

# The Effects of *GSTM1* and *GSTT1* Polymorphisms on Micronucleus Frequencies in Human Lymphocytes *In vivo*

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## Abstract

The influence of genetic polymorphisms in *GSTM1* and *GSTT1* genes on micronucleus frequencies in human peripheral blood lymphocytes was assessed through a pooled analysis of data from seven laboratories that did biomonitoring studies using the *in vivo* cytokinesis-block micronucleus assay. A total of 301 nonoccupationally exposed individuals (207 males and 94 females) and 343 workers (237 males and 106 females) occupationally exposed to known or suspected genotoxic substances were analyzed by Poisson regression. The results of the pooled analysis indicate that the *GSTT1* null subjects had lower micronucleus frequencies than their positive counterparts in the total population (frequency ratio, 0.55; 95% confidence interval, 0.33-0.89). The protective effect of this genotype is reversed with increasing age, with a frequency ratio of

1.33 (95% confidence interval, 1.06-1.68) in subjects aged 60 years. A significant overall increase in micronucleus frequency with age and gender ( $P < 0.001$  and  $P = 0.024$ , respectively) was observed, females having higher micronucleus frequencies than males, when occupationally exposed ( $P = 0.002$ ). Nonoccupationally exposed smokers had lower micronucleus frequencies than nonsmokers ( $P = 0.001$ ), whereas no significant difference in micronucleus level was observed between smokers and nonsmokers in the occupationally exposed group ( $P = 0.79$ ). This study confirms that pooled analyses, by increasing the statistical power, are adequate for assessing the involvement of genetic variants on genome stability and for resolving discrepancies among individual studies. (Cancer Epidemiol Biomarkers Prev 2006;15(5):1038-42)

## Introduction

The micronucleus frequency in cultured peripheral blood lymphocytes is extensively used as a biomarker of genotoxic

exposure and early biological effect in human biomonitoring studies (for review, see refs. 1-5). Micronuclei may originate from a lagging acentric chromosomal fragment or from a whole chromosome failing to engage with the mitotic spindle. Consequently, the micronucleus assay provides a measure of both chromosome breakage and chromosome loss. Because micronucleus formation requires nuclear division, the scoring of those cells that have completed nuclear division is a prerequisite for the correct interpretation of the observed micronucleus frequencies. This is achieved by scoring micronuclei in binucleated cells using the cytokinesis-block micronucleus technique (6, 7).

The number of published articles measuring micronuclei in human populations has dramatically increased in the last decades (8), and the assay has been very successful despite a certain extent of heterogeneity in the laboratory protocol and especially in the scoring criteria. In recent years, international efforts, such as the Human Micronucleus project (<http://www.humn.org>), contributed to improve the reliability of the assay, providing guidelines on scoring criteria and analyzing major sources of variability (5, 9-12).

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Many studies have been done to investigate the effect of occupational mutagens on micronucleus frequency, but only a few of them have considered the effect of genetic polymorphisms of genes involved in the metabolism of mutagens. The major difficulty in the design of these studies, especially for rare polymorphisms, is the large population size required. To overcome this limitation, pooled analyses are a powerful tool, and recent literature has specifically addressed this issue (13).

The aim of the present study was to determine through the pooled analysis of a large number of individuals genotyped for *GSTM1* and *GSTT1* the influence of these common polymorphisms on micronucleus frequency in peripheral blood lymphocytes of the general population and of groups occupationally exposed to known or suspected genotoxic substances.

## Materials and Methods

**Subjects, Laboratories, Studies, and Data.** Data on *GSTM1*, *GSTT1* genotypes, and micronucleus frequencies of the same subjects were collected from seven European laboratories. The database included data from articles already published (14-20), and from an unpublished study done at the University of Pisa, Italy (partially published in refs. 21, 22).<sup>17</sup> Data from 343 workers (237 males and 106 females) exposed to occupational genotoxins (styrene, pesticides, traffic fumes, and organic solvents) and from 301 nonoccupationally exposed individuals (207 males and 94 females) were considered for the pooled analysis. All studies used the cytokinesis-block micronucleus assay in peripheral blood lymphocytes (6). Methods of genotyping were described in the original articles (14-22). Deviations from the Hardy-Weinberg equilibrium were not tested because heterozygous *GSTM1* and *GSTT1* individuals could not be distinguished from wild type using PCR followed gel electrophoresis. *GSTM1* and *GSTT1* genotypes were coded as null or positive depending upon the presence of at least one functional allele.

Individual characteristics of subjects included in the analysis (e.g., age, gender, smoking status, presence of disease, exposure to genotoxic agents, laboratory protocols for genotyping and the micronucleus assay, scoring criteria, and micronucleus frequency) were collected from the participating laboratories through a detailed questionnaire adapted from a previous Human Micronucleus project (12). Age ranged from 16 to 64 years (mean = 39.1 years; SD = 9.4 years) in the nonoccupationally exposed population and from 18 to 64 years (mean = 39.0 years; SD = 9.8 years) in the occupationally exposed population and was comparable for males and females. All individuals examined were Caucasians. Information on alcohol consumption and diet was not available for all studies and, when collected, was too heterogeneous to allow correct standardization in the pooled analysis. Therefore, it was not included in the present study.

**Statistical Methods.** The influence of genotype, age, gender, exposure status, and smoking status on the frequencies of micronucleated cells per 1,000 binucleated cells was determined using Poisson regression analysis (13). A scale variable was introduced into the model to account for overdispersion. To take interlaboratory variation into account, a mixed regression model was used; therefore, normalization of interlaboratory data was not necessary (23, 24). The model included genotype, gender, exposure (occupationally exposed or nonoccupationally exposed), and smoking (classified as smoker or nonsmoker) as fixed factors; age as a continuous covariate; and a term for each study as a random factor. All

possible two-way interactions among genotypes, age, gender, exposure, or smoking were tested. In the presence of significant interaction terms, main effects were kept in the model even when not significant.

All analyses were first done in the total population and thereafter stratified by occupational exposure. Frequency ratio (FR), its 95% confidence interval (95% CI), and the corresponding *P*-values were estimated. For categorical variables, the FR indicates the proportional increase of the micronucleus frequency in the study group; for example, a FR of 1.21 for females versus males means a 21% increase of micronucleus frequency in females. For continuous variables, the FR represents the proportional increase of micronucleus frequency due to the increase of one unit of the variable evaluated; for example, a FR for age of 1.02 means a 2% increase of micronucleus frequency per year of age. The statistical analyses were carried out using statistical routines developed in R version 2.2.0. (<http://www.r-project.org/>).

## Results

The distribution of study subjects by genotype, exposure to genotoxic agents, gender, and smoking status is shown in Table 1. In the total population, the FR estimated by Poisson regression analysis, including age, gender, smoking status, and exposure as potential confounding factors (Table 2), showed a significant increase of micronuclei with age ( $P < 0.001$ ) and in females ( $P = 0.017$ ). The gender effect was more pronounced in occupationally exposed females compared with exposed males (FR, 1.26; 95% CI, 1.08-1.47). Smokers had lower micronucleus frequencies than nonsmokers ( $P = 0.004$ ), but when coexposed to occupational genotoxins, smokers and nonsmokers had comparable micronucleus levels (FR, 1.02; 95% CI, 0.93-1.13; derived from Table 2). The results of the Poisson logistic regression, taking into account the effect of *GSTM1* and *GSTT1* polymorphisms, are summarized in Table 3. This analysis confirmed the overall increase in micronucleus frequency with age and gender ( $P < 0.001$  and  $P = 0.024$ , respectively) and the overall decrease in micronucleus frequency in smokers compared with nonsmokers ( $P = 0.005$ ). The previously described interactions of occupational exposure with gender and smoking status were also confirmed ( $P = 0.002$  and  $P = 0.019$ , respectively). *GSTT1* null subjects showed a lower micronucleus frequency compared with their positive counterparts ( $P = 0.016$ ). In addition, the *GSTT1* genotype was shown to influence micronucleus frequencies in an age-dependent way ( $P = 0.011$ ). To illustrate the effect of age as an effect modifier, micronucleus frequencies were calculated for three reference age values (20, 40, and 60 years), corresponding to approximately the mean and the range of the data distribution. A lower micronucleus frequency was found in *GSTT1* null subjects aged 20 years (FR, 0.74; 95% CI, 0.56-0.96), and a higher micronucleus frequency was found in *GSTT1* null subjects aged 60 years (FR, 1.33; 95% CI, 1.06-1.68), compared with their positive counterparts (derived from Table 3). At age 40, the *GSTT1* null genotype had no significant effect on micronucleus frequencies (FR, 0.99; 95% CI, 0.90-1.09). The influence of *GSTM1* genotype on micronucleus frequencies was only borderline significant ( $P = 0.09$ ).

Stratified analysis according to occupational exposure also showed an increase in micronucleus frequency with age and gender, as observed for the total population (Tables 2 and 3). Significantly lower micronucleus frequencies were found in smokers compared with nonsmokers for the nonoccupationally exposed individuals ( $P \leq 0.001$ ), whereas no such association was observed in the occupationally exposed group ( $P = 0.8$ ). The age-dependent influence of *GSTT1* genotype on the micronucleus frequencies was found only for the occupationally exposed group ( $P = 0.048$ ). In addition, occupationally

<sup>17</sup> L. Migliore, personal communication.

**Table 1. Distribution of study subjects by genotype, exposure to genotoxic agents, gender, and smoking status**

	<i>GSTM1</i> (NS/S, M/F)			<i>GSTT1</i> (NS/S, M/F)		
	Positive	Null	Total	Positive	Null	Total
Nonoccupationally exposed	77/65, 98/44 (22.0%)	65/94, 109/50 (24.6%)	142/159, 207/94 (46.6%)	106/133, 164/75 (37.1%)	36/26, 43/19 (9.6%)	142/159, 207/94 (46.7%)
Occupationally exposed	73/83, 110/46 (24.1%)	101/88, 128/61 (29.3%)	174/171, 238/107 (53.4%)	147/139, 196/90 (44.4%)	27/30, 41/16 (8.9%)	174/169, 237/106 (53.2%)
Total	150/148, 208/90 (46.1%)	166/182, 237/111 (53.9%)	316/330, 445/201 (100%)	253/272, 360/165 (81.5%)	63/56, 84/35 (18.5%)	316/328, 444/200 (100%)

Abbreviations: NS/S, nonsmokers/smokers; M/F, males/females.

exposed subjects carrying both *GSTM1* and *GSTT1* null genotypes showed lower micronucleus frequencies than their positive counterparts (FR, 0.72; 95% CI, 0.53-0.98).

## Discussion

The modifying effect of genetic polymorphisms in *GSTM1* and *GSTT1* genes on the micronucleus frequency in human lymphocytes was investigated through a pooled analysis of biomonitoring studies done on occupationally exposed populations. To insure the highest statistical power to the study, we restricted the evaluation to those polymorphisms that are more commonly evaluated in population studies and that have the highest frequency in the general population.

Glutathione *S*-transferases (GSTs) are considered primarily detoxification enzymes, although metabolic activation involving GST-mediated glutathione conjugation has also been described (e.g., for some chlorinated substrates; ref. 22). Detoxification by glutathione conjugation can represent a minor (e.g., styrene oxide) or a major (e.g., smoking) metabolic pathway for many genotoxic agents.

The results of this study suggest that the circumstances in which the *GSTM1* and/or *GSTT1* null genotype modifies micronucleus frequencies depend on the presence of exposure to mutagens and on age. *GSTM1* operates in the detoxification of several compounds, such as benzo( $\alpha$ )pyrene and styrene-7,8-oxide, that produce bulky adducts, but does not have a high affinity for substrates resulting from free radical attack on lipid or DNA (25-28). *GSTT1* catalyzes the conjugation of relatively small molecules, such as methylene chloride, ethylene dibromide, and the epoxides derived from isoprene (29). Given the importance of GSTs in the detoxification of electrophilic carcinogens, *GSTM1* and *GSTT1* null genotypes

have become the object of much research because homozygous deletions of *GSTM1* and *GSTT1* are expected to result in an impaired ability to detoxify carcinogenic compounds and may place *GSTM1* and/or *GSTT1* null individuals at increased cancer risk (30).

In our study, the *GSTT1* null genotype was associated with a significantly lower level of micronucleated cells in the total population; interestingly, the protective effect of the *GSTT1* null genotype was reversed in older-age classes in occupationally exposed subjects and in the total population. The effect of the *GSTM1* null genotype on micronucleus frequencies was small and only borderline significant. However, subjects carrying both *GSTM1* and *GSTT1* null genotypes showed lower micronucleus frequencies than their positive counterparts when coexposed to occupational genotoxins ( $P = 0.039$ ).

To the best of our knowledge, this study is the first one to report about decreased frequencies of micronuclei in *GSTT1* null individuals. However, a decreased micronucleus frequency in *GSTM1* null subjects was already described by Falck et al. (22) in pesticide-exposed and unexposed floriculturists. Moreover, in a recent review, Parl (30) noted that the relative risk of breast cancer for Caucasian women with the *GSTM1*<sup>+/+</sup> genotype compared with women with the *GSTM1*<sup>-/-</sup> genotype was 2.82 (31). As an explanation for this result, he suggested that the combined conjugation activities of all GSTs may lead to glutathione depletion and thereby become counterproductive. Similar considerations may also apply for *GSTT1* null individuals. Whether chronic exposure to relatively low levels of genotoxins as encountered in the studied occupational settings could lead to depletion of the glutathione pool and explain the higher frequency of micronuclei in carriers of both *GSTM1*- and *GSTT1*-positive genotypes needs further investigation.

**Table 2. Poisson regression analysis of the total, nonoccupationally exposed, and occupationally exposed populations without taking into account genetic polymorphisms**

Population	Variable	FR (95% CI)	P
Total population ( $n = 646$ )	Age	1.02 (1.01-1.02)	<0.001*
	Smoking status <sup>†</sup> (Smoker)	0.84 (0.75-0.95)	0.004*
	Gender <sup>‡</sup> (female)	1.20 (1.03-1.39)	0.017*
	Exposure <sup>§</sup> (exposed)	1.03 (0.90-1.18)	0.68
	Exposure $\times$ gender <sup>¶</sup> (exposed/female)	1.26 (1.08-1.47)	0.004*
	Exposure $\times$ smoking <sup>¶</sup> (exposed/smoker)	1.22 (1.05-1.42)	0.011*
Nonoccupationally exposed population ( $n = 301$ )	Age	1.01 (1.01-1.02)	<0.001*
	Smoking status <sup>†</sup> (smoker)	0.83 (0.75-0.92)	<0.001*
	Gender <sup>‡</sup> (female)	1.21 (1.04-1.41)	0.014*
Occupationally exposed population ( $n = 345$ )	Age	1.01 (1.01-1.02)	<0.001*
	Smoking status <sup>†</sup> (smoker)	1.02 (0.92-1.13)	0.76
	Gender <sup>‡</sup> (female)	1.48 (1.25-1.77)	<0.001*

\*Statistically significant level.

<sup>†</sup>Reference category: nonsmokers.

<sup>‡</sup>Reference category: males.

<sup>§</sup>Reference category: nonoccupationally exposed.

<sup>¶</sup>Interaction term.

**Table 3. Poisson regression analysis of the total, nonoccupationally exposed, and occupationally exposed populations taking into account genetic polymorphisms**

Total population (n = 644)			Nonoccupationally exposed population (n = 301)			Occupationally exposed population (n = 343)		
Variable	FR (95% CI)	P	Variable	FR (95% CI)	P	Variable	FR (95% CI)	P
Age	1.01 (1.01-1.02)	<0.001*	Age	1.01 (1.01-1.02)	<0.001*	Age	1.01 (1.01-1.02)	<0.001*
<i>GSTM1</i> <sup>†</sup> : null	0.94 (0.87-1.01)	0.09	<i>GSTM1</i> <sup>†</sup> : null	0.98 (0.88-1.08)	0.68	<i>GSTM1</i> <sup>†</sup> : null	0.95 (0.85-1.06)	0.34
<i>GSTT1</i> <sup>‡</sup> : null	0.55 (0.33-0.89)	0.016*	<i>GSTT1</i> <sup>‡</sup> : null	1.07 (0.95-1.21)	0.28	<i>GSTT1</i> <sup>‡</sup> : null	0.51 (0.23-1.11)	0.09
Smoking status <sup>§</sup> : smoker	0.85 (0.75-0.95)	0.005*	Smoking status <sup>§</sup> : smoker	0.84 (0.75-0.93)	0.001*	Smoking status <sup>§</sup> : smoker	0.99 (0.89-1.10)	0.79
Gender <sup>  </sup> : female	1.19 (1.02-1.38)	0.024*	Gender <sup>  </sup> : female	1.21 (1.04-1.41)	0.015*	Gender <sup>  </sup> : female	1.49 (1.25-1.77)	<0.001*
Exposure status <sup>¶</sup> : exposed	1.03 (0.90-1.19)	0.64				<i>GSTT1</i> × age <sup>**</sup> : age/ <i>GSTT1</i> null	1.02 (1.00-1.04)	0.048*
<i>GSTT1</i> × age <sup>**</sup> : age/ <i>GSTT1</i> null	1.02 (1.00-1.03)	0.011*				<i>GSTM1</i> × <i>GSTT1</i> <sup>**</sup> : <i>GSTM1</i> null/ <i>GSTT1</i> null	0.72 (0.53-0.98)	0.039*
Exposure × gender <sup>**</sup> : exposed/female	1.28 (1.10-1.50)	0.002*						
Exposure × smoking <sup>**</sup> : exposed/smoker	1.20 (1.03-1.40)	0.019*						

\*Statistically significant level.

†Reference genotype: *GSTM1* positive.‡Reference genotype: *GSTT1* positive.

§Reference category: nonsmokers.

||Reference category: males.

¶Reference category: nonoccupationally exposed.

\*\*Interaction term.

Another important finding of this analysis is the observation that women occupationally exposed to genotoxic agents are at higher risk for micronucleus induction than occupationally exposed males. This issue should be further addressed on a larger scale and for specific exposures to define adequate preventive measures in occupational settings.

The significantly lower frequency of micronuclei in nonoccupationally exposed smokers compared with nonsmokers reported in Tables 2 and 3 confirms the results of Bonassi et al. (9), who in a larger database of 3,501 subjects found a small decrease in micronuclei frequencies in current smokers (all smoking levels combined) among nonoccupationally exposed subjects (FR, 0.95; 95% CI, 0.92-0.99). However, when stratifying by level of smoking, they also found a significant increase in micronuclei frequencies in nonoccupationally exposed heavy smokers (>30 cigarettes per day). In our study, smokers and nonsmokers showed comparable micronucleus levels when coexposed to occupational genotoxins, which might be the result of the combined exposure. Standardized information on the amount of smoking was not available for each study included in this pooled analysis, and we could therefore not stratify by smoking level.

In conclusion, the results of this pooled analysis indicate that *GSTT1* null subjects had lower micronucleus frequencies than their positive counterparts in the total population. The individual data sets included in this pooled analysis revealed that the influence of *GST* genotype on micronucleus frequencies was statistically significant only in two (17, 19) of the eight studies and only for *GSTM1*. Our finding confirms that pooled analyses, by increasing the statistical power, are adequate for assessing the involvement of genetic variants on genome stability and for resolving discrepancies among individual studies.

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## References

- Kirsch-Volders M, Elhajouji A, Cundari E, Van Hummelen P. The *in vitro* micronucleus test: a multi-endpoint assay to detect simultaneously mitotic delay, apoptosis, chromosome breakage, chromosome loss and non-disjunction. *Mutat Res* 1997;392:19-30.
- Kirsch-Volders M, Sofuni T, Aardema M, et al. Report from the *In vitro* Micronucleus Assay Working Group. *Environ Mol Mutagen* 2000;35:167-72.
- Surrallés J, Natarajan AT. Human lymphocytes micronucleus assay in Europe. An international survey. *Mutat Res* 1997;392:165-74.
- Fenech M. Important variables that influence base-line micronucleus frequency in cytokinesis-blocked lymphocytes-a biomarker for DNA damage in human populations. *Mutat Res* 1998;404:155-65.
- Fenech M, Holland N, Chang WP, Zeiger E, Bonassi S. The Human MicroNucleus Project: an international collaborative study on the use of the micronucleus technique for measuring DNA damage in humans. *Mutat Res* 1999;428:271-83.
- Fenech M, Morley AA. Measurement of micronuclei in lymphocytes. *Mutat Res* 1985;147:29-36.
- Kirsch-Volders M, Fenech M. Inclusion of micronuclei in non-divided mononuclear lymphocytes and necrosis/apoptosis may provide a more comprehensive cytokinesis block micronucleus assay for biomonitoring purposes. *Mutagenesis* 2001;16:51-8.
- Bonassi S, Ugolini D, Kirsch-Volders M, Stromberg U, Vermeulen R, Tucker JD. Human population studies with cytogenetic biomarkers: review of the literature and future perspectives. *Environ Mol Mutagen* 2005;45:258-70.
- Bonassi S, Neri M, Lando C, et al. Human MicroNucleus project. Effect of smoking habit on the frequency of micronuclei in human lymphocytes: results from the Human MicroNucleus project. *Mutat Res* 2003;543:155-66.
- Fenech M, Bonassi S, Turner J, et al. Human MicroNucleus project. Intra- and inter-laboratory variation in the scoring of micronuclei and nucleoplasmic bridges in binucleated human lymphocytes. Results of an international slide-scoring exercise by the HUMN project. *Mutat Res* 2003;534:45-64.
- Fenech M, Chang WP, Kirsch-Volders M, Holland N, Bonassi S, Zeiger E. Human MicroNucleus project. HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures. *Mutat Res* 2003;534:65-75.
- Bonassi S, Fenech M, Lando C, et al. Human MicroNucleus project: International database comparison for results with the cytokinesis-block micronucleus assay in human lymphocytes: I. Effect of laboratory protocol, scoring criteria, and host factors on the frequency of micronuclei. *Environ Mol Mutagen* 2001;37:31-45.
- Taioli E, Bonassi S. Methodological issues in pooled analysis of biomarker studies. *Mutation Research Review* 2002;512:85-92.
- Godderis L, De Boeck M, Haufroid V, et al. Influence of genetic polymorphisms on biomarkers of exposure and genotoxic effects in styrene-exposed workers. *Environ Mol Mutagen* 2004;44:293-303.
- Laffon B, Pasaro E, Mendez J. Evaluation of genotoxic effects in a group of workers exposed to low levels of styrene. *Toxicology* 2002;171:175-86.

16. Laffon B, Perez-Cadahia B, Pasaro E, Mendez J. Effect of epoxide hydrolase and glutathione *S*-transferase genotypes on the induction of micronuclei and DNA damage by styrene-7,8-oxide *in vitro*. *Mutat Res* 2003; 536:49–59.
17. Leopardi P, Zijno A, Marcon F, et al. Analysis of micronuclei in peripheral blood lymphocytes of traffic wardens: effects of exposure, metabolic genotypes, and inhibition of excision repair *in vitro* by ARA-C. *Environ Mol Mutagen* 2003;41:126–30.
18. Lucero L, Pastor S, Suarez S, et al. Cytogenetic biomonitoring of Spanish greenhouse workers exposed to pesticides: micronuclei analysis in peripheral blood lymphocytes and buccal epithelial cells. *Mutat Res* 2000;464: 255–62.
19. Pitarque M, Vaglenov A, Nosko M, et al. Sister chromatid exchanges and micronuclei in peripheral lymphocytes of shoe factory workers exposed to solvents. *Environ Health Perspect* 2002;110:399–404.
20. Teixeira JP, Gaspar J, Silva S, et al. Occupational exposure to styrene: modulation of cytogenetic damage and levels of urinary metabolites of styrene by polymorphisms in genes CYP2E1, EPHX1, GSTM1, GSTT1 and GSTP1. *Toxicology* 2004;195:231–42.
21. Scarpato R, Hirvonen A, Migliore L, Falck G, Norppa H. Influence of GSTM1 and GSTT1 polymorphisms on the frequency of chromosome aberrations in lymphocytes of smokers and pesticide-exposed greenhouse workers. *Mutat Res* 1997;389:227–35.
22. Falck GC, Hirvonen A, Scarpato R, Saarikoski ST, Migliore L, Norppa H. Micronuclei in blood lymphocytes and genetic polymorphism for GSTM1, GSTT1 and NAT2 in pesticide-exposed greenhouse workers. *Mutat Res* 1999;441:225–37.
23. Blettner M, Sauerbrei W, Schlehofer B, Scheuchenpflug T, Friedenreich C. Traditional reviews, meta-analyses and pooled analyses in epidemiology. *Int J Epidemiol* 1999;28:1–9.
24. Munafò MR, Flint J. Meta-analysis of genetic association studies. *Trends Genet* 2004;20:439–44.
25. Salama SA, Sierra-Torres CH, Oh HY, Hamada FA, Au WW. Variant metabolizing gene alleles determine the genotoxicity of benzo[a]pyrene. *Environ Mol Mutagen* 2001;37:17–26.
26. Shield AJ, Sanderson BJ. Role of glutathione *S*-transferase mu (GSTM1) in styrene-7,8-oxide toxicity and mutagenicity. *Environ Mol Mutagen* 2001;37: 285–9.
27. Bernardini S, Hirvonen A, Jarventaus H, Norppa H. Influence of GSTM1 and GSTT1 genotypes on sister chromatid exchange induction by styrene in cultured human lymphocytes. *Carcinogenesis* 2002;23:893–97.
28. Ketterer B. Glutathione *S*-transferases and prevention of cellular free radical damage. *Free Radic Res* 1998;28:647–58.
29. van Bladeren PJ. Glutathione conjugation as a bioactivation reaction. *Chem Biol Interact* 2000;129:61–76.
30. Parl FF. Glutathione *S*-transferase genotypes and cancer risk. *Cancer Lett* 2005;221:123–9.
31. Roodi N, Dupont WD, Moore JH, Parl FF. Association of homozygous wild-type glutathione *S*-transferase M1 (GSTM1) genotype with increased breast cancer risk. *Cancer Res* 2004;64:1233–36.