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Joint effect among *p53, CYP1A1, GSTM1* polymorphism combinations and smoking on prostate cancer risk: an exploratory genotype-environment interaction study

Luis A. Quiñones[1], Carlos E. Irarrázabal[1], Claudio R. Rojas[1], Cristian E. Orellana[1], Cristian Acevedo[2], Christian Huidobro[2], Nelson E. Varela[1], Dante D. Cáceres[3]

[1]*Laboratory of Chemical Carcinogenesis and Pharmacogenetics, Faculty of Medicine, Biomedical Science Institute (ICBM), University of Chile, Independencia 1027, Santiago, Chile*
[2]*Department of Urology, National Cancer Corporation, Chile, Capellán Abarzúa 027, Providencia, Santiago, Chile*
[3]*Epidemiology Division, School of Public Health, Faculty of Medicine, University of Chile, Independencia 939, Santiago, Chile*

Abstract

Aim: To assess the role of several genetic factors in combination with an environmental factor as modulators of prostate cancer risk. We focus on allele variants of low-penetrance genes associated with cell control, the detoxification processes and smoking. **Methods:** In a case-control study we compared people carrying *p53cd72 Pro* allele, *CYP1A1 M1* allele and *GSTM1 null* genotypes with their prostate cancer risk. **Results:** The joint risk for smokers carrying *Pro** and *M1*, Pro** and *GSTM1null* or *GSTM1 null* and *CYP1A1 M1** variants was significantly higher (odds ratio [OR]: 13.13, 95% confidence interval [CI]: 2.41–71.36; OR: 3.97, 95% CI: 1.13–13.95 and OR: 6.87, 95% CI: 1.68–27.97, respectively) compared with that for the reference group, and for non-smokers was not significant. OR for combinations among *p53cd*72, *GSTM1* and *CYP1A1 M1* in smokers were positively and significantly associated with prostate cancer risk compared with non-smokers and compared with the putative lowest risk group (OR: 8.87, 95% CI: 1.25–62.71). **Conclusion:** Our results suggest that a combination of *p53cd72, CYP1A1, GSTM1* alleles and smoking plays a significant role in modified prostate cancer risk on the study population, which means that smokers carrying susceptible genotypes might have a significantly higher risk than those carrying non-susceptible genotypes.

Keywords: *p53cd72*; *GSTM1; CYP1A1*; genetic polymorphism; prostate cancer; risk; smoking

1 Introduction

Prostate cancer (PCa) is one of the most frequent malignant neoplasms in men; however, the rate of this disease shows remarkable worldwide variation. Many studies indicate that environmental and genetic factors play a significant role in the etiology of this disease [1,2]. Given the multicausal etiology of PCa, synergistic interactions among genetic and other risk factors might have significant effects on PCa risk, especially gene–gene (*G*×*G*) and gene–environment (*G*×*E*) interactions. It is well known that in the carcinogenic process there are mul-

Correspondence to: Dr Dante D. Cáceres, Epidemiology Division, School of Public Health, University of Chile, Independencia 939, Santiago, Chile.
Tel: +56-2-678-6396, Fax: +56-2-737-7121
E-mail: dcaceres@med.uchile.cl

tiple points at which genetically-determined host characteristics and/or environmental factors might influence individual susceptibility, through effects on metabolic activation, DNA-repair capacity, and other cellular processes. Polymorphic genes implicated in cancer etiology can have significant effects on increasing or reducing differential susceptibility to environmental cancer [3].

The *p53* gene is one of the most mutated tumor-suppressor genes in human neoplasms, and it has been referred as the "emergency brake gene" because of its tumor-preventing apoptotic and cell-cycle-checkpoint functions in physiologically stressful situations [4]. The wild type *p53* gene polymorphism at codon 72 (*p53cd72*) produces a protein with an arginine (*Arg*: CGC) or proline (*Pro*: CCC) genotype. This polymorphism related to changes in the function of the p53 protein is strongly associated with the tumor formation process. The wild-type *p53* gene suppresses cellular transformation with activated oncogenes, therefore inhibiting the growth of malignant cells [5].

In contrast, human cytochrome P450 (CYP) phase I enzymes function in a wide variety of metabolic pathways involving endogenous and exogenous compounds, such as steroids and environmental xenobiotics. The *CYP1A1* gene encodes by benzo(a)pyrene hydroxylase and it is primarily expressed in the liver but has also been detected in prostate tissue [6]. *CYP1A1* activates benzo-(a)pyrene into epoxides and phenolic products that are mutagenic and carcinogenic; therefore, higher catalytic activity might predispose patients to cancer risk by increasing carcinogenic compounds in target sites such as the prostate and lung tissues [6, 7]. Three restriction fragment length polymorphic (RFLP) variants have received the most attention: *MspI* RFLP (*CYP1A1**2A), *MspI* RFLP (*CYP1A1**2C) and (*CYP1A1**3). The rare *Val* and *M2* alleles of the *CYP1A1* gene might increase individual cancer risk by heightening aryl hydrocarbon hydroxylase (AHH) enzyme inducibility [8]. Alternatively, among the phase II enzymes involved in the metabolism of xenobiotic compounds the *GST* family catalyze the conjugation of glutathione to numerous potentially genotoxic compounds, including aliphatic aromatic heterocyclic radicals, epoxides, or arene oxides. Individual differences in the detoxification of reactive chemicals through the *GST* pathways are frequently the result of deletion of the *GST* genes, particularly *GSTM1* and *GSTT1* [9]. Individuals who have inherited susceptible variants (homozygous deletions of *GSTM1* or *GSTT1*) of the metabolizing genes might have an increased body burden of reactive metabolites from cigarette smoke, causing increased risk of the development of PCa [10, 11]. However, contradictory findings have been reported in recent studies [12-15].

It is possible that individual variations in biotransformation activities on both phase I and phase II enzymes in coordination with *p53* activity regulate the effect of DNA toxic metabolites and might be partially responsible for host susceptibility to chemical exposure, which is related to PCa.

Our overall aim in the present study is to assess the role of several genetic factors in combination with an environment factor as modulators of PCa risk by focusing on allele variants of low-penetrance genes associated with cell control and detoxification processes, and smoking. We determine the relationships between *GSTM1 deletion, Msp1 CYP1A1* polymorphism*, p53cd72* polymorphism and smoking on PCa risk.

2 Materials and methods

2.1 Sample subjects

Cases for this study were recruited in a voluntary screening performed in Santiago's Metropolitan Area, Chile, by the National Cancer Corporation. Prostate specific antigen (PSA) and digital rectal examination (DRE) were carried out by urologists [16]. All people with suspected PCa (PSA = 4 ng/dL or altered DRE, or both) were biopsied and histologically confirmed. A total of 60 PCa cases and 117 controls were included in the present study. The controls were men attending the respiratory service of the Clinical Hospital of University of Chile, with similar demographic characteristics to the PCa cases. All study subjects provided informed consent for participation in this research under a protocol approved by the Ethics Committee for Studies on Human Beings at the University of Chile.

2.2 Blood samples

Blood samples were collected from all of the participants at time of inclusion. The samples were processed at the Laboratory of Chemical Carcinogenesis and Pharmacogenetics at the Faculty of Medicine of the University of Chile to obtain genomic DNA from peripheral leucocytes using the method of Miller [17]. The genomic DNA was checked for purity at 260/280 nm ab-

sorption and re-purified with phenol/chloroform protocol if required. DNA was stored at –30ºC until use.

2.3 Genotyping methods

After DNA extraction, DNA samples were analyzed for *GSTM1*, *CYP1A1* and *p53* genetic polymorphisms. Polymerase chain reaction (PCR) based RFLP was used to examine the polymorphisms of interest. All samples were submitted to separate amplifications followed by digestion with restriction enzymes.

2.4 Polymerase chain reaction (PCR) amplification detection

For the *CYP1A1 MspI* site, PCR amplification was carried out using previously described primers C44 and C47 yielding a fragment of 340 bp [7]. *GSTM1 null* variant was determined using primers described by Ambrosone *et al.* [18] simultaneously with *Msp1* primers as internal control for amplification. *p53cd72* genetic polymorphism was determined using the primers described by de la Calle-Martin *et al.* [19]. The different genotypes were observed using 2% agarose or 6% polyacrylamide gel electrophoresis.

2.5 Nomenclature used to genetic polymorphisms

The following nomenclature was used to describe the different polymorphic variants [20]. For the *p53cd72* polymorphisms the possible genotypes are *Pro/Pro, Arg/Pro* and *Arg/Arg*. For the *CYP1A1*2A*, the reference allele is called wild type (*Wt*), and rare allele is called *M1*. The *GSTM1 1*/1** and *GSTM1 1*/*2* are referred to as *present* variant (homozygous and heterozygous), with *GSTM1 2*/*2* genotype used to indicate the homozygous *null* variant. For *GST*, null and present denominations will be used. From this point forward, *M1** and *Pro** will be used for *Wt/M1, M1/M1,* and *Pro/Pro* and *Arg/Pro* genotypes, with the objective to increase precision.

2.6 Analytic methodology

Genotype frequencies and 95% confidence interval (CI) for *GSTM1* were calculated as the proportion of individuals with a given genotype divided by the total number of participants. For *p53cd72* and *CYP1A1*, allele frequencies and 95% CI were calculated as the number of alleles divided by the number of chromosomes, and the test for Hardy–Weinberg equilibrium was conducted. To explore the possible associations between *GSTM1, CYP1A1* and *p53cd72* genetic polymorphisms and PCa risk, and to evaluate the putative modification by these genotypes of the effect of smoking, we cross-classified the data using a 2 by 4 table, as described by different authors, for a case-control design [21]. The relationship between these polymorphic genes and smoking and PCa risk was examined using odds ratio (OR), with 95% CI using Woolf's method in an unconditional logistic model. All associations were evaluated using a priori low-risk bivariate genotype combinations (*Arg/Arg, Wt/Wt* and *GSTM1 present*) in non-smokers as a common reference group. Finally, the ORs are presented unadjusted and adjusted by age. All statistical analyses were performed with Stata version 7.0 software (STATA Corporation; College Station, TX, USA)

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3 Results

Characteristics of participants, genotype and allelic frequencies and OR for these polymorphisms and PCa in the present study are described in Table 1. Both the PCa and the control groups had a similar age distribution. Smoking frequency was higher in the PCa cases compared with that in the controls and PCa cases had a significant risk of PCa compared with the controls (OR: 2.59, 95% CI: 1.35–4.95). Allele frequencies for the *Pro* and *M1* allele were higher in the PCa cases compared with that in the controls. We did not find significant differences in genotype frequencies for *GSTM1* and *CYP1A1* between the PCa cases and the controls subjects. A higher prevalence of *Pro/Pro* genotype in the PCa cases compared with that in the control subjects was observed. ORs for PCa associated with *GSTM1* and *CYP1A1* genotypes were close to the null value. For the different genotypes of *p53cd72,* only *Pro/Pro* genotype was positively associated with PCa (OR: 2.89, 95 % CI: 1.17–7.10). We did not observe significant departures from the Hardy-Weinberg equilibrium from *p53cd72* and *CYP1A1* genotypes among the PCa cases (P = 0.356; P = 0.096) or the controls (P = 0.621; P = 0.706), respectively.

Table 2 shows the results of the distribution of the joint effect between gene-gene polymorphisms and smoking risk factor on PCa. The joint age-adjusted OR for smokers carrying *Pro* and M1** variants was 13.13 (95% CI: 2.41–71.36) and for non-smokers was 2.25 (95% CI: 0.44–13.48), compared with the reference group (*Arg/Arg* + *Wt/Wt* + *non-smokers*). In contrast, those smokers and non-smokers carrying only *Pro** variants had a lower,

Table 1. Age, smoking habits, allele and genotypic frequencies for polymorphisms in *p53cd72*, *GTSM1* and *CYP1A1* of the subjects studied. SD: standard deviation; OR: odds ratio; CI: confidence interval; *Pro**: Pro/Pro + Arg/Pro; *M1**: M1/M1+Wt/M1; *Wt*: Wild type.

Subjects	Cases (60)			Controls (117)			*P*–value	
Age (years)	60.70 ± 12.85			60.36 ± 14.25			0.564	
	n	Frequency	95% CI	*n*	Frequency	95% CI	OR	95% CI
Smokers								
Yes	40	66.67	53.31–78.31	51	43.59	34.44–53.06	2.59	1.35–4.95
No	20	33.33	21.68–46.68	66	56.41	46.93–65.55		
Allele								
P53cd72								
Pro	52	43.33	34.31–52.68	71	30.34	24.52–36.66	1.77	1.12–2.80
Arg	68	56.67	47.31–65.68	163	69.23	62.88–75.08		
CYP1A1								
M1	54	45.00	35.90–54.34	72	30.76	24.91–37.11	1.84	1.17–2.90
Wt	66	55.00	45.66–64.09	162	69.23	62.88–75.08		
Genotypic variant								
P53cd72								
*Pro**	38	63.33	51.39–75.52	58	49.57	40.51–58.63	1.76	0.93–3.33
Pro/Pro	14	23.33	12.63–34.03	13	11.11	5.41–16.80	2.89	1.17–7.10
Arg/Pro	24	40.00	27.60–52.39	45	38.46	29.64–47.27	1.43	0.71–2.87
Arg/Arg	22	36.67	24.47–48.86	59	50.43	41.36–59.48	1.00	—
χ^2*: 5.55 P = 0.062*								
GSTM1								
Null	22	36.67	24.47–48.86	36	30.77	22.40–39.13	1.30	0.68–2.51
Present	38	63.33	51.39–75.22	81	69.23	60.86–77.59	1.00	—
χ^2*: 0.62 P = 0.429*								
CYP1A1								
*M1**	46	76.66	65.96–87.36	59	50.43	41.36–59.48	1.27	0.48–3.31
M1/M1	14	23.33	12.36–34.03	58	49.57	40.51–58.63	0.39	0.14–1.13
Wt/M1	38	63.33	51.14–75.52	46	39.32	30.46–48.17	1.34	0.50–3.58
Wt/Wt	8	13.33	4.73–21.93	13	11.11	5.41–16.80	1.00	—
χ^2*: 11.69 P = 0.003*								

non-significant risk (OR: 4.16, 95% CI: 0.75–22.96 and OR: 1.51, 95% CI: 0.21–10.59). However, smokers carrying *M1** and *Arg/Arg* genotypic variants had a higher significant risk compared with the reference group (OR: 8.74, 95% CI: 1.58–48.39), but was not significant in non-smokers (OR: 2.71, 95% CI: 0.48–15.35). The joint age-adjusted OR for smokers carrying *Pro** and *GSTM1 null* polymorphism was 3.97 (95% CI: 1.13–13.95) and for non-smokers was 0.80 (95% CI: 0.19–3.28) compared with the reference group (*Arg/Arg* + *GSTM1 null* + *non-smokers*). Conversely, those smokers carrying *Pro** and *GSTM1* present variants had a significant risk, 3.07 (95% CI: 1.01–9.37), but non-smokers had a non-significant risk, 0.95 (95% CI: 0.28–3.19). Smokers carrying *Arg/Arg* and *GSTM1 null* genotypes showed an increased but non-significant risk compared with the reference group (OR: 4.73, 95% CI: 0.89–25.18). Similarly, the risk for non-smokers was not significant (OR: 0.57, 95% CI: 0.10–3.29). However, the joint effect between the *GSTM1 null* and *CYP1A1 M1** in smokers was significantly associated with PCa risk: 6.87 (95% CI: 1.68–27.97). In contrast, in non-smokers the risk was not significant: 1.37 (95% CI: 0.35–5.46). The age-adjusted OR for smokers carrying the *GSTM1 null* and *Wt/wt* genotypes were high but not significant: 2.69 (95% CI: 0.52–14.08). Those subjects carrying *GSTM1* present and *M1** genotypes had a significant Pca risk for smokers but not for non-smokers (OR: 5.00, 95% CI: 1.47–

Table 2. Odds Ratios (ORs) for bivariate combinations of *p53cd72, GSTM1, CYP1A1* genotypes and smoking factor and PCa. ***P*-value: fisher exact test; SD: standard deviation; CI: confidence interval; P*ro**: Pro/Pro + Arg/Pro; *M1**: M1/M1+Wt/M1; +: smoker; - : non-smoker; MH: Mantel and Haenszel pooled OR estimator; *Wt*: Wild type; Ref: reference low risk group.

Gene-gene variants		Smoking	Cases	Controls		Unadjusted			Adjusted by age		
						OR	95 % CI	*P*-value	OR	95 % CI	*P*-value
P53cd72	*CYP1A1*										
Arg/Arg	*Wt/wt*	–	2	13		Ref			Ref		
Arg/Arg	*Wt/wt*	+	0	18		—	—	0.213**	—	—	—
Arg/Arg	*M1**	–	7	18		2.52	0.46–15.99	0.248	2.71	0.48–15.35	0.258
Arg/Arg	*M1**	+	13	10		8.45	1.43–67.17	0.004	8.74	1.58–48.39	0.013
*Pro**	*Wt/wt*	–	3	13		1.50	0.22–11.70	0.632	1.51	0.21–10.59	0.679
*Pro**	*Wt/wt*	+	9	14		4.17	0.83–30.27	0.052	4.16	0.75–22.96	0.102
*Pro**	*M1**	–	8	22		2.36	0.45–14.36	0.273	2.25	0.44–13.48	0.302
*Pro**	*M1**	+	18	9		13.00	2.07–116.88	<0.001	13.13	2.41–71.36	0.003
Test of homogeneity chi2(6) = 12.47 *P* = 0.052					MH	3.44	1.79–6.62	<0.001			
p53cd72	*GSTM1*										
Arg/Arg	*Present*	–	7	20		Ref			Ref		
Arg/Arg	*Present*	+	8	25		0.91	0.27–2.75	0.881	0.92	0.28–2.99	0.896
Arg/Arg	*Null*	–	2	11		0.52	0.10–3.22	0.460	0.57	0.10–3.29	0.533
Arg/Arg	*Null*	+	5	3		4.76	0.69–14.13	0.059	4.73	0.89–25.18	0.068
*Pro**	*Present*	–	7	21		0.95	0.29–3.17	0.937	0.95	0.28–3.19	0.931
*Pro**	*Present*	+	16	15		3.05	1.00–8.73	0.048	3.07	1.01–9.37	0.048
*Pro**	*Null*	–	4	14		0.82	0.35–4.49	0.779	0.80	0.19–3.28	0.760
*Pro**	*Null*	+	11	8		3.93	1.36–15.11	0.030	3.97	1.13–13.95	0.031
Test of homogeneity chi2(6) = 8.84 *P* = 0.1827					MH	1.68	0.98– 2.56	0.055			
GSTM1	*CYP1A1*										
Present	*Wt/wt*	–	5	17		Ref			Ref		
Present	*Wt/wt*	+	5	27		0.62	0.15–2.55	0.513	0.63	0.16–2.49	0.508
Present	*M1**	–	9	24		1.27	0.35–4.55	0.707	1.29	0.36–4.58	0.692
Present	*M1**	+	19	13		4.96	1.32–18.65	0.008	5.00	1.47–17.05	0.010
Null	*Wt/wt*	–	0	9		—	—	0.124**	—	—	—
Null	*Wt/wt*	+	4	5		2.72	0.48–15.16	0.234	2.69	0.52–14.08	0.239
Null	*M1**	–	6	16		1.27	0.31–5.10	0.730	1.37	0.35–5.46	0.652
Null	*M1**	+	12	6		6.80	1.40–32.96	<0.005	6.87	1.68–27.97	0.007
Test of homogeneity chi2(6) = 13.02 *P* = 0.0427					MH	1.86	1.11–3.11	0.015			

17.05 and OR: 1.29, 95% CI: 0.36–4.58, respectively).

We performed the test of homogeneity for *p53cd72-CYP1A1, p53cd72-GSTM1, GSTM1-CYP1A1* and smoking status, which clearly indicated that the Mantel–Henszel OR for Pca differs depending on whether an individual smoked and on polymorphisms combinations (OR: 3.44, 95% CI: 1.79–6.62; OR: 1.68, 95% CI: 0.98–2.56; OR: 1.86, 95% CI: 1.11–3.11, respectively).

4 Discussion

The *p53 Pro* allele has recently been reported to be associated with genetically determined susceptibility to smoking-related lung cancer in the Chilean population [22]. Few studies have reported an association of *p53cd72* polymorphism with PCa risk [23,24], however the findings have been inconsistent. Phase I enzymes on cytochrome P450 (CYP) is the major enzyme system in xenobiotic metabolism and plays a critical role in metabolic activation of many environmental chemicals. Together with phase II metabolizing enzymes on glutathione S-transferase (GST) are responsible for detoxification process,

even though they might also be involved in bioactivation of some carcinogenic compounds. This is true in the case of polycyclic aromatic hydrocarbons (PAH), which are important carcinogenic components of tobacco smoke. Individual variation in the genes encoding these enzymes could be modifying the effect of specific environmental risk factors and, therefore, could influence susceptibilities to cancer [25].

In this study, we used a case-control design to assess the joint effects of *p53cd72, CYP1A1, GSTM1* polymorphism and smoking habit on PCa risk. We observed that those subjects who are smokers carrying high-risk genotypic variants have an increased PCa risk compared with non-carrying susceptible variant subjects. In general terms, smoking has a synergistic effect on overall risk, which can be explained by the carcinogenic compounds of cigarette smoke that can be differentially biotransformed by *CYP1A1* and/or *GSTM1* (e.g. benzo-[a]pyrene).

The increased risks observed for smokers carrying susceptible genotypes of *CYP1A1* and *p53* (OR: 13.13), *GSTM1* and *p53* (OR: 3.97), and *CYP1A1* and *GSTM1* (OR: 6.87) might be explained by the metabolic function of these biotransformation enzymes, which might act in a coordinated but contrary pathway. Whereas *CYP1A1* produces the reactive benzo(a)pyrene diol epoxide, which can initiate a tumoral process, *GSTM1* detoxifies it by GSH conjugation [6, 26]. However, people who have *M1* allele and *GSTM1* deletion cannot properly detoxify the carcinogenic metabolites. This situation could be worse if *p53* function is decreased or deleted, which apparently occurs with the *p53cd72 Pro* allele [27]. Our results support this asseveration (OR: 8.87, 95% CI: 1.25–62.71). In contrast, there is evidence that benzo(a)-pyrene diol epoxide is able to inactivate *p53* antioncogen, providing indirect evidence of the potential relationship between *CYP1A1* and *GSTM1* biotransformation enzymes and *p53* antioncogen [27]. An interesting additional research hypothesis is related to the role of *CYP1A1* and *GSTM1* in steroid metabolism, taking account the structural similarities between these hormones and PAH, and the participation of testosterone in prostate cancer. This issue can explain, in part, the observed positive associations with the *CYP1A1* gene, even though this topic should be further investigated.

A limitation of the present study is the small numbers of cases of PCa. Hence, it is likely that the relationships between these polymorphisms and smoking can be explained by chance. Therefore, the presence of positive associations for *CYP1A1, GSTM1* and *p53* polymorphisms in smokers and PCa risk must be determined in a bigger study.

In conclusion, our results suggest that a combination of *p53cd72, CYP1A1, GSTM1* genetic polymorphisms and smoking play a significant role in modified prostate cancer risk on the study population, which means that smokers carrying susceptible genotypes might have a significantly higher risk of PCa than those carrying non-susceptible genotypes.

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