Available online at www.sciencedirect.com
SCIENCE DIRECT®
Cancer Letters 211 (2004) 199–207
CANCER Letters
www.elsevier.com/locate/canlet

Investigation of genetic polymorphisms and smoking in a bladder cancer case–control study in Argentina

Lee E. Moore[a,1], John K. Wiencke[b], Michael N. Bates[a], Shichun Zheng[b], Omar A. Rey[c], Allan H. Smith[a,*]

[a]*School of Public Health, Arsenic Health Effects Research Program, University of California, 140 Warren Hall, Berkeley, CA 94720-7360, USA*
[b]*University of California San Francisco Cancer Center, San Francisco, CA, USA*
[c]*Facultad de Medicina, Universidad Catolica de Córdoba, Córdoba, Argentina*

Received 13 January 2004; received in revised form 15 April 2004; accepted 16 April 2004

Abstract

We investigated the role of glutathione S-transferase (GST) enzymes (M1, T1), methylenetetrahydrofolate (MTHFR) 677 and 1298, and the NAD(P)H:quinone oxidoreductase (NQO1) polymorphisms in a population-based bladder cancer case–control study in Argentina. Buccal cell DNA was obtained from 106 cases and 109 controls. The strongest evidence was for an interaction between NQO1 genotype and smoking. For ever smoking vs. never smoking the odds ratio was 8.6 (95% confidence interval (CI) 2.7–27), in the CC genotype, and 1.3 (95% CI 0.5–3.5) in the CT and TT genotypes combined. Also, elevated bladder cancer risks associated with GSTM1 and GSTT1 null genotypes were found in smokers. Having both null polymorphisms conferred the highest risks. The MTHFR 677 CT and TT polymorphisms appeared protective against bladder cancer.

Keywords: Tobacco; MTHFR; GSTM1; GSTT1; NQO1; Bladder cancer

1. Introduction and background

Bladder cancer incidence and mortality rates vary about 10-fold worldwide [1–3]. The highest rates are found in North America and Western Europe, and are lower in Eastern Europe and many parts of Asia [1]. In Argentina much less is known about bladder cancer incidence. Except for a population-based registry in Concordia, the country lacks population-based cancer registries and, consequently, cancer incidence rates are not available. However, cancer mortality data by region are available through death certificates. Bladder cancer mortality has been reported to be approximately 6.7/100,000 and 1.1/100,000 in males and females, respectively [4]. These are similar to rates reported in Western Europe. Geographic variation in bladder cancer rates could be explained by differences in exposure to carcinogens, or differences in genetic susceptibility.

* Corresponding author. Tel.: +1-510-843-1736; fax: +1-510-843-5539.
E-mail address: ahsmith@berkeley.edu (A.H. Smith).
[1] Present address: National Cancer Institute, Bethesda, MD, USA.

0304-3835/$ - see front matter © 2004 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.canlet.2004.04.011

Ecological mortality studies in Argentina have produced evidence of increased bladder cancer mortality in a region in the Province of Córdoba where some well water has been contaminated with levels of arsenic often above 100 μg/l, and sometimes above 2000 μg/l [5]. We conducted a bladder cancer case–control study in Córdoba to further investigate this association [6]. The other main known risk factor for bladder cancer is tobacco smoke.

To date there have been no published studies of bladder cancer risk and genetic susceptibility in any South American population. In this study, polymorphisms in the glutathione S-transferase (GST) M1 and T1 genes, the methylene tetrahydrofolate reductase (MTHFR) gene, and the NAD(P)H:quinone oxidoreductase (NQO1) gene were investigated.

There are two common low-function polymorphic variants of MTHFR: the T variant at nucleotide 677 (MTHFR C677T) and the C variant at nucleotide 1298 (MTHFR A1298C). Variant C677T has been associated with higher baseline homocysteine levels in serum and is associated with increased risks of vascular disease and neural tube defects [7–9]. In contrast, this same low-function variant has also been reported to be protective for colon cancer [10–12], adult acute lymphocytic leukemia [13], and certain subtypes of pediatric leukemia [14]. Findings for bladder cancer have been inconsistent [15,16].

A C → T polymorphism in the NQO1 gene, at position 609 in exon 6, was also examined in relation to bladder cancer and for interaction with smoking. In laboratory animal studies the wild-type polymorphism of this gene has been shown to protect against carcinogenicity of benzo(a)pyrene, (a component of tobacco smoke) [17]. NQO1 also protects cells from oxidative damage by preventing the generation of reactive oxygen species. The exon 6 polymorphism of NQO1 was shown to reduce its activity. This could increase cancer risk [18].

Epidemiological studies have consistently shown that bladder cancer is strongly associated with cigarette smoking. Since our case–control study showed the expected increased risks with smoking, but not with direct measures of arsenic exposure [6], here we focus on whether the GSTM1, GSTT1, MTHFR677/1298 and NQO1 gene polymorphisms interacted with tobacco smoking in the causation of bladder cancer.

2. Methods

2.1. Study design, subjects, and data collection

Cases and controls were obtained from a bladder cancer case–control study, involving 114 case–control pairs, in two largely rural counties of Córdoba province, Argentina. The design and the results of this study are published elsewhere [6]. Briefly, cases were identified from 1996 to 2000 by pathologists and urologists in the area. All incident bladder cancer cases were between 20 and 80 years of age and were histologically confirmed. Controls, individually matched to cases on sex, year of birth, and county of residence, were identified from voter registration lists. All cases in the study were present on the voter registration lists. Detailed data on demographic factors, including date of birth and consumption of tobacco products, were collected by questionnaire. As a source of genomic DNA, buccal cells were obtained from all cases and controls, as previously described [19]. Cells were collected by rubbing the inside of each subject's cheeks with two pre-moistened tongue depressors for 30 s. Each depressor was then placed into 25 ml of 1 × –Tris–HCl at pH 7.8, and frozen at −20 °C at the end of each day.

2.2. Laboratory analysis

Upon arrival in the United States, buccal cells were centrifuged and DNA was extracted using the QIAmp DNA Mini kit, according to manufacturer's instructions [catalogue no. 51304, QIAGEN, Valencia, California, USA]. PCR was performed with a Gene Amp PCR 9600 thermal cycler (Perkin Elmer) in 50 μl reaction volumes. Each PCR reaction contained 1.5 mM $MgCl_2$, 0.2 mM deoxynucleoside triphosphate, 2.5 units Taq polymerase in 1 × PCR buffer (Perkin Elmer), and 0.4 μM each of forward and reverse primers. Amplifications were performed with 50 ng of purified buccal cell DNA. Genotyping for the GSTM1, GSTT1, MTHFR 677, 1298, and NQO1 polymorphisms were performed as previously described [20].

2.3. Statistical analyses

To calculate odds ratios (OR) and 95% confidence intervals (CI) a combination of logistic regression and exact methods was used (Stata 7.0, College Station, TX). Exact methods were used whenever expected numbers in any cell were less than five. Comparison of regression models, with and without interaction between polymorphisms and smoking, was conducted using a likelihood ratio test. Ordered logistic regression was used to test for trend in analyses of gene activity.

Multivariate adjustment variables were age (approximate tertiles: ≥65, 66–75, 76–84 years) and gender. Because the two polymorphisms at codons 677 and 1298 of the MTHFR gene are in negative disequilibrium [21], each of these polymorphisms was adjusted for the other when examined individually. When the polymorphisms were examined together, they were also considered by their level of MTHFR activity [22,23]. Two trichotomous variables with an activity level for each allelic variant were generated for each polymorphism of the MTHFR gene.

3. Results

DNA extraction and genotype analyses were completed for 106 (96%) cases and 109 (93%) controls. All samples with available DNA for analysis were successfully genotyped for all polymorphisms.

In this susceptibility study, the mean ages of cases and controls were similar, 68.1 and 68.4 years, respectively. There were more male than female cases (82 and 18%, respectively) and more cases than controls ever smoked tobacco (75 and 55%, respectively).

In Table 1, univariate conditional logistic regression analyses of each polymorphism with bladder cancer risk are presented. These revealed slightly elevated risks of bladder cancer for

Table 1
Univariate analyses of polymorphisms and bladder cancer

Polymorphism	Cases *N* (%)	Controls *N* (%)	OR[a]	95% CI[b]	*P*-value 2-tailed
GSTM1					
Active[c]	52 (49.1)	60 (55.0)	1.00	–	
Null	54 (50.9)	49 (45.0)	1.27	0.74–2.24	0.38
GSTT1					
Active[c]	89 (84.0)	97 (89.0)	1.00	–	
Null	17 (16.0)	12 (11.0)	1.54	0.71–3.41	0.28
MTHFR 677					
CC[c]	45 (42.5)	32 (29.4)	1.00	–	
CT	42 (39.6)	59 (54.1)	0.49	0.25–0.92	0.03
TT	19 (17.9)	18 (16.5)	0.74	0.29–1.85	0.52
CT or TT	61 (57.5)	77 (70.6)	0.52	0.27–0.98	0.05
MTHFR 1298					
AA[c]	52 (49.0)	55 (50.9)	1.00	–	
AT	45 (42.4)	45 (41.7)	0.92	0.50–1.70	0.80
TT	9 (8.5)	8 (7.4)	0.90	0.24–2.82	0.86
NQO1					
CC[c]	62 (58.5)	61 (56.5)	1.00	–	
CT	35 (33.0)	40 (37.0)	0.86	0.48–1.53	0.61
TT	9 (8.5)	7 (6.5)	1.26	0.44–3.61	0.66

[a] All odds ratios presented are unadjusted, except for the MTHFR gene. Each MTHFR polymorphism (677 and 1298) is adjusted for the other because of lack of independent assortment.
[b] Confidence interval.
[c] Reference group.

individuals carrying either the GSTM1 or GSTT1 polymorphic variant (null) genotypes, compared to those carrying the corresponding active allele(s). However, there are wide confidence intervals. A lower risk of bladder cancer was observed in individuals carrying either the CT or TT MTHFR 677 polymorphisms compared to those carrying the CC genotype (OR = 0.52, 95% CI 0.27–0.98). However, there was some inconsistency in that the strongest risk reduction was observed in the CT heterozygotes rather than the TT homozygotes. An inconsistent pattern of risks was observed for the NQO1 polymorphic groups.

In Table 2, bladder cancer risks associated with combinations of the GSTM1 and GSTT1 polymorphisms, based on enzyme activity patterns, were examined. The risk with having both inactive variants was greater than having either one alone (OR = 1.84; 95% CI: 0.62–5.56; *P* for trend = 0.17). Subjects were also grouped by their estimated MTHFR enzyme activity levels. As in previous studies, there was no subject carrying the 677TT genotype combined with the 1298CC genotype [13,14,22,23]. Relative to the CC/AA high activity combination, bladder cancer risks were reduced in individuals carrying other polymorphic combinations (OR = 0.40; 95% CI: 0.15–1.07).

Table 3 examines the interaction between genotype and smoking status. The effects of smoking are examined separately within the genotypes. Odds ratios for smoking, both unadjusted and adjusted for age and gender, are shown. Expected increases in risk associated with smoking are found for almost all genotypes. However, only for the NQO1 gene is there evidence of a gene–environment interaction. Subjects with the CC genotype appear particularly susceptible to bladder cancer risk associated with smoking (OR = 8.6; 95% CI 2.7–27), whereas those with the CT or TT genotypes only have a relatively small elevation in risk, if any (OR = 1.3; 95% CI 0.5–3.5). Some differences in magnitude of risk associated with smoking were apparent between polymorphism variants of other genes. However, statistically, there was no evidence of interaction.

Table 4 presents separate analyses for ever- and never-smokers. Never-smokers carrying the variant CT and TT NQO1 alleles had higher bladder cancer

Table 2
Combinations of polymorphisms and bladder cancer risk

	Activity[a]	Cases *N* (%)	Controls *N* (%)	OR[b]	95% CI[c]	*P*-value 2-tailed
GST M1/GST T1						
Active/Active[d]	High	44 (41.1)	54 (50.0)	1.00	–	–
Active/null	Medium	45 (42.1)	43 (39.1)	1.28	0.72–2.30	0.40
Null/active	Medium	8 (8.4)	6 (5.5)	1.64	0.53–5.07	0.38
Null/null	Low	9 (8.4)	6 (5.5)	1.84	0.62–5.56	0.28
				P for trend = 0.17		
MTHFR 677/1298						
CC/AA[d]	Highest	14 (13.2)	6 (5.6)	1.00	–	–
CC/AC	High	22 (20.8)	18 (16.7)	0.52	0.17–1.64	0.27
CC/CC	Medium	9 (8.5)	8 (7.4)	0.48	0.13–1.85	0.29
CT/AA	Medium	19 (17.9)	34 (31.5)	0.24	0.08–0.73	0.01
CT/AC	Medium	23 (21.7)	25 (23.2)	0.39	0.13–1.20	0.10
TT/AA	Low	19 (17.9)	15 (13.9)	0.54	0.17–1.75	0.31
TT/AC	Low	–	2 (1.9)	–	–	–
				P for trend = 0.15		
	Combined[e]	92	102	0.40	0.15–1.07	0.07

[a] Activity levels for the MTHFR gene based on results reported by van der Put [22] and Weisberg [23].
[b] All odds ratios presented are unadjusted, except for the MTHFR gene. Each MTHFR polymorphism (677 and 1298) is adjusted for the other allele because of lack of independent assortment.
[c] Confidence interval.
[d] Reference group.
[e] All allelic combinations other than the reference group.

Table 3
Relative risk estimates associated with smoking, after stratification by genotype

Gene	Polymorphism 1					Polymorphism 2				
	Cases *N* (%)	Controls *N* (%)	OR	95% CI[a]	*P* 2-tailed	Cases *N* (%)	Controls *N* (%)	OR	95% CI[a]	*P* 2-tailed
GST M1			*Wild type (active)*					*Null*		
Never smoked[b]	15 (29)	25 (42)	1.00	–	–	12 (22)	24 (49)	1.00	–	–
Smoked (unadj)	37 (71)	35 (58)	1.76	0.80–3.88	0.16	42 (78)	25 (51)	3.36	1.43–7.87	0.005
Smoked (adj)[c]			2.31	0.91–5.92	0.08			5.26	1.73–16.0	0.003
			P for interaction between genotype and smoking = 0.25							
GST T1			*Wild type (active)*					*Null*		
Never smoked[b]	25 (28)	44 (45)	1.00	–	–	2 (12)	5 (42)	1.00	–	–
Smoked (unadj)	64 (72)	53 (55)	2.13	1.15–3.92	0.02	15 (88)	7 (58)	5.36	0.83–34.7	0.08
Smoked (adj)[c]			3.02	1.43–6.37	0.004			9.14	0.77–108	0.08
			P for interaction between genotype and smoking = 0.41							
MTHFR-677			*CC*					*CT + TT*		
Never smoked[b]	13 (29)	12 (38)	1.00	–	–	14 (23)	37 (48)	1.00	–	–
Smoked (unadj)	32 (71)	20 (62)	1.60	0.60–4.28	0.35	47 (77)	40 (52)	3.02	1.43–6.37	0.004
Smoked (adj)[c,d]			2.51	0.78–8.07	0.12			3.97	1.56–10.1	0.004
			P for interaction between genotype and smoking = 0.22							
MTHFR-1298			*AA*					*AC + CC*		
Never smoked[b]	14 (27)	27 (49)	1.00	–	–	13 (24)	21 (40)	1.00	–	–
Smoked (unadj)	38 (73)	28 (51)	2.60	1.16–5.85	0.02	41 (76)	32 (60)	2.03	0.88–4.70	0.10
Smoked (adj)[c,d]			4.81	1.62–14.3	0.005			2.47	0.93–6.59	0.07
			P for interaction between genotype and smoking = 0.58							
NQO1			*CC*					*CT + TT*		
Never smoked[b]	10 (16)	30 (49)	1.00	–	–	17 (39)	18 (38)	1.00	–	–
Smoked (unadj)	52 (84)	31 (51)	5.03	2.17–11.7	<0.001	27 (61)	29 (62)	0.99	0.42–2.30	0.97
Smoked (adj)[c]			8.58	2.73–27.0	<0.001			1.31	0.49–3.53	0.59
			P for interaction between genotype and smoking = 0.006							

[a] Confidence interval.
[b] Reference group.
[c] Odds ratios adjusted for age and gender.
[d] Each MTHFR polymorphism (677 and 1298) is adjusted for the other because of lack of independent assortment.

risks than those carrying the wild-type (CC) allele (OR = 3.32; 95% CI: 1.18–9.39). In smokers, the pattern was different, with the NQO1 C → T variants being associated with a lower bladder cancer risk (OR = 0.55; 95% CI: 0.22–1.09). Bladder cancer risks were elevated in smokers (but not in never-smokers) carrying the GSTM1 and GSTT1 null genotypes (OR = 1.59; 95% CI: 0.80–3.14 and OR = 1.79; 95% CI: 0.68–4.73, respectively). Overall, the protective effect of the MTHFR 677 variants was stronger in never-smokers than in smokers (OR = 0.32; 95% CI: 0.10–1.02 and OR = 0.64; 95% CI: 0.28–1.42, respectively).

4. Discussion

Results from this study, although small, support other evidence that genetic polymorphisms in detoxification enzymes can modify bladder cancer risk, and in particular, risk from tobacco smoking. However, it is clear from Tables 3 and 4 that smoking is a more important risk factor than genotype.

The statistically strongest results from this study suggest that there is an interaction between NQO1 polymorphisms and smoking, with the CC wild type being associated with the highest risk (Table 3). Results of some previous studies have suggested

Table 4
Relative risk estimates associated with genotype, after stratification by smoking status

	Never-smokers					Ever-smokers				
	Cases *N* (%)	Controls *N* (%)	OR[a]	95% CI[b]	*P* 2-tailed	Case *N* (%)	Controls *N* (%)	OR[a]	95% CI[b]	*P* 2-tailed
GST M1										
WT[c]	15 (55.6)	25 (51.0)	1.00	–	–	37 (46.8)	35 (58.3)	1.00	–	
Null	12 (44.4)	24 (49.0)	0.78	0.29–2.09	0.62	42 (53.2)	25 (41.7)	1.59	0.80–3.14	0.18
GST T1										
WT[c]	25 (92.6)	44 (89.8)	1.00	–	–	64 (81.0)	53 (88.3)	1.00	–	
Null	2 (7.4)	5 (10.2)	0.76	0.12–4.50	0.76	15 (19.0)	7 (11.7)	1.79	0.68–4.73	0.24
MTHFR-677										
CC[c]	13 (48.1)	12 (24.5)	1.00			32 (40.5)	20 (33.3)	1.00	–	
CT	6 (22.2)	28 (57.1)	0.21	0.06–0.74	0.02	36 (45.6)	31 (51.7)	0.64	0.28–1.45	0.28
TT	8 (29.6)	9 (18.4)	0.92	0.20–4.18	0.92	11 (13.9)	9 (15.0)	0.62	0.18–2.07	0.44
CT + TTs			0.32	0.10–1.02	0.05			0.64	0.28–1.42	0.27
MTHFR-1298										
AA[c]	14 (51.9)	27 (56.2)	1.00	–		38 (48.1)	28 (46.7)	1.00	–	
AC	11 (40.7)	19 (39.6)	1.11	0.35–3.48	0.85	34 (43.0)	26 (43.3)	0.82	0.38–1.80	0.63
CC	2 (7.4)	2 (4.2)	2.72	0.27–27.37	0.40	6 (8.9)	6 (10.0)	0.62	0.16–2.46	0.50
AC + CCs			1.21	0.40–3.66	0.74			0.80	0.37–1.73	0.58
NQO1										
CC[c]	10 (37.0)	30 (62.5)	1.00			52 (65.8)	31 (51.7)	1.00	–	
CT	15 (55.6)	15 (31.3)	3.39	1.16–9.88	0.03	20 (25.3)	25 (41.7)	0.47	0.22–0.99	0.05
TT	2 (7.4)	3 (6.2)	2.00	0.29–13.74	0.30	7 (8.9)	4 (6.7)	1.03	0.28–3.82	0.97
CT + TTs			3.32	1.18–9.39	0.23			0.55	0.22–1.09	0.09

[a] Odds ratios adjusted for age and gender. Each MTHFR polymorphism (677 and 1298) is adjusted for the other because of lack of independent assortment.
[b] Confidence interval.
[c] Reference group.

the variant NQO1 polymorphism is associated with higher bladder cancer risk [24,25]. However, consistent with the present study, a recent bladder cancer case–control study of Korean men (218 cases and 199 controls) found that it was the wild-type CC genotype that was associated with increased bladder cancer risk and the risk was higher in smokers [26].

We examined the relationship between one-carbon metabolism and bladder cancer. One-carbon metabolism is divided into two main branches: one consists of reactions involving purine and thymidine synthesis; the other involves production of methionine and S-adenosylmethionine (SAM) for protein and polyamine synthesis and methylation reactions. In this study, individuals with polymorphic variants of MTHFR, other than the high activity wild types, were at reduced risk of bladder cancer. The low activity 677 TT allele has been associated both with reduced risks for cancers at a number of sites [10,11,13,14,27] and with increased risks for some cancer types [28–31], including cancers of the urinary tract [16]. A study showing no association with bladder cancer has also been reported [15]. There are currently two hypotheses for a role of the low activity variant of this gene in carcinogenesis. Increased cancer risks may be associated with insufficient methylation of DNA, which can promote carcinogenesis by the derepression of proto-oncogenes or by increasing genomic instability [32–36]. On the other hand, lowered risks may be caused by the increased fidelity of DNA synthesis afforded by the greater availability of the MTHFR substrate 5,10-CH_2–THF for DNA synthesis, particularly the increased availability of methyl groups for conversion of uracil to thymidine

[13,14,37]. An inadequate thymidine supply can result in increased incorporation of uracil into DNA, resulting in strand breaks [37]. It is possible that both mechanisms play a role.

GSTM1 and GSTT1 genes are involved in phase II detoxification of polycyclic aromatic hydrocarbons found in cigarette smoke. In this study, elevated risks occurred in smokers carrying the GSTM1/GSTT1 null genotype, compared to smokers with the non-null genotype. This is generally consistent with results found elsewhere. Two recent meta-analyses [38,39] found that the GST M1 null genotype was associated with an overall 40–50% increase in bladder cancer risk. An overall 27% increase was observed in the present study. One of the meta-analyses [39] found some evidence for an interaction between the GSTM1 null genotype and ever smoking in relation to bladder cancer risk. The results of the present study are consistent with this.

Published results concerning the GSTT1 polymorphism and bladder cancer are less consistent [40–44]. In our study, lack of GSTT1 activity was associated with an overall 54% increase in bladder cancer risk. Risks were elevated only in smokers with the null polymorphism (Table 4). Bladder cancer risks were highest in individuals carrying both of the GSTM1 and GSTT1 inactive polymorphisms. This finding is similar to those observed in a recent study of gene–environment interactions and bladder cancer risk conducted in Bresola, Italy [45].

Although some of the results presented in this study are novel, the study has some limitations. First, the sample size is small, limiting the precision of the odds ratios. Second, we did not have information regarding folate intake, a micronutrient that is involved in MTHFR metabolism and can affect risks for some types of cancer. There is some evidence that consumption of fruits and vegetables (which contain folate) is protective against bladder cancer [46]. However, a study in smokers only found no relation between folate intake specifically and bladder cancer risk [47]. The same study found no protective effect for fruits and vegetables. Also, we cannot entirely rule out the possibility that some of our results could be caused by confounding. This could be by another gene that was in linkage disequilibrium with one of the genes examined in this study, or by another exposure that was highly correlated with tobacco smoking in the study population and also a cause of bladder cancer.

In conclusion, the strongest evidence produced by this study was for an interaction between NQO1 genotype and smoking in affecting bladder cancer risk. Also, elevated bladder cancer risks associated with GSTM1 and GSTT1 null genotypes were found in smokers. Having both null polymorphisms conferred the highest risks. The MTHFR 677 CT and TT polymorphisms appeared protective against bladder cancer in both smokers and non-smokers.

Acknowledgements

The authors thank Marita Ubencel for interviewing and buccal cell collection, and the pathologists and urologists of Córdoba for case identification. Primary support for this work was provided by grant No. P42-ES04705 from the National Institute of Environmental Health Science (NIEHS). Additional support was received from NIEHS grant P30-ES01896, the Center for Occupational and Environmental Health. L.E.M. was the recipient of American Cancer Society Fellowship No. PF4440.

References

[1] D.T. Silverman, N. Rothman, S.S. Devesa, Bladder cancer, in: K.N. Syrigos, D.G. Skinner (Eds.), Bladder Cancer: Biology, Diagnosis, and Management, Oxford University Press, New York, NY, 1999, pp. 11–55.
[2] D.M. Parkin, S. Whelan, J. Ferlay, L. Teppo, D.B. Thomas, Cancer Incidence in Five Continents, vol. VIII, IARC, Lyon, France, 2002, IARC Publication no. 155.
[3] D.M. Parkin, R. Steinitz, M. Khlat, J. Kaldor, L. Katz, J. Young, Cancer in Jewish migrants to Israel, Int. J. Cancer 45 (1990) 614–621.
[4] E.L. Matos, D.M. Parkin, D.I. Loria, M. Vilensky, Geographical patterns of cancer mortality in Argentina, Int. J. Epidemiol. 19 (1990) 860–870.
[5] C. Hopenhayn-Rich, M.L. Biggs, A. Fuchs, R. Bergoglio, E.E. Tello, H. Nicolli, A.H. Smith, Bladder cancer mortality associated with arsenic in drinking water in Argentina, Epidemiology 7 (1996) 117–124.
[6] M.N. Bates, O.A. Rey, M.L. Biggs, C. Hopenhayn, L.E. Moore, D. Kalman, et al., Case–control study of bladder cancer and exposure to arsenic in Argentina, Am. J. Epidemiol. 159 (2004) 381–389.
[7] L.B. Bailey, J.F. Gregory, Polymorphisms of methlenetetrahydrofolate reductase and other enzymes: metabolic

significance, risks and impact on folate requirement, J. Nutr. 129 (1999) 919–922.
[8] H. Refsum, P.M. Ueland, Recent data are not in conflict with homocysteine as a cardiovascular risk factor, Curr. Opin. Lipidol. 9 (1998) 533–539.
[9] G.N. Welch, J. Loscalzo, Homocysteine and atherothrombosis, N. Engl. J. Med. 338 (1998) 1042–1050.
[10] J. Chen, E. Giovannucci, K. Kelsey, E.B. Rimm, M.J. Stampfer, G.A. Colditz, et al., A methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer, Cancer Res. 1 (56) (1996) 4862–4864.
[11] J. Ma, M.J. Stampfer, E. Giovannucci, C. Artigas, D.J. Hunter, C. Fuchs, et al., Methylenetetrahydrofolate reductase polymorphism, dietary interaction and risk of colorectal cancer, Cancer Res. 15 (57) (1997) 1098–1102.
[12] M.L. Slattery, J.D. Potter, W. Samowitz, D. Schaffer, M. Leppert, Methylenetetrahydrofolate reductase, diet, and risk of colon cancer, Cancer Epidemiol. Biomarkers Prev. 8 (1999) 513–518.
[13] C.F. Skibola, M.T. Smith, E. Kane, E. Roman, S. Rollinson, R.A. Cartwright, G. Morgan, Polymorphisms in the methylenetetrahydrofolate reductase gene are associated with susceptibility to acute leukemia in adults, Proc. Natl Acad. Sci. USA 96 (1999) 12810–12815.
[14] J.L. Wiemels, R.N. Smith, G.M. Taylor, O.B. Eden, F.E. Alexander, M.F. Greaves, Methylenetetrahydrofolate reductase (MTHFR) polymorphisms and risk of molecularly defined subtypes of childhood acute leukemia, Proc. Natl Acad. Sci. USA 98 (2001) 4004–4009.
[15] F. Kimura, A.R. Florl, C. Steinhoff, K. Golka, R. Willers, H.H. Seifert, W.A. Schulz, Polymorphic methyl group metabolism genes in patients with transitional cell carcinoma of the urinary bladder, Mutat. Res. 458 (2001) 49–54.
[16] B.T. Heijmans, J.M. Boer, H.E. Suchiman, C.J. Cornelisse, R.G. Westendorp, D. Kromhout, et al., A common variant of the methylenetetrahydrofolate reductase gene (1p36) is associated with an increased risk of cancer, Cancer Res. 63 (2003) 1249–1253.
[17] D.J. Long, R.L. Waikel, X.J. Wang, L. Perlaky, D.R. Roop, A.K. Jaiswal, NAD(P)H:quinone oxidoreductase 1 deficiency increases susceptibility to benzo(a)pyrene-induced mouse skin carcinogenesis, Cancer Res. 60 (2000) 5913–5915.
[18] R.D. Traver, D. Siegel, H.D. Beall, R.M. Phillips, N.W. Gibson, W.A. Franklin, D. Ross, Characterization of a polymorphism in NAD(P)H: quinone oxidoreductase (DT-diaphorase), Br. J. Cancer 75 (1997) 69–75.
[19] L.E. Moore, J.K. Wiencke, C. Eng, S. Zheng, A.H. Smith, Evaluation of buccal cell collection protocols for genetic susceptibility studies, Biomarkers 6 (2001) 448–454.
[20] S. Zheng, X. Ma, P.A. Buffler, M.T. Smith, J.K. Wiencke, Whole genome amplification increases the efficiency and validity of buccal cell genotyping in pediatric populations, Cancer Epidemiol. Biomarkers Prev. 10 (2001) 697–700.
[21] K. Stegmann, A. Ziegler, E.T. Ngo, N. Kohlschmidt, B. Schroter, Z. Ermert, M.C. Koch, Linkage disequilibrium of MTHFR genotypes 677C/T-1298A/C in the German population and association studies in probands with neural tube defects (NTD), Am. J. Med. Genet. 5 (87) (1999) 23–29.
[22] N.M. van der Put, F. Gabreels, E.M. Stevens, J.A. Smeitink, F.J. Trijbels, T.K. Eskes, et al., A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects?, Am. J. Hum. Genet. 62 (1998) 1044–1051.
[23] I. Weisberg, P. Tran, B. Christensen, S. Sibani, R. Rozen, A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity, Mol. Genet. Metab. 64 (1998) 169–172.
[24] W.A. Schulz, A. Krummeck, I. Rosinger, P. Eickelmann, C. Neuhaus, T. Ebert, et al., Increased frequency of a null-allele for NAD(P)H: quinone oxidoreductase in patients with urological malignancies, Pharmacogenetics 7 (1997) 235–239.
[25] S.J. Park, H. Zhao, M.R. Spitz, H.B. Grossman, X. Wu, An association between NQO1 genetic polymorphism and risk of bladder cancer, Mutat. Res. 536 (2003) 131–137.
[26] J.Y. Choi, K.M. Lee, S.H. Cho, S.W. Kim, H.Y. Choi, S.Y. Lee, et al., CYP2E1 and NQO1 genotypes, smoking and bladder cancer, Pharmacogenetics 13 (2003) 349–355.
[27] F. Kimura, K.H. Franke, C. Steinhoff, K. Golka, H.C. Roemer, A.G. Anastasiadis, W.A. Schulz, Methyl group metabolism gene polymorphisms and susceptibility to prostatic carcinoma, Prostate Nov 1 (45) (2000) 225–231.
[28] S.J. Lee, S.H. Cho, S.K. Park, S.W. Kim, M.S. Park, H.Y. Choi, et al., Combined effect of glutathione S-transferase M1 and T1 genotypes on bladder cancer risk, Cancer Lett. 177 (2002) 173–179.
[29] H. Shen, Y. Xu, Y. Zheng, Y. Qian, R. Yu, Y. Qin, et al., Polymorphisms of 5,10-methylenetetrahydrofolate reductase and risk of gastric cancer in a Chinese population: a case–control study, Int. J. Cancer 95 (2001) 332–336.
[30] R.G. Ziegler, S.J. Weinstein, T.R. Fears, Nutritional and genetic inefficiencies in one-carbon metabolism and cervical cancer risk, J. Nutr. 132 (2002) 2345S–2349S.
[31] B. Shannon, S. Gnanasampanthan, J. Beilby, B. Iacopetta, A polymorphism in the methylenetetrahydrofolate reductase gene predisposes to colorectal cancers with microsatellite instability, Gut 50 (2002) 520–524.
[32] P.W. Laird, R. Jaenisch, The role of DNA methylation in cancer genetic and epigenetics, Annu. Rev. Genet. 30 (1996) 441–464.
[33] Z. Siegfried, S. Eden, M. Mendelsohn, X. Feng, B.Z. Tsuberi, H. Cedar, DNA methylation represses transcription in vivo, Nat. Genet. 22 (1999) 203–206.
[34] R.Z. Chen, U. Pettersson, C. Beard, L. Jackson-Grusby, R. Jaenisch, DNA hypomethylation leads to elevated mutation rates, Nature 395 (1998) 89–93.
[35] B. Jurgens, B.J. Schmitz-Drager, W.A. Schulz, Hypomethylation of L1 LINE sequences prevailing in human urothelial carcinoma, Cancer Res. 56 (1996) 5698–5703.
[36] A.R. Florl, R. Lower, B.J. Schmitz-Drager, W.A. Schulz, DNA methylation expression of LINE-1 and HERV-K

provirus sequences in urothelial and renal cell carcinomas, Br. J. Cancer 80 (1999) 1312–1321.

[37] B.C. Blount, M.M. Mack, C.M. Wehr, J.T. MacGregor, R.A. Hiatt, G. Wang, et al., Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage, Proc. Natl Acad. Sci. USA 94 (1997) 3290–3295.

[38] L.E. Johns, R.S. Houlston, Glutathione S-transferase mu1 (GSTM1) status and bladder cancer risk: a meta-analysis, Mutagenesis 15 (2000) 399–404.

[39] L.S. Engel, E. Taioli, R. Pfeiffer, M. Garcia-Closas, P.M. Marcus, Q. Lan, et al., Pooled analysis and meta-analysis of glutathione S-transferase M1 and bladder cancer: a HuGE review, Am. J. Epidemiol. 156 (2002) 95–109.

[40] S.Z. Abdel-Rahman, W.A. Anwar, W.E. Abdel-Aal, H.M. Mostafa, W.W. Au, GSTM1 and GSTT1 genes are potential risk modifiers for bladder cancer, Cancer Detect. Prev. 22 (1998) 129–138.

[41] M. Kempkes, K. Golka, S. Reich, T. Reckwitz, H.M. Bolt, Glutathione S-transferase GSTM1 and GSTT1 null genotypes as potential risk factors for urothelial cancer of the bladder, Arch. Toxicol. 71 (1996) 123–126.

[42] J. Brockmöller, R. Kaiser, R. Kerb, I. Cascorbi, V. Jaeger, I. Roots, Polymorphic enzymes of xenobiotic metabolism as modulators of acquired P53 mutations in bladder cancer, Pharmacogenetics 6 (1996) 535–545.

[43] I. Georgiou, I.F. Filiadis, Y. Alamanos, I. Bouba, X. Giannakopoulos, D. Lolis, Glutathione S-transferase null genotypes in transitional cell bladder cancer: a case–control study, Eur. Urol. 37 (2000) 660–664.

[44] J. Salagovic, I. Kalina, V. Habalova, M. Hrivnak, L. Valansky, E. Biros, The role of human glutathione S-transferases M1 and T1 in individual susceptibility to bladder cancer, Physiol. Res. 48 (1999) 465–471.

[45] R.J. Hung, P. Boffetta, C. Malaveille, A. Hautefeuille, P. Brennan, F. Donato, et al., GSTs, NATs, SULT1A1, CYP1B1 genetic polymorphisms, interactions with environmental exposures and bladder cancer risk in Bresola, Italy (Abstract), Proc. Am. Assoc. Cancer Res. 44 (2003) 1281.

[46] C. Steinmaus, S. Nunez, A.H. Smith, Diet and bladder cancer: a meta-analysis of six dietary variables, Am. J. Epidemiol. 151 (2000) 693–702.

[47] D.S. Michaud, P. Pietinen, P.R. Taylor, M. Virtanen, J. Virtamo, D. Albanes, Intakes of fruits and vegetables, carotenoids and vitamins A, E, C in relation to the risk of bladder cancer in the ATBC cohort study, Br. J. Cancer 87 (2002) 960–965.