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Ethnic susceptibility to lung cancer: differences in *CYP2E1*, *CYP1A1* and *GSTM1* genetic polymorphisms between French Caucasian and Chilean populations ☆

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Abstract

Many investigators have reported an association between genetic polymorphisms of cytochromes *P*-450 *CYP2E1*, *CYP1A1* or *glutathione S-transferase Mu* (*GSTM1*) and susceptibility to lung cancer. However, pronounced interethnic variations have been described in the frequencies of these polymorphisms, especially between Asians and Caucasians. The present study was set up to establish *CYP2E1* (c1, c2 and C, D), *CYP1A1* (m1, m2 and Ile, Val) and *GSTM1* (null) allelic frequencies in Chileans ($n = 96$) who are an admixture of Native Americans and Caucasians (Spaniards). The rare allele frequencies were found to be 0.15 (c2), 0.21 (C), 0.23 (m2), 0.32 (Val) and 0.21 ('null' genotype). These values are significantly higher than those of Caucasians except for the *GSTM1* 'null' genotype and suggest differences in susceptibility to lung cancer between both populations.

Keywords: *CYP2E1*; *CYP1A1*; *Glutathione S-transferase Mu*; Genetic polymorphism; Chile

1. Introduction

Several enzymes involved in human xenobiotic metabolism are subject to genetic polymorphisms and many investigators have reported an association between these polymorphisms and susceptibility to cancer [1]. Among these enzymes, Phase I cytochrome *P*-450s, CYP2E1 and CYP1A1, involved in the bioactivation of a large number of procarcinogens, and glutathione *S*-transferase Mu (GSTM1), a Phase II detoxifying enzyme, have been extensively studied. About 50% of the Caucasian population inherit two deficient alleles (*GSTM*1*0) and are devoid of GST activity. This makes these subjects more susceptible to develop lung, bladder, skin or colon cancer [2]. Several polymorphisms associated with inducibility phenotypes have also been described for CYP1A1. They include a polymorphism in the 3′-flanking region of *CYP1A1* detected by the presence of a *Msp* I restriction site [3]. This polymorphism was later found in linkage disequilibrium with a mutation in exon 7 of the gene, which resulted in an Ile to Val mutation in the active site of the enzyme [4]. Mutations were also detected in the 5′-flanking region and in intron 6 of the *CYP2E1* gene using *Pst* I/*Rsa* I and *Dra* I restriction enzymes, respectively [5]. Genetic polymorphisms of *CYP2E1* and *CYP1A1* genes have been shown to be associated with lung cancer which is

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an excellent model for studying gene–environment interactions. This association is stronger when the *GSTM1* null allele coexists with *CYP2E1* or *CYP1A1* mutant alleles [6,7].

However, profound ethnic differences in *CYP2E1* and *CYP1A1* allele frequencies have previously been reported, especially between Asians, Caucasians and Africans [8]. They have been suggested to explain ethnic differences in disease [9]. According to recent information from the Chilean Ministry of Health, the mortality for tracheal, bronchial and lung cancer is the second cause of death by cancer in the country. In 1996, the national rate for these types of cancers per 100 000 inhabitants was 13.0, but there are no official data about their incidence. As Chileans are descendants of both Caucasians (Spaniards), and Native Americans, the present study was carried out in order to establish the frequencies of the polymorphic genotypes of *CYP2E1*, *CYP1A1* and *GSTM1*. This could give an insight into the susceptibility of this population towards lung cancer.

2. Methods

2.1. Subjects

Blood samples were obtained from 96 Chileans (both sexes, 27–87 years old), unrelated and living in Santiago. The Chilean population is a hybrid population of biparental origin: native South-Amerindians (Araucanians) and Caucasians (Spanish conquerors). The aboriginal admixture Araucanos/Caucasian (Spaniards) was found to be 22% as determined by ABO blood markers [10]. All samples were obtained following informed written consent and the approval of the ethical committee. After DNA extraction, they were analyzed for *CYP2E1* (*Rsa* I/*Pst* I, *Dra* I), *CYP1A1* (*Msp* I, exon 7) and *GSTM1* genetic polymorphisms. In addition, DNA of 81 French Caucasoid subjects, randomly selected from a control population previously checked for *CYP2E1* and *CYP1A1* (*Msp* I) polymorphisms [11] were analyzed for *CYP1A1* exon 7 and *GSTM1* polymorphism.

2.2. Genotyping

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. All samples were submitted to separate amplifications followed by digestion with appropriate restriction enzymes.

2.2.1. PCR amplification

2.2.1.1. CYP2E1

For the *Pst* I/*Rsa* I sites, DNA amplification was performed as described by Hayashi et al. [5] using Taq polymerase (Eurogentec, Seraing, Belgium), to yield a 410 bp fragment containing both the *Pst* I and the *Rsa* I polymorphic sites in the 5′-flanking region. For the *Dra* I polymorphism, primers described by Hirvonen et al. [12] were used and yielded fragments of 393 bp.

2.2.1.2. CYP1A1

For the *Msp* I site, PCR amplification was carried out using primers C44 and C47, described by Hayashi et al. [5] and yielded a fragment of 340 bp. For the exon 7 polymorphism (substitution of Ile^{462} for Val^{462}) primers described by Cantlay et al. [13] were used. The downstream primer incorporated a mismatched base to engineer a *Nco* I restriction enzyme site in the PCR products derived from the Ile^{462} allele of the gene. This restriction site is lost in the Val^{462} allele of the gene. A *Nco* I restriction enzyme site, located upstream of the mutation in either genotypes, serves as a positive control for PCR product digestion. Fragments of 322 bp were yielded.

2.2.1.3. GSTM1

CYP1A1 and *GSTM1* genetic polymorphisms were determined simultaneously using primers described by Ambrosone et al. [14] for *GSTM1* and those described by Hayashi et al. [3] for *CYP1A1*. The presence of the *GSTM1* gene was determined by the presence of a band (273 bp) while the null genotype was determined by the lack of a band using agarose electrophoresis. The *CYP1A1* amplification served as an internal control.

2.2.2. Digestion by restriction enzymes

The PCR products were subjected to restriction enzyme digestion *Pst* I, *Rsa* I, *Dra* I for *CYP2E1* and *Msp* I and *Nco* I for *CYP1A1* (GIBCO BRL,

Table 1
Genotype frequencies for *CYP2E1* and *CYP1A1* and *GSTM1* polymorphisms in Chileans

		Genotype		
		n (%)	*n* (%)	*n* (%)
CYP2E1	*Pst* I	c1c1	c1c2	c2c2
	$n = 89$	64 (71.9)	23 (25.8)	2 (2.2)
	Dra I	DD	CD	CC
	$n = 93$	60 (64.5)	26 (28)	7 (7.7)
CYP1A1	*Msp* I	m1m1	m1m2	m2m2
	$n = 78$	46 (58.9)	28 (35.9)	4 (5.1)
	Nco I	Ile/Ile	Ile/Val	Val/Val
	$n = 90$	39 (43.3)	44 (48.9)	7 (7.8)
GSTM1		plus	Null	
	$n = 89$	70 (78.6)	19 (21.4)	

Life Technologies, Inc., Gaithersburg, MD) at 37°C for 1 h. The samples were then analyzed by electrophoresis in polyacrylamide (6%) gel (Biorad Lab., Richmond, CA).

3. Results

Little information is available on *CYP2E1*, *CYP1A1* and *GSTM1* polymorphisms in the Chilean population and this study contributes to establish a picture of the normal genotype frequencies in the Latin-American population which is quite complex given the high degree of interracial admixture. Chileans constitute a recently admixed population consisting of Native Americans and Caucasians (Spaniards). The Amerindian admixture index was found to be 0.22 by ABO blood group distribution [10]. Genotype frequencies for *CYP2E1*, *CYP1A1* and *GSTM1* are reported in Table 1. These distributions are consistent with the Hardy–Weinberg equilibrium model. As shown in Table 2, rare allele frequencies were 0.15 (c2) and 0.215 (C) for *Pst* I and *Dra* I *CYP2E1* polymorphisms, 0.231 (m2) and 0.322 for *Msp* I and Val *CYP1A1* polymorphisms, respectively. In addition, 21.3 % of the population was homozygote for the null variant allele of *GSTM1*. This contrasts with the 40–60% prevalence in Caucasians or in Asians. The data concerning the exon 7 and *GSTM1* polymorphism frequencies in the French population (Table 2) are similar to those reported by others [13,15,16]. When compared with frequencies of Caucasian populations, great differences appeared for each polymorphism in the Chilean population which was closer in similarity to that in Asians (Table 2).

4. Discussion

As frequencies of the rare CYP alleles are higher in Chileans than in Caucasians, and given the percentage of Amerindian admixture of this population, this suggests that Native Americans display much higher frequencies of the rare alleles than Caucasians. Very recently, Munoz et al. [17] reported frequencies of 0.250 (c2), 0.256 (C), 0.768 (m2), 0.833 (Val) for rare *CYP2E1* and *CYP1A1* alleles, respectively, in the Mapuche population which constitutes one of the most important aboriginal populations of Chile. We had also found in another study [18] that full Native Americans belonging to Sioux and Navajo tribes ($n = 85$) displayed higher frequencies of these

Table 2
Rare allele frequencies in different ethnic groups

Population	*CYP2E1*		*CYP1A1*		*GSTM1*
	c2 (*Pst* I)	C (*Dra* I)	m2 (*Msp* I)	Val (*Nco* I)	null/null
Chileans	0.150 (178)	0.215 (186)	0.231 (156)	0.322 (180)	0.213 (178)
Caucasians	0.031 (242) [11]	0.079 (252) [11]	0.08 (424) [11]	0.068 (162)	0.471 (102)
Asians	0.28 (240) [8]	0.24 (238) [8]	0.33 (750) [3]	0.20 (750) [3]	0.31 (168) [27]
Chi square test with Yates' correction					
Chileans vs. Caucasians	$P < 0.00001$	$P < 0.00005$	$P < 0.00001$	$P < 0.00001$	$P < 0.002$
Chileans vs. Asians	$P = 0.002$	$P = 0.56$	$P = 0.17$	$P = 0.001$	$P = 0.055$

alleles than Caucasians (c2 = 0.12, C = 0.17 and m2 = 0.54). These frequencies are close to those of Asians. It is believed that Native Americans originate from a single migration of Asians to Beringia 40 000 years ago despite the recent finding of a new genetic marker suggesting a 'definite, if ancient, link between Eurasians and Native Americans' [19]. Distributions of *CYP2E1* genotypes in Chileans are similar to those observed in Mexicans [20] who have also Native Americans and Spaniards in their ancestry.

The *Dra* I *CYP2E1* polymorphism was partially linked with the *Pst* I/*Rsa* I polymorphism (data not shown) as previously shown in Asians, in Caucasians [8] or in Mexicans [20]. The two *Msp* I and exon 7 *CYP1A1* polymorphisms are usually but not necessarily linked in Asians or in Caucasians. However, no linkage was observed in Chileans. Because of the recent admixture of this population, disequilibrium between the closely located polymorphisms of *CYP1A1* can be expected to vary.

CYP1A1 and *GSTM1* are of critical importance for the activation of polycyclic aromatic hydrocarbons (PAHs) into DNA binding metabolites. PAHs are present not only in tobacco smoke but also in the urban environment [21]. PAHs have been reported in higher levels in Santiago than in other cities of the world [22–24] and produce dramatic metabolic changes of cytochrome *P*-450 monooxygenases [21,25]). Combined mutated *CYP1A1* and *GSTM1* null allele genotype is a potential predictor of genetic susceptibility to lung cancers in populations where frequencies of the mutant *CYP1A1* alleles are high. Reports concerning the influence of the *CYP2E1* genotype on lung cancer are somewhat controversial. *Rsa* I and *Dra* I mutant alleles are considered either as a protective factor against cancer [20] or as a risk factor [26], especially when associated with the *GSTM1* null allele [6]. However, the *CYP2E1* and *CYP1A1* polymorphisms were shown to be closely associated with susceptibility to lung cancer in populations with low smoking exposure where environmental carcinogens from sources other than tobacco smoke such as air pollutants and diet might contribute to human pulmonary carcinogenesis [7,26]. By combining genotypes from different genes of interest, the identification of risk groups can be made more specific and can reveal factors of importance in the development of specific types of cancer [6]. It is likely that the *CYP* and *GST* mutations studied to date are only a part of the total spectrum of allelic mutations.

Taken altogether, these data show that genetic polymorphisms in Chilean people are close, although different, to those of Asians. Because of the relatively high frequencies of *CYP1A1* and *CYP2E1* mutated alleles, the Chilean population appears to be more susceptible to lung cancer than Caucasians when exposed to carcinogens but considering the lower frequency of the *GSTM1* null allele, they might be better protected. Therefore, the susceptibility of the Chilean population to lung cancer needs to be further investigated.

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