Short Communication

Urinary Isothiocyanate Excretion, *Brassica* Consumption, and Gene Polymorphisms among Women Living in Shanghai, China

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Abstract

Alternative measures of *Brassica* vegetable consumption (*e.g.*, cabbage) may clarify the association between *Brassica* and cancer risk. *Brassica* isothiocyanates (ITCs) are excreted in urine and may provide a sensitive and food-specific dietary biomarker. However, the persistence of ITCs in the body may be brief and dependent on the activity of several Phase II enzymes, raising questions about the relationship between a single ITC measure and habitual dietary patterns. This study investigates the association between urinary ITC excretion and habitual *Brassica* consumption, estimated by a food frequency questionnaire, among healthy Chinese women enrolled in the Shanghai Breast Cancer Study. Participants (n = 347) completed a validated food frequency questionnaire querying habitual dietary intake during the prior 5 years and provided a fasting first-morning urine specimen. Genetic deletion of glutathione *S*-transferases (*GSTM1*/*GSTT1*), and single nucleotide substitutions in *GSTP1* (A313G) and NAD(P)H:quinone oxidoreductase 1 (*NQO1*: C609T), were identified from blood DNA. Urinary ITC excretion levels were marginally higher with the *GSTT1*-null or *GSTP1-G/G* genotypes (P = 0.07, P = 0.05, respectively). Mean habitual *Brassica* intake was 98.3 g/day, primarily as bok choy, and *Brassica* intake significantly increased across quartile categories of ITC levels. The association between habitual *Brassica* intake and urinary ITC levels was stronger among women with *GSTT1*-null or *GSTP1-A/A* genotypes, or *NQO1* T-allele, and the interaction was statistically significant across *GSTP1* genotype. In conclusion, a single urinary ITC measure, in conjunction with markers of Phase II enzyme activity, provides a complementary measure of habitual *Brassica* intake among Shanghai women.

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Introduction

Enzymatic degradation of the glucosinolates from *Brassica* vegetables (*e.g.*, cabbage) generates isothiocyanates (ITCs) and other agents with potential to induce Phase II enzymes and apoptosis and stabilize cellular proliferation, possibly reducing cancer risk (1, 2). However, it is difficult to evaluate the association between habitual *Brassica* vegetable consumption and cancer in epidemiological analyses. Most food frequency questionnaires (FFQs) do not query the less common, but potentially potent, *Brassica* vegetables. Furthermore, FFQs do not capture variability in glucosinolate exposure because of cooking practices, cultivar, storage conditions, and myrosinase and gut microflora activities (3–5).

Conjugated urinary ITCs have been measured after consumption of glucosinolate rich foods (6), and urinary ITC levels correlate favorably with recent glucosinolate exposure (7) and decrease with vegetable cooking (3). Two epidemiological studies have found greater urinary ITC levels protectively associated with breast and lung cancer (8, 9). However, excretion of urinary ITCs after a single *Brassica* meal peaks within 24 h, and ITCs are removed within 3 days (7). Therefore, inference from a single urinary ITC biomarker to a habitual pattern of *Brassica* intake may be limited to analyses of study populations with high-level and steady-state *Brassica* vegetable consumption. In Singapore, Seow *et al.* (10) found that urinary ITC levels were favorably associated with habitual *Brassica* intake as measured by FFQ. Furthermore, homozygous deletion of the Phase II enzyme *GSTT1* modified the ITC and *Brassica* association. This study extends the evaluation of urinary ITC as a biomarker of habitual *Brassica* intake to women living in Shanghai, China, and potential modification by *GSTP1*, *GSTM1*, *GSTT1*, and *NQO1* genetic polymorphisms.

Materials and Methods

Participants. The present study is limited to a subset of controls recruited as a part of the Shanghai Breast Cancer Study (SBCS), and SBCS details have been reported (11). The controls of the SBCS were recruited from women randomly selected from the female general Shanghai population using the Shanghai Resident Registry, a registry of all adult residents in urban Shanghai. Eligible women had to live at the registered address between 1996 and 1998 and were between 25 and 64 years of age. A total of 1724 eligible population controls were recruited (90.3% recruitment; refusals: n = 166, 9.6%; death or prior cancer diagnosis: n = 2, 0.1%). Demographics and other data were measured by a structured questionnaire. Habitual dietary intake was measured using a validated food frequency questionnaire (12), which included the five most commonly consumed *Brassica* vegetables (green cabbage, nappa, Chinese cabbage and bok choy, cauliflower, and white turnip). Nutrient scores were computed using the Chinese Food Composition Table (13).

A fasting first-morning urine sample was collected from

99.8% of subjects. After collection, 125 mg of ascorbic acid were added to ~100 ml of urine to prevent oxidation of labile compounds, and samples were placed on ice during transport to the central laboratory for processing and storage at −70°C. Urinary ITC levels were measured in urine specimens collected from a sample of 348 women. These participants were selected as controls in a case–control subgroup study and were matched to breast cancer cases by age group (5 years) and menopausal status. A comparison of SBCS controls with our sub-study population found that our substudy was generally comparable with the SBCS control population (8).

Laboratory Analysis. The method of urinary ITC analysis has been reported previously (6). Briefly, triplicate aliquots of 100 μl of clarified urine were mixed with 10 mM 1,2-benzenedithiol (Lancaster Synthesis, Inc., Windham NH) and degassed 0.1 mM potassium phosphate (pH 8.5). The reaction mixtures incubated (2 h at 65°C) in capped high-performance liquid chromatography vials, were cooled and then were centrifuged (2800 rpm, 20 min) before detection of the cyclocondensation product 1,3-benzendithiol-2-thione by reverse-phase high-performance liquid chromatography [Waters μBondapak C_{18} (150 × 3.9 mm); Waters C_{18} guard column], wavelength, 365 nm; isocratic mobile phase methanol/water, 7:3). The interbatch coefficient of variation [(*CV* = SD/mean) * 100] was 3.40%. The intrabatch coefficient of variation was 9.64, 6.64, 5.57, 5.11, and 3.84% at ITC concentrations of 2, 5, 10, 15, and 100 μM, respectively. Urinary creatinine levels were measured using a Vitros 500 Clinical Chemistry Analyzer (Johnson & Johnson Clinical Diagnostics, Rochester, NY). Urines were analyzed in two batches, separated by ~18 months.

Genotyping Methods. Genomic DNA was extracted from buffy coat fractions (Puregene DNA isolation kit; Gentra Systems, Minneapolis, MN). A multiplex PCR protocol conclusively identified the null genotype for the *GSTM1* and *GSTT1* genes (14). The *Albumin* gene was used as an internal control. The *GSTP1* A313G polymorphism, leading to an Ile104Val substitution, was determined by PCR-RFLP method, as reported previously (15). The *NQO1* C609T polymorphism was evaluated by the PCR-RFLP method as described previously (8).

Statistical Analysis. Total habitual *Brassica* intake was calculated as the sum of the five *Brassica* vegetables reported by the FFQ. The ITC exposure index was calculated using estimated *Brassica* vegetable ITC levels reported by Jiao *et al.* (16), where watercress ITC values were used for turnip intake. Urinary ITC concentrations were standardized to urinary creatinine, to control for variability in urine volume, and the distribution of natural log-transformed ITC values approached normality (Shapiro-Wilk $P = 0.14$). One subject with a highly influential urinary ITC value was excluded. Normality of the residuals of the general linear models was assessed and confirmed using Shapiro-Wilk tests. Natural log-transformed ITC levels were compared across genotypes, age categories, or menopausal status in a general linear model adjusted for laboratory batch. Geometric mean ITC levels are reported.

Epidemiological studies categorize continuous dietary intake measures before investigating diet-disease relationships. Urinary ITC exposure categories were determined at the quartiles of the ITC distribution, and average habitual *Brassica* intake was calculated for each ITC category. Trend tests were performed by regressing an ordinal variable representing the ITC category on normalized (natural log-transformed) *Brassica* intake. Normality of the residuals was confirmed.

Modification of the *Brassica* and urinary ITC associations by Phase II enzyme genotypes was assessed by further including cross-product term of a genotype by ITC continuous value (nontransformed) with main effects of genotype and ITC variables, in the general linear model predicting *Brassica* intake. Partial Spearman rank correlations, adjusted for laboratory batch, described the monotonic association between individual-level urinary ITC levels and *Brassica* intake. All of the analyses were performed using SAS 8.02 (SAS Institute, Cary, NC).

Results

Participants included healthy female Shanghai residents (n = 347), with an average age and body mass index of 47.6 years (range, 30–64 years) and 23.0 (range, 15.7–42.2), respectively. The majority of participants were premenopausal [n = 221 (64%)], married [n = 326 (94%)], and nonsmokers [n = 340 (98%)], and had less than a high school education [less than high school, n = 202 (58%); high school, n = 114 (33%); more than high school, n = 31 (9%)].

There was no evidence that urinary ITC excretion was modified by *GSTM1* deletion (Tables 1 and 2). ITC levels were marginally higher with homozygous *GSTT1* deletion (P = 0.07), and subjects with the *G/G* genotype of GSTP1 had marginally higher urinary ITC levels compared with subjects with the *A/A* or *G/A* genotypes (P = 0.05 and P = 0.08, respectively). Mean urinary ITC levels did not substantively differ across the *NQO1 C/C*, *C/T*, or *T/T* genotypes, or when we combined subjects with the NQO1 *T*-allele to increase sample size within strata.

Participants reported consuming 98 g/day of *Brassica* vegetables, primarily as bok choy (75 g/day). Urinary ITC levels were categorized, and average *Brassica* consumption generally increased across categories (Table 3). Trends were statistically significant with total *Brassica*, bok choy and green cabbage intakes, and a marginal pattern was evident with turnip intake. The associations between urinary ITC and nappa or cauliflower intakes were inconsistent. These patterns were not affected by simultaneous control for Phase II enzyme genotypes described in Table 2.

The trends between ITC and *Brassica* were more consistent among women with *GSTT1/M1*-null, *NQO1-C/T* or *-T/T*, or *GSTP1-A/A* genotypes, although variability due to *GSTM1* genotype was trivial (Table 4). The interaction between reported *Brassica* intake, urinary ITC levels, and *GSTP1* genotype was statistically significant, with a trend between ITC and *Brassica* observed only among subjects with the *GSTP1 A/A* genotype. This interaction persisted after further adjustment for the remaining Phase II enzyme genotypes (P = 0.03). With only 11 subjects homozygous for the *G*-allele, it was not possible to consider the *G/G* genotype separately.

At an individual level, urinary ITC levels were weakly correlated with *Brassica* intake ($r_s = 0.16$, $P < 0.01$; adjusted for batch) and the ITC Index, a calculated estimate of dietary ITC exposure ($r_s = 0.16$, $P < 0.01$). The *Brassica* intake and urinary ITC correlation was somewhat stronger among subjects

Table 1 Urinary isothiocynates (ITC) levels (μmol/ml/mg creatinine) among Chinese women in Shanghai

	n	Mean	SE	Minimum	Maximum
ITC[a]	347	2.25	1.07	0.00	86.33

[a] ITC: creatinine standardized natural-log-transformed ITC values were approximately normally distributed (Shapiro-Wilk = 0.14) and geometric ITC values reported.

Table 2 Urinary isothiocynates (ITC) levels (μmol/ml/mg creatinine) by Phase II enzyme genotypes among Chinese women in Shanghai

	n	ITC	SE	$P_{difference}$[a]
GSTM1				
Present	145	1.95	1.11	
Null	167	2.10	1.11	0.62
GSTT1				
Present	193	1.79	1.13	
Null	117	2.34	1.10	0.07
GSTP1				
A/A	221	1.93	1.09	
G/A	81	2.01	1.15	0.74
G/G	11	4.20	1.46	0.05
NQO1				
C/C	97	2.00	1.14	
C/T	157	2.04	1.12	0.92
T/T	42	1.80	1.22	0.66

[a] $P_{difference}$ comparing natural log-transformed ITC values to the homozygous-null genotype of *GSTM1* or *GSTT1*, the *A/A* genotype of *GSTP1*, or the *C/C* genotype of *NQO1*. ITC values and *P*s adjusted for laboratory batch, and geometric mean ITC values and SEs reported. Missing genotype data: *NQO1* ($n = 51$), *GSTP1* ($n = 33$), *GSTT1* ($n = 37$), *GSTM1* ($n = 35$).

with the *GSTP1 A/A* (*A/A*: $r_s = 0.19$, $P < 0.01$; *G/A* or *G/G*: $r_s = 0.04$, $P = 0.72$), *NQO1 T*-allele (*T/T* or *C/T*: $r_s = 0.20$, $P < 0.01$, *C/C*: $r_s = 0.01$, $P = 0.88$), or *GSTT1*-null (Null: $r_s = 0.19$, $P < 0.01$; positive: $r_s = 0.09$, $P = 0.21$) genotypes, but was stable with participant age, menopausal status, and *GSTM1* deletion.

Discussion

Several investigations suggest that *Brassica* vegetable consumption reduces the risk of lung, colon, bladder, prostate, and breast cancers (17–19). The urinary ITC biomarker provides a measure of internalized glucosinolate exposure, the class of phytochemicals with purported biological action, while avoiding dietary assessment errors associated with participant recall and report of past dietary patterns. Urinary ITC levels have been inversely associated with breast or lung cancer risk (8, 9). However, in either a case–control or a cohort study, it must be assumed that a biomarker measured at a single point in time reflects a long-term pattern. In our investigation, habitual *Brassica* consumption significantly increased with urinary ITC excretion, suggesting that this biomarker provides an additional measure of overall habitual *Brassica* consumption within Shanghai women.

Our FFQ included five commonly consumed *Brassica* vegetables in the typical Shanghai diet, as was validated against repeated 24-h dietary recall surveys (10). Bok choy was the predominant *Brassica*, and the associations between urinary ITC levels and either total *Brassica*, bok choy, or the ITC index were similar. Green cabbage and turnip intakes also were associated with urinary ITC levels. Although reported intakes of green cabbage and turnip were low, Jiao *et al.* (16) suggest that the relatively higher ITC content of these vegetables may contribute more ITCs. Furthermore, there was no evidence of an association between cauliflower or nappa intakes and urinary ITC levels, consistent with a lower ITC content (16). FFQs are not a "gold-standard" dietary measure, and weak correlations may be explained by the validity of the FFQ, as well.

Brassica ITCs induce Phase II enzymes, and, in turn, Phase II enzymes conjugate ITCs leading to excretion (20). Seow *et al.* (10) interviewed participants for dietary intake during the prior 12 months and measured urinary ITC levels in spot samples collected ~16 months after the interview. They found greater urinary ITC levels with greater *Brassica* intake and the *GSTT1*-positive genotype, primarily among subjects with high *Brassica* intake, and ITC levels were not associated with *GSTM1* or *GSTP1* (A313G) genotypes (10). We collected fasting first-morning urine specimens and also found ITC levels unaffected by *GSTM1* genotype. However, urinary ITC excretion was marginally higher with the *GSTT1*-null and *GSTP1-A/A* or *-A/G* genotypes, and trends between ITC levels and habitual *Brassica* intake was more consistent within subjects with the *GSTT1*-null, *GSTP1-A/A*, and *NQO1-C/T* or *-T/T* genotypes. Urinary ITC levels may better reflect a time-weighted

Table 4 Reported *Brassica* (g/day) intake across quartile (Q) of urinary isothiocynate (ITC) excretion, stratified by Phase II enzyme genotype among Chinese women in Shanghai

	ITC Quartiles				P_{trend}	P_{int}[b]
	Q1	Q2	Q3	Q4		
GSTM1						
Present	82.9	91.2	97.9	97.0	0.08	0.91
Null	95.2	86.9	99.6	119.3	0.05	
GSTT1						
Present	92.3	96.2	95.4	108.6	0.11	0.26
Null	82.4	84.6	104.4	106.8	0.05	
GSTP1						
A/A	83.2	91.3	92.7	118.5	<0.01	0.02
G/A or *G/G*	103.7	85.6	111.9	92.8	0.55	
NQO1						
C/C	92.4	101.8	103.9	98.0	0.35	0.65
C/T or *T/T*	90.4	87.0	99.7	117.4	0.01	

[a] P_{trend} using log-transformed *Brassica* intake to meet analysis assumptions, and adjusted for batch.
[b] P_{int}, *P* interaction by regressing log-transformed *Brassica* on ITC levels and genotype, with an interaction term, while adjusting for batch.

Table 3 Mean *Brassica* intake across quartiles of urinary isothiocynate (ITC) excretion among Chinese women in Shanghai

	Mean[a]	SD	ITC Quartile (Q)				P_{trend}[b]
			Q1 ($n = 86$)	Q2 ($n = 87$)	Q3 ($n = 87$)	Q4 ($n = 87$)	
Brassica (g/day)	98.3	70.8	88.7	89.5	99.7	109.1	<0.01
Bok choy	75.3	64.6	68.1	70.4	74.8	86.3	<0.01
Turnip	4.1	6.9	3.1	3.7	4.7	5.3	0.06
Cabbage	6.7	11.9	6.0	3.9	7.1	7.7	0.03
Nappa	8.1	5.8	8.3	7.4	8.9	6.1	0.90
Cauliflower	3.9	5.9	3.2	4.2	4.1	3.7	0.50
ITC index	10.2	8.6	8.8	8.8	10.9	11.7	<0.01

[a] Average vegetable intakes (arithmetic scale).
[b] Trend tests performed on log-transformed food group intake, and adjusted for laboratory batch.

index of *Brassica* consumed during the prior several days among subjects with low-activity *GSTT1*-null and *NQO1 T*-allele (21) genotypes. The interaction with GSTP1 was statistically significant, and the *GSTP1 A/A* and *G/G* genotypes are known to have differential activities in polycyclic aromatic hydrocarbon catalysis (22–24). A recent *in vitro* analysis suggests the *G/G* genotype has less activity toward an ITC called sulforaphane (25), and perhaps the rate of intracellular ITC conjugation and excretion is more stable with the *GSTP1-A/A* genotype. Reasons for inconsistencies with Seow *et al.* (10) are unclear but may include differences in urine collection protocols, dietary assessment methods, types or amounts of *Brassica* consumed, or genetic profiles between the Shanghai and Singapore study procedures and populations.

In summary, *Brassica* consumption may contribute to the observed variability in cancer incidence and mortality across countries. However, analyses based solely on FFQ data may be limited by reporting errors, constrained food lists, and natural variability in glucosinolate profiles. We found the urinary ITC levels measured from a first-morning urine sample, with Phase II enzyme genotyping, provides a complementary measure of habitual *Brassica* consumption among Shanghai residents.

References

1. Hong, C., Firestone, G. L., and Bjeldanes, L. F. Bcl-2 family-mediated apoptotic effects of 3,3′-diindolylmethane (DIM) in human breast cancer cells. Biochem. Pharmacol., *63:* 1085–1097, 2002.

2. Basten, G. P., Bao, Y., and Williamson, G. Sulforaphane and its glutathione conjugate but not sulforaphane nitrile induce UDP-glucuronosyl transferase (UGT1A1) and glutathione transferase (GSTA1) in cultured cells. Carcinogenesis (Lond.), *23:* 1399–1404, 2002.

3. Conaway, C. C., Getahun, S. M., Liebes, L. L., Pusateri, D. J., Topham, D. K., Botero-Omary, M., and Chung, F. L. Disposition of glucosinolates and sulforaphane in humans after ingestion of steamed and fresh broccoli. Nutr. Cancer, *38:* 168–178, 2000.

4. Vallejo, F̂., Tomás-Barberán, F., Garcia-Viguera, C. Health promoting compounds of broccoli as influenced by refrigerated transport and retail sale. J. Agricul. Food Chem. 51: 3029–3034, 2003.

5. Fahey, J. W., Zalcmann, A. T., and Talalay, P. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry, *56:* 5–51, 2001.

6. Chung, F-L., Jiao, D., Getahun, S. M., and Yu, M. C. A urinary biomarker for uptake of dietary isothiocyanates in humans. Cancer Epidemiol. Biomark. Prev., *7:* 103–108, 1998.

7. Shapiro, T. A., Fahey, J. W., Wade, K. L., Stephenson, K. K., and Talalay, P. Human metabolism and excretion of cancer chemoprotective glucosinolates and isothiocyanates of *Cruciferous* vegetables. Cancer Epidemiol. Biomark. Prev., *7:* 1091, 1998.

8. Fowke, J. H., Chung, F-L., Jin, F., Qi, D., Cai, Q., Conaway, C. C., Cheng, J. R., Shu, X., Gao, Y-T., and Zheng, W. Urinary isothiocyanate levels, Brassica, and human breast cancer. Cancer Res., *63:* 3980–3986, 2003.

9. London, S. J., Yuan, J-M., Chung, F-L., Gao, Y-T., Coetzee, G. A., Ross, R. K., and Yu, M. C. Isothiocyanates, glutathione *S*-transferase M1 and T1 polymorphisms, and lung-cancer risk: a prospective study of men in Shanghai, China. Lancet, *356:* 724–729, 2000.

10. Seow, A., Shi, C-Y., Chung, F-L., Jiao, D., Hankin, J. H., Lee, H-P., Coetzee, G. A., and Yu, M. C. Urinary total isothiocyanate (ITC) in a population-based sample of middle-aged and older Chinese in Singapore: relationship with dietary total ITC and glutathione *S*-transferase M1/T1/P1 genotypes. Cancer Epidemiol. Biomark. Prev., *7:* 775–781, 1998.

11. Zheng, W., Xie, D-W., Jin, F., Cheng, J-R., Dai, Q., Wen, W-Q., Shu, X-O., and Gao, Y-T. Genetic polymorphism of cytochrome P450–1B1 and risk of breast cancer. Cancer Epidemiol. Biomark. Prev., *9:* 147–150, 2000.

12. Shu, X., Yang, G., Jin, F., Liu D., Kushi, L., Wen, W-Q., Gao, Y-T., and Zheng, W. Validity and reproducibility of the food frequency questionnaire used in the Shanghai Women's Health Study. Eur. J. Clin. Nutr., in press, 2003. Paper should be out January 2004

13. Chinese Academy of Medical Sciences Food Composition Tables. Beijing: People's Health Publishing House, 1991.

14. Arand, M., Muhlbauer, R., Hengstler, J., Jager, E., Fuchs, J., Winkler, L., and Oesch, F. A multiplex polymerase chain reaction protocol for the simultaneous analysis of the glutathione *S*-transferase *GSTM1* and *GSTT1* polymorphisms. Ann. Biochem., *236:* 184–186, 1996.

15. Zhao, M., Lewis, F., Gustafson, D. R., Wen, W-Q., Cerhan, J. R., and Zheng, W. No apparent association of GSTP1 A(313)G polymorphism with breast cancer risk among postmenopausal Iowa women. Cancer Epidemiol. Biomark. Prev., *10:* 1301–1302, 2001.

16. Jiao, D., Yu, M., Hankin, J. H., Low, S-H., and Chung', F-L. Total isothiocyanate contents in cooked vegetables frequently consumed in Singapore. J. Agric. Food Chem., *46:* 1055–1058, 1998.

17. Kristal, A. R., and Lampe, J. W. Brassica vegetables and prostate cancer risk: a review of the epidemiological evidence. Nutr. Cancer, *42:* 1–9, 2002.

18. Verhoeven, D. T., Goldbohm, R. A., van Poppel, G., Verhagen, H., and van den Brandt, P. A. Epidemiological studies of *Brassica* vegetables and cancer risk. Cancer Epidemiol. Biomark. Prev., *5:* 733–748, 1996.

19. Michaud, D. S., Spiegelman, D., Clinton, S. K., Rimm, E. B., Willett, W. C., and Giovannucci, E. L. Fruit and vegetable intake and incidence of bladder cancer in a male prospective cohort. J. Natl. Cancer Inst. (Bethesda), *91:* 605–613, 1999.

20. Zhang, Y., and Callaway, E. C. High cellular accumulation of sulphoraphane, a dietary anticarcinogen, is followed by rapid transporter-mediated export as a glutathione conjugate. Biochem. J., *364:* 301–307, 2002.

21. Moran, J. L., Siegel, D., and Ross, D. A potential mechanism underlying the increased susceptibility of individuals with a polymorphism in NAD(P)H:quinone oxidoreductase 1 (NQO1) to benzene toxicity. Proc. Natl. Acad. Sci. USA, *96:* 8150–8155, 1999.

22. Hu, X., Pal, A., Krzeminski, J., Amin, S., Awasthi, Y. C., Zimniak, P., and Singh, S. V. Specificities of human glutathione *S*-transferase isozymes toward anti- diol epoxides of methylchrysenes. Carcinogenesis (Lond.), *19:* 1685–1689, 1998.

23. Sundberg, K., Johansson, A. S., Stenberg, G., Widersten, M., Seidel, A., Mannervik, B., and Jernstrom, B. Differences in the catalytic efficiencies of allelic variants of glutathione transferase P1–1 towards carcinogenic diol epoxides of polycyclic aromatic hydrocarbons. Carcinogenesis (Lond.), *19:* 433–436, 1998.

24. Hu, X., Xia, H., Srivastava, S. K., Pal, A., Awasthi, Y. C., Zimniak, P., and Singh, S. V. Catalytic efficiencies of allelic variants of human glutathione *S*-transferase P1–1 toward carcinogenic anti-diol epoxides of benzo[*c*]phenanthrene and benzo[*g*]chrysene. Cancer Res., *58:* 5340–5343, 1998.

25. Lin, H. J., Johansson, A. S., Stenberg, G., Materi, A. M., Park, J. M., Dai, A., Zhou, H., Gim, J. S. Y., Kau, I. H., and Hardy, S. I. Naturally occurring Phe151Leu substitution near a conserved folding module lowers stability of glutathione transferase P1–1. Biochim. Biophys. Acta, *1649:* 16–23, 2003.