*, 1990(112)	Polish	67	22	Phenotyping (Isoniazid)	70/45	2.8 (1.1–7.5)
Hayes *et al*, 1993(113)	Chinese	38	43	Phenotyping (Dapsone) Genotyping (*NAT2*5;6;7*)	13/23	0.3 (0.1–1.1) (Phenotype) 0.5 (0.2–1.6) (Genotype)
Lee *et al*, 1994(24)	Korean	98	84	Phenotyping (Isoniazid)	16/17	1.0 (0.4–2.1)
Dewan *et al*, 1995(114)	Indian	77	80	Phenotyping (Isoniazid)	60/35	3.3 (1.8–6.6)
Ishizu *et al*, 1995(115)	Japanese	71	91	Phenotyping (Isoniazid)	28/14	2.4 (1.1–5.2)
Risch *et al*, 1995(116)	British	189	59	Genotyping (*NAT2*5A,B,C; 6A; 7B*)	67/44	2.6 (1.4–4.7)
Brockmoller *et al*, 1996(20)	German	374	373	Genotyping (*NAT2*5A,B,C; 6A; 7B*)	62/58	1.2 (0.9–1.6)
Golka *et al*, 1996(117)	German	196	0	Genotyping (*NAT2*5A,B,C; 6A;7B*)	55/*[2]	No significant excess of NAT2 slow in cases who worked in chemical production(P=0.123)or chemical or rubber industry (P=0.141)

Table 4. Previous reports of association between NAT2 polymorphisms and urothelial cancer(continued)

Author, year of publication (reference number)	Population	Number of cases[1]	Number of controls	Phenotyping and genotyping (drug used for phenotyping or mutant allele)	Frequencies of NAT2 slow cases/controls(%)	Crude OR or results OR (95%CI)
Okkels *et al*, 1997(118)	Danish	254	242	Genotyping (*NAT2*5A,B,C; 6A; 7*)	61/56	1.2 (0.9−1.7)
Peluso *et al*, 1998(119)	Italian	114	46	Genotyping (*NAT2*5A; 6A; 7A*)	67/57	1.5 (0.8−3.1)
Schnakenberg *et al*, 1998(120)	German	60	154	Genotyping (*NAT2*5A,B,C; 6A,B; 7B; 13*)	70/61	1.5 (0.8−2.8)
Su *et al*, 1998(121)	Taiwanese	27	60	Genotyping (*NAT2*5; 6; 7*)	30/12	3.3 (1.0−9.9)
Taylor *et al*, 1998(122)	American	230	203	Genotyping (*NAT2*5; 6; 7; 14*)	53/54	1.0 (0.7−1.4)
Filiadis *et al*, 1999(123)	Greek	89	147	Genotyping (*NAT2*5; 6; 7*)	58/38	2.3 (1.3−3.9)
Hsieh *et al*, 1999(97)	Taiwanese	74	184	Genotyping (*NAT2*5; 6; 7; 14*)	21/24	0.8 (0.4−1.6)
Inatomi *et al*, 1999(124)	Japanese	85	146	Genotyping (*NAT2*5; 6; 7*)	20/7	4.2 (1.8−10.8)
Katoh *et al*, 1999(94)	Japanese	116[3]	122	Genotyping (*NAT2*5; 6; 7*)	19/6	3.8 (1.6−9.0)
Kim *et al*, 2000(80)	Korean	112	219	Genotyping (*NAT2*5; 6; 7*)	7/11	0.6 (0.3−1.4)
Martone *et al*, 2000(81)	Italian	44	0	Genotyping (*NAT2*5A,B; 6A,B; 7A,B*)	80/*[2]	No association between p53 mutation and NAT2 polymorphism among cancer cases
Peluso *et al*, 2000(83)	Italian	123	54	Genotyping (*NAT2*5A; 6A; 7A*)	68/56	1.7 (0.9−3.3)
Schnakenberg *et al*, 2000(84)	German	157	223	Genotyping (*NAT2*5A,B,C; 6A,B; 7B; 13*)	73/66	1.3 (0.9−2.1)
Cascorbi *et al*, 2001(125)	German	425	343	Genotyping (*NAT2*5A,B,C; 6A; 12A; 14B*)	63/57	1.3 (1.0−1.8)
Kontani *et al*, 2001(126)	Japanese	149	163	Genotyping (*NAT2*5A,B,C; 6A; 7B*)	7/6	1.1 (0.4−2.7)

[1]Cases were bladder cacer patients, [2]There were no controls, [3]Cases were 96 bladder cancer, 7 renal pelvis cancer and 13 ureter cancer patients

possesses G to A polymorphism that results in an Arg213 to His amino acid substitution, and the His213 allele has been shown to have low activity and low thermal stability. Studies by Hung *et al.* [132] in a north Italian population have reported a marginal protective effect of *SULT1A1* Arg213His polymorphism. However, Tsukino *et al.* reported there is no significant association between urothelial cancer and *SULT1A1* Arg213His polymorphism in Japanese [133].

Conclusion

A number of independent studies have now demonstrated the importance of polymorphisms in xenobiotic metabolism as risk factors in the development of urothelial cancer associated with chemical exposure. It has been revealed that *GSTM1* null genotype or *NAT2* slow acetylator genotype may be associated with a small increase in urothelial cancer risk. Associations between other polymorphisms of metabolic enzymes and urothelial cancer were not well-known or inconsistent. One of the reasons for these discrepancies may be insufficient study power or bad study design or a bias against publishing the absence of correlations. International Agency for Research on Cancer (IARC) [15] and Bartsch *et al.* [134] provided state-of-the art reviews of the application of biomarkers and the design and analysis of molecular epidemiological studies. The prerequisites for proper study design and conduct include: (a) clear definition of representative study populations and controls; (b) a sample size adequate to provide enough statistical power; (c) proper documentation (or measurement) of exposure; (d) avoidance of confounding because of use of study subjects of mixed ethnic background; and (e) study only of gene polymorphisms that have been shown to lead to altered phenotypic expression. The rigor and size of study designs will need to increase, as multiple comparison and power issue dictate. In parallel with these studies, a clearer understanding of the genetic bias of the polymorphisms has emerged, together with more accurate and less invasive methods for screening of populations.

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