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Research Article

CYP1A1, *CYP2E1*, *GSTM1*, *GSTT1*, *GSTP1*, and *TP53* polymorphisms: do they indicate susceptibility to chronic obstructive pulmonary disease and non-small-cell lung cancer?

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Abstract

Gene polymorphisms of phase I (*CYP1A1* and *CYP2E* of cytochrome P,) and phase II (*GSTM1*, *GSTT1* and *GSTP1* of glutathione-S-transferase,) enzymes and the *TP53* tumor suppressor gene were studied as markers in a sample of 262 Brazilians of European descent, the sample consisting of 97 patients with non-small-cell lung cancer (NSCLC), 75 patients with chronic obstructive pulmonary disease (COPD) and 90 control individuals. For NSCLC, we found no significant relationship between any of the markers studied and susceptibility to this disease. With respect to COPD, although the distribution of the *CYP1A1*, *GSTM1, GSTP1, GSTT1* and *TP53* genotypes was similar to that of the controls the frequency of the *CYP2E1*1A/*5B* heterozygote was about 6 times higher in COPD patients than in controls (OR= 6.3; CI = 1.1-35.5 for p = 0.04). Individuals who presented the *GSTT1* null phenotype and *GSTP1 Ile/Val* genotype had a risk about four times higher (OR= 4.0; CI = 1.2–14.6 for p = 0.02) of having COPD than individuals without these genotypes, the same being true for individuals having the *GSTT1* null phenotype and *CYP1A1*1A/*2A* genotype (OR= 3.7; 1.1–14.6 for p = 0.04).These results suggest that the *CYP2E1* and *GSTT1 + GSTP* or *GSTT1 + CYP1A1* polymorphisms may be predictive of susceptibility to COPD, at least in this population of European ancestry.

Key words: *CYPs*, *GSTs* and *TP53* polymorphisms, chronic obstructive pulmonary disease, non-small-cell lung cancer, genetic susceptibility.

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Introduction

Chronic obstructive pulmonary disease (COPD) and non-small-cell lung cancer (NSCLC) are directly associated with cigarette smoking, although only a small proportion of smokers develop these diseases (Murray and Nadel, 1994). Susceptibility to cigarette smoke might be associated with genetic variability of the genes involved in chemical carcinogen metabolism, a large fraction of chemical carcinogens being biotransformed to more toxic metabolites by phase I activation enzymes (*e.g.* cytochrome P, coded for by *CYP* genes) or to non-toxic compounds by phase II detoxification enzymes (*e.g.* glutathione-S-transferase, coded for by *GST* genes). The rate of these competing metabolic pathways is an important determinant of DNA damage (Autrup, 2000), although the cellular response to DNA damage is mediated by another group of genes, *i.e.*, the tumor suppressor genes (Murata *et al*., 1998).

Many enzymes involved in either activation or detoxification of chemical carcinogen metabolism are polymorphically expressed, with the alleles presenting different enzymatic activities and some of them having been associated with susceptibility to cancer (Autrup, 2000).

Among the tumor suppressor genes, *TP53* has a key and potent role in the cellular response to DNA damage (Bennett *et al.,* 1999). An unusual spectrum of *TP53* mutations resulting in loss or disruption of tumor suppressor function has been described in several human cancer tissues (Bennett *et al.,* 1999), and some *TP53* polymorphisms seem also to be related to susceptibility to cancer (Murata *et al*., 1998).

It has been hypothesized (Autrup, 2000; Murata *et al*., 1998; Ishii *et al.,* 1999) that individuals who are environ-

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mentally exposed to cigarette carcinogens and who also have genetic variants of tumor suppressor genes and/or genes for phase I or phase II enzymes have a higher risk of developing lung cancer or COPD, although there is no clear consensus on the effect of these polymorphisms on lung diseases (Bartsch *et al*., 2000).

The study reported in this paper investigated seven genetic polymorphisms of cytochrome P activation enzymes (genes *CYP1A1, CYP2E1*) and glutathione-S-transferase detoxification enzymes (genes *GSTM1*, *GSTT1*, *GSTP1)* as well as the *TP53* tumor suppressor gene in patients with COPD and NSCLC and in a control sample (all Brazilians of European origin) in order to evaluate the role of these genetic markers in the prediction of susceptibility to these pulmonary pathologies.

Sample population and methods

A total of 262 Brazilians of European origin were investigated, the sample consisting of 97 patients with NSCLC, 75 with COPD and 90 control individuals with no symptoms of these diseases. For the NSCLC group, only previously untreated NSCLC patients with a diagnosis of cancer confirmed by histology (according to WHO, 1982 guidelines) were included in the study, while the COPD group consisted of patients whose diagnosis had been confirmed by pulmonary function tests and radiography according to the guidelines of the European Respiratory Society (Siafakas *et al*., 1995). Blood samples were collected from patients from August 1998 to July 2001 at a general hospital (*Santa Casa de Misericórdia de Porto Alegre*, Porto Alegre, Rio Grande do Sul, Brazil). Information about patient smoking habits was obtained and quantified as previously described (Sugimura *et al.,* 1995). The hospital ethics committee approved this investigation, and the subjects were previously informed about this research and signed an informed consent sheet.

The control group consisted of adults who came to our laboratory for paternity tests and were apparently healthy, although no detailed data about their health conditions were obtained. The control group was representative of the Porto Alegre (1.360.033 inhabitants) population in terms of sex and age distribution (http://www.ibge.gov.br/) and although no data about smoking habits were obtained for this group the frequency of smokers in this group would be expected to be lower than that of the patients group. Data about *TP53* polymorphism and *CYP1A1*2C* allele distribution in this sample have been described previously (Gaspar *et al.,* 2001; 2002 b).

Genomic DNA was isolated from whole blood by the salting out method (Miller *et al.,* 1995) and seven polymorhic markers (Table 1) investigated for gene polymorphisms by genotyping using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The *CYP1A1* gene was genotyped using the procedure of Hayashi *et al.* (1991) and Cascorbi *et al.* (1996) and the *TP53* gene by the method of Gaspar *et al.* (2001). The *GSTM1*, *GSTT1* and *CYP2E1* genes were typed by a multiplex PCR method using a reaction mixture consisting of 100 ng of genomic DNA, 15 pmol of *GSTM1* and *GSTT1* primers, 7.5 pmol of *CYP2E1* primers, 10 mM Tris HCl, 4 mM $MgCl_2$, 50 mM KCl, 150 mM dNTPs and 1.0 U of *Taq* DNA polymerase in a total volume of 25 μL. The amplification protocol consisted of initial denaturation at 94 °C for 5 min, 6 touchdown cycles of 1 min at 94 °C followed by 2 min at 59 °C (decreasing to 54 °C at a rate of 1 °C per cycle) and 1 min at 72 °C, and 30 cycles at 94 °C for 1 min followed by 1 min at 55 °C and 1 min at 72 °C, plus a final extension of 5 min at 72 °C. An aliquot of the amplification product was subjected to horizontal agarose gel (4%) electrophoreses to verify the presence or absence of *GSTM* and *GSTT* fragments, the *CYP2E1* product being used as an internal control for this reaction. A second aliquot of the amplified product was then subjected to the *PstI* and *RsaI* enzymes to establish the *CYP2E1* haplotypes (Gaspar *et al.,* 2002 a). Primer sequences were those re-

Table 1 - Characteristics of the cytochrome P (*CYP1A1*, *CYP2E1*), glutathione-S-transferase (*GSTM1*, *GSTT1*, *GSTP1*) and tumor suppressor (*TP53*) polymorphisms investigated in this study.

Loci	Gene location	Allele or genotype	Phenotype[a]	Method	References
CYP1A1[b] (*Msp*I)	3' flanking	*CYP1A1*2A*	*Msp*I +	PCR-RFLP	Hayashi *et al.,* 1991;
(Ile/Val)	Exon 7	*CYP1A1*2C*	*Bsr*DI – (Val)	PCR-RFLP	Cascorbi *et al.,* 1996
CYP2E1[b]	5' flanking	*CYP2E1*1A*	*Pst*I –; *Rsa*I +	PCR-RFLP	Kato *et al*., 1992
		*CYP2E1*5B*	*Pst*I +; *Rsa*I –		
GSTM1[c]	Whole gene	*GSTM1* (–)	No amplification	Multiplex-PCR	Bell *et al.,* 1993
GSTT1[c]	Whole gene	*GSTT1* (–)	No amplification	Multiplex-PCR	Pemble *et al.,* 1994
GSTP1	Exon 5	**Ile*	*Bsma*I –	Multiplex-PCR	Harries *et al*., 1991
		**Val*	*Bsma*I +		
TP53	Exon 4	**Arg*	*Bst*UI +	PCR-RFLP	Gaspar *et al*., 2001
		**Pro*	*Bst*UI –		

[a]Plus and minus signs indicate the presence or absence of the restriction site; [b]Allele nomenclature as recommended in http:// www.imm.ki.se/CYPalleles; [c] *GSTM1* (–) and *GSTT1* (–) indicate gene deletion.

ported by Kato *et al.* (1992), Bell *et al.* (1993) and Pemble *et al.* (1994).

Gene frequencies were estimated by gene counting and Hardy-Weinberg equilibrium was evaluated by the chi-squared test for goodness of fit adjusted for small samples, when appropriate. Heterogeneity between groups was estimated by the Mann-Whitney or Fisher tests. Due to the difference in age and sex between control and patient groups the comparisons between NSCLC and controls as well as between COPD and controls were adjusted by age and sex, using multiple linear regression models. The Odds ratio test (OR) with a 95% confidence interval (CI) was employed to verify possible effects of genetic markers on lung diseases. The tests were carried out for each independent locus and considering all combinations of two simultaneous loci. Statistical analyses were performed using the programs for epidemiological analysis (PEPI) software version 2.0 (Gahlinger and Abramson, 1995) or SPSS® for Windows version 10 (SPSS incorporation).

Results

Table 2 presents data on the age and gender of the patient and controls as well as patient smoking status and tumor histology classification. No difference was observed between the NSCLC and COPD groups in relation to smoking status, mean age or sex distribution. However, the frequency of males was higher in the NSCLC and COPD patients ($p < 0.001$) and they were older than the controls ($p < 0.001$). With respect to tumor histology, about 50% of NSCLC patients presented adenocarcinoma, while the others had squamous cell carcinoma.

The genotype distributions and allele frequencies are shown in Table 3, no deviation from Hardy-Weinberg expectations occurred either in regard to polymorphism or sample group and the genotype and allele distributions were similar for males and females in all groups (NSCLC and COPD patients and controls).

The frequencies of the *TP53*Pro*, *CYP1A1*2A*, *CYP1A1*2C*, *CYP2E1*5B,* and *GSTP1*Val* alleles and of the *GSTM1* null and *GSTT1* null phenotypes in the control sample were similar to those verified for European populations (Cascorbi *et al*., 1996; Harries *et al.,* 1991; Rebbeck, 1997; Rannug *et al.,* 1995; Aynacioglu *et al.,* 1998).

No significant differences in genotype distribution were detected between controls and NSCLC patients, considering the sample as a whole, the histological cell type groups and smoking status. Although the frequency of *CYP1A1*2C*, *GSTP1*Val* and *TP53*Pro* carriers was higher in cancer patients than in controls, the differences were not statistically significant.

Genotype distributions for *CYP1A1*, *GSTM1, GSTP1, GSTT1* and *TP53*, were similar between COPD patients and controls, but the frequency of heterozygous *CYP2E1*1A/*5B* was about 6 times higher in COPD patients than in controls (OR= 6.3; CI = 1.1 - 35.5 for $p = 0.04$).

The analyses considering two loci simultaneously indicated two significant associations, both involving the *GSTT1* null phenotype (Table 4). Individuals who presented both the *GSTT1* null phenotype and *GSTP1 Ile/Val* genotype had a four times higher risk (OR = 4.0; CI = 1.2 - 14.6 for $p = 0.02$) of having COPD than individuals without these characteristics, the same being true for individuals having both the *GSTT1* null phenotype and *CYP1A1*1A/*2A* genotype who had a COPD risk about

Table 2 - Main characteristics of the patients sampled in this study.

Variable	NSCLC (n = 97)	COPD (n = 75)	Controls (n = 90)
Mean age in years (SE; range)[1]	65.3 (0.9; 39-81)	64.3 (1.3; 41-87)	31.9 (1.0; 16-59)
Gender, n (%) [2]			
Male	67 (69.1)	61 (81.3)	47 (52.2)
Female	30 (30.9)	14 (18.7)	43 (47.8)
Tumor histology (%)			
Adenocarcinoma	49 (50.5)	–	–
Squamous cell carcinoma	48 (49.5)	–	–
Smoking status, n (%; range)			
≤ 40 pack-years[3]	49 (50; 13-40)	30 (40; 4-40)	–
>40 pack-years[3]	48 (50; 41-70)	45 (60; 50-70)	–
Mean years smoked (SE; range) [4]	41 (1.5; 13-70)	41 (1.5; 20-75)	–
Mean pack-years (SE; range)[4]	27 (1.8; 5-70)	31 (2.0; 4-70)	–

[1]Mann-Whitney test, NSCLC x Controls (C) and COPD x C: $p < 0.001$; NSCLC x COPD: not significant (ns). [2]Fisher's exact test, NSCLC x C and COPD x C: $p < 0.05$; NSCLC x COPD: ns. [3]Fisher's exact test, NSCLC x COPD: ns. [4]Mann-Whitney test, NSCLC x COPD: ns. NSCLC = non-small-cell lung cancer. COPD = chronic obstructive pulmonary disease.

Table 3 - Allele frequencies (%) of cytochrome P (*CYP1A1*, *CYP2E1*), glutathione-S-transferase (*GSTM1*, *GSTT1* and *GSTP1*) and tumor suppressor (*TP53*) genotypes in patients with non-small-cell lung cancer (NSCLC) or chronic obstructive pulmonary disease (COPD).

Genotype and allele frequencies (in bold)	Controls (n = 90)	NSCLC (n = 97)	NSCLC *vs.* control odds ratio (95% CI)	COPD (n = 75)	COPD *vs.* Control Odds ratio (95% CI)
CYP1A1 (MspI)[1]					
**1A/*1A*	71.1	67.0		65.3	
**1A/*2A*	23.3	28.9	1.9 (0.4-4.0)	33.3	1.6(0.7-2.4)
**2A/*2A*	5.6	4.1		1.3	
CYP1A1*2A	**.166**	**.185**		**.180**	
CYP1A1 (Ile/Val)[2]					
**1A/*1A*	81.1	72.2		78.7	
**1A/*2C*	15.6	24.7	2.5(0.6-1.3)	21.3	0.6(0.2-2.5)
**2C/*2C*	3.3	3.1		–	–
CYP1A1*2C	**.111**	**.155**		**.107**	
CYP2E1					
**1A/*1A*	96.7	90.7		86.7	
**1A/*5B*	3.3	9.3	4.8 (0.2-9.3)	13.3	6.3 (1.1-35.5) *
CYP2E1*5B	**.016**	**.046**		**.067**	
GSTM1					
Null	50.0	51.5	0.7 (0.2-2.0)	37.3	0.6 (0.2-1.9)
GSTT1					
Null	21.1	22.7	1.1 (0.3-4.0)	40.0	0.9 (0.8-1.0)
GSTP1[3]					
Ile/Ile	52.2	42.3		46.7	
Ile/Val	40.0	46.4	2.9 (0.9-8.9)	46.7	1.9 (0.6-6.1)
Val/Val	7.8	11.3		6.6	
GSTP1*Val	**.278**	**.345**		**.300**	
TP53[4]					
Arg/Arg	44.4	48.5		57.3	
Arg/Pro	48.9	39.2	0.5 (0.2-1.5)	38.7	0.3 (0.1-1.1)
Pro/Pro	6.7	12.4		4.0	
TP53*Pro	**.312**	**.320**		**.233**	

[1]Comparison between *CYP1A1*1A* homozygous x carriers of at least one *CYP1A1*2A* allele; [2]comparison: *1A/*1A x others; [3]comparison: *Ile*/*Ile* x others; [4]comparison: *Arg*/*Arg* x others; * Significant association - CYP2E1**1A*/**5B*, COPD *vs.* Control, p = 0.038.

four times higher (OR = 3.7; 1.1 - 14.6 for p = 0.04) than individuals without these attributes.

Discussion

The differences observed in age and sex distribution between controls and patients were to be expected since both NSCLC and COPD are generally associated with older people (Murray and Nadel, 1994). Despite these differences, age and sex did not affect the association results because the data were adjusted for these parameters during the statistical analyses. Another variable is the smoking status of patients and controls, but since we had no information about the smoking habits of the individuals in the control group we could not correct for this factor, although it is to be expected that the frequency of smokers in the patient group should be higher than that among the general population. These undefined variables mean that it is possible that some of our negative results may represent underestimates of the role of the *TP53*, *CYP* or *GST* markers on NSCLC and COPD.

Previous data on the role of the *TP53*, *CYP* or *GST* markers in lung cancer are conflicting, but, in general, no associations have been described for European populations, although positive correlations between these markers and lung cancer have been reported for groups of Asians. These differences are probably due to ethnic and/or environmental heterogeneity as well as to gene/environment and gene/gene interactions (Murata *et al*., 1998; Ishii *et al.,* 1999; Bartsch *et al*., 2000; Rebbeck, 1997; Indulski and Lutz, 2000).

Table 4 - Combined loci effects comparing patients with chronic obstructive pulmonary disease (COPD) and controls (%).

Combined loci		COPD (n = 75)	Controls (n = 90)	Odds ratio (95% CI)
GSTP1	*GSTT1*[1]			
Ile/Ile	(+)	28.0	39.5	
	(–)	19.0	12.8	2.0 (0.7-6.0)
Ile/Val	(+)	27.0	33.7	1.1 (0.5-2.7)
	(–)	20.0	7.0	4.0 (1.2-14.6) *
Val/Val	(+)	5.0	4.7	1.6 (0.3-9.7)
	(–)	1.0	2.3	0.8 (0.01-16.5)
CYP1A1 (MspI)				
**1A/*1A*	(+)	41.3	55.8	
	(–)	24.0	16.3	2.1 (0.8-5.2)
**1A/*2A*	(+)	17.3	16.3	1.1 (0.4-3.1)
	(–)	16.1	5.8	3.7 (1.1-14.6) *
**2A/*2A*	(+)	1.3	5.8	0.3 (0.006-3.0)

[1](+) = *GSTT1* wild type, (–) = *GSTT1* null phenotype; * Significant associations: *GSTT1* (–) and *GSTP1Ile/Val*, p = 0.019; *GSTT1* (–) and *CYP1A1*1A/*2A*, p = 0.036. GST = glutathione-S-transferase. CYP = cytochrome P.

Data about COPD are scarce and as far as we know this is the first investigation reporting *CYP1A1*, *CYP2E1*, *GSTT1*, *GSTP1* and *TP53* gene polymorphisms in COPD patients in populations of European origin. Studies analyzing some of these genes in relation to the risk of COPD have previously been performed on Asian populations, Ishii *et al.* (1999) having studied *GSTP1* polymorphisms in Japan and found an association between the 105Ile/Ile genotype and COPD pathogenesis but Yim *et al.* (2000) found no association between *GSTM1* and *GSTT1* markers and COPD in Koreans.

Our data suggest that the *CYP2E1*1A/*5B* genotype and *GSTT1* null phenotype in association with the *CYP1A1-Msp*I or *GSTP1* loci could be predictive of susceptibility to COPD. These associations are clearly explained by the biological role of these enzymes, because phase I enzymes (CYP1A1, CYP2E1) activate procarcinogens to highly reactive intermediates, with the enzyme generated by the *CYP1A1*2A* and *CYP2E1*5B* alleles having higher activity on some toxic compounds than the wild allele (Landi *et al.,* 1994; Watanable *et al.*, 1994). The oxygen reactive species generated by phase I enzymes are converted to inactive derivatives by phase II enzymes (GSTT1 and GSTP1*)*, with the *GSTT1* null phenotype presenting no activity and *GSTP1*Val* allele reduced activity (Autrup, 2000; Rebbeck, 1997; Indulski and Lutz, 2000).

The interaction between two phase II deficient enzymes (GSTT1 null and GSTP1 Val) or a phase I hyperactive enzyme (CYP1A1 2A) and a phase II absent enzyme (GSTT1 null) results in a larger amount of toxic compounds that might have a role in the initiation or progression of COPD (Rebbeck, 1997; Indulski and Lutz, 2000; Watson *et al*., 1998; Walter *et al*., 2000).

Chronic obstructive pulmonary disease (COPD) is a complex disease in which multiple loci are involved and only the joint analysis of several markers could provide new clues for prediction of its occurrence. The associations discussed in this paper demonstrate the importance of genetic variation in phase I and II enzyme genes in susceptibility to COPD. However, more data on other populations are needed to confirm our findings before these polymorphisms can be used as predictive factors for assessing the risk of developing COPD.

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