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Association Between *p53 codon 72* Genetic Polymorphism and Tobacco Use and Lung Cancer Risk

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Abstract Lung cancer (LCa) is the leading cause of death by cancer in men. Genetic and environmental factors play a synergistic role in its etiology. We explore in 111 lung cancer cases and 133 unrelated noncancer controls the gene-environment interaction (G × E) between *p53cd72* polymorphism variants and smoking and the effect on LCa risk in two kinds of case-control designs. We assessed the interaction odds ratio (IOR) using an adjusted unconditional logistic model. We found a significant and positive interaction association between *Pro** allele carriers and smoking habits in both case-control and case-only designs: IOR = 3.90 (95% confidence interval [CI] = 1.10–13.81) and 3.05 (95% CI = 1.63–5.72), respectively. These exploratory results suggest a synergistic effect of the smoking habit and the susceptibility of the *Pro* allele on lung cancer risk compared with each risk factor alone.

Keywords Lung cancer · Case control · Case only · Gene-environment interaction · Genetic polymorphisms · Risk effect modification · Synergistic effect

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Introduction

After skin cancer, lung cancer (LCa) is the most frequent malignant neoplasm in humans and the most common cause of cancer mortality worldwide [1]. Given the multicausal etiology of this cancer, synergistic interactions among risk factors may have significant effects on LCa risk, especially gene–environment interactions (G × E) [2]. Within the causal network of carcinogenesis, there are multiple points at which genetically determined host characteristics or environmental factors might influence an individual's susceptibility via effects on metabolic activation, DNA-repair capacity, and other cellular processes. Polymorphic low-penetrance genes implicated in cancer etiology can have profound effects on increasing or reducing the differential susceptibility to environmental cancer [3, 4]. The *p53* gene is one of the most mutated genes in human tumors and has been referred to as the "emergency brake" because of its tumor-preventing apoptotic and cell-cycle-checkpoint functions in physiologically stressful situations [5, 6]. This gene is an important component in the response to DNA damage, participating in the DNA-repair process and preventing mutations and aneuploidy that result from cellular replication. Therefore, the wild-type *p53* gene suppresses cellular transformation by activated oncogenes, thus inhibiting the growth of malignant cells [7, 8]. On the other

hand, polymorphic variants of the *p53* tumor-suppressor gene produce a guanine–cytosine change (G > C) at codon 72 resulting in arginine–proline (*Arg > Pro*) amino acid substitution. The functional impact of this *p53* polymorphism has been reported and the *Arg/Arg* genotype seems to induce apoptosis with faster kinetics and to suppress transformation more efficiently than the *Pro/Pro* genotype [9]. Several studies have reported that the *p53cd72* polymorphism of the tumor-suppressor gene is associated with LCa risk [10–13]. However, other studies have reported nonsignificant differences between LCa and healthy controls in relation to genotypic polymorphic frequencies [11, 14–17].

It is well known that tobacco smoke has thousands of compounds, some of which are considered carcinogenic, the most important ones being the polycyclic aromatic hydrocarbons (PAH) and the nicotine-derived nitrosamines. Recent studies have indicated that there is a strong coincidence of G-to-T transversion hotspots in lung cancers and sites of preferential formation of PAH adducts along the *p53* gene. Xiao and Singh report that *p53* plays a significant role in the regulation of cellular response to benzo[a]pyrene, one of the most important carcinogenic compounds of tobacco smoke [18]. The available data suggest that *p53* mutations in lung cancer can be attributed to direct DNA damage from cigarette smoke carcinogens rather than to selection of pre-existing endogenous mutations [19, 20]. Experimental studies carried out in yeast assay have shown that smoking may cause mutations in *p53* by formation of PAH o-quinones, which produce reactive oxygen species [21].

In the present study we propose that smokers with the polymorphic variant of *p53* would have a differentiated risk of lung cancer. To test this hypothesis, we explored in our study population the interaction between *p53cd72* genetic variants and tobacco use as a modifying factor for the risk of lung cancer. In addition, to evaluate lung cancer risk in the Chilean population, this study might help to understand interethnic differences in the distribution of polymorphic proteins as well as the function of single rare alleles and smoking with respect to lung cancer susceptibility.

Material and Methods

Sample Subjects

A hospital-based case–control study was carried out using 111 lung cancer patients histologically determined and 133 controls, with hospital admission between 1998 and 2001 [22]. Cases and controls were recruited from the "Hospital del Tórax" of Chile (referral hospital for respiratory diseases). Controls were people who were admitted for pulmonary diseases not related to lung cancer. All study subjects provided informed consent for participation in this research which was done under a protocol approved by the Ethics Committee for Studies on Human Beings of the Faculty of Medicine at the University of Chile.

Laboratory Assays

Genotyping Methods and PCR Amplification Detection

After extraction, DNA samples were analyzed for *p53* genetic polymorphisms. PCR-based restriction fragment length polymorphism (RFLP) was used to examine the polymorphisms of interest [22]. *p53cd72* genetic polymorphism was determined using the primers described by De la Calle-Martin et al. [23].

Analytic Methodology

Allele frequencies for *p53cod72 Pro* and *Arg* were calculated as the number of alleles divided by the number of chromosomes, and tests for Hardy–Weinberg equilibrium were conducted by comparing observed and expected genotype frequencies using a χ^2 test [24]. The extent of tobacco smoke exposure was assessed by the smoking index (SI) (cigarettes/day × 365). A smoker was defined as a person with a SI of 800. Both present and former smokers at the time of the analysis were considered smokers [25].

To explore the possible risk effect modification between *p53cd72* polymorphism and smoking habit on LCa risk, we computed the odds ratios (OR) of the effect measures using an extended 2-by-2 table design [26], considering as a reference group *a priori* low-risk susceptibility combination (*Arg/Arg p53cd72* genotype and nonsmoker). In this analysis we presented the genetic polymorphisms in three genotypes (*Arg/Arg*, *Arg/Pro*, *and Pro/Pro*) and in a collapsed way [*Pro** (*Pro/Pro* + *Arg/Pro*)], respectively. Later, these ORs were combined to assess departures from a multiplicative interactions model in a case-control and a case-only design. We used the following formulas to compute the interaction odds ratio (IOR):

To case-control design: $\mathrm{IOR_{cc}} = [\mathrm{OR_{ge}}/\mathrm{OR_g} \times \mathrm{OR_e}]$

To case-only design: $\mathrm{IOR_{co}} = [\mathrm{OR_{ge}}/\mathrm{OR_g} \times \mathrm{OR_e}] \times Z$

where $\mathrm{OR_{ge}}$ is the joint OR for disease among smokers with the susceptibility genotypes, $\mathrm{OR_g}$ is the OR for disease among nonsmokers with the susceptibility genotypes, $\mathrm{OR_e}$ is the OR for disease among smokers without the susceptibility genotypes, and Z is the OR between exposure and genotype in the controls (assumed to be 1 based on the assumption of independence in the case-only design;

therefore, $IOR_{cc} = IOR_{co}$). The IOR_{co} provides an estimate of the ratio of the joint effect divided by the product of the individual effects of the gene and environmental factors [27]. The 95% CI estimates for IORs were computed as described by Hosmer and Lemeshow [28]. The precision of these ORs was evaluated by computing the confidence limits ratio (CLR) [29]. In order to test the assumption of independence of factors in the study population, we performed a control-only analysis [26]. Finally, the ORs and IORs are presented unadjusted and adjusted by gender and age using an unconditional logistics model. All statistical analyses were performed using STATA 7.0 software (StataCorp. LP, College Station, TX) [30]. All statistical tests were two-sided.

Results

Participants' characteristics, genotype, allelic frequencies, and distribution of cell types of LCa and ORs for these polymorphisms are given in Table 1. Both groups had a similar gender distribution. Smoking frequency was higher in cases compared with controls; however, they had a nonsignificant risk of LCa compared with controls (OR = 1.45, 95% CI = 0.84–2.53). Allele frequencies for the *Pro* allele were similar in cases compared with controls. We did not observe significant departures from Hardy–Weinberg equilibrium for *p53cd72* genotypes among cases and controls ($p = 0.1873$). Similar frequencies for *Pro/Pro*, *Arg/Pro*, and *Arg/Arg* genotypes in cases and controls were observed. There were nonsignificant differences for LCa risk between cases and control carriers of susceptibility genotypes.

Joint ORs and IORs between *p53cd72* genotypes and smoking with respect to LCa are presented in Table 2. We describe the adjusted results because they were similar to the unadjusted findings. Compared with unexposed people without the susceptibility genotype (low-risk susceptibility combination), a modest, nonsignificant increase in LCa risk was observed for smokers who were carriers of two copies of the susceptibility allele *Pro/Pro*: $OR_{ge} = 1.27$ (95% CI = 0.47–3.42), and a lesser, also nonsignificant association was observed for carriers of one copy of *Arg/Pro*: $OR_{ge} = 0.98$ (95% CI = 0.43–2.22). There also was a nonsignificant relationship between and LCa risk and nonsmokers with two copies of the susceptibility allele *Pro/Pro*: $OR_g = 0.74$ (95% CI = 0.22–2.54). In contrast, when only one allele was present in nonsmokers, a protecting significant effect was observed: *Arg/Pro*: $OR_g = 0.26$ (95% CI = 0.10–0.68). In the collapsed model, there was a nonsignificant association with LCa risk among smoker carriers of the risk allele compared with nonsmokers without the susceptibility genotype. In contrast, nonsmoker carriers of the risk allele had a significant inverse association with LCa compared with the low-risk reference group (*Pro**: OR = 0.35, 95% CI = 0.15–0.83).

G × E interactions odds ratios (IOR) for *p53cd72* genotypes and smoking for LCa patients calculated using case-only and control-only designs are presented in

Table 1 Age, gender, allele, genotypic frequencies, and distribution cell types of lung cancer and odds ratios for polymorphisms in *p53cd72* of the subjects studied

	Cases (111)	Controls (133)	OR (95% CI)	*p* value
Age, mean (SD)	61.79 (12.83)	56.75 (11.80)		0.0017
Gender, *n* (%)	76 (68)	87 (65)		0.6138
Smoking, *n* (%)				
Smokers	71 (64)	73 (55)	1.45 (0.84–2.53)	0.1511
Nonsmokers	40 (36)	60 (45)		
Allele *p53cd72*				
Pro	128	167	0.81 (0.55–1.18)	0.2490
Arg	94	99		
Genotypic variant				
Arg/Arg	42 (38)	54 (41)	Reference	
Arg/Pro	44 (40)	59 (44)	0.96 (0.52–1.74)	0.8833
Pro/Pro	25 (22)	20 (15)	1.61 (0.74–3.49)	0.1907
*Pro**	69 (66)	79 (59)	0.19 (0.64–1.94)	0.6599
Cell types				
Squamous	40 (36)	–	–	–
Adenocarcinoma	22 (20)	–	–	–
Large-cell carcinoma	14 (13)	–	–	–
Others	7 (6)	–	–	–
Not determined	28 (25)	–	–	–

SD = standard deviation; OR = odds ratio; CI = confidence interval; *Pro** = *Pro/Pro* + *Arg/Pro*

Table 2 Joint odds ratios (ORs) and interaction odds ratios (IOR) for G × E interactions of simple combinations of *p53cd72* genotypes and smoking for LCa risk in a case-control design

Genotype	Smoking	Cases	Controls	ORs	Unadjusted model			Adjusted model		
					OR	95% CI	CLR	OR	95% CI	CLR
p53 Codon 72										
Pro/Pro	+	18	12	OR_{ge}	1.36	0.53–3.52	6.64	1.27	0.47–3.42	7.30
Pro/Pro	−	7	8	OR_g	0.80	0.24–2.59	10.80	0.74	0.22–2.54	11.55
Arg/Pro	+	33	27	OR_{ge}	1.11	0.50–2.45	4.90	0.98	0.43–2.22	5.20
Arg/Pro	−	11	32	OR_g	0.31	0.13–0.80	6.20	0.26	0.10–0.68	6.80
Arg/Arg	+	20	34	OR_e	0.53	0.24–1.21	5.04	0.44	0.19–1.07	5.63
Arg/Arg	−	22	20	Ref	1			1		
Total		111	133							
Collapsed model										
Pro (PP/AP)	+	51	39	OR_{ge}	1.19	0.53–2.65	5.00	1.07	0.50–2.30	4.60
Pro (PP/AP)	−	18	40	OR_g	0.41	0.16–1.01	6.31	0.35	0.15–0.83	5.53
	Multiplicative scale expected (IOR)				Unadjusted interaction odds ratios			Adjusted interaction odds ratios		
	($OR_g \times OR_e$)		($OR_g \times OR_e$)[a]		IOR_{cc}	95% CI	CLR	IOR_{cc}	95% CI	CLR
Pro/Pro	0.42		0.33		3.20	0.86–11.93	13.90	3.90	1.10–13.81	12.55
Arg/Pro	0.16		0.11		6.76	3.39–13.50	3.50	8.57	4.58–16.04	3.50
Collapsed model	0.21		0.15		5.67	3.00–10.73	3.58	5.48	2.78–10.80	3.90

OR = odds ratio; OR_{ge} = odds ratio gene-environment; OR_g = odds ratio gene-only; OR_e = odds ratio environment-only; IOR_{cc} = interaction odds ratio case-control

[a] Adjusted by gender and age; (+) = yes; (−) = No; CLR = confidence limits ratio

Table 3 G × E interactions odds ratios (IOR) for *p53cd72* genotypes and smoking for lung cancer patients using case-only and control-only designs

Genotype	Smoking	No smoking	Unadjusted model			Adjusted model[a]		
			IOR_{co}	95% CI	CLR	IOR_{co}	95% CI	CLR
Case-only								
Pro/Pro	18	7	2.83	1.54–5.42	3.52	3.05	1.63–5.72	3.51
Arg/Pro	33	11	3.30	1.76–6.20	3.52	3.26	1.74–6.12	3.52
Arg/Arg	20	22	1.0			1.0		
Collapsed model	51	18	3.12	1.29–7.61	5.90	2.98	1.29–6.88	5.33
Control-only								
Pro/Pro	12	8	0.88	0.50–1.65	3.30	1.03	0.55–1.93	3.51
Arg/Pro	27	32	0.50	0.40–1.39	3.50	0.53	0.40–1.41	3.52
Arg/Arg	34	20	1.0			1.0		
Collapsed model	39	40	0.57	0.27–1.23	4.56	0.67	0.32-1.41	4.41

IOR_{co} = interaction odds ratio case-only; CLR = confidence limits ratio

[a] Adjusted by age and gender

Tables 2 and 3, respectively. When we assessed IOR effects for cigarette smoking and *p53cd72* polymorphism on LCa risk in a case-control design, a significant departure from the multiplicative effect was detected between the *Pro/Pro* and *Arg/Pro* genotypes and smoking (*Pro/Pro*: IOR_{cc} = 3.90, 95% CI = 1.10–13.81; *Arg/Pro*: IOR_{cc} = 8.57, 95% CI = 4.58–16.04). Similar results were observed in a case-only design (*Pro/Pro*: IOR_{co} = 3.05, 95% CI = 1.63–5.72; *Arg/Pro*: IOR_{co} = 3.26, 95% CI = 1.74–6.12). In contrast, no association was observed between smoking and *p53cd72* polymorphism when assessed by a control–only design (*Pro/Pro*: IOR_{co} = 1.03, 95% CI = 0.55–1.93; *Arg/Pro*: IOR_{co} = 0.53, 95% CI = 0.40–1.41).

Discussion

Gene polymorphisms that are important to apoptosis will increasingly be recognized as clues to individual susceptibility to cancer, explaining why individuals with shared environmental exposures do not always have equivalent cancer morbidity and mortality.

In the present study, we explored G × E interactions as a risk effect modification between the *p53cd72 Pro* allele and smoking on LCa risk using two different case–control designs. When these factors were evaluated in a joint way in a stratified and in a collapsed analysis using a case–control design, we did not find a clear relationship between the *Pro* allele and the smoking habit. Smoker carriers of two copies of the risk allele presented an increased but nonsignificant LCa risk compared with nonsmokers. In contrast, a protective effect on LCa risk in nonsmokers carrying one copy of *Pro* was observed: *Arg/Pro* ($OR_g = 0.26$, 95% CI = 0.10–0.68). On the other hand, when the subjects were smokers, the effect was null.

Several studies have found an association between the *p53cd72 Pro/Pro* genotype and LCa susceptibility among smokers [10–12, 31–33]. Fan et al. [32] reported that the combination of susceptibility genotypes homozygous *Pro/Pro* and heterozygous *Arg/Pro* (OR = 1.45, 95% CI = 1.01–2.06) was associated with an higher risk of adenocarcinoma compared with *Arg/Arg* genotype after adjustment for relevant variables. Lung adenocarcinoma risk increased with the presence of one or both variant alleles across smoking strata. In this study we observed a similar trend, but the risk was moderately low and nonsignificant (*Pro** = 1.07, 95% CI = 0.50–2.30), probably because of the small sample size or the different histologic type. For example, Liu et al. reported a different risk level when they stratified by histologic subtype. They report that the *Pro** allele carrier patients had an increased risk of LCa (OR = 1.36, 95% CI = 1.1–1.7), especially the adenocarcinoma cell type compared with wild-type patients (*Arg/Arg*). On the other hand, no relationship was observed in patients with squamous cell carcinoma (OR = 1.04, 95% CI = 0.8–1.4) [34]. Szymanowska et al. reported that the *p53 codon 72 Pro* allele may increase the risk of non-small-cell lung cancer (NSCLC) (OR = 1.28, 95% CI = 0.91–1.80) [35]. Similarly, Hu et al. reported that *p53 Pro* allele is associated with an increased frequency of *p53* mutations in NSCLC [36].

When we evaluated the G × E interaction effect between the *p53* polymorphism and smoking on LCa risk, both approaches (case-control and case-only) revealed a significant synergistic effect between these factors compared with each risk factor alone, suggesting that there is a significantly increased LCa risk when the susceptibility genotype and smoking are present simultaneously. Popanda et al. reported that the *p53cd72 Pro* polymorphism increases the risk for squamous cell carcinoma mainly in heavy smokers (OR = 3.84, 95% CI = 1.46–10.1). They suggested that the observed interaction with smoking is biologically plausible as, similar for the *p53cd72 Pro* variant, decreased apoptosis and extended G1 cell cycle arrest are reported after carcinogen exposure [37]. Zhang et al. reported a significant interaction between MDM2 GG and *p53 Pro/Pro* genotypic polymorphisms and smoking (OR = 10.41, 95% CI = 5.26–20.58) [38]. On the other hand, Metakidou et al. [39], in a systematic review and meta-analysis performed on *p53* polymorphisms and lung cancer risk, reported a nonclear relationship between these factors; however, they could not assess the relationship with tobacco exposure. Also, they concluded that most of studies that they reviewed had insufficient power to detect an association between *p53* polymorphisms and LCa risk. Recently, Schabath et al. reported on the multigenetic effects of variant alleles from *p53* exon 4 (*p53cd72*) and introns 3 and 6, and from *p73*, and on their interaction with smoking, resulting in a significantly increased risk for lung cancer in a Caucasian population [40]. Xiao and Singh [18] concluded that interaction of benzo[a]pyrene and p53 is an important regulating factor in human lung cancer cells.

Others kinds of cancer have been evaluated in relation to *p53cd72* and tobacco use. Hong et al. reported that a significant interaction between the *p53 Pro/Pro* genetic polymorphism and smoking was related to the risk of esophageal squamous cell carcinoma (OR = 5.29, 95% CI = 2.91–9.61) [41]. Kuroda et al. found a significant urothelial cancer risk in smoker carriers of the *Pro/Pro* genotype compared with subjects who never smoked (OR = 2.28, 95% CI = 1.12–4.66) [42].

In conclusion, our results suggest that a combination of the *p53cd72* rare allele (*Pro**) and a smoking habit plays a significant role in LCa risk in the studied population compared with each factor alone.

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