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CYP1A1, *CYP2E1* and *GSTM1* genetic polymorphisms. The effect of single and combined genotypes on lung cancer susceptibility in Chilean people

L. Quiñones[a], D. Lucas[b,*], J. Godoy[a], D. Cáceres[a], F. Berthou[b], N. Varela[a], K. Lee[a], C. Acevedo[a], L. Martínez[a], A.M. Aguilera[a], L. Gil[a]

[a]*Faculty of Medicine, Laboratory of Environmental Toxicology and School of Public Health, University of Chile, Santiago, Chile*
[b]*Faculty of Medicine, Laboratory. Biochimie-Nutrition EA948, B.P. 29285 Brest, France*

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Abstract

CYP1A1, *CYP2E1* and *GSTM1* polymorphisms were evaluated in Chilean healthy controls and lung cancer patients. In the Chilean healthy group, frequencies of *CYP1A1* variant alleles for *MspI* (m2 or *CYP1A1*2A*) and ile/val (val or *CYP1A1*2B*) polymorphisms were 0.25 and 0.33, respectively. Frequencies of variant alleles C (*CYP2E1*6*) and c2 (*CYP2E1*5B*) for *CYP2E1* were 0.21 and 0.16, respectively and frequency for *GSTM1*(−) was 0.24. The presence of variant alleles for *GSTM1*, *MspI* and Ile/val polymorphisms was more frequent in cases than in controls. However, frequencies for the c2 and C alleles were not significantly different in controls and in cases. The estimated relative risk for lung cancer associated to a single mutated allele in *CYP1A1*, *CYP2E1* or *GSTM1* was 2.41 for m2, 1.69 for val, 1.16 for C, 0.71 for c2 and 2.46 for *GSTM1*(−). The estimated relative risk was higher for individuals carrying combined *CYP1A1* and *GSTM1* mutated alleles (m2/val, OR = 6.28; m2/*GSTM1*(−), OR = 3.56) and lower in individuals carrying *CYP1A1* and *CYP2E1* mutated alleles (m2/C, OR = 1.39; m2/c2, OR = 2.00; val/C, OR = 1.45; val/c2, OR = 0.48; not significant). The OR values considering smoking were 4.37 for m2, 4.05 for val, 3.47 for *GSTM1*(−), 7.38 for m2/val and 3.68 for m2/*GSTM1*(−), higher values than those observed without any stratification by smoking. Taken together, these findings suggest that Chilean people carrying single or combined *GSTM1* and *CYP1A1* polymorphisms could be more susceptible to lung cancer induced by environmental pollutants such as polycyclic aromatic hydrocarbons.

Keywords: CYP1A1; CYP2E1; GSTM1; Polymorphisms; Lung cancer risk; Molecular epidemiology

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) present in smoked foods, tobacco smoke and ubiquitous in urban environments in large cities, are believed to be responsible for an elevated risk in certain cancers, specially lung cancer. PAHs are metabolized to reactive DNA binding diol epoxides by phase I enzymes cytochrome P4501A1 (CYP1A1) and detoxified by phase II enzymes, including glutathione S-transferase (GSTM1) [1,2] before reaching their target. It is possible that individual variations in metabolic activities in each phase or in the coordination of these two phases regulate the clearance of DNA toxic metabolites and

* Corresponding author. Laboratory of Biochemistry-EA948, Faculty of Medicine, 22, Avenue C. Desmoulins, BP-815 – 29285 Brest Cedex, France. Tel.: +33-2-98-016-465; fax: +33-2-98-016-603.

E-mail address: daniele.lucas@univ-brest.fr (D. Lucas).

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might be partially responsible for individual host susceptibility to PAHs exposure-related cancer.

CYP1A1 is an enzyme with aryl hydrocarbon hydroxylase activity (AHH). CYP1A1 high activity has been shown to be associated with high lung cancer risks [3–5], The entire *CYP1A1* gene has been sequenced [6] and two separate point mutations have been reported: one in the 3′- non-coding region, *MspI* polymorphism[1] [7] and the other within exon 7, ile/val polymorphism [8]. More recently, two other CYP1A1 polymorphisms have been reported, m3 allele[1] also in the 3′-flanking region which seems to be specific to people of African descent [9,10] and m4 allele[1] located in exon 7, that exchanges threonine 461 with asparagine and that has been found present in German, Polish and Turkish populations [11,12]. Though the relationship of these mutations with AHH inducibility has not been fully established [4,5], it has been suggested that homozygotes of variant allele of each mutation are correlated with an enhanced susceptibility to lung cancer, specially in Japanese smokers [7]. However, contradictory data have been published on this topic and a recent meta-analysis by Houlston et al. [13] did not show significant associations between CYP1A1 genetic polymorphisms and lung cancer.

On the other hand, CYP2E1 has been proposed as a possible cancer marker enzyme due to its activity toward several carcinogenic xenobiotics as for example ethanol, benzene, N-nitrosodimethylamine, styrene, butadiene and urethane [14–17]. Two point mutations have been identified upstream of the CYP2E1 gene in the regulatory elements which are completely linked (*RsaI* and *PstI)*[1]. Other mutations have been identified within intron 6 of the gene (*DraI)*[1] [18].

GSTM1 is one of the enzymes belonging to a family of glutathione transferases that is polymorphic with a deficient activity in approximately 50% of the Caucasian population [19]. Lack of this detoxification activity has been demonstrated to be caused by an inherited homozygous deletion of the gene [20] which has been related to ethnic variation [21].

Polymorphisms in *CYP1A1*, *CYP2E1* and *GSTM1* have been associated to an increase of cancer risk in lung, bladder, liver, pharynx, larynx, skin, rectum and colon [22–33].

In Chile the mortality rate for lung cancer has increased from 2 /100 000 inhabitants in 1935 to 13/100 000 in 1996, although the rate of mortality in some mining regions might reach 35/100 000. The rate of mortality for lung, bronchia and trachea is the second cause of mortality by cancer with around 1700 cases a year. Santiago, Chile which is a highly polluted city specially in winter, has recently been declared a saturated zone to ozone, carbon monoxide and particulate matter (PM10). In a previous work, we have shown that organic extracts from Santiago's airborne particles are highly mutagenic and contain high levels of carcinogenic PAHs, including benzo(a)pyrene [34–37]. In this work, we have investigated the distribution of single and combined genotypes of *CYP1A1* (*MspI* and ile/val), *CYP2E1* (C and c2) and *GSTM1* in two Chilean groups (healthy controls and lung cancer). We have also studied how the associated risk for smoking related lung cancers is modified by the combination of *CYP1A1*, *CYP2E1* and *GSTM1* polymorphisms.

2. Materials and methods

2.1. Study design and sample size

In order to evaluate the relationship between genetic polymorphisms and lung cancer risk, a case-control study was designed. The following criteria were taken in account to calculate the sample size: (a) 15% expected frequency for the allele c2 (the less frequent allele) in the chilean population [37]; (b) CI 95%$(1-\alpha)$; (c) a β value of 20%; (d) a relationship 1:2 between cases and controls and a risk three times higher for lung cancer for the individuals carrying unfavorable alleles in the cases. This gives a size of 122 controls and 61 cases.

2.2. Study population

Lung cancer patients and controls were recruited from the Santiago of Chile area. Blood samples were obtained from 174 healthy Chilean controls

[1] According to the recommended nomenclature of the human polymorphic genes, *CYP1A1* wild type allele: *CYP1A1*1A*; *MspI* or *m2 allele*: *CYP1A1*2A*; *Val* allele: *CYP1A1*2B*; *CYP1A1 m3* allele: *CYP1A1*3 CYP1A1 m4* allele: *CYP1A1*4*; *CYP2E1* wild type allele: *CYP2E1*1A*; *c2 (PstI/RsaI)* allele: *CYP2E1*5B*; *C (DraI)* allele: *CYP2E1*6*. (http://www.imm.ki.se/CYPalleles).

Table 1
General characteristics of the healthy control and lung cancer patient groups

	Healthy controls ($n = 174$)	Lung cancer ($n = 60$)
Age (years)	50.30 ± 14.43[a]	62.78 ± 11.02[a]
Gender (M/F)	88/86	50/10
Smoking status (%smokers)	90 (52%)	60 (100%)
Histological type		Adenocarcinoma (8.30%)
		Squamous cell ca (46.9%)
		Large cell ca (17.2%)
		Small cell ca (2.10%)
		Others (10.6%)
		Non-classified (14.9%)

[a] The data are presented as mean ± SD.

and 60 unrelated lung cancer patients living in Santiago. Table 1 summarizes their characteristics. All samples were obtained following informed written consent, previously approved by the ethics committee. Both controls and cancer patients were interviewed regarding smoking habits, alcohol drinking, use of oral contraceptives or hormones, incidence of past records of cancer in related family members, and exposure to occupational, outdoor and indoor carcinogenic pollutants. Lung cancer patients were all previously diagnosed histologically and their medical records were available from the Hospital del Torax, Santiago. The histological type was determined only in 51 of 60 cases. The most abundant was the squamous cells, followed by the large cells. Extent of tobacco smoke exposure was assessed by smoking index (SI) (cigarettes/day × 365). A smoker was defined as a person with an SI of 800. Both present smokers and former smokers at the time of the analysis were considered as smokers.

2.3. Genotyping methods

Polymerase chain reaction (PCR) based restriction fragment length polymorphism (RFLP) was used to examine the polymorphisms of interest. DNA was isolated from peripheral blood samples, collected on EDTA [38]. All samples were submitted to separate amplifications followed by digestion with appropriate restriction enzymes.

2.4. PCR amplification

2.4.1. CYP1A1

For the *MspI* site, PCR amplification was carried out using previously described primers C44 and C47 [8] yielding a fragment of 340 bp. The exon 7 polymorphism (substitution of Ile 462 for val 462), was determined as previously described [39]. The downstream primer incorporated a mismatched base to engineer a *NcoI* restriction enzyme site in the PCR products derived from the Ile 462 allele of the gene. This restriction site was lost in the val 462 allele of the gene. A *NcoI* restriction enzyme site, located upstream of the mutation in both genotypes, served as a positive control for PCR product digestion. Fragments of 322 bp were yielded.

2.4.2. CYP2E1

For the *DraI* site, detection was carried out using the primers described by Hirvonen et al. [40], yielding a 373 bp fragment. For the *RsaI* and *PstI* polymorphisms, primers yielding a 413 bp fragment [41] were used to amplify between −1372 and −960 nucleotides upstream of the gene to detect both mutations.

2.4.3. GSTM1

GSTM1 genetic polymorphism was determined simultaneously with *MspI* primers as an internal control of amplification, as described by Ambrosone et al. [42]. *GSTM1* null genotype was assessed by the absence of a 273 bp fragment, using agarose electrophoresis.

2.5. Digestion by restriction enzymes

The PCR products were subjected to restriction enzyme digestion at 37°C for 1 h with *MspI* and *NcoI* for *CYP1A1*, and *DraI*, *RsaI* and *PstI* for

CYP2E1 (GIBCO BRL, Life Technologies, Inc., Gaithersburg, MD, USA). The samples were then analyzed by polyacrylamide (6%) gel electrophoresis (Biorad Lab., Richmond, CA, USA).

2.6. Statistical analysis

Statistical analysis was performed by the Epi Info 6.0 and SPSS 8.0 programs. Univariate logistic regression (simple) and multivariate regression(combined) were performed. Control and cancer groups were adjusted by age and sex. The association of histological type of cancer with any single, or with combined genotypes, was estimated by the Chi-square test and Fisher's exact test.

3. Results

Allele distributions and frequencies for the *MspI* and ile/val polymorphisms in *CYP1A1*, *DraI* and *RsaI/PstI* in *CYP2E1* and the *GSTM1* genotypes in healthy controls and lung cancer patients are shown in Table 2. In the Chilean healthy group, frequency of the m2 variant allele (0.25) was higher than in Caucasians [43] and lower than in Asians [8,44]. Frequency of the val variant allele (0.33) was the highest reported, higher than in Caucasians and Asians. For *MspI* and ile/val *CYP1A1* genotypes, the presence of variant allele was more frequent in cases than in controls (1.5-fold). Similarly, the frequency of homozygous deletions of *GSTM1* loci was 1.8-fold higher in lung cancer patients. However, *RsaI/PstI* and *DraI* polymorphism frequencies were similar in both groups.

Lung cancer risk associated with polymorphisms in *GSTM1* and *CYP1A1* genes in reference to the wild-type genotype is shown in Table 3. For the *MspI* polymorphism, the heterozygous genotype was present in 53% of cancer patients and in 27% of controls (OR = 4.10, $P = 0.0001$), whereas the homozygous mutant genotype was present in 18% of the cancer patients and in 11% of controls (OR = 3.36, $P = 0.009$). Regarding the ile/val polymorphism, the heterozygous genotype was present in 58% of cancer patients and in 43% of controls (OR = 2.42, $P = 0.01$), whereas the homozygous mutant genotype was present in 17% of cancer group and in 12% of controls (OR = 2.57, $P = 0.057$). The *DraI* heterozygous genotype was present in 38% of cancer patients and in 31% of controls (OR = 1.33, $P = 0.39$) whereas the homozygous mutant genotype was present in 3.5% of cancer group and in 5.4% of controls, (OR = 0.69, $P = 0.65$). The *RsaI/PstI* heterozygous genotypes

Table 2
Distribution and allele frequencies of *CYP1A1*, *CYP2E1* and *GSTM1* polymorphisms in chilean healthy controls and lung cancer patients[a]

	Group	*N*	Genotype			Allele frequencies
			m1m1	m1m2	m2m2	
CYP1A1 (*MspI*)	Healthy control	140	86	38	16	m1 = 0.75, m2 = 0.25
	Lung cancer	55	16	29	10	m1 = 0.56, m2 = 0.44
Ile/val			ile/ile	ile/val	val/val	
	Healthy control	120	54	52	14	Ile = 0.67, Val = 0.33
	Lung cancer	60	15	35	10	Ile = 0.54, Val = 0.46
CYP2E1 (*DraI*)			DD	CD	CC	
	Healthy control	129	82	40	7	D = 0.79, C = 0.21
	Lung cancer	58	34	22	2	D = 0.78, C = 0.22
(*RsaI/PstI*)			c1c1	c1c2	c2c2	
	Healthy control	148	105	40	3	c1 = 0.84, c2 = 0.16
	Lung cancer	59	45	14	0	c1 = 0.88, c2 = 0.12
GSTM1			GSTM1 (+)		GSTM1 (−)	
	Healthy Control	174	133		41	+ = 0.76, − = 0.24
	Lung Cancer	58	33		25	+ = 0.57, − = 0.43

[a] According to the recommended nomenclature of the human polymorphic genes, *CYP1A1* wild type allele: *CYP1A1*1A; MspI or m2 allele: CYP1A1*2A; Val allele: CYP1A1*2B. CYP2E1* wild type allele: *CYP2E1*1A; c2 (PstI/RsaI) allele: CYP2E1*5B; C (DraI) allele:CYP2E1*6.*

Table 3
Lung cancer risk associated with polymorphism in *GSTM1*, *CYP2E1* and *CYP1A1* genes in reference to the wild-type genotype

Gene	Genotype	Cancer		Control		OR_c[a]	CI 95%	χ^2
		n	%	*n*	%			*P* value
CYP1A1	*Msp1*							
	m1/m1	16	29.1	86	61.4	1.0		
	m1/m2	29	52.7	38	27.1	4.1	1.8–9.0	0.0001
	m2/m2	10	18.2	16	11.4	3.3	1.2–9.7	0.009
	Ile/val							
	ile/ile	15	25.0	54	45.0	1.0		
	ile/val	35	58.3	52	43.3	2.4	1.1–5.3	0.01
	val/val	10	16.7	14	11.7	2.5	0.8–7.8	0.057
CYP2E1	*Dra1*							
	D/D	34	58.6	58.60	63.6	1.0		
	C/D	22	37.9	40	31.0	1.3	0.6–2.7	0.390
	C/C	2	3.5	7	5.4	0.6	0.09–3.9	0.650
	Rsa1/Pst1							
	c1/c1	45	76.3	105	71.0	1.0		
	c1/c2	14	23.7	40	27.0	0.8		0.570
	c2/c2	0	0.0	3	2.0	–	0.3–1.7	0.340[b]
GSTM1	*GSTM1(+)*[c]	33	56.9	133	76.4	1		
	GSTM1(−)[d]	25	43.1	41	23.6	2.46	1.3–4.8	0.004

[a] OR_c, crude odds ratios.
[b] The result was obtained through the Fischer exact test due to absence of c2/c2 in the cancer group.
[c] GSTM1 present.
[d] GSTM1 null.

were present in 24% of cancer patients and in 27% of controls (OR = 0.82, $P = 0.57$) whereas the homozygous mutant genotype was absent in the cancer group and present in 2% of controls; the *P* value obtained through the Fischer exact test (to evaluate small samples or absence of one in the comparison matrix values) was 0.34. The null *GSTM1* genotype was found in 43% of cases and 24% of controls; the OR was 2.46 ($P = 0.004$).

The estimated relative risks for lung cancer in Chilean patients associated to single or combined *CYP1A1*, *CYP2E1* and *GSTM1* genotypes are shown in Table 4. The OR values related to the presence of variant alleles were 2.41 for m2 and 1.69 for val. The odds ratios ranged between 1.70 and 6.28 for all combinations between *CYP1A1* and *GSTM1* polymorphisms and were lower when the variant alleles of these polymorphisms were combined with *CYP2E1* variant alleles. The risk was higher in individuals with combined m2 and val alleles (OR = 6.28). The m2 and *GSTM1*(−) combination showed a higher risk than each separate allele. However, the combination val/*GSTM1*(−) showed a lower OR value (1.70, not significant) than each separate allele. The lowest observed OR value was for the combination of val and c2 alleles with an OR = 0.48, not significant.

In order to control the confounding factor 'smoking habit', a stratified analysis was performed. Table 5 displays the single and combined effects of genotypes only in smokers from healthy control and lung cancer groups, since non-smokers had no significant statistics (null risk, data not shown). The ORs for single genotypes were 4.37, 4.05 and 3.47 for m2, val and *GSTM1*(−), respectively, and for combined genotypes were: m2/val OR = 7.38 and m2/*GSTM1*(−), OR = 3.68. For the C/c2 combined genotypes the OR value was 0.59 (not significant). These results, in which cigarette smoking has been controlled as a confounding factor, clearly show that lung cancer risk is mostly related to the presence of unfavorable genotypes which might give an estimation of the individual response to carcinogen exposure. All these results suggest that the balance of altered genotypes in *CYP1A1* and *GSTM1* will determine the response of Chileans to PAHs exposure.

Table 4
Single or combined *CYP1A1*, *CYP2E1* and *GSTM1* genotypes and estimated relative risk for lung cancer in Chilean patients adjusted by age and sex

Single or combined genotype[a]	Cases *n*	Controls *n*	OR_a[b]	95% CI	χ^2 *P* value
m2	39	54	2.41	1.48–3.93	0.0001
Val	45	66	1.69	1.05–2.72	0.009
GSTM1(−)	25	41	2.46	1.25–4.82	0.004
C	24	47	1.16	0.65–2.08	0.519
c2	14	43	0.71	0.35–1.41	0.438
m2/val	24	15	6.28	2.39–18.34	0.0001
m2/GSTM1(−)	16	17	3.56	1.38–9.83	0.0004
m2/c2	9	12	2.00	0.37–9.54	0.12
m2/C	11	15	1.39	0.49–4.82	0.49
val/GSTM1(−)	8	11	1.70	0.74–4.88	0.06
val/c2	3	15	0.48	0.25–1.72	0.17
val/C	9	18	1.45	0.55–3.67	0.48
C/c2	9	31	0.56	0.19–1.46	0.19
C/GSTM1(−)	6	8	3.01	0.49–19.4	0.07
c2/GSTM1(−)	7	5	2.54	0.40–18.55	0.20

[a] Rare alleles are considered either including heterozygous or homozygous.
[b] OR_a, adjusted odds ratios.

4. Discussion

In this work *CYP1A1*, *CYP2E1* and *GSTM1* genotypes have been analyzed in 60 Chilean lung cancer patients and 174 healthy controls. The frequencies of m2 and val alleles in *CYP1A1* and the null genotype for *GSTM1* were higher in lung cancer patients whereas frequencies for C and c2 alleles in the *CYP2E1* gene were not significantly different.

Contradictory data have been published concerning *CYP1A1* and *CYP2E1* polymorphisms and their relationships with lung cancer susceptibility [13,45,46]. For example, no association was found between CYP1A1 polymorphisms and lung cancer in Caucasians while Asians showed associations limited to the homozygous recessive *MspI* genotype or the heterozygous exon 7 genotype [45]. An association between *CYP1A1* hyperinducibility and lung cancer was

Table 5
Risk analysis for single and combined *CYP1A1*, *CYP2E1* and *GSTM1* genotypes and lung cancer in smokers from healthy control and lung cancer groups, adjusted by age and sex[a]

Single or combined genotype[b]	Cases *n*	Controls *N*	OR_a[c]	95% CI	χ^2 *P* value
m2	39	26	4.37	1.78–10.82	0.0003
Val	45	36	4.05	1.54–8.37	0.0017
C	24	27	1.07	0.44–3.01	0.55
c2	14	22	0.78	0.23–2.73	0.80
GSTM1(−)	25	17	3.47	1.53–13.29	0.001
m2/val	24	7	7.38	2.79–26.3	0.00002
m2/GSTM1(−)	16	8	3.68	1.31–10.40	0.003
C/c2	9	20	0.59	0.09–5.67	0.12

[a] Some combinations were not analyzed because of the small number of individuals carrying both rare alleles.
[b] Rare alleles are considered as heterozygous or homozygous.
[c] OR_a, adjusted odds ratios.

reported [3–5], but not between *CYP1A1* hyperinducibility and *MspI* polymorphism in Caucasians [4]. However, most of the studies performed in Caucasians have not been large enough to compensate for the very low frequency of the m2 allele in this population compared to Asians. Another reason for these discrepancies could be related to differences in linkage or genetic associations between alleles in different populations as shown in Africans which display no linkage between *MspI* and Ile-val polymorphisms in contrast to Asians or Caucasians [47].

In a previous work [37] we have reported that frequencies of m2 and val alleles were three and five times higher in a healthy Chilean control group than in a healthy French control group, whereas the frequency for the null genotype for *GSTM1* was almost half as high as in the French group. Although in the Chilean healthy group the frequency of the m2 allele was higher than in Caucasians, it was lower than in Asians [8]. On the other hand, the frequency of the val allele in the Chilean healthy group was the highest reported, higher than for Caucasians, Africans and Asians [8]. Therefore, as shown by Garte et al. [10] and Kihara et al. [48], *CYP1A1* and *GSTM1* mutations vary among ethnic groups.

In Chileans, the genotypes most associated to the lung cancer were those of *Msp1* polymorphism (m1m2: OR = 4.10, $P = 0.0001$; m2m2: OR = 3.36, $P = 0.009$) and the presence of the null genotype of GSTM1 gene (OR = 2.46, $P = 0.004$). According to the literature, a higher frequency of the null genotype for *GSTM1* in lung cancer patients was found, suggesting that the deletion of the *GSTM1* gene causes the loss of detoxification of the ultimate carcinogen resulting in higher risk for lung cancer. In addition, our data suggest an association between *GSTM1(−)* or *MspI* polymorphisms and squamous cell carcinoma, as recently reported [49]. The association values found for *GSTM1* and *MspI* were close to significant ($\chi^2 = 3.12$, $P = 0.08$ and 2.26, $P = 0.013$, respectively).

The estimated relative risk for combined unfavorable genotypes was particularly high for combined *m2* and val variant alleles in *CYP1A1* (OR = 6.28, $P = 0.0001$). The enzyme expressed from the val type has been described as presenting higher activity and mutagenicity towards benzo(a)pyrene than that corresponding to the ile type [50]. These observations might indicate that the val *CYP1A1* genotype could increase and/or activate procarcinogens resulting in higher risk for lung cancer. Although lower, the estimated relative risk for the combination between the null genotype for *GSTM1* and m2 variant allele in *CYP1A1* was still high (OR = 3.56, $P = 0.008$). Association between hyperinducibility and *GSTM1* deletion was shown to highly increase risk of lung cancer [5].

Cigarette smoking enhanced the association between lung cancer and unfavorable polymorphisms in *CYP1A1* and *GSTM1* genes. The OR values for the mutated alleles in smokers showed that the major cancer risk was for individuals carrying unfavorable genotypes. These results suggest that the presence of altered *CYP1A1* and *GSTM1* genotypes in Chilean smokers makes these individuals at higher risk for lung cancer. The highest odds ratio was observed for smokers carrying m2 and val alleles (OR = 7.38, $P = 0.00002$) suggesting that smokers presenting both mutations might have higher capacity of *CYP1A1* gene transcriptions and hence higher capacity for carcinogen activation. Stücker et al. [5] recently reported that there was no interaction between smoking and *CYP1A1* inducibility.

The frequency of *CYP1A1* altered genotypes in the Chilean studied groups suggests that Chilean individuals might be more susceptible to PAHs exposures than Caucasian and Afro-American groups, although this will depend on the balance of unfavorable genotypes including other polymorphic enzymes in each ethnia. This might be important in relationship to smoking and exposure to urban PAHs adsorbed in particulate matter. Almost 40% of the Chilean population live in Santiago and this city has shown elevated mutagenicity and high levels of carcinogenic PAHs in outdoor airborne particles [34–36] thus possibly representing a high risk for lung cancer to Santiago inhabitants.

Our results suggest that the Chilean population, which represents different degrees of mixture between native Americans and Caucasians (mostly Spaniards), shows genetic polymorphisms in metabolic genes which are closer, although different from Asians and might be important in lung cancer susceptibility. Recently, Taioli et al. [33] found a significant increased risk for lung adenocarcinoma among people carrying the *CYP1A1*- m3 polymorphism, although they did not observe any association of the Afro-

American specific polymorphism with overall lung cancer risk. They observed that subjects carrying the m3 polymorphism smoked significantly less than patients without this mutation, suggesting that some polymorphisms in metabolic genes might play an important role in cancer risk at low levels of PAHs exposure. Although this mutation is present only in Afro-Americans, and might not be present in Chileans, from a public health point of view, this example might be relevant if a similar effect is observed for other genotypes, since a large part of the general population is exposed to low levels of environmental carcinogens and the frequencies of polymorphisms in metabolic genes are relatively high compared to single cancer susceptibility genes.

Our data, in addition to evaluating lung cancer risk in the Chilean population, might help to understand inter-ethnic differences in the distribution of polymorphic enzymes as well as the function of simultaneous polymorphisms in metabolic genes in each subject and lung cancer susceptibility. To our knowledge, this is the first study relating *CYP1A1*, *CYP2E1* and *GSTM1* gene polymorphisms with lung cancer risk in a Chilean population, a region where environmental and ethnical factors might play an important role in the etiology of the disease.

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References

[1] D. Nebert, D.D. Petersen, A. Puga, Human Ah locus polymorphism and cancer: inducibility of CYP1A1 and other genes by combustion products and dioxin, Pharmacogenetics 1 (1991) 68–78.

[2] V. Daniel, Glutathione S-transferases: gene structure and regulation of expression, Crit. Rev. Biochem. Mol. Biol. 28 (1993) 173–207.

[3] R. Kouri, C. McKinney, D. Slomiany, D. Snodgrass, N. Wray, T. McLmore, Positive correlation between high aryl hydrocarbon hydroxylase activity and primary lung cancer as analyzed in cryopreserved lymphocytes, Cancer Res. 42 (1982) 5030–5037.

[4] M. Jacquet, V. Lambert, E. Baudoux, M. Muller, P. Kremers, J. Gielen, Correlation between P450 CYP1A1 inducibility, Msp1 genotype and lung cancer incidence, Eur. J. Can 32A (1996) 1701–1706.

[5] I. Stücker, M. Jacquet, I. de Waziers, S. Cénée, Ph. Beaune, P. Kremers, D. Hémon, Relation between inducibility of CYP1A1, GSTM1 and lung cancer in a French population, Pharmacogenetics 10 (2000) 617–627.

[6] K. Kawajiri, J. Watanabe, O. Gotoh, Y. Tagashira, K. Sowaga, Y. Fujii-Kuriyama, Structure and drug inducibility of human cytochrome P450c gene, Eur. J. Biochem. 159 (1986) 219–225.

[7] K. Kawajiri, K. Nakachi, K. Imai, A. Yoshü, N. Shinada, J. Watanabe, Identification of genetically high risk individuals to lung cancer by DNA polymorphisms of the cytochrome P4501A1 gene, FEBS Lett. 263 (1990) 131–133.

[8] S. Hayashi, J. Watanabe, K. Nakachi, K. Kawajiri, P.C.R. detection, of an A/G polymorphism within exon 7 of the CYP1A1 gene, Nucleic Acids res 19 (1991) 4797.

[9] F. Crofts, G.N. Cosma, D. Currie, E. Taioli, P. Toniolo, S.J. Garte, A. novel, CYP1A1 gene polymorphism in African-Americans, Carcinogenesis 14 (1993) 29–31.

[10] S. Garte, J. Trachman, F. Crofts, P. Toniolo, J. Buxbaum, S. Bayo, E. Taioli, Distribution of composite CYP1A1 genotypes in africans, african-americans and caucasians, Hum. Hered. 46 (1996) 121–127.

[11] L. Cascorbi, J. Brockmoller, L. 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.

[12] A.S. Aynacioglu, I. Cascorbi, P.M. Ozikiewicz, I. Roots, High frequency of CYP1A1 mutations in a Turkish population, Arch. Toxicol. 72 (1998) 215–218.

[13] R.S. Houlston, CYP1A1 polymorphisms and lung cancer risk: a meta-analysis, Pharmacogenetics 10 (2000) 105–114.

[14] I. Johansson, M. Ingelman-Sundberg, Benzene metabolism by ethanol acetone and benzene inducible cytochrome P-450 IIE1 in rat and rabbit liver microsomes, Cancer Res. 48 (1988) 5387–5390.

[15] P. Thomas, S. Bandiera, S. Maines, D. Ryan, W. Levin, Regulation of cytochrome P-450j a high-affinity N-nitrosodimethylamine demethylase, in rat hepatic microsomes, Biochemistry 26 (1987) 2280–2289.

[16] C. Yang, J. Yoo, H. Ishizaki, J. Hong, Cytochrome P450IIE1: roles in nitrosamine metabolism and mechanism of regulation, Drug. Metabol. Rev 22 (1990) 147–159.

[17] F. Guengerich, D. Kim, M. Iwasaki, Role of human cytochrome P450IIE1 in the oxidation of many low molecular weight cancer suspects, Chem. Res. Toxicol. 4 (1991) 158178.

[18] A. Rannug, A. Alexandrie, I. Persson, M. Ingelman-Sundberg, Genetic polymorphism of cytochromes P450 1A1, 2D6 and 2E1: regulation and toxicological significance, J. Occup. Med. 37 (1995) 25–36.

[19] S. Harada, M. Abbey, M. Tanaka, D. Agarwal, H. Goedde, Liver glutathione S-transferase polymorphisms in Japanese and its pharmacogenetic importance, Human Genet. 75 (1987) 322–325.
[20] J. Seidegard, W.R. Vorachek, R.W. Pero, W.R. Pearson, Hereditary differences in the expression of the human glutathione transferase active on trans-stilbene oxide are due to a gene deletion, Proc. Natl. Acad. Sci. USA 85 (1998) 7293–7297.
[21] H.J. Lin, C.-Y. Han, D.A. Bernstein, W. Hsiao, B.K. Lin, S. Hardy, Ethnic distribution of the glutathione transferase Mu 1-1 (GSTM1) null genotype in 1473 individuals and application to bladder cancer susceptibility, Carcinogenesis 15 (1994) 1077–1081.
[22] F. Uematsu, H. Kikuchi, T. Ohmachi, I. Sagami, M. Motomiya, Y. Kamataki, Two common RFLPs of the human CYP2E gene, Nucleic Acids Res. 19 (1991) 2803.
[23] S. London, A. Daly, J. Cooper, C. Carpenter, W. Navidi, L. Ding, J. Idle, Lung cancer risk in relation to the CYP2E1 RsaI genetic polymorphism among african-americans and caucasians in Los Angeles Country, Pharmacogenetics 6 (1996) 151–158.
[24] M. Yu, A. Gladek-Tarborough, S. Chiamprasert, R. Santella, Y. Liaw, C. Chen, Cytochrome P450 2E1 and glutathione S-transferase M1 polymorphisms and susceptibility to hepatocellular carcinoma, Gastroenterology 109 (1995) 1266–1273.
[25] M. Tsutsumi, Y. Matsuda, A. Takada, Role of ethanol-inducible cytochrome P-450 2E1 in the development of hepatocellular carcinoma by the chemical carcinogen, N-nitrosodimethylamine, Hepatology 18 (1993) 1483–1489.
[26] M. Ingelman-Sundberg, I. Johansson, H. Yin, Y. Terelius, E. Eliasson, P. Clot, Ethanol-inducible cytochrome P4502E1: genetic polymorphism, regulation, and possible role in the etiology of alcohol-induced liver disease, Alcohol 10 (1993) 447–452.
[27] D. Bell, J. Taylor, D. Paulson, C. Robertson, J. Mohler, G. Lucier, Genetic risk and carcinogen exposure: a common inherited defect of the carcinogen-metabolism gene glutathione S-transferase M1 (GSTM1) that increases the susceptibility to bladder cancer, J. Natl. Cancer Inst. 85 (1993) 1159–1164.
[28] G.I. Murray, P.J. Paterson, R.J. Weaver, S.W.B. Ewen, W.T. Melvin, M.D. Burke, The expression of cytochrome P-450, epoxide hydrolase, and glutathione s-transferase in hepatocellular carcinoma, Cancer 71 (1993) 36–43.
[29] J. Tanita, S. Tsuchida, J. Hozawa, K. Sato, Expression of glutathione s-transferase-p in human squamous cell carcinomas of the pharynx and larynx, Cancer 72 (1993) 569–576.
[30] A.K. Alexandrie, M.1. Ingelman-Sunberg, J. Seidegard, G. Tornlin, 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.
[31] A. Heagerty, D. Fitzgerald, A. Smith, B. Bowers, P. Jones, A. Fryer, L. Zhao, J. Alldersea, R. Strange, Glutathione S-transferase GSTM1 phenotypes and protection against cutaneous malignancy, Lancet 343 (1994) 266–268.
[32] C.E. Szarka, G.R. Pfeiffer, S.T. Hum, L.C. Everley, A.M. Balshem, D.F. Moore, Glutathione s-transferase activity and glutathione s-transferase mu expression in subjects with risk for colorectal cancer, Cancer Res. 55 (1995) 2789–2793.
[33] 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.
[34] M. Adonis, L. Gil, Polycyclic aromatic hydrocarbons levels and mutagenicity of inhalable particulate matter in Santiago, Chile. Inhalation Toxicol. 12 (2000) 1173–1183.
[35] L. Gil, M. Adonis, Polycyclic aromatic hydrocarbons levels and mutagenic activities of organic extracts from airborne particles in santiago of Chile, Indoor + Built Environ. 5 (1996) 155–164.
[36] L. Gil, L. King, M. Adonis, Trends of polycyclic aromatic hydrocarbons levels and mutagenicity in Santiago's Inhalable airborne particles in the period 1992 -1996, Inhalation Toxicol. 12 (2000) 1185–1204.
[37] L. Quiñones, F. Berthou, N. Varela, B. Simon, L. Gil, D. Lucas, "Ethnic susceptibility to lung cancer: differences in CYP2E1, CYP1A1 and GSTM1 genetic polymorphisms between French Caucasian and Chilean Populations", Cancer Lett. 141 (1999) 167–171.
[38] D. Lucas, C. Ménez, C. Girre, F. Berthou, P. Bodenez, I. Joannet, E. Hispard, L. Bardou, J. Ménez, Cytochrome P4502E1 genotype and chlorzoxazone metabolism in healthy and alcoholic caucasian subjects, Pharmacogenetics 5 (1995) 298–304.
[39] A. Cantlay, D. Lamb, M. Gillooly, J. Norman, D. Morrison, C.A.D. Smith, Association between the CYP1A1 gene polymorphism and susceptibility to emphysema and lung cancer, J. Clin. Pathol. Mol. Pathol. 48 (1995) M210–M214.
[40] A. Hirvonen, K. Husgafuel-Pursiainen, S. Anttila, A. Karjailainen, H. Vainio, The human CYP2E1 gene and lung cancer: DraI and RsaI restriction fragment length polymorphisms in a Finnish study population, Carcinogenesis 14 (1993) 85–88.
[41] S. Hayashi, J. Watanabe, K. Kawajiri, Genetic polymorphisms in the 5′-Flanking region change transcriptional regulation of the human cytochrome P450IIE1 gene, J. Biochem. 110 (1991) 559–565.
[42] C. Ambrosone, J. Freudenheim, S. Oraham, J. Marshall, J. Vena, J. Brasure, R. Laughlin, T. Nemoto, A. Michalek, A. Harrington, T. Ford, P. Shields, Cytochrome P4501A1 and Glutathione S-transferase (Ml) genetic polymorphisms and postmenopausal breast cancer risk, Cancer Res. 55 (1995) 3483–3485.
[43] D. Lucas, C. Menez, F. Floch, Y. Gourlaouen, O. Sparfel, I. Joannet, P. Bodénez, J. Jezequel, F. Berthou, L. Bardou, J. Ménez, Cytochrome P4502E1 and P4501A1 genotypes and susceptibility to cirrhosis or upper digestive tract cancer in alcoholic caucasians, Alcohol Clin. Exp. Res. 20 (1996) 1033–1037.
[44] E. Stephens, J. Taylor, N. Kaplan, C. Yang, L. Hsieh, G. Lucier, D. Bell, Ethnic variation in the CYP2E1 gene: polymorphism analysis of 695 African-Americans, European-Americans and Taiwanese, Pharmacogenetics 4 (1994) 58–63.

[45] A. d'Errico, E. Taioli, X. Chen, P. Vineis, Genetic metabolic polymorphisms and the risk of cancer: a review of the literature, Biomarkers 1 (1996) 149–173.

[46] N. Wong, F. Rae, K.J. Simpson, G.D. Murray, D.J. Harrison, Genetic polymorphisms of cytochtome P4502E1 and susceptibility to alcoholic liver disease and hepatocellular carcinoma in a white population: a study and literature review, including meta-analysis. , J. Clin. Pathol: Mol. Pathol. 53 (2000) 88–93.

[47] S. Garte, The role of ethnicity in cancer susceptibility gene polymorphisms: the example of CYP1A1, Carcinogenesis 19 (1998) 1329–1332.

[48] M. Kihara, M. Kihara, K. Noda, Risk of smoking for squamous and small cell carcinomas of lung modulated by combinations of CYP1A1 and GSTM1 gene polymorphisms in Japanese population, Carcinogenesis 16 (1995) 2331–2336.

[49] 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 (21) (1998) 1858–1863.

[50] S.I. Hayashi, J. Watanabe, K. Kawajiri, High susceptibility to lung cancer analyzed in terms of combined genotypes of P450IA1 and Mu-class glutathione s-transferase genes, Jpn. J. Cancer Res. 83 (1992) 866–870.