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Polymorphisms in *GSTT1* and *p53* and urinary transitional cell carcinoma in south-western Taiwan: A Preliminary Study

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Little is known about the relevance of genetic polymorphisms to arsenic-related bladder cancer. A preliminary case-control study was conducted to explore the association between genetic polymorphisms of *GSTT1*, *p53* codon 72 and bladder cancer in southern Taiwan, a former high arsenic exposure area. Fifty-nine urinary transitional cell carcinoma (TCC) patients from a referral centre in south-western Taiwan and 81 community controls matched on residence were recruited from 1996 to 1999. A questionnaire was administered to obtain arsenic exposure and general health information. Genotypes of *p53* codon 72 and *GSTT1* were analysed by polymerase chain reaction-restriction fragment length polymerase. The combined variant genotypes (heterozygous or homozygous variant) of *p53* codon 72 and *GSTT1* null were observed in 29% of cases and in 44% of controls, respectively. In this preliminary study, bladder cancer risk was slightly elevated for subjects carrying the variant genotype of *p53* codon 72 or in subjects carrying the *GSTT1* null genotype. Variants in *p53* codon 72 increased the risk of bladder cancer among smokers. However, the results were not statistically significant and larger confirmatory studies are needed to clarify the role of candidate gene polymorphisms and bladder cancer risk in arsenic exposed populations.

Keywords: bladder cancer, arsenic, environmental disease, genetic polymorphisms.

Introduction

In epidemiological studies, a single-base change of *p53* codon 72 of exon 4 has been associated with an increased risk of lung cancer (To-Figueras *et al.* 1996, Wang *et al.* 1999, Fan *et al.* 2000, Pierce *et al.* 2000), bladder cancer (Oka *et al.* 1991, Kempkes *et al.* 1996, Soulitzis *et al.* 2002), hepatocellular carcinoma (Yu *et al.* 1999), ovarian cancer (Buller *et al.* 1997), acute myelogenous leukaemia (Zhang *et al.* 1992), and breast cancer (Wang-Gohrke *et al.* 1998). A mutation in *GSTT1* is associated with arsenic (As) methylation ability (Chiou *et al.* 1997), myelodysplastic syndromes (Chen *et al.* 1996), breast cancer (Siegelmann-Danieli and Buetow 2002), and hereditary non-polyposis colorectal cancer (Moisio *et al.*

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1998). In this preliminary study, we assess the possible joint association between germline polymorphisms in *p53* codon 72, *GSTT1,* and the risk of bladder cancer in the blackfoot disease (BFD) endemic area of south-western Taiwan.

As is ubiquitous in the earth's crust and biosphere. Humans may be exposed to As via ingestion, inhalation or skin absorption (a very minor route). Previous epidemiological studies have shown that inorganic As exposure may lead to cancers of the liver, kidney, bladder, prostate, lymphoid, skin, lung, colon and nasal cavity, as well as to a peripheral vascular occlusion known as BFD, ischaemic heart disease, hyperpigmentation, hyperkeratosis and other adverse health effects (Chiou *et al.* 1995, Chan and Huff 1997).

The *p53* gene is located on the short arm of chromosome 17 and it encodes a nuclear phosphoprotein involved in the inhibition of cell proliferation (Finley *et al.* 1989) by preventing cells from entering the S-phase (Martinez *et al.* 1991). Soulitzis *et al.* (2002) evaluated the risk of bladder cancer in relation to genotype of *p53* codon 72, and found that the homozygosity for arginine at residue 72 was associated with an increased risk for bladder cancer ($p < 0.001$; OR = 4.69; 95% CI = 2.13–10.41).

The *GSTT1* gene is located on the long arm of chromosome 22. *GSTT1* function is lost when both alleles are present. Lee *et al.* (1989) reported that the elevation of intracellular GSH (glutathione) levels and GST (glutathione S-trasferase) activity in the Chinese hamster ovary cells (SA7) may be responsible for resistance to arsenite.

Salagovis *et al.* (1999) also found that the *GSTT1* null genotype did not modify the risk of smoking-associated bladder cancer. Georgiou *et al.* (2000) found the *GSTT1* null genotype was not statistically associated with bladder cancer in non-As-related disease. While several studies have examined the *GSTT1* (Chiou *et al.* 1997, Salagovic *et al.* 1999) or *p53* codon 72 (Soulitzis *et al.* 2002) genotypes in patients with bladder cancer, no study has been explored the potential joint effect of *p53* codon 72 and *GSTT1* polymorphisms in bladder cancer patients and controls.

Several epidemiological studies in Taiwan have related prevalence, incidence and risk of bladder cancer to As exposure via drinking water. A significantly higher incidence and mortality rate for transitional cell carcinoma (TCC) of the urinary bladder, up to 30 times greater than those in other regions of Taiwan, have been reported from the BFD endemic area (Su *et al.* 1985, Chen *et al.* 1986, Chen and Wang 1990, Chiang *et al.* 1993). In this exploratory study, we assessed polymorphisms of *p53* codon 72 and *GSTT1* as well as estimates of cumulative As exposure in the bladder cancer patients and controls in south-western Taiwan, a former BFD endemic area.

Materials and methods

Study design

The research protocol was approved by the Institutional Review Boards of the Harvard School of Public Health and National Cheng-Kung University (NCKU). From January 1996 to December 1999, a case-control study was conducted in south-western Taiwan. Fifty-nine newly diagnosed bladder cancer patients and 81 community controls matched on residence, all over age 30 years, were recruited from the NCKU Medical Center. The NCKU Medical Center is the main medical referral centre for cancer diagnosis and treatment for residents in Tainan City and its surrounding rural communities.

Cases were newly diagnosed urinary transitional cell carcinoma (TCC) patients. The pathological diagnosis was performed at the NCKU Pathology Department using the International Classification of Diseases, Version 9 (ICD-9, code 188). Since As exposure occurred mainly from consumption of drinking water, it was necessary to estimate As exposure over time. The cumulative As exposure index (CAE) (Chiou *et al.* 1995) was defined as: CAE $=\Sigma$[(average As concentration of artesian well water in mg $l^{-1})_i \times$(duration of consuming artesian well water in years)$_i$: unit of village]. The average As concentration of artesian well water was estimated from questionnaire data based upon the village in which they lived 30 years ago and the average As level in well water for each village, obtained from the Taiwan Provincial Institute of Environmental Sanitation Survey of 83 656 wells between 1974 and 1976 (Chen and Wang 1990).

At the time of blood collection at the NCKU Medical Center, trained interviewers administered a questionnaire to each subject. All interviewers were blinded to exposure status and study hypotheses. Information collected from the questionnaire included demographic information, personal habits, disease history, diet information recalled over the past year, and other relevant questions. Persons who failed to complete or refused to answer the questionnaire were excluded. Blood samples were obtained on 74% subjects with completed questionnaires.

Sample analysis

p53 *BstUI polymorphism.* A polymerase chain reaction-restriction fragment length polymerase (PCR-RFLP) analysis of the codon 72 of the *p53* gene originally described by Ara *et al.* (1990) was used to identify *p53* BstUI genotypes. The two primers were 5′-TTGCCGTCCCAAGCAATGGATGA-3′ and 5′-TCTGGGAAGGGACAGAAGATGAC-3′. Each PCR reaction mixture (50 μl) contained 10 pmol of each primer, 2.0 mM $MgCl_2$, 200 mM each dNTP, 1 unit *Taq* polymerase and 100–300 ng genomic DNA. Reaction mixtures were pre-incubated for 5 min at 94°C. PCR conditions were 94°C for 30 s and 55°C for 1 min, followed by 72°C for 1 min for 35 rounds. After confirmation of an amplified fragment of the expected size (199 bp) on an agarose gel, the PCR products were digested with 2 units restriction enzyme BstUI (Biolabs, New England, ME, USA) at 60°C for 16 h. DNA fragments electrophoresis through a 2% agarose gel and stained with ethidium bromide. For wild-type (*Arg*/*Arg*), the *Arg* allele is cleaved by BstUI, and yields two small fragments (113 and 86 bp). For homozygous variant (*Pro*/*Pro*), the *Pro* allele is not cleaved by BstUI at codon 72, and has a single band (199 bp). The heterozygous (*Arg*/*Pro*) has three bands (199, 113 and 86 bp).

GSTT1 *and* GSTM1

The *GSTT1* genetic polymorphisms were evaluated using multiplex PCR techniques, modified from a *GSTM1* technique by Zhong *et al.* (1993). Four primers were used: (1) 5′-CGCCATCTTGTGCTA-CATTGCCCG-3′ (final reaction concentration 25 pM), (2) 5′-ATCTTCTCCTCTTCTGTCTC-3′ (50 pM), (3) 5′-TTCCTTACTGGTCCTCACATCTC-3′ (25 pM) and (4) 5′-TCACCGGAT-CATGCCAGCA-3′ (25 pM). Each 25 μl of the PCR reaction mixture contained 3.0 mM $MgCl_2$, 200 M each dNTP, 1.25 units *taq* polymerase and approximately 100 ng genomic DNA. Reaction mixtures were pre-incubated for 5 min at 94°C. Initial amplification involved the following PCR conditions: 94°C for 15 s, 67°C for 15 s and 72°C for 22 s for two cycles. Then the main amplification involved the following PCR conditions: 94°C for 30 s and 62°C for 30 s, followed by 72°C for 45 s for 37 rounds, followed by final extension 72°C for 5 min. Primers (1) and (2) amplified a 157 bp fragment as an internal control; primers (3) and (4) amplified a 480 bp fragment in the presence of the *GSTT1* allele. DNA fragments were electrophoresed through a 2% agarose gel and stained with ethidium bromide.

Statistical analysis

We used multiple logistic regression models to estimate the multivariate OR (and 95% CI) of CAE, *p53* codon 72, and *GSTT1* polymorphisms associated with bladder cancer. We also assessed whether age, gender, BMI, smoking status, alcohol consumption and education status modified the association between *p53* codon 72, *GSTT1* polymorphisms, and the risk of bladder cancer. To control for potential confounding, we adjusted for the following risk factors for bladder cancer in the multivariate models: age, gender, body-mass index (BMI $=$kg m^{-2}), CAE, cigarette smoking, alcohol consumption, hair dye usage, and education status in the multivariate models.

Results

Table 1 shows the demographic characteristics and multivariate odds ratio for bladder cancer cases and controls. The risk of bladder cancer did not increase with

Table 1. Characteristics and multivariate analyses of bladder cancer cases and controls, 1996–1999.

	Cases ($n=59$)		Controls ($n=81$)		
Variable	*n*	%	*n*	%	Multivariate OR[a] (95% CI)
Age (years)					
>30–50	4	7	5	6	1.00
>50–70	33	57	49	61	1.19 (0.18–7.66)
>70	21	36	26	33	0.73 (0.10–5.25)
Gender:					
Male	43	73	56	69	0.96 (0.25–3.72)
Female	16	27	25	31	1.00
Live in BFD area:					
Yes	5	9	11	14	1.08 (0.21–5.47)
No	53	91	68	86	1.00
BMI (kg m^{-2})					
<18.5	3	5	5	7	0.54 (0.10–2.89)
18.5–23.0	29	53	36	51	1.00
>23.0	23	42	30	42	0.89 (0.36–2.17)
Cigarette smoking:					
Yes	35	60	37	46	3.98 (0.99–16.06)
No	23	40	43	54	1.00
Alcohol consumption:					
Yes	13	22	16	20	0.92 (0.29–2.93)
No	45	78	64	80	1.00
Hair dye:					
Yes	23	40	27	34	0.70 (0.30–1.64)
No	35	60	53	66	1.00
Education status:					
Illiterate	15	26	16	20	1.00
Elementary school	25	43	41	51	0.44 (0.14–1.34)
High school and above	18	31	23	29	0.75 (0.20–2.92)

[a]Models adjusted for cumulative arsenic exposure (CAE), age, gender, BMI, hair dye usage, cigarette smoking, alcohol consumption, and education.

age and is similar for males and females. Smokers had an elevated risk of bladder cancer as compared with non-smokers (multivariate OR = 3.98; 95% CI = 0.99–16.06; $p = 0.05$). In this small sample, residence in the BFD endemic area, BMI, smoking status, alcohol consumption, hair dye usage, and education status were not significantly associated with the risk of bladder cancer.

CAE did not differ between males and females. It was different between cases and controls in the age group of 30–50 years but not statistically significant. The average CAE was 7.4 and 11.0 mg l^{-1} $year^{-1}$ for cases and controls, respectively (data not shown).

The variant genotype of *p53* codon 72 (*AP* and *PP*) was not significantly associated with bladder cancer (*AP* and *PP* versus *AA*: multivariate OR = 1.12, 95% CI = 0.40–30.18) as compared with *AA* genotype (table 2). There was no significantly increased risk for *GSTT1* null individuals (null versus present: multivariate OR = 1.21, 95% CI = 0.53–2.73). The risk of bladder cancer was

Table 2. Genotypes of *p53* codon 72 and *GSTT1* in bladder cancer cases and controls.

	Cases	Controls	
Genotype	*n* (%)	*n* (%)	OR (95% CI)
p53 codon 72			
AA	22 (37)	21 (26)	1.00
AP+PP	37 (63)	60 (74)	1.12 (0.40–30.18)
GSTT1			
Present	30 (46)	30 (37)	1.00
Null	32 (54)	51 (63)	1.21 (0.53–2.73)

	Non-smokers		Smokers	
	Cases/controls	OR (95% CI)	Cases/controls	OR (95% CI)
p53 codon 72				
AA	10/13	1.00	12/8	1.00
AP+PP	13/30	0.78 (0.19–3.18)	23/29	2.62 (0.44–5.51)
GSTT1				
Present	13/30	1.00	18/20	1.00
Null	10/13	1.50 (0.43–5.26)	17/17	1.07 (0.36–3.13)

	Males		Females	
	Cases/controls	OR (95% CI)	Cases/controls	OR (95% CI)
p53 codon 72				
AA	16/11	1.00	6/10	1.00
AP+PP	27/45	0.99 (0.26–3.77)	10/15	2.03 (0.29–14.00)
GSTT1				
Present	23/32	1.00	9/19	1.00
Null	20/24	1.02 (0.40–2.61)	7/6	2.43 (0.42–14.08)

[a]Models adjusted for cumulative arsenic exposure (CAE), age, gender, BMI, hair dye usage, cigarette smoking, alcohol consumption, and education.

higher among smokers with *AP* and *PP* genotype of *p53* codon 72 (*AP* and *PP* versus *AA*: multivariate OR = 2.62, 95% CI = 0.44–15.51) than smokers with the *AA* genotype. However, no elevated risk was observed among non-smokers. Females with *AP* and *PP* genotype of *p53* codon 72 tended to have higher risk of bladder cancer than did females with *AA* genotype (*AP* and *PP* versus *AA*: multivariate OR = 2.03, 95% CI = 0.29–14.00). No increased risk was observed among males with the variant *p53* codon 72 polymorphism. In addition, females with *GSTT1* null had a higher risk of bladder cancer than did females with *GSTT1* present (null versus present: multivariate OR = 2.43, 95% CI = 0.42–14.08).

Analysis of a joint effect between *p53* codon 72 and *GSTT1* on bladder cancer revealed that subjects with the variant (*AP+PP*) genotype *p53* codon 72 and *GSTT1* present had a higher risk of bladder cancer (multivariate OR = 2.10, 95% CI = 0.75–5.90) than had those with wild-type genotypes of both genes (table 3). There was a non-statistically significant elevated risk observed for subjects with both *GSTT1* null and either the wild-type or variant genotype of *p53* codon 72.

Males with the combined variant genotype (*AP* and *PP*) of *p53* codon 72 and *GSTT1* present or *GSTT1* null had a higher risk of bladder cancer compared with those males with wild-type *p53* codon 72 and *GSTT1* present (table 3). However,

Table 3. Gene interaction on the risk of bladder cancer by gender and smoking status.

	Multivariate OR (95% CI)[a]
Total (Cases = 59, Controls = 81)	
p53 (*AA*) & *GSTT1* present	1.00
p53 (*AA*) & *GSTT1* null	1.36 (0.28–6.73)
p53 (*AP*+*PP*) & GSTT1 present	2.10 (0.75–5.90)
p53 (*AP*+*PP*) & *GSTT1* null	1.30 (0.44–3.86)
Male (Cases = 43, Controls = 56)	
p53 (*AA*) & *GSTT1* present	1.00
p53 (*AA*) & *GSTT1* null	0.97 (0.13–7.57)
p53 (*AP*+*PP*) & *GSTT1* present	2.93 (0.78–11.02)
p53 (*AP*+*PP*) & *GSTT1* null	2.55 (0.65–9.92)
Female (Cases = 16, Controls = 25)	
p53 (*AA*) & *GSTT1* present	1.00
p53 (*AA*) & *GSTT1* null	N/A[b]
p53 (*AP*+*PP*) & *GSTT1* present	1.37 (0.15–12.65)
p53 (*AP*+*PP*) & *GSTT1* null	0.14 (0.01–1.91)

[a]Multivariate models controlled for cumulative arsenic exposure (CAE), age, gender, BMI, hair dye usage, cigarette smoking, alcohol consumption, and education.
[b]N/A, data not available due to small sample size.

no elevated risk of bladder cancer was observed for males with the *AA* genotype of *p53* codon 72 and *GSTT1* null compared with those males with *AA* genotype of *p53* codon 72 and *GSTT1* present. Females with the variant genotype of *p53* codon 72 and either *GSTT1* genotype did not have a higher risk of bladder cancer compared with males with wild-type *p53* codon 72 and *GSTT1* present.

Discussion

In this preliminary study in south-western Taiwan, we observed a consistently higher risk of bladder cancer for subjects with variant genotypes of *p53* codon 72 and *GSTT1* present compared with those with other genotype combinations. The variant genotype of each gene alone did not predict the risk of bladder cancer. Thus, the combined variant genotypes of *p53* codon 72 and *GSTT1* present appear to elevate the risk of bladder cancer, while the role of variant *GSTT1* alone is not conclusive.

Several studies have reported a significant relationship between the *GSTM1* null genotype and the risk of bladder cancer (Lee *et al.* 1989, Zhang *et al.* 1992, Salagovic *et al.* 1999, Georgiou *et al.* 2000), but not with *GSTT1* null (Georgiou *et al.* 2000, Miller *et al.* 2001). However, Salagovis *et al.* (1999) (Slovak Republic) and Abdel-Rahman *et al.* (1998) found a significant association between *GSTT1* null genotype and bladder cancer. In a Japanese population (Katoh *et al.* 1999), no increased bladder cancer risk was observed for *GSTT1* null (OR = 0.83; 95% CI = 0.45–1.52). Similar results were observed in a German population (Kempkes *et al.* 1996) for *GSTM1* null genotype (OR = 1.81; 95% CI = 1.10–2.98). However, they found a significant relationship between *GSTT1* null genotype and the risk of bladder cancer among non-smokers (OR = 3.84; 95% CI = 1.21–12.23), consistent with our results.

We found no statistically significant association between *p53* codon 72 genotypes and the risk of bladder cancer, consistent with the results from studies of Toruner *et al.* (2001b) (Turkey) and Chen *et al.* (2000) (Taiwan). All these studies have similar sample sizes. Soulitzis *et al.* (2002) (Greece) is the only team reporting that individuals carrying *AA* (wild) genotype on *p53* codon 72 have an increased risk of bladder cancer (OR = 4.69, 95% CI = 2.13–10.41), in contrast to the results from this and other studies (Chen *et al.* 2000, Toruner *et al.* 2001).

We found a consistently higher risk of bladder cancer (although not statistically significant) for subjects with combined variant (*AP* and *PP*) genotypes of *p53* codon 72 and *GSTT1* present compared with those with wild-type of *p53* codon 72 and *GSTT1* present. Larger studies are needed to confirm this result.

Smoking is known as an important risk factor of bladder cancer, especially for men (Anon, 2002), and it is related to the risk of bladder cancer. Hair dye usage, BMI, gender and alcohol consumption were not significantly associated with bladder cancer. For the relationship between cigarette smoking and the frequency of *p53* codon 72, Toruner *et al.* (2001) found that stratification of the data by tobacco exposure did not result in a significant difference in *p53* codon 72 genotype frequencies. The same team (Toruner *et al.* 2001) found that in individuals with the combined risk factors of cigarette smoking and the *GSTM1* null genotype, the risk of bladder cancer is 2.81 times (95% CI = 1.23–6.35) that of persons who both carry the *GSTM1*-present genotype and do not smoke. No studies to date have reported a significant relationship between smoking habit and *GSTT1*.

There are significant limitations to our study. First, the sample size is small. These findings should be considered preliminary. Second, the average As well water level in each village (gathered from questionnaire data) was used to calculate CAE. Using the average As level in each village did not allow us to evaluate an individual dose–response relationship between As exposure and the risk of bladder cancer, and this estimation might lead to non-differential misclassification of exposure and subsequent underestimation of the association between CAE and the risk of bladder cancer. In addition, since we matched cases and controls on residence, there may be overmatching on As exposure, leading to underestimation of an As exposure (but probably not genotype) effect. On the other hand, the observed difference between cases and controls may result mainly from genetic differences. Recall bias is a potential confounder for case-control studies. Therefore, we validated most of the information obtained from questionnaires (e.g. gender, age, occupation, and residence) from the household registration office. Selection bias is unlikely to affect this study because the NCKU medical centre covers approximately 80% of all cancer cases requiring specialists in the region. However, other variables, such as smoking and As exposure, are more prone to recall bias and should be validated in future studies in Taiwan. Moreover, cases and controls were identified by ICD-9 and their exposure history and genotype status was blind to the team that recruited and interviewed them.

Bladder cancer is a human disease with complex determinants. Therefore, a relatively modest risk contributed from genetic polymorphisms might be due to incomplete penetrance and phenocopy effects. The role of genetic polymorphisms in metabolism and in cell cycle regulation for bladder cancer risk in As exposed

populations needs to be examined further. Additional studies are needed to evaluate variables such as nutrition, tumour cell type (Guo *et al.* 1997), and the generalizibility of our findings to other populations. Larger studies are needed to evaluate the role of *p53* codon 72, and *GSTT1* genetic polymorphisms, other polymorphisms, environmental exposure to As, and bladder cancer risk.

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References

Abdel-Rahman, S. Z., Anwar, W. A., Abdel-Aal, W. E., Mostafa, H. M. and Au, W. W. 1998, GSTM1 and FSTT1 genes are potential risk modifiers for bladder cancer. *Cancer Detection and Prevention*, **22**, 129–138.

Anon. 2002, Bladder cancer. *Harvard Mens' Health Watch*, **6**, 3–6.

Ara, S., Lee, P. S. Y., Hansen, M. F. and Saya, H. 1990, Codon 72 polymorphism of the TP53 gene. *Nucleic Acids Research*, **18**, 4961.

Buller, R. E., Sood, A., Fullenkamp, C., Sorosky, J., Powills, K. and Anderson, B. 1997, The influence of the p53 condon 72 polymorphism on ovarian carcinogenesis and prognosis. *Cancer Gene Therapy*, **4**, 239–245.

Chan, P. C. and Huff, J. 1997, Arsenic carcinogenesis in animals and in humans: mechanistic, experimental, and epidemiological evidence. *Environmental, Carcinoma and Ecotoxicology Reviews*, **C15**, 83–122.

Chen, C. J., Chuang, Y. C., You, S. L., Lin, T. M. and Wu, H. Y. 1986, A retrospective study on malignant neoplasms of bladder, lung and liver in blackfoot disease endemic area in Taiwan. *British Journal of Cancer*, **53**, 399–405.

Chen, C. J. and Wang, C. J. 1990, Ecological correlation between level in well water and age-adjusted mortality from malignant neoplasms. *Cancer Research*, **50**, 5470–5474.

Chen, H., Sandler, D., Taylor, J. A., Shore, D. L., Liu, E., Bloomfield, C. and Bell, D. A. 1996, Increased risk for myelodysplastic syndrome. *Lancet*, **347**, 295–296.

Chen, W. C., Tsai, F. J., Wu, J. Y., Wu, H. C., Lu, H. F. and Li, C. W. 2000, Distribution of p53 codon 72 polymorphism in bladder cancer — profile form is prominent in invasive tumor. *Urology Research*, **28**, 293–296.

Chiang, H. S., Guo, H. R., Hong, C. L., Lin, S. M. and Lee, E. F. 1993, The incidence of bladder cancer in the black foot disease endemic area in Taiwan. *British Journal of Urology*, **71**, 274–278.

Chiou, H. Y., Hsueh, Y. M., Hsieh, L. L., Hsu, L. I., Hsu, Y. H., Hsieh, F. I., Wei, M. L., Chen, H. C., Yang, H. T., Leu, L. C., Chu, T. H., Chen-Wu, C., Yang, M. H. and Chen, C. J. 1997, Arsenic methylation capacity, body retention, and null genotypes of glutathione S-transferase M1 and T1 among current arsenic-exposed residents in Taiwan. *Mutation Research*, **386**, 197–207.

Chiou, H. Y., Hsueh, Y. M., Liaw, K. F., Horng, S. F., Chiang, M. H., Pu, Y. S., Lin, J. S. N., Huang, C. H. and Chen, C. J. 1995, Incidence of internal cancers and ingested inorganic arsenic: a seven-year follow-up study in Taiwan. *Cancer Research*, **55**, 1296–1300.

Fan, R., Wu, M. T., Miller, D., Wain, J. C., Kelsey, K. T., Wiencke, J. K. and Christiani, D. C. 2000, The p53 codon 72 polymorphism and lung cancer risk. *Cancer Epidemiology, Biomarkers and Prevention*, **9**, 1037–1042.

Finley, C. A., Hinds, P. W. and Levine, A. J. 1989, The p53 proto-oncogene can act as a suppressor of transformation. *Cell*, **57**, 1083–1093.

Georgiou, I., Filiadis, I. F., Alamanos, Y., Bouba, I., Giannakopoulos, X. and Lolis, D. 2000, Gluathione S-transferase null genotypes in transitional cell bladder cancer. *European Urology*, **37**, 660–664.

Guo, H. R., Chiang, H. S., Hu, H., Lipsitz, S. R. and Monson, R. R. 1997, Arsenic in drinking water and incidence of urinary cancers. *Epidemiology*, **8**, 545–550.

Katoh, T., Nakano, M., Yoshioka, M., Inatomi, H., Matsumoto, T. and Kawamoto, T. 1999, Urothelial cancer risk in relation to genotypes of glutathione S-transferase (GST) M1, T1, P1 and N-acetyltransferasse 2 (NAT2) and tobacco smoking. *Journal of Occupational Health*, **41**, 12–18.

KEMPKES, M., GOLKA, K., REICH, S. and RECKWITZ, T. 1996, Glutathione S-transferase GSTM1 and GSTT1 null genotypes as potential risk factors for urethelial cancer of the bladder. *Arch Toxicology*, **71**, 123–126.

LEE, T. C., WEI, M. L., CHANG, W. J., HO, I. C., LO, J. F., JAN, K. Y. and HUNG, H. 1989, Elevation of glutathione levels and glutathione S-transferase activity in arsenic-resistant Chinese hamster ovary cells. *In Vitro Cellular and Developmental Biology*, **25**, 442–448.

MARTINEZ, J., GEORGOFF, I., MARTINEZ, J. and LEVINE, A. 1991, Cellular localization and cell cycle regulation by a temperature-sensitive p53 protein. *Genes Development*, **5**, 151–159.

MILLER, M. C. R., MOHRENWEISER, H. W. and BELL, D. A. 2001, Genetic variability in susceptibility and response to toxicants. *Toxicology Letters*, **120**, 269–280.

MOISIO, A. L., SISTONEN, P., MECKLIN, J. P., JARVINEN, H. and PELTOMAKI, P. 1998, Genetic polymorphisms in carcinogen metabolism and their association to hereditary nonopolyposisi colon cancer. *Gastroenterology*, **115**, 1387–1394.

OKA, K., ISHKAWA, J., BRUNER, J. M., TAKAHASHI, R. and SAYA, H. 1991, Detection of loss of heterozygosity in the p53 gene in renal cell carcinoma and bladder cancer using the polymerase chain reaction. *Molecular Carcinogenesis*, **4**, 10–13.

PIERCE, L. M., SIVARAMAN, L., CHANG, W., LUM, A., DONLON, T., SEIFRIED, A., WILKENS, L. R., LAU, A. F. and MARCHAND, L. L. 2000, Relationships of TP53 codon 72 and HRAS1 polymorphisms with lung cancer risk in an ethnically diverse population. *Cancer Epidemiology, Biomarkers and Prevention*, **9**, 1199–1204.

SALAGOVIC, J., KALINA, I., HABALOVA, V., HRIVNAK, M., VALANSKY, L. and BIROS, E. 1999, The role of human glutathione S-transferases M1 and T1 in individual susceptibility to bladder cancer. *Physiological Research*, **48**, 465–471.

SIEGELMANN-DANIELI, N. and BUETOW, K. H. 2002, Significance of genetic variation at the glutathione S-transferase M1 and NAD(P)H:quinone oxidoreductase 1 detoxification genes in breast cancer development. *Oncology*, **62**, 39–45.

SOULITZIS, N., SOURVINOS, G., DOKIANAKIS, D. N. and SPANDIDOS, D. A. 2002, p53 codon 72 polymorphism and its association with bladder cancer. *Cancer Letters*, **179**, 175–183.

SU, I. J., CHEN, W. J., HUANG, M. H. and LIN, R. S. 1985, High frequency of transitional cell carcinoma of the urinary tract in an endemic area of chronic arsenicism in Taiwan. *Journal of the Chinese Oncology Society*, **1**, 29–36.

TO-FIGUERAS, J., GENE, M., GOMEZ-CATALAN, J., GALAN, C., FIRVIDA, J., FUENTES, M., RODAMILANS, M., HUGUET, E., ESTAPE, J. and CORBELLA, J. 1996, Glutathione-S-transferase M1 and codon 72 p53 polymorphism in a north-western Mediterranean population and their relation to lung cancer susceptibility. *Cancer Epidemiology, Biomarker and Prevention*, **5**, 337–342.

TORUNER, G. A., AKYERLI, C., UCAR, A., AKI, T., ATSU, N., OZEN, H., TEZ, M., CETINKAYA, M. and OZCELIK, T. 2001a, Polymorphisms of glutathione S-transferase genes (GSTM1, GSTP1 and GSTT1) and bladder cancer susceptibility in the Turkish population. *Archives of Toxicology*, **75**, 459–464.

TORUNER, G. A., UCAR, A., TEZ, M., CETINKAYA, M., OZEN, H. and OZCELIK, T. 2001b, p53 codon 72 polymorphism in bladder cancer — no evidence of association with increased risk or invasiveness. *Urology Research*, **29**, 393–395.

WANG, N. M., TSAI, C. H., YEH, K. T., CHEN, S. J. and CHANG, J. G. 1999, p53 codon 72Arg polymorphism is not a risk factor for carcinogenesis in the Chinese. *International Journal of Molecular Medicine*, **4**, 249–252.

WANG-GOHRKE, S., REBBECK, T. R., BESENFELDER, W., KREIENBERG, R. and RUNNEBAUM, I. B. 1998, p53 germline polymorphisms are associated with an increased risk for breast cancer in German women. *Anticancer Research*, **18**, 2095–2099.

YU, M. W., YANG, S. Y., CHIU, Y. H., CHIANG, Y. C., LIAW, Y. F. and CHEN, C. J. 1999, A p53 genetic polymorphism as a modulator of hepatocellular carcinoma risk in relation to chronic liver disease, familial tendency, and cigarette smoking in hepatitis B carriers. *Hepatology*, **29**, 697–702.

ZHANG, W., HU, G. and DEISSEROTH, A. 1992, Polymorphism at codon 72 of the p53 gene in human acute myelogenous leukemia. *Gene*, **117**, 271–275.

ZHONG, S., WYLLIE, A. H., BARNES, D., WOLF, C. R. and SPURR, N. K. 1993, Relationship between the GSTM1 genetic polymorphism and susceptibility to bladder, breast and colon cancer. *Carcinogenesis*, **14**, 1821–1824.