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Metabolic gene polymorphisms and lung cancer risk in non-smokers An update of the GSEC study

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Abstract

Background: Since genetic factors may play an important role in lung cancer development at low dose carcinogen exposure, non-smokers are a good model to study genetic susceptibility and its interaction with environmental factors.
Materials and methods: We evaluated the role of the metabolic gene polymorphisms *CYP1A1MspI*, *CYP1A1Ile*^{462}*Val*, *GSTM1*, and *GSTT1* in non-smoker lung cancer patients from the International Collaborative Study on Genetic Susceptibility to Environmental Carcinogens (GSEC). Non-smokers (defined as subjects who never smoked on a regular basis) were selected from the GSEC database. We pooled the raw data from 21 case-control studies for a total of 2764 Caucasians (555 cases and 2209 controls) and 383 Asians (113 cases and 270 controls). Tests of heterogeneity and of inclusion bias were performed.
Results: A significant association between lung cancer and *CYP1A1Ile*^{462}*Val* polymorphism was observed in Caucasians (adjusted OR = 2.04, 95% CI 1.17–3.54). *GSTT1* deletion seems to be a risk factor for lung cancer in Caucasian non smokers only when the analysis was restricted to studies including healthy controls (adjusted OR = 1.66, 95% CI 1.12–2.46). A protective effect on lung cancer was observed with the combination of *CYP1A1 wild type*, *GSTM1 null*, *and GSTT1 non-null* genotypes. None of the analysed polymorphisms were associated with lung cancer in Asian non-smokers.
Discussion: Our analysis confirms previous findings that *CYP1A1Ile*^{462}*Val* polymorphism may play a role in lung carcinogenesis in Caucasian non-smokers.

Keywords: Pooled analysis; Epidemiology; Genetic susceptibility

1. Introduction

Lung cancer represents the main cause of avoidable death in the world [1]. Adenocarcinoma is increasing in frequency and accounts for almost half of lung cancers in some countries [2], and is now the most common histologic subtype in the United States [3]. The development of lung cancer is strongly associated with both active and passive cigarette smoking [4–7], and the risk increases with exposure over a lifetime [8]. However, polycyclic aromatic hydrocarbons (PAH) and other carcinogenic compounds (such as NNK) found in tobacco smoke are also present in ambient air and diet [9–11]. For this reason, other environmental sources of PAHs, such as second hand smoking [12], dietary factors [13–15], indoor exposure to fumes from cooking oils [15–17], coal or wood combustion [15,17], and occupational exposure [18] have been considered as risk factors for lung cancer in non-smokers, although the association with each of these factors are usually moderate, possibly because the exposure doses are low. Since genetic factors may play an important role at low dose carcinogen exposure [19,20], non-smokers are a good model to study genetic susceptibility and its interaction with environmental factors.

Most chemicals that initiate lung cancer, including PAHs, require bioactivation; individual differences in the ability to bioactivate (phase I) and detoxify (phase II) carcinogens may be relevant in interindividual differences in susceptibility to carcinogenesis.

Polymorphisms in the phase I CYP1A1 gene have received particular interest because this enzyme plays a central role in the metabolic activation of several procarcinogens, such as PAHs [21–23]. There are several known CYP1A1 polymorphisms: one of them (known as *MspI*, and located in the non-coding 3′-flanking region of the gene) consists of a T to C transition. Another CYP1A1 polymorphism, known as *Ile-Val* consists of an A to G transition at the exon 7 heme binding region, resulting in a replacement of isoleucine by valine, and an increase in microsomal enzyme activity [24–26]. A previous pooled analysis from our group [27] found a significant association between both *MspI* and *Ile-Val* polymorphisms and 302 lung cancer in Caucasian non-smokers.

The glutathione *S*-transferases (GSTs) phase II enzymes play a major role in detoxification of many carcinogens, such as those from cigarette smoke. The currently identified GSTs are categorized based on biochemical characteristics [28] into four main classes. *GSTM1* is a μ class isoenzyme which catalyses the formation of polycyclic aromatic hydrocarbon epoxides of benzo(a)pyrene to prevent the formation of diolepoxide, its terminal metabolite and the most important mutagen. The complete deletion of *GSTM1* results in the loss of function [29,30]. In our previous

pooled analysis [27] we found no significant association between this variant and lung cancer risk in non-smokers. *GSTT1* is involved in the detoxification of several carcinogens, such as 1,3-butadiene and ethylene oxide, present both in tobacco smoke and ambient air [31]. A complete deletion of the *GSTT1* gene, corresponding to null *GSTT1* activity [31], is less frequent among Caucasians (13–26%) than Asians (35–52%) [32]. The role of *GSTT1* deletion as a susceptibility factor for lung cancer is not well established [33–38], either in smokers or in non-smokers [39,40].

The interaction between *CYP1A1* polymorphisms and *GSTM1* deletion seems to increase lung cancer risk [41,42]. Our previous pooled analysis [27] found a statistically significant increased risk of lung cancer for non-smokers carrying both the *Ile-Val* polymorphism and the *GSTM1* deletion.

In this analysis, we have updated our previously published work [27], based on the data set of International Collaborative Study on Genetic Susceptibility to Environmental Carcinogens (GSEC), by adding additional studies, including those on Asian subjects, and extending the analysis to *GSTT1* deletion.

2. Material and methods

2.1. Study population

Subjects were recruited from the International Collaborative Study on Genetic Susceptibility to Environmental Carcinogens (GSEC). The design of this collaborative project is explained in detail elsewhere [43].

Lung cancer case-control studies that included never-smoker subjects with at least one of the studied polymorphisms (*CYP1A1MspI*, *CYP1A1Ile*^{462}*Val*, *GSTM1*, *GSTT1*) were selected. In order to avoid the potential confounding effect of ethnicity, only Caucasian and Asian subjects were included, and analysed separately. Subjects of other ethnicities (19 African–American cases from two studies and 7 cases from other ethnicities) were excluded because the data were not sufficient to perform a pooled analysis. Since the youngest case was 17 years old for Caucasians and 21 years old for Asians, seven Caucasian subjects under the age of 17 and four Asian subjects under the age of 21 were excluded among the controls.

The final sample consisted of 555 Caucasian cases and 2209 Caucasian controls from 21 studies, and 113 Asian cases and 270 Asian controls from 4 studies. Details on number of studies and number of subjects available for each genotype are included in (Table 1). We updated our previous analysis [27] by adding seven new studies of Caucasians for a total of 253 new cases and 585 new controls, and by extending the analysis to include *GSTT1* deletion and Asian subjects.

Among the 21 studies in Caucasians, 12 recruited controls from the general population, 6 from hospital patients and 2 from a combination of healthy and hospitalised subjects (Table 2). Among four studies on Asians, three recruited controls from the general population, one from hospital patients (Table 2). Only one study [44] included data for *GSTT* genotype, therefore no pooled-analysis could be performed for this polymorphism. For both Caucasians and Asians, information on other variables such as age and sex were available for almost all participants, while information on ETS exposure and area of residence were available only for a subset of subjects (19 and 33%, respectively) (Table 1).

Non-smokers were defined as subjects who had smoked less than 100 cigarettes in their lifetime, although the precise definition of never-smoking status varied slightly among the studies. The cases were all histologically confirmed lung cancer patients.

2.2. Statistical analysis

GSTM1 and *GSTT1* were dichotomized into the null genotype and the non-null type, whereas *CYP1A1MspI* and *CYP1A1Ile*^{462}*Val* polymorphisms were categorised into two groups, based on the absence or presence of the polymorphic allele (wild type homozygous versus the combined heterozygous plus the variant homozygous genotype).

Study-specific crude odds ratios (OR) and 95% confidence intervals (CI) of lung cancer for each polymorphism were estimated.

Since the data could be affected by inclusion bias, Egger's test and funnel plots were performed for Caucasians. The funnel plot shows the study-specific log effect estimates against their standard errors. If a study is small (with larger standard errors), it should scatter more widely around the true effect, whereas if it is

Table 1
Summary of the main characteristics of the sample

	Caucasians					Asians			
	CYP1A1 (MspI)	*CYP1A1 ($Ile^{462}Val$)*	*GSTM1*	*GSTT1*	Total	*CYP1A1 (MspI)*	*CYP1A1 ($Ile^{462}Val$)*	*GSTM1*	Total
Studies included in the analysis	9	8	20	8	21	2	3	3	4
Cases	165	175	531	378	555	46	60	93	113
Controls	519	723	1981	1026	2209	138	212	210	270
Mean age (median)	53.1 (54)	50.9 (49)	55.0 (57)	53.6 (55)	54.9 (57)	54.3 (58)	54.5 (57)	53.1 (55)	55.0 (57)
N of males/total (%)	378/684 (55%)	486/898 (54%)	1237/2512 (49%)	677/1404(48%)	1396/2764 (51%)	89/184 (48%)	169/272 (62%)	115/303 (38%)	194/384 (51%)

large, it should scatter narrowly around the true effect. If there is inclusion bias, an asymmetry or skewness of the plot should be observed. Egger's test was used as a regression-based test for detecting skewness in the funnel plot [45].

In order to estimate if the differences in study-specific ORs were greater than could be expected by chance, Cochran Q test [46] and Breslow–Day's test [47] were performed.

For each polymorphism, Mantel–Haenszel pooled ORs adjusted for study, and ORs adjusted for study, sex and age (using multiple logistic regression models) were performed separately for Caucasian and Asian studies. Studies in which the OR could not be estimated because of one or more zero-cell in the four-fold table (one study [38,70]) for *CYP1A1MspI*, two studies [38,70,78,79] for *CYP1A1Ile^{462}Val* and five studies (unpublished, [48,66,73,75]) for *GSTT1*, all on Caucasian subjects) were excluded from the pooled analysis. Moreover, ORs were calculated separately for studies with healthy controls and studies with hospital controls. Studies with a combination of healthy and hospitalised subjects were not considered in this analysis. For Asians, no analysis according to type of controls was performed, because of the small number of studies.

To study the possible combined effect of the polymorphisms on lung cancer risk, a gene–gene interaction analysis between Phases 1 and 2 polymorphisms was performed. ORs were estimated using multivariate logistic regression models.

The statistical analyses were performed using SAS, Version 8e. Funnel plots, Cochran's test and Egger's test were obtained using STATA package.

3. Results

The associations between each polymorphism and lung cancer for each study are shown in Table 2. The study-specific ORs for *CYP1A1MspI* polymorphism variants ranged from 0.3 to 5.15. Among the nine studies in Caucasians, six suggested that subjects carrying the *CYP1A1MspI* variant allele had a greater risk of developing lung cancer in comparison with homozygous wild-type subjects, although for only one study [48] was the association statistically significant. No heterogeneity was found among the nine

Table 2
Study-specific odds ratios and confidence intervals for *CYP1A1*, *GSTM1* and *GSTT1* polymorphisms

Reference	Cases/controls	Source of controls	*CYP1A1 MspI* (T/C, C/C vs. T/T) crude OR (95% CI)	*CYP1A1 Ile^{462}Val* (Ile/Val, Val/Val vs. Ile/Ile) crude OR (95% CI)	*GSTM1* (null vs. present) crude OR (95% CI)	*GSTT1* (null vs. present) crude OR (95% CI)
Caucasians						
[63,64]	8/22	Hospital	–	–	0.75 (0.11–5.11)	–
[65]	23/143	Healthy	–	1.71 (0.6–5.12)	1.76 (0.72–4.34)	–
[48]	12/201	Healthy, hospital	5.15 (1.54–17.23)	10.93 (2.64–45.18)	1.28(0.39–4.18)	–
[50]	16/30	Healthy	0.6 (0.04–9.16)	7.29 (1.52–35.03)	0.47 (0.13–1.68)	–
[unpublished]	104/73	Healthy	1.23 (0.57–2.66)	1.45 (0.51–4.14)	1.03 (0.56-1.9)	1.62 (0.82–3.17)
[66]	27/138	Hospital	–	–	0.81 (0.35–1.85)	–
[67–69]	28/182	Healthy	0.3 (0.01-6.38)	2.04 (0.36–11.56)	1.01 (0.4–2.56)	–
[38,70]	19/225	Healthy	–	–	0.68 (0.26–1.79)	1.38 (0.38–5.03)
[37]	26/236	Healthy	–	–	1.23 (0.54-2.79)	0.2 (0.03–1.5)
[42]	8/54	Healthy	0.96 (0.1–9.06)	2.08 (0.2–21.83)	0.44 (0.09–2.18)	–
[71,72]	7/164	Healthy	–	–	2.01 (0.38–10.64)	–
[39]	123/123	Healthy, hospital	–	–	1.51 (0.91–2.51)	0.62 (0.36–1.08)
[73]	2/43	Hospital	–	–	0.77 (0.05–13.27)	
[74]	9/148	Healthy	2.05 (0.48–8.7)	1.73 (0.2–15.19)	1.01 (0.26–3.9)	1.03 (0.12–8.79)
[75]	18/98	Healthy	–	–	1.7 (0.58–5)	
[76]	46/126	Hospital	–	–	0.69 (0.35-1.37)	0.78 (0.31–1.96)
[unpublished]	4/27	Healthy	1.3 (0.1-17.73)	–	0.8 (0.1–6.54)	
[77]	10/68	Hospital	–	–	11.4 (1.37–95.04)	1.19 (0.28–5.10)
[78,79]	13/9	Hospital	1.5 (0.19–11.93)	–	0.84 (0.13–5.26)	
[80]	46/39	Healthy	–	–	0.84 (0.36–1.99)	2.58 (0.89–7.51)
[49]	6/60	Healthy, hospital	3.53 (0.54–23.06)	0.9 (0.09–8.57)	–	–
Asians						
[44]	17/57	Hospital		6.75 (1.37–33.26)	–	–
[81]	30/96	Healthy	0.79 (0.32–1.92)	1.02 (0.43–2.43)	1.22 (0.5–3.01)	–
[42]	18/68	Healthy	0.67 (0.22–2.05)	0.57 (0.2–1.63)	0.44 (0.15–1.32)	–
[82]	48/49	Healthy	–	–	1.34 (0.6–2.98)	–

studies on *CYP1A1MspI* polymorphism: the *p*-values for Breslow–Day's test and for Cochran's test were, respectively, 0.51 and 0.59. Both studies on Asians suggested a protective effect of the *CYP1A1MspI* variant allele, although none of the ORs were statistically significant. For *CYP1A1Ile*^{462}*Val* polymorphism variants, the study-specific estimates varied from 0.57 (0.9 for Caucasians only) to 10.93. Except for two studies (one in Caucasians [49] and one in Asians [42]), all the ORs suggested a higher risk of lung cancer for subjects who carried the *CYP1A1Ile*^{462}*Val* variant allele in comparison with homozygous wild-type subjects. The results from two studies [48,50] were statistically significant. No heterogeneity was found among the eight Caucasian studies (*p*-value for Breslow–Day's test: 0.23, *p*-value for Cochran's test: 0.3).

The study-specific ORs for *GSTM1* and for *GSTT1* null genotype spread around the null value: in the 20 Caucasian studies on *GSTM1*, 10 produced an OR greater than 1.0, 10 an OR smaller than 1.0; among the 8 Caucasian studies on *GSTT1*, 5 produced an OR greater than 1.0, and 3 an OR smaller than 1.0. None of the associations were significant. Among the three Asian studies on *GSTM1*, two showed an OR greater than 1.0, one showed an OR smaller than 1.0. The assumption of homogeneity among the 20 Caucasian studies on *GSTM1* and among the eight Caucasian studies evaluating *GSTT1* was confirmed by both Breslow–Day's test and Cochran's test (*p*-values for *GSTM1* were, respectively, 0.54 and 0.69; for *GSTT1* were, respectively, 0.12 and 0.15).

Funnel plots for *CYP1A1Msp1*, *CYP1A1Ile*^{462}*Val*, *GSTM1* and *GSTT1* polymorphisms are presented in Fig. 1a–d. For all polymorphisms, no evidence of participation bias was found (*p*-values of Egger's test were 0.61 for *CYP1A1Msp1*, 0.87 for *CYP1A1Ile*^{462}*Val*, 0.86 for *GSTM1* and 0.87 for *GSTT1*).

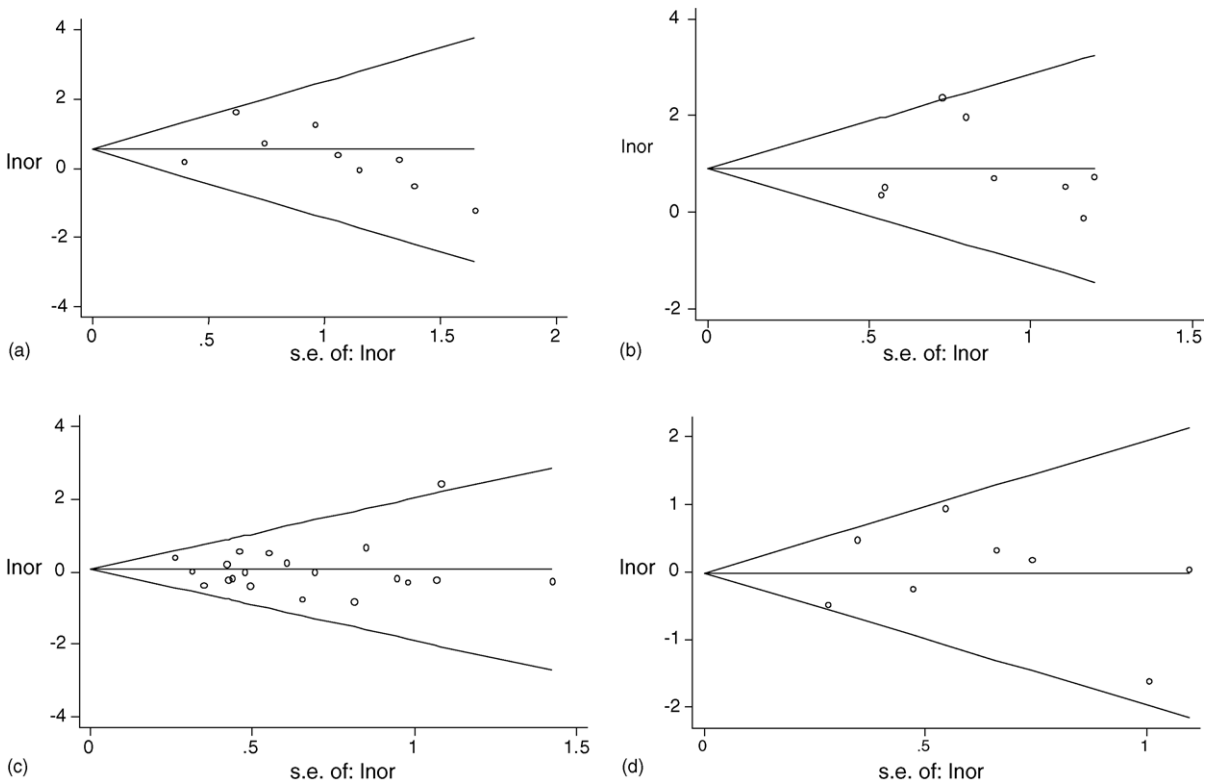

Fig. 1. Begg's funnel plot with pseudo 95% confidence limits of (a) *CYP1A1 MspI* (b) *CYP1A1 Ile*^{462}*Val* (c) *GSTM1* (d) *GSTT1* polymorphisms (log ORs of lung cancer and their standard errors) for Caucasians.

In Table 3, the pooled odds ratios and 95% confidence intervals for *CYP1A1*, *GSTM1* and *GSTT1* polymorphisms are presented. A borderline association was observed for *CYP1A1Msp1* polymorphism (adjusted OR = 1.65, 95% CI 0.98–2.77), which was reduced when the analysis was restricted to studies including healthy controls. A significant association between lung cancer and the *CYP1A1Ile*^{462}*Val* polymorphism (adjusted OR = 2.04, 95% CI 1.17–3.54) was observed in Caucasians, with 78% power (two-sided test; $\alpha = 0.05$). This effect was reduced in the analysis restricted to healthy controls (adjusted OR = 1.56, 95% CI 0.87–1.38).

No association between *GSTM1* or *GSTT1* and lung cancer was found in the whole set of non-smokers, while a significant association between lung cancer and *GSTT1* was observed in studies including healthy controls (adjusted OR = 1.79, 95% CI 1.18–2.68).

An additional analysis was performed on the different histologic types: lung adenocarcinoma comprised 252 cases, whereas lung squamous cell carcinoma comprised 64 of the 555 lung cancer Caucasian cases. The results are presented in Table 3; for adenocarcinoma, the results were similar to those previously presented on lung cancer as a whole. For squamous cell carcinoma, a significant association between *CYP1A1Msp1* polymorphism and lung cancer was found, but the number of cases was very small.

For Asian studies, no association was found between *CYP1A1Msp1*, *CYP1A1Ile*^{462}*Val*, *GSTM1* and lung cancer, but the statistical power to obtain an OR = 1.5, with a two-side test ($\alpha = 0.05$) is low (12% for *CYP1A1Msp1*, 23% for *CYP1A1Ile*^{462}*Val* and 30% for *GSTM1*).

The combined effect of three genes (*CYP1A1*, *GSTM1* and *GSTT1*) in Caucasians is presented in Table 4. A protective effect on lung cancer was observed with the combination of *CYP1A1 wild type*, *GSTM1 null*, *and GSTT1 non-null* genotypes. Adjusted ORs were 0.34 (95% CI 0.15–0.76) for the combination including *CYP1A1Msp1*, and 0.29 (95% CI 0.13–0.62) for *CYP1A1Ile*^{462}*Val*.

4. Discussion

This study includes a large number of non-smoking lung cancer cases, with three metabolic gene polymorphisms tested. The results of our analysis confirm previous evidence of an association between lung cancer risk and the *CYP1A1Ile*^{462}*Val* polymorphism in Caucasians, although the effect was weaker and no longer statistically significant if the analysis was restricted to healthy controls. The positive association could be due to the increase in microsomal enzyme activity for subjects carrying the Val variant allele [24–26]. As in our previous analysis [27], a borderline association was observed for *CYP1A1MspI* polymorphism and lung cancer. A similar result in Caucasian non-smokers was found in another pooled analysis conducted on part of these data [55]. In agreement with our previous analysis [27] and what has been already published [39,40,51,56–58], no association was found for *GSTM1* polymorphism and lung cancer risk, either in Caucasians or in Asians.

A significant association between lung cancer and *GSTT1* polymorphism was observed only when the analysis was restricted to studies including healthy controls. Three studies in Caucasian non-smoker not included in our analysis found no association between lung cancer and the *GSTT1* polymorphism, either when the design was a population-based study [56,58] or a hospital-based study [39]. It has to be pointed out, however, that one of the population-based studies only included a small number of non-smoking cases (eleven) [56]. Another population-based study found a protective effect of *GSTT1* polymorphism in never-smoker lung cancers [59].

In general, population controls are considered more representative of the general population, however in the present analysis the number of subjects in the stratified analysis according to the source of controls is very small, therefore the results may be a chance finding.

Although in our previous analysis [27] we reported publication biases for both *CYP1A1MspI* and *GSTM1* polymorphisms, this result was not confirmed in the present. For *GSTM1* deletion, the difference was probably due to inclusion of several new studies ($n = 7$), while for *CYP1A1MspI* polymorphism only 1 study was added, but it contained the largest number of cases ($n = 128$).

Looking at the combined effect of phase I and phase II polymorphisms on lung cancer, a protective effect was observed with the combination of *CYP1A1 wild type*, *GSTM1 null*, and *GSTT1 non-null* genotypes. This was seen when using either the MspI or the Ile/Val poly-

Table 3
Pooled odds ratios and confidence intervals for *CYP1A1*, *GSTM1* and *GSTT1* polymorphisms

	Cases	Controls	OR[a] (95% CI)	Adjusted OR[b] (95% CI)	Healthy controls		Hospital controls	
					OR[a] (95% CI)	Adj OR[b] (95% CI)	OR[a] (95% CI)	Adj OR[b] (95% CI)
Caucasians								
CYP1A1 MspI								
T/T	122	417	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	–	–
T/C, C/C	43	102	1.63 (0.99–2.7)	1.65(0.98–2.77)[c]	1.20 (0.65–2.2)	1.24(0.65–2.36)	–	–
CYP1A1 Ile^{462}Val								
Ile/Ile	143	656	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	–	–
Ile/Val, Val/Val	32	67	2.31(1.37–3.88)	2.04(1.17-3.54)[d]	2.08(1.16-3.72)	1.68(0.91–3.1)	–	–
GSTM1								
Present	242	965	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Null	289	1016	1.09(0.88–1.35)	1.03(0.84-1.26)[e]	1.03(0.77-1.37)	1.05(0.80–1.38)	0.97(0.61–1.50)	0.92(0.59–1.45)
GSTT1								
Present	281	835	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Null	97	191	0.96(0.69–1.32)	1.23(0.91–1.66)[f]	1.35(0.85–2.13)	1.79(1.18–2.68)	0.88(0.4–1.91)	0.84(0.38–1.89)
Asians								
CYP1A1 MspI								
T/T	18	43	1.00 (ref.)	1.00 (ref.)	–	–	–	–
T/C, C/C	28	95	0.74(0.37–1.49)	1.03(0.47–2.26)	–	–	–	–
CYP1A1 Ile^{462}Val								
Ile/Ile	30	116	1.00 (ref.)	1.00 (ref.)	–	–	–	–
Ile/Val, Val/Val	30	96	1.23(0.69–2.19)	1.30(0.71–2.38)	–	–	–	–
GSTM1								
Present	42	92	1.00 (ref.)	1.00 (ref.)	–	–	–	–
Null	51	118	1.00(0.6–1.67)	0.98(0.58–1.66)	–	–	–	–
Adenocarcinoma (Caucasians)								
CYP1A1 MspI								
T/T	59	358	1.00 (ref.)	1.00 (ref.)	–	–	–	–
T/C, C/C	19	89	1.39(0.73–2.64)	1.59(0.81–3.1)	–	–	–	–
CYP1A1 Ile^{462}Val								
Ile/Ile	46	411	1.00 (ref.)	1.00 (ref.)	–	–	–	–
Ile/Val, Val/Val	15	58	2.83(1.38–5.79)	2.51(1.21–5.19)	–	–	–	–
GSTM1								
Present	119	781	1.00 (ref.)	1.00 (ref.)	–	–	–	–
Null	117	818	0.91 (0.68–1.22)	0.83 (0.62–1.12)	–	–	–	–
GSTT1								
Present	130	642	1.00 (ref.)	1.00 (ref.)	–	–	–	–
Null	30	154	0.62(0.39–0.99)	0.62(0.39–0.99)	–	–	–	–
Squamous Cell Carcinoma (Caucasians)								
CYP1A1 MspI								
T/T	2	308	1.00 (ref.)	1.00 (ref.)	–	–	–	–
T/C, C/C	4	69	7.25(1.18–44.66)	15.47(2.30–103.91)	–	–	–	–
CYP1A1 Ile^{462}Val								
Ile/Ile	15	561	1.00 (ref.)	1.00 (ref.)	–	–	–	–
Ile/Val, Val/Val	4	53	2.02(0.66–6.15)	3.16(0.92–10.89)	–	–	–	–
GSTM1								
Present	28	668	1.00 (ref.)	1.00 (ref.)	–	–	–	–
Null	35	700	1.30(0.78–2.18)	1.23(0.73–2.06)	–	–	–	–
GSTT1								
Present	35	582	1.00 (ref.)	1.00 (ref.)	–	–	–	–
Null	8	148	0.61(0.26–1.39)	0.81(0.36–1.80)	–	–	–	–

[a] Mantel-Haenszel OR adjusted for study.
[b] OR adjusted for study, sex and age.
[c] $p = 0.06$.
[d] $p = 0.01$.
[e] $p = 0.77$.
[f] $p = 0.18$.

Table 4
Gene–gene interaction between *CYP1A1*, *GSTM1* and *GSTT1*

	GSTM1	*GSTT1*	Cases	Controls	OR (95% CI)
CYP1A1 MspI					
T/T	Present	Present	31	87	1.00 (Ref.)
T/T	Present	Null	9	23	0.41 (0.13–1.27)
T/T	Null	Present	28	101	0.34 (0.15–0.76)
T/T	Null	Null	19	17	0.89 (0.31–2.51)
T/C, C/C	Present	Present	7	21	0.85 (0.27–2.7)
T/C, C/C	Present	Null	3	5	0.90 (0.11–7.37)
T/C, C/C	Null	Present	11	27	0.52 (0.18-1.54)
T/C, C/C	Null	Null	4	5	0.59 (0.09–3.77)
CYP1A1 Ile^{462}Val					
Ile/Ile	Present	Present	35	100	1.00 (Ref.)
Ile/Ile	Present	Null	9	26	0.44 (0.15–1.3)
Ile/Ile	Null	Present	29	115	0.29 (0.13–0.62)
Ile/Ile	Null	Null	23	16	1.11 (0.41–3.01)
Ile/Val, Val/Val	Present	Present	2	8	0.67 (0.11–4.06)
Ile/Val, Val/Val	Present	Null	2	0	–
Ile/Val, Val/Val	Null	Present	7	9	2.08 (0.51–8.54)
Ile/Val, Val/Val	Null	Null	1	2	0.14 (0.01–2.33)

ORs are adjusted for study, sex and age (Caucasians).

morphism as the CYP1A1 variant (Table 4). This result could be explained by a peculiar metabolic pathway of the carcinogens that are responsible for lung cancer in non-smokers: such carcinogens could be preferentially activated by phase I enzymes (*CYP1A1*), and then preferentially detoxified by phase II enzyme *GSTT1*. The wild type *CYP1A1* could be responsible for a decreased activation, while the deletion of *GSTM1* could increase the amount of products in the preferential pathway involving *GSTT1*. However, the result could also be due to chance, given the large amount of comparisons performed in this analysis.

In Asians, no association was observed between non-smoking lung cancer and *CYP1A1MspI*. A similar result was observed recently in non-smoking Japanese women [51], in Chinese non-smokers [52] and in Japanese light smokers [53,54]. The analysis of the data from Asian populations suggests also a lack of association between *CYP1A1Ile^{462}Val* polymorphism and risk of lung cancer, as already reported in another study on Chinese subjects [52], however the power of the present pooled analysis is still small to draw any conclusion. We could not perform a pooled analysis on *GSTT1* in Asians because only one study had information on this polymorphism. The role of *GSTT1* polymorphism in Asian is still controversial: a study [60] found no association between *GSTT1* polymorphism and adenocarcinoma in Japanese non-smokers, while recently [40] such an association was found in non-smoking Chinese subjects from Hong Kong.

Caucasian and Asian results on associations between metabolic gene polymorphisms and non-smoker lung cancers may differ because of differences in environmental and dietary factors between ethnic groups. However, the real issue may be the lack of statistical power of studies conducted in Asian populations.

The mechanisms underlying the associations described in this paper have not yet been studied in detail. While in lung cancer among smokers it has been hypothesized that polymorphisms in Phases 1 and 2 genes may induce specific DNA damage through adducts formation, this aspect has not been investigated in non-smokers lung cancer [61].

The GSEC database contains individual data on 55,000 subjects. Such large number of data allows to perform the largest study on lung cancer in non-smokers, who represent less than 10% of lung cancer cases, and to perform a combined analysis on the effects of the three different gene polymorphisms. The dataset include information on several variables, such as sex, age and ethnicity, which allowed us to perform

separate analyses for Caucasians and Asians, and to adjust the results for the confounding effect of study, sex and age. The availability of information on potential confounding variables makes the pooled-analyses preferable over the meta-analysis [62].

Several types of bias could affect the results of our pooled analysis and they were discussed in our previous study [27]. Among these limitations, heterogeneity and inclusion bias could affect our results. However, the statistical analysis showed no evidence of inclusion bias in our data. Another limitation is the possible confounding effect of the different source of controls in different studies. We checked this factor by stratifying the analysis according to healthy or hospitalised controls, and found some heterogeneity in the associations.

The presence of misclassification in the definition of smoking status, and in genotyping laboratory analysis is also possible. A misclassification of the Exon 7 polymorphism could not be avoided in the present analysis, as previously discussed [27], since the included studies could not distinguish the Ile/Val polymorphism from the close Thr461Asn. Despite their close distance, we found no linkage disequilibrium between these two polymorphisms, while a significant linkage between the *CYP1A1Msp1* and *CYP1A1Ile*^{462}*Val* polymorphism in the studies that tested both the Ile/Val polymorphism and the Thr461Asn polymorphisms. However, the fact that most of the studies did not distinguish between the Ile/Val polymorphism and the close Thr461Asn prevented us from testing the role of the allele consisting of both the *CYP1A1Msp1* and *CYP1A1Ile*^{462}*Val* polymorphisms in non-smokers lung cancer risk.

Other risk factors, such as second hand smoking, air pollution, radon exposure and diet could be important in the study of lung cancer in non-smokers, however we have very few subjects with information on second hand smoking and area of residence, and no information at all on diet and on radon exposure. For Caucasian subjects, the possible confounding effect of second hand smoking and area of residence was investigated. The adjustment of the data for these variables in multivariate logistic models did not change substantially the results. One population in our sample [65] was living in a highly exposed area: however, they represent a small group of the whole data set (only 23 cases, 7 of which with reported high level of exposure to PAHs), therefore we don't think that they could affect strongly our results.

In conclusion, we found an association between *CYP1A1Ile*^{462}*Val* polymorphism and lung cancer in a large sample of Caucasian non-smokers. A protective effect of the combination of *CYP1A1 wild type*, *GSTM1 null*, and *GSTT1 non-null* polymorphisms is reported, which could be due to different metabolic pathways involved in the metabolism of carcinogens in non-smoker lung cancers.

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References

[1] J. Ferlay, F. Bray, P. Pisani, D.M. Parkin, GLOBOCAN 2000: Cancer Incidence, Mortality and Prevalence Worldwide (1.0), Cancer Base no. 5, IARC, Lyon, France, 2001.
[2] M. Palmarini, H. Fan, Retrovirus-induced ovine pulmonary adenocarcinoma, an animal model for lung cancer, JNCI 93 (2001) 1603–1614.
[3] L.T. Nordquist, G.R. Simon, A.C. Antor, W.M. Alberts, G. Bepler, Improved survival in never-smokers vs current smokers with primary adenocarcinoma of the lung, Chest 126 (2004) 347–351.
[4] International Agency of Research on Cancer, Tobacco Smoke and Involuntary Smoking IARC Monographs of Carcinogenic Risks to Humans, vol. 83, IARC, Lyon, France, 1994.
[5] W.J. Blot, J.F. Fraumeni, Cancers of the lung and pleura, in: D. Schottenfeld, J.G. Searle, J.F. Fraumeni (Eds.), Cancer Epidemiology and Prevention, Oxford University Press, New York, NY, 1996, pp. 637–665.
[6] J.A. Baron, T.E. Rohan, Tobacco, in: J.G. Schottenfeld, J.F. Searle, Fraumeni (Eds.), Cancer Epidemiology and Prevention, Oxford University Press, New York, NY, 1996, pp. 269–288.
[7] P. Vineis, M. Alavanja, P. Buffler, E. Fontham, S. Franceschi, Y.T. Gao, P.C. Gupta, A. Hackshaw, E. Matos, J. Samet, F. Sitas, J. Smith, L. Stayner, K. Straif, M.J. Thun, H.E. Wichmann, A.H. Wu, D. Zaridze, R. Peto, R. Doll, Tobacco and cancer: recent epidemiological evidence, J. Natl. Cancer Inst. 96 (2004) 99–106.
[8] R. Doll, R. Peto, K. Wheatley, R. Gray, I. Sutherland, Mortality in relation to smoking: 40 years' observations on male British doctors, BMJ 309 (1994) 901–11.

[9] International Agency of Research on Cancer, IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Tobacco Smoking, IARC, Lyon, France, 1986.
[10] ICF Clement, U.S. Environmental Protection Agency, Toxicological Profile for B(a)P, Oak Ridge National Laboratory, Oak Ridge, TN, 1990.
[11] D. Phillips, M. Castegnaro, Standardization and validation of DNA adducts postlabelling methods: report of interlaboratory trials and production of recommended protocols, Mutagenesis 14 (1999) 301–315.
[12] A.K. Hackshaw, M.R. Law, N.J. Wald, The accumulated evidence on lung cancer and environmental tobacco smoke, BMJ 315 (1997) 980–988.
[13] E.T. Fontham, Protective dietary factors and lung cancer, Int. J. Epidemiol. 19 (Suppl. 1) (1990) S32–S42.
[14] M.C. Alavanja, R.C. Brownson, J. Benichou, Estimating the effect of dietary fat on the risk of lung cancer in nonsmoking women, Lung Cancer 14 (Suppl. 1) (1996) S63–S74.
[15] Y.C. Ko, C.H. Lee, M.J. Chen, C.C. Huang, W.Y. Chang, H.J. Lin, H.Z. Wang, P.Y. Chang, Risk factors for primary lung cancer among non-smoking women in Taiwan, Int. J. Epidemiol. 26 (1997) 24–31.
[16] T.J. Wang, B.S. Zhou, J.P. Shi, Lung cancer in nonsmoking Chinese women: a case-control study, Lung Cancer 14 (Suppl. 1) (1996) S93–S98.
[17] Y.T. Gao, Risk factors for lung cancer among nonsmokers with emphasis on lifestyle factors, Lung Cancer 14 (Suppl. 1) (1996) S39–S45.
[18] M. Lang, O. Pelkonen, Metabolism and chemical carcinogenesis, in: P. Vineis, N. Malats, M. Lang, A. d'Errico, N. Caporaso, J. Cuzick, P. Boffetta (Eds.), Metabolic Polymorphisms and Susceptibility to Cancer, vol. 148, IARC Scientific Publications, Lyon, 1999, pp. 13–22.
[19] P. Vineis, H. Bartsch, N. Caporaso, A.M. Harrington, F.F. Kadlubar, M.T. Landi, C. Malaveille, P.G. Shields, P. Skipper, G. Talaska, Genetically based *N*-acetyltransferase metabolic polymorphism and low-level environmental exposure to carcinogens, Nature 369 (1994) 154–156.
[20] S.J. Garte, C. Zocchetti, E. Taioli, Gene-environment interactions in the application of biomarkers of cancer susceptibility in epidemiology, IARC Monogr. 142 (1997) 251–264.
[21] M.E. McManus, W.M. Burgess, M.E. Veronese, A. Huggett, L.C. Quattrochi, R.H. Tukey, Metabolism of 2-acetylaminofluorene and benzo(a)pyrene and activation of food-derived heterocyclic amine mutagens by human cytochromes P-450, Cancer Res. 50 (1990) 3367–3376.
[22] T. Shimada, C.-H. Yun, H. Yamazaki, J.-C. Gautier, P.H. Beaune, F.P. Guengerich, Characterization of human lung microsomal cytochrome P-450 1A1 and its role in the oxidation of chemical carcinogens, Mol. Pharmacol. 41 (1992) 856–864.
[23] F.P. Guengerich, T. Shimada, Oxidation of toxic and carcinogenic chemicals by human cytochrome P-450 enzymes, Chem. Res. Toxicol. 4 (1991) 391–407.
[24] G. Cosma, F. Crofts, E. Taioli, P. Toniolo, S.J. Garte, Relationship between genotype and function of the human CYP1A1 gene, J. Toxicol. Environ. Health 40 (1993) 309–316.
[25] F. Crofts, E. Taioli, J. Trachman, G.N. Cosma, D. Curie, P. Toniolo, S.J. Garte, Functional significance of different human CYP1A1 genotypes, Carcinogenesis 15 (1994) 2961–2963.
[26] C. Kiyohara, T. Hirohata, S. Inutsuka, The relationship between aryl hydrocarbon hydroxylase and polymorphisms of the CYP1A1 gene, Jpn. J. Cancer Res. 87 (1996) 18–24.
[27] R.J. Hung, P. Boffetta, J. Brockmoller, et al., CYP1A1 and *GSTM1* genetic polymorphisms and lung cancer risk in Caucasian non-smokers: a pooled analysis, Carcinogenesis 24 (2003) 875–882.
[28] J.D. Hayes, D.J. Pulford, The glutathione *S*-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance, Crit. Rev. Biochem. Mol. Biol. 30 (1995) 445–600.
[29] S. Zhong, A.F. Howie, B. Ketterer, J. Taylor, J.D. Hayes, G.J. Beckett, C.G. Wathen, C.R. Wolf, N.K. Spurr, Glutathione *S*-transferase mu locus: use of genotyping and phenotyping assays to assess association with lung cancer susceptibility, Carcinogenesis 12 (1991) 1533–1537.
[30] S.E. Pemble, S.R. Schroeder, S.R. Spencer, D.I. Meyer, E. Hallier, H.M. Bolt, B. Ketterer, J.B. Taylor, Human glutathione *S*-transferase theta (*GSTT1*): cDNA cloning and the characterization of a genetic polymorphism, Biochem. J. 300 (1994) 271–276.
[31] S. Landi, Mammalian class theta GST and differential susceptibility to carcinogens: a review, Mutat. Res. 463 (2000) 247–283.
[32] S. Garte, L. Gaspari, A.K. Alexandrie, et al., Metabolic gene polymorphism frequencies in control populations, Cancer Epidemiol. Biomarkers Prev. 10 (2001) 1239–1248.
[33] M. Deakin, J. Elder, C. Hendricks, D. Peckham, D. Baldwin, C. Pantin, N. Wild, P. Leopard, D.A. Bell, P. Jones, H. Duncan, K. Brannigan, J. Alldersea, A.A. Fryer, R.C. Strange, Glutathione *S*-transferase *GSTT1* genotypes and susceptibility to cancer: studies of interactions with *GSTM1* in lung, oral, gastric and colorectal cancers, Carcinogenesis 17 (1996) 881–884.
[34] R. El-Zein, J.B. Zwischenberger, T.G. Wood, S.Z. Abdel-Rahman, C. Brekelbaum, W.W. Au, Combined genetic polymorphism and risk for development of lung cancer, Mutat. Res. 381 (1997) 189–200.
[35] K.T. Kelsey, M.R. Spitz, Z.-F. Zuo, J.K. Wiencke, Polymorphisms in the glutathione *S*-transferase class mu and theta genes interact and increase susceptibility to lung cancer in minority populations (Texas, United States), Cancer Causes Control 8 (1997) 554–559.
[36] J. To-Figueras, M. Gene, J. Gomez-Catalan, M.C. Galan, M. Fuentes, J.M. Ramon, M. Rodamilans, E. Huguet, J. Corbella, Glutathione *S*-transferase M1 (*GSTM1*) and T1 (*GSTT1*) polymorphisms and lung cancer risk among Northwestern Mediterraneans, Carcinogenesis 18 (1997) 1529–1533.
[37] J. Salagovic, I. Kalina, J. Stubna, V. Habalova, M. Hrivnak, L. Valansky, A. Kohut, E. Biros, Genetic polymorphism of glutathione *S*-transferases M1 and T1 as a risk factor in lung and bladder cancers, Neoplasma 45 (1998) 312–317.
[38] S.T. Saarikoski, A. Voho, M. Reinikainen, S. Anttila, A. Karjalainen, C. Malaveille, H. Vainio, K. Husgafvel-Pusiainen, A.

Hirvonen, Combined effect of polymorphic GST genes on individual susceptibility to lung cancer, Int. J. Cancer 77 (1998) 516–521.
[39] N. Malats, A.M. Camus-Radon, F. Nyberg, W. Ahrens, V. Constantinescu, A. Mukeria, S. Benhamou, H. Batura-Gabryel, I. Bruske-Hohlfeld, L. Simonato, A. Menezes, S. Lea, M. Lang, P. Boffetta, Lung cancer risk in nonsmokers and *GSTM1* and *GSTT1* genetic polymorphism, Cancer Epidemiol. Biomarkers Prev. 9 (2000) 827–833.
[40] M. Chan-Yeung, K.C. Tan-Un, M.S. Ip, K.W. Tsang, S.P. Ho, J.C. Ho, H. Chan, W.K. Lam, Lung cancer susceptibility and polymorphisms of glutathione-*S*-transferase genes in Hong Kong, Lung Cancer 45 (2004) 155–160.
[41] G.B. Smith, P.A. Harper, J.M. Wong, M.S. Lam, K.R. Reid, D. Petsikas, T.E. Massey, Human lung microsomal cytochrome P4501A1 (CYP1A1) activities: impact of smoking status and CYP1A1, aryl hydrocarbon receptor, and glutathione *S*-transferase M1 genetic polymorphisms, Cancer Epidemiol. Biomarkers Prev. 10 (2001) 839–853.
[42] L. Le Marchand, L. Sivaraman, L. Pierce, A. Seifried, A. Lum, L.R. Wilkens, A.F. Lau, Associations of CYP1A1, *GSTM1*, and CYP2E1 polymorphisms with lung cancer suggest cell type specificities to tobacco carcinogens, Cancer Res. 58 (1998) 4858–4863.
[43] E. Taioli, International Collaborative Study on genetic susceptibility to environmental carcinogens, Cancer Epidemiol. Biomarkers Prev. 8 (1999) 727–728.
[44] H. Sugimura, K. Wakai, K. Genka, K. Nagura, H. Igarashi, K. Nagayama, A. Ohkawa, S. Baba, B.J. Morris, S. Tsugane, Y. Ohno, C. Gao, Z. Li, T. Takezaki, K. Tajima, T. Iwamasa, Association of Ile462Val (Exon 7) polymorphism of cytochrome P450 IA1 with lung cancer in the Asian population: further evidence from a case-control study in Okinawa, Cancer Epidemiol. Biomarkers Prev. 7 (1998) 413–417.
[45] M. Egger, D. Smith, M. Schneider, C. Minder, Bias in meta-analysis detected by a simple, graphical test, BMJ 315 (1997) 629–34.
[46] W.G. Cochran, The combination of estimates from different experiments, Biometrics 10 (1954) 101–129.
[47] N.E. Breslow, N.E. Day, The analysis of case-control studies Statistical Methods in Cancer Research, vol. 1, IARC Scientific Publications, Lyons, 1980.
[48] I. Cascorbi, J. Brockmoller, I. Roots, A C4887A polymorphism in exon 7 of human CYP1A1: population frequency, mutation linkages, and impact on lung cancer susceptibility, Cancer Res. 56 (1996) 4965–4969.
[49] E. Taioli, J. Ford, J. Trachman, Y. Li, R. Demopoulos, S. Garte, Lung cancer risk and CYP1A1 genotype in African Americans, Carcinogenesis 19 (1998) 813–817.
[50] C.M. Dresler, C. Fratelli, J. Babb, L. Everley, A.A. Evans, M.L. Clapper, Gender differences in genetic susceptibility for lung cancer, Lung Cancer 30 (2000) 153–160.
[51] C. Kiyohara, K. Wakai, H. Mikami, K. Sido, M. Ando, Y. Ohno, Risk modification by CYP1A1 and *GSTM1* polymorphisms in the association of environmental tobacco smoke and lung cancer: a case-control study in Japanese nonsmoking women, Int. J. Cancer 107 (2003) 139–144.
[52] N. Song, W. Tan, D. Xing, D. Lin, CYP 1A1 polymorphism and risk of lung cancer in relation to tobacco smoking: a case-control study in China, Carcinogenesis 22 (2001) 11–16.
[53] K. Nakachi, K. Imai, S. Hayashi, J. Watanabe, K. Kawajiri, Genetic susceptibility to squamous cell carcinoma of the lung in relation to cigarette smoking dose, Cancer Res. 51 (1991) 5177–5180.
[54] T. Okada, K. Kawashima, S. Fukushi, T. Minakuchi, S. Nishimura, Association between a cytochrome P450 CYPIA1 genotype and incidence of lung cancer, Pharmacogenetics 4 (1994) 333–340.
[55] L. Le Marchand, C. Guo, S. Benhamou, C. Bouchardy, I. Cascorbi, M.L. Clapper, S. Garte, A. Haugen, M. Ingelman-Sundberg, M. Kihara, A. Rannug, D. Ryberg, I. Stucker, H. Sugimura, E. Taioli, Pooled analysis of the CYP1A1 exon 7 polymorphism and lung cancer (United States), Cancer Causes Control 14 (2003) 339–346.
[56] V. Nazar-Stewart, T.L. Vaughan, P. Stapleton, J. Van Loo, B. Nicol-Blades, D.L. Eaton, A population-based study of glutathione *S*-transferase M1, T1 and P1 genotypes and risk for lung cancer, Lung Cancer 40 (2003) 247–258.
[57] J. Schneider, U. Bernges, M. Philipp, H.J. Woitowitz, *GSTM1*, *GSTT1*, and GSTP1 polymorphism and lung cancer risk in relation to tobacco smoking, Cancer Lett. 208 (2004) 65–74.
[58] A.K. Alexandrie, F. Nyberg, M. Warholm, A. Rannug, Influence of CYP1A1, *GSTM1*, *GSTT1*, and NQO1 genotypes and cumulative smoking dose on lung cancer risk in a Swedish population, Cancer Epidemiol. Biomarkers Prev. 13 (2004) 908–914.
[59] S.M. Hou, S. Falt, F. Nyberg, Glutathione *S*-transferase T1-null genotype interacts synergistically with heavy smoking on lung cancer risk, Environ. Mol. Mutagen. 38 (2001) 83–86.
[60] N. Sunaga, T. Kohno, N. Yanagitani, H. Sugimura, H. Kunitoh, T. Tamura, Y. Takei, S. Tsuchiya, R. Saito, J. Yokota, Contribution of the NQO1 and *GSTT1* polymorphisms to lung adenocarcinoma susceptibility, Cancer Epidemiol. Biomarkers Prev. 11 (2002) 730–738.
[61] K. Alexandrov, I. Cascorbi, M. Rojas, G. Bouvier, E. Kriek, H. Bartsch, CYP1A1 and *GSTM1* genotypes affect benzo[*a*]pyrene DNA adducts in smokers' lung: comparison with aromatic/hydrophobic adduct formation, Carcinogenesis 23 (2002) 1969–1977.
[62] M. Blettner, W. Sauerbrei, B. Schlehofer, T. Scheuchenpflug, C. Friedenreich, Traditional reviews, meta-analyses and pooled analyses in epidemiology, Int. J. Epidemiol. 28 (1999) 1–9.
[63] I. Roots, J. Brockmoller, N. Drakoulis, R. Loddenkemper, Mutant genes of cytochrome P-450IID6, glutathione *S*-transferase class Mu, and arylamine N-acetyltransferase in lung cancer patients, Clin. Investig. 70 (1992) 307–319.
[64] J. Brockmoller, R. Kerb, N. Drakoulis, M. Nitz, I. Roots, Genotype and phenotype of glutathione *S*-transferase class mu isoenzymes mu and psi in lung cancer patients and controls, Cancer Res. 53 (1993) 1004–1011.
[65] D. Butkiewicz, K.J. Cole, D.H. Phillips, C.C. Harris, M. Chorazy, *GSTM1*, GSTP1, CYP1A1 and CYP2D6 polymorphisms in lung cancer patients from an environmentally polluted region of Poland: correlation with lung DNA adduct levels, Eur. J. Cancer Prev. 8 (1999) 315–323.

[66] A. Gsur, G. Haidinger, P. Hollaus, I. Herbacek, S. Madersbacher, K. Trieb, N. Pridun, A. Mohn-Staudner, N. Vetter, C. Vutuc, M. Micksche, Genetic polymorphisms of CYP1A1 and *GSTM1* and lung cancer risk, Anticancer Res. 21 (2001) 2237–2242.
[67] T. Tefre, D. Ryberg, A. Haugen, D.W. Nebert, V. Skaug, A. Brogger, A.L. Borresen, Human CYP1A1 (cytochrome P(1)450) gene: lack of association between the Msp I restriction fragment length polymorphism and incidence of lung cancer in a Norwegian population, Pharmacogenetics 1 (1991) 20–25.
[68] D. Ryberg, A. Hewer, D.H. Phillips, A. Haugen, Different susceptibility to smoking-induced DNA damage among male and female lung cancer patients, Cancer Res. 54 (1994) 5801–5803.
[69] D. Ryberg, V. Skaug, A. Hewer, D.H. Phillips, L.W. Harries, C.R. Wolf, D. Ogreid, A. Ulvik, P. Vu, A. Haugen, Genotypes of glutathione transferase M1 and P1 and their significance for lung DNA adduct levels and cancer risk, Carcinogenesis 18 (1997) 1285–1289.
[70] A. Hirvonen, K. Husgafvel-Pursiainen, S. Anttila, H. Vainio, The *GSTM1* null genotype as a potential risk modifier for squamous cell carcinoma of the lung, Carcinogenesis 14 (1993) 1479–1481.
[71] S.J. London, A.K. Daly, J.B. Leathart, W.C. Navidi, J.R. Idle, Lung cancer risk in relation to the CYP2C9*1/CYP2C9*2 genetic polymorphism among African–Americans and Caucasians in Los Angeles County, California, Pharmacogenetics 6 (1996) 527–533.
[72] S.J. London, A.K. Daly, J. Smart, Lung cancer risk in relation to genetic polymorphisms of microsomal epoxide hydrolase among African-Americans and Caucasians in Los Angeles County, Lung Cancer 28 (2000) 147–155.
[73] S.J. Lewis, N.M. Cherry, R.M. Niven, P.V. Barber, A.C. Povey, *GSTM1*, *GSTT1* and GSTP1 polym*orphisms and lung cancer risk, Cancer Lett 180 (2002) 165–171.
[74] A.K. Alexandrie, M.I. Sundberg, J. Seidegard, G. Tornling, A. Rannug, Genetic susceptibility to lung cancer with special emphasis on CYP1A1 and *GSTM1*: a study on host factors in relation to age at onset, gender and histological cancer types, Carcinogenesis 15 (1994) 1785–1790.
[75] E. Reszka, W. Wasowicz, K. Rydzynski, N. Szeszenia-Dabrowska, W. Szymczak, Glutathione *S*-transferase M1 and P1 metabolic polymorphism and lung cancer predisposition, Neoplasma 50 (2003) 357–362.
[76] A. Risch, H. Wikman, S. Thiel, P. Schmezer, L. Edler, P. Drings, H. Dienemann, K. Kayser, V. Schulz, B. Spiegelhalder, H. Bartsch, Glutathione-*S*-transferase M1, M3, T1 and P1 polymorphisms and susceptibility to non-small-cell lung cancer subtypes and hamartomas, Pharmacogenetics 11 (2001) 757–764.
[77] A. Ruano-Ravina, A. Figueiras, L. Loidi, J.M. Barros-Dios, *GSTM1* and *GSTT1* polymorphisms, tobacco and risk of lung cancer: a case-control study from Galicia, Spain, Anticancer Res. 23 (2003) 4333–4337.
[78] B. Schoket, D.H. Phillips, S. Kostic, I. Vincze, Smoking-associated bulky DNA adducts in bronchial tissue related to CYP1A1 MspI and *GSTM1* genotypes in lung patients, Carcinogenesis 19 (1998) 841–846.
[79] B. Schoket, G. Papp, K. Levay, G. Mrackova, F.F. Kadlubar, I. Vincze, Impact of metabolic genotypes on levels of biomarkers of genotoxic exposure, Mutat Res. 482 (2001) 57–69.
[80] M.R. Spitz, C.M. Duphorne, M.A. Detry, P.C. Pillow, C.I. Amos, L. Lei, M. de Andrade, X. Gu, W.K. Hong, X. Wu, Dietary intake of isothiocyanates: evidence of a joint effect with glutathione *S*-transferase polymorphisms in lung cancer risk, Cancer Epidemiol. Biomarkers Prev. 9 (2000) 1017–1020.
[81] I. Persson, I. Johansson, Y.C. Lou, Q.Y. Yue, L.S. Duan, L. Bertilsson, M. Ingelman-Sundberg, Genetic polymorphism of xenobiotic metabolizing enzymes among Chinese lung cancer patients, Int. J. Cancer 81 (1999) 325–329.
[82] Q. Lan, X. He, D.J. Costa, L. Tian, N. Rothman, G. Hu, J.L. Mumford, Indoor coal combustion emissions, *GSTM1* and *GSTT1* genotypes, and lung cancer risk: a case-control study in Xuan Wei, China, Cancer Epidemiol. Biomarkers Prev. 9 (2000) 605–608.