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Relationship among metabolizing genes, smoking and alcohol used as modifier factors on prostate cancer risk: Exploring some gene–gene and gene–environment interactions

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Abstract. *Background*: Prostate cancer (PCa) is one of the most common male cancers, but the burden of this disease shows remarkable worldwide variation. The role of susceptibility low penetrance genes and environmental factors in the etiology of (PCa) is unclear, but may involve, in some cases, multiple alleles at multiple loci and environmental factors. *Study Objectives*: To assess whether *CYP1A1*, *GSTM1*, *GSTT1* susceptibility genotypes, smoking status and alcohol consumption factors contribute to PCa risk, gene–gene and gene–environment interactions were analyzed. *Design and Participants*: We explored interactions on a multiplicative scale conducting a population-based case–control and a case–only study on 103 incident PCa patients and 132 unrelated controls. *Main Results*: The interaction odds ratios (IOR) for PCa risk were increased in men who had both susceptibility genotypes *GST* (*M1*; *T1*) *null and CYP1A1-M1** in a case–control and case-only design (IOR_{cc}: 1.11; 95% CI: 0.12–10.02; IOR_{cc}: 6.23; 95%, CI: 0.51–75.89; IOR_{co}: 2.80; 95% CI: 0.44–17.45 and IOR_{co}: 2.65; 95%, CI: 0.30–25.40). No clear evidence for interaction on a multiplicative scale between smoking status, alcohol consumption and genetic polymorphisms in PCa risk was observed. *Conclusions*: Our findings suggest that the interaction between genetic polymorphisms in *GST* (*T1*; *M1*) and *CYP1A1-M1** would play a significant role as a modifying factor on PCa risk in Chilean people. However, these preliminary exploratory results should be confirmed in a larger study.

Key words: Case–control, Case-only, Gene–environment interaction, Gene–gene interaction, Prostate cancer

Introduction

Prostate cancer (PCa) is the second most common neoplasm diagnosed in men after skin cancer, and the second leading cause of death in men following lung cancer. In the US about 30% of all new male cancer cases are PCa and more than 40,000 men die from the disease each year [1]. PCa rarely occurs before 55 years old and in most cases is diagnosed in men over 65 years old. It is estimated that PCa diagnosis has increased over 70% in the last 30 years, possibly explained by factors such as early detection and life expectancy [2]. PCa deaths in Chile rank fourth, following gastric, lung, and breast cancer, in the general population and are the second cause of death among males. About 1300 people die annually from this disease [3].

Different epidemiologic studies made in Chile indicate that the PCa incidence had increased considerably since 1990, attributed fundamentally to the use of the prostate specific antigen (PSA), in the diagnosis of this illness [4]. In Chile, due to the lack of appropriate records, the real incidence of PCa is not known with accuracy. However, the rate of mortality by PCa is estimated at of $16.2 \times 100{,}000$ men, exceeded only by stomach and lung cancer [5]. In a study carried out in the city of Valdivia in the south of Chile between the years 1990 and 2000, Corti and coworkers found a significant increase in the annual incidence of PCa, up to 55 per 100,000 in 1999. The most affected people are over 65 years old, with an age adjusted incidence of 763 per 100,000 men over 65 years old. They also found that the routine use of PSA and free/total PSA, since 1997, has contributed to detection at disease in younger men [6]. On the other hand, Szot determined that the PCa mortality rate in Santiago in 1999 (40% of Chile's population lives in Santiago) was $16.79 \times 100{,}000$ men, and in those over 65-year-old the leading cause of mortality due to cancer, followed by gastric and lung cancer, respectively [7].

Recent studies indicate that both genetic (high and low penetrance genes) and/or environmental factors,

such as family antecedents of PCa (father, brother, uncle), ethnicity, age, diet and life style are of importance in the etiology of PCa [2, 8–11]. Lichtenstein et al. [12] in a study of cancer in monozygotic and dizygotic twins concluded that genetic factors accounted for approximately 42% of PCa with the remaining risk attributed to environmental factors. An environmental role in PCa is strongly suggested by the 30-fold variation internationally in disease incidence and the observation that PCa rates among immigrants tend to approach those of the host country [13]. Among these environmental factors we identified the exposure to some chemicals that are metabolized by cellular enzymes (CYP and GST family) into reactive or inert metabolites. Relationships between low-penetrance genes that encode these metabolizing enzymes and environmental exposure to xenobiotic compounds have been extensively studied as risk factors for cancer [11, 14, 15]. Many of these genes are polymorphic and can have profound effects on increasing or reducing the metabolic capabilities of the enzymes and can contribute to differential susceptibility to environmental cancer [16–19].

The CYP family of enzymes functions in a wide variety of metabolic pathways involving both endogenous and exogenous compounds, such as steroids and environmental xenobiotics. The *CYP1A1* gene encodes aryl hydrocarbon hydroxylase (AHH), which is primarily expressed in the liver but has been detected in prostate tissue [20]. For example, *CYP1A1* activates aromatic hydrocarbons such as benzo(*a*)pyrene into epoxides and phenolic products that are mutagenic and carcinogenic, therefore, higher catalytic activity may predispose patients to cancer risk by increasing carcinogenic compounds in target sites such as the prostate and lung tissues [10, 19, 21, 22]. Three restriction fragment length polymorphic (RFLP) variants have received the most attention; *Msp*I RFLP (*CYP1A1**2A), *MspI* RFLP (*CYP1A1**2C) and *CYP1A1**3 [23]. The rare *Val* and *M2* alleles of the *CYP1A1* gene may increase individual cancer risk by heightening AHH enzyme inducibility [24]. On the other hand, the GST family catalyzes 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 via the GST pathways are frequently the result of deletion of the GST genes, particularly *GSTM1* and *GSTT1* [25, 26]. Individuals who have inherited susceptible versions (homozygous deletions of *GSTM1* or *GSTT1*) of the metabolizing genes may have increased body burdens of reactive metabolites from cigarette smoke, causing increased risk for the development of PCa [27, 28].

Our overall aim is to explore the role of gene–gene and gene–environment interactions as modifying factor on PCa risk by focusing on polymorphisms in low-penetrance genes associated with the metabolism of xenobiotic compounds. We determined relationships between *GSTM1*, *GSTT1*, *CYP1A1*2A* gene polymorphisms, and alcohol and tobacco use.

Methods

Study designs, subjects selection and genotyping analyses

An unmatched case–control and a case-only study were conducted. Selection of the subjects, personal characteristics, smoking exposure assessment and genotyping methods are explained in detail elsewhere [29]. Briefly, from November to December of 1999 a voluntary screening for PCa was administered to 1700 individuals 45–85 years old in Santiago's Metropolitan Area, Chile. PSA and digital rectal examination (DRE) by urologists was administered. All people with suspect findings (PSA $\geqslant$4 ng/dl or altered DRE, or both) were biopsied and histologically confirmed. Controls were randomly selected from men histologically confirmed without PCa. A total of 235 subjects were selected; 103 PCa cases and 132 unrelated controls. Both control and cancer patients were interviewed regarding smoking habits, alcohol consumptions, use of oral contraceptives or hormones, incidence of cancer in related family members, and exposure to occupational, outdoor, and indoor carcinogenic pollutants. PCa were all previously diagnosed histologically. All individuals were tested for PSA antigen using IMMULITE® generation PSA assays [30]. The extent of tobacco use was assessed by the smoking index (SI) (cigarettes/day × 365) [31]. A smoker was defined as a person with an SI of 800 or higher. Alcohol users were defined as a person that consumed at least one cup of wine or beer per day during the last year. PCR based RFLP was used to examine the polymorphisms of interest. 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.

Polymorphisms nomenclature

The following nomenclature was used to describe the different polymorphic variants [32, 33]. For the *CYP1A1* polymorphisms the genotype is *CYP1A1*2A Wt/Wt (Wild type)* for the reference group. The *CYP1A1*2A* allele has a 3801T > C exchange (allele also known as *MspI*), the reference allele is called *Wt*, polymorphism is called *M1*. The *GSTM1 1*/1** and *GSTM1 1*/*2* are referred to as carriers '*present variant*' (homozygous and heterozygous), with *GSTM1 2*/*2* genotype used to indicate the homozygous '*null variant*'. Similarly, the *GSTT1* carriers include the genotypes *GSTT1 1*/1** and *1*/*2* (*present variant*), with the *GSTT1 *2/*2* (*null variant*) genotype indicating the deleted phenotype.

From this point forward, *M1** will be used for both *Wt/M1* and *M1/M1* genotypes, and *Wt** will be used for *Wt/Wt* genotypes. For GST '*null and present*' designations will be used.

Analytical methods

Genotype frequencies for *GSTM1*, *GSTT1*, and *CYP1A1-M1** were calculated as the proportion of individuals with a given genotype divided by the total number of participants. For *CYP1A1* (*M1*;*Wt*), allele frequencies 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 [34]. χ^2 tests were used to compare the prevalence of *GST*s *and CYP1A1* genotypes among cases and controls. Genetic polymorphisms and environmental exposure were considered as dichotomous factors. In order to evaluate multiple combinations of gene–gene and gene–environment factors and their association with PCa risk, extended 2×2 tables were used [35, 36]. Gene–gene effects of *GST*s *and CYP1A1* genotypes were estimated using '*a priori*' low-risk susceptibility genotype combinations (*GST present* and *CYP1A1 Wt** variants) as a common referent groups. The relationship between genetic polymorphism and environmental factors and PCa risk was examined by odds ratios (ORs), with 95% confidence limits using unconditional logistic regression. All risk models were adjusted for age. To assess for gene–gene $(G \times G)$ and gene–environment $(G \times E)$ interactions on a multiplicative scale, we used an interaction odds ratios (IORs), called synergy index (SIM) in a case–control and, case-only odds ratio (COR) in a case-only design, respectively [37].

For $G \times G$ and $G \times E$ interactions the following formulae were used:

$$SIM : \mathrm{IOR_{CC}} = [\mathrm{OR_{gg;ge}}/\mathrm{OR_{g(-);g}} \times \mathrm{OR_{(-)g;e}}]$$

$$COR : \mathrm{IOR_{CO}} = [\mathrm{OR_{gg;ge}}/\mathrm{OR_{g(-);g}} \times \mathrm{OR_{(-)g;e}}], \times \mathrm{Z_{gg;ge}}$$

where, $\mathrm{IOR_{cc}}$ and $\mathrm{IOR_{co}}$ are IORS by case-control and case–only study, respectively. In these formulae $\mathrm{OR_{gg}}$ and $\mathrm{OR_{ge}}$ are the joint OR for disease among smokers with the susceptibility genotypes and, among individuals carrying both susceptibility genotypes, respectively. $\mathrm{OR_g}$ and $\mathrm{OR_{g(-)}}$ are the OR for disease among non-smokers with the susceptibility genotypes and, among individuals carrying one susceptibility and one wild-type genotype, respectively. $\mathrm{OR_e}$ and $\mathrm{OR_{(-)g}}$ are the OR for disease among smokers without the susceptibility genotypes, and among individuals carrying one wild-type and one susceptibility genotype. Finally, Z is the OR between exposure and genotype or between both genes in the control group in the case-only design [38]. If the genotypes and/or exposure are independent, this factor becomes unity and the OR obtained from a case–only study becomes simply the SIM on a multiplicative scale derived from a regular case–control study, therefore SIM = COR [36, 37]. In the present study we assumed independence between genes and environmental factors. All statistical analyses were performed using STATA 7.0 software [39].

Results

Characteristics of subjects and polymorphic genotypic frequencies are described in Table 1. Briefly, the prevalence of smokers and drinkers was similar in both groups, 20.4, 24.2 and 27.8, 32.6% in cases and controls respectively. Age and familiar prostate antecedents were significantly higher in the case group ($p = 0.007$). Positive digital examination (%) and PSA antigen levels were significantly higher in cases compared with controls. We did not observe significant departures from the Hardy–Weinberg equilibrium for *CYP1A1* genotypes among cases ($p = 0.97$) and controls ($p = 0.82$). On the other hand, we observed a higher significant difference in genotype frequencies for *GSTM1 null and CYP1A1-M1** in cases compared with controls, respectively ($p = 0.02$ and $p = 0.006$). However, we did not observe differences for *GSTT1 null* (p=0.197). Allele frequencies for the *CYP1A1 M1* allele were significantly higher in cases (38%) compared with controls (26%) ($p = 0.005$). We did not observe significant departures from the Hardy–Weinberg equilibrium for *CYP1A1* genotypes among cases (p=0.97) and controls ($p = 0.82$) (calculations not shown).

Joint ORs for gene–gene combinations and IORs for *GST*S and *CYP1A1* genotypes and PCa risk are presented in Table 2. Compared with the reference lowest risk group (multiple combinations of *GSTM1 null*, *GSTT1 null* and *CYP1A1 Wt**), ORs for *GSTM1* (*null* and *present*) and *GSTT1 null* were not associated with PCa risk. In contrast, OR for *GSTM1 null* and *GSTT1 present* genotypes were significantly associated with PCa risk ($\mathrm{OR_{g(-)}}$: 2.00; 95% CI: 1.03–3.87). ORs combinations for *GSTM1* (*null* and *present*) and *CYP1A1-M1** genotypes were positively associated with PCa risk ($\mathrm{OR_{gg}}$: 3.35; 95% CI: 1.42–7.88 and $\mathrm{OR_{(-)g}}$: 1.97; 95% CI: 1.00–3.90) compared with the referent group. However, OR combination for *GSTM1 null* and *CYP1A1* (*Wt**) was not significant. OR combination for *GSTT1 null* and *present* and *CYP1A1-M1** genotypes showed a non-significant increased PCa risk cancer compared with the referent group. In contrast, OR for *GSTT1 null* and *CYP1A1 Wt** showed an inverse relation with PCa risk, however, this association did not achieve statistical significance. The SIM case–control

Table 1. General characteristics and genotypic frequencies for polymorphisms GSTM1, GSTT1, and CYP1A1 (M1*) of the subjects studied

Subjects	Cases	N = 103	Controls	N = 132	p-Value
Age (years)(SD)	68.7	(7.3)	63.3	(7.4)	0.001
PSA (ng/dl)(SD)	35.7	(84.9)	6.3	(5.3)	0.01
DRE (+) (%)	56	54.0	30	22.7	0.001
Smokers (%)	21	20.4	32	24.2	0.48
Drinkers (%)	28	27.8	43	32.6	0.37
Familial antecedents of PCa risk					
Father (%)	10	9.7	9	6.8	0.41
Brother (%)	10	9.7	3	2.3	0.01
Uncle (%)	0	0	6	4.5	0.01
Mother (Paternal) (%)	43	41.7 %	22	16.7	0.001
χ^2: 12.07 p = 0.007					
Genotypic variant	N	%	N	%	
GSTM1					
Null	37	36.3	30	22.7	0.023
Present	65	63.7	102	77.3	
(missing)	(1)		(0)		
GSTT1					
Null	6	6.0	14	10.9	0.197
Present	94	94.0	115	89.1	
(missing)	(3)		(3)		
CYP1A1 (M1*)					
M1*	63	61.8	57	43.8	0.006
M1/M1	14	13.8	11	8.4	0.049
Wt/M1	49	48.0	46	35.4	0.015
Wt/Wt	39	38.2	73	56.2	
(missing)	(1)		(2)		

PSA = Prostate Specific Antigen; DRE = Digital Rectal Examination; (sd) = standard deviation; % = Genotype frequency χ^2 *M1* = Wt/M1 and M1/M1*; *Null and M1** = Susceptibility genotypes; χ^2 = chi square.

and case–only design for *GSTM1-GSTT1* and *GSTT1-CYP1A1* were more than one indicating more than multiplicative effect between these genes (IOR_{cc}: 1.11; 95% CI: 0.12–10.02; OR_{co}: 2.80; 95% CI: 0.44–17.45 and IOR_{cc}: 6.23; 95% CI: 0.51–75.89; IOR_{co}: 2.65; 95% CI: 0.30–25.40, respectively).

Joint ORs for gene–environment combinations and IORs for GST, *CYP1A1* genotypes, and smoking and alcohol use and PCa risk are presented in Table 3. An increased PCa risk among people with susceptibility *GSTM1* and *CYP1A1* genotypes, and environmental factors (OR_{ge}) were observed compared with unexposed people without the susceptibility genotype. However, these risk differences were not-statistically significant. In contrast, high PCa risk among unexposed people with susceptibility genotypes (*GSTM1 null* or *CYP1A1-M1**) was observed (OR_g: 2.16; 95% CI: 1.07–4.31; OR_g: 2.07; 95% CI: 1.10–3.90 for smoking, and OR_g: 1.72; 95% CI: 0.81–3.65; OR_g: 1.67; 95% CI: 0.86–3.27). *GSTT1* susceptibility genotypes for both exposed and unexposed environmental factors were close to the nullity. The SIM case–control and case-only design for *GSTM1*-smoking and *CYP1A1*-smoking were less than one indicating a non-multiplicative effect between these genes and smoking risk (IOR_{cc}: 0.47; 95% CI: 0.12–1.91; IOR_{co}: 0.65; 95% CI: 0.23–1.87 and IOR_{cc} 0.69 95% CI: 0.19–2.53; IOR_{co}: 0.82 95% CI: 0.30–2.32, respectively). In contrast, IORs for case–control and case-only for *GSTT1*-smoking were more than one indicating more than multiplicative effect in smokers with susceptibility genotype (IOR_{cc}: 2.47; 95% CI: 0.27–22.30 and IOR_{co}: 2.00; 95% CI: 0.34–11.74, respectively). Similarly, more than a multiplicative interaction effect between *GSTM1*-alcohol and *CYP1A1*-alcohol in both designs were observed (IOR_{cc}: 1.30; 95% CI: 0.39–4.32; IOR_{co}: 1.55; 95% CI: 0.63–3.81 and IOR_{cc}: 1.51; 95% CI: 0.45–5.01; IOR_{co}: 1.04; 95% CI: 0.42–2.61, respectively). On the other hand, a non-multiplicative effect between drinking and susceptibility genotype was observed in both designs (IOR_{cc}: 0.16; 95% CI: 0.01–1.95 and IOR_{co}: 0.52; 95% CI: 0.06–4.70, respectively).

Discussion

Many reports have been written about gene–environment interaction and its association with cancer susceptibility, especially the relationship between polymorphic chemical metabolizing genes and environmental carcinogens [14, 16, 17, 40, 41]. Chemical

Table 2. Gene-gene interactions for multiple combinations of *GSTM1*, *GSTT1* and *CYP1A1* (*M1**) genotypes and prostate cancer risk

Genotype combinations		Cases	Controls	OR		95% CI
GSTM1	*GSTT1*					
Null	Null	3	5	OR_{gg}	0.71	0.15–3.32
Null	Present	33	24	$OR_{g(-)}$	2.00	1.03–3.87
Present	Null	2	9	$OR_{(-)g}$	0.32	0.06–1.62
Present	Present	61	91	Ref	1	–
χ^2: 8.5 $p = 0.03$						
Multiplicative Interaction (SIM)						
Case-control design				IOR_{cc}	1.11	0.12–10.02
Case-only design				IOR_{co}	2.80	0.44–17.45
GSTM1	*CYP1A1*					
Null	*M1**	23	14	OR_{gg}	3.35	1.42–7.88
Null	*Wt**	14	16	$OR_{g(-)}$	1.88	0.80–4.66
Present	*M1**	40	43	$OR_{(-)g}$	1.97	1.00–3.90
Present	*Wt**	24	56	Ref	1	–
χ^2: 12.0 $p = 0.007$						
Multiplicative Interaction (SIM)						
Case-control design				IOR_{cc}	0.90	0.27–2.98
Case-only design				IOR_{co}	0.96	0.41–2.25
GSTT1	*CYP1A1*					
Null	*M1**	4	5	OR_{gg}	1.49	0.75–2.92
Null	*Wt**	1	9	$OR_{g(-)}$	0.17	0.02–1.74
Present	*M1**	57	50	$OR_{(-)g}$	1.40	0.31–6.22
Present	*Wt**	37	63	Ref	1	–
χ^2: 6.89 $p = 0.07$						
Multiplicative Interaction (SIM)						
Case-control design				IOR_{cc}	6.23	0.51–75.89
Case-only design				IOR_{co}	2.65	0.30–25.40

*Null and M1** = Susceptibility genotypes; *M1** = *Wt/M1 and M1/M1*; *Wt** = *Wt/Wt*; OR = Odds Ratio adjusted by age; 95% CI = Confidence interval; Ref = Reference low risk group; IOR_{cc} and IOR_{co} = Interaction Odds Ratio case–control and case-only, respectively; SIM = Synergy Index.

carcinogens generally require activation to reactive forms in order to produce reactive species, such as DNA adducts, this being mainly catalyzed by phase I enzymes of the CYP family. On the other hand, phase II enzymes, such as the GST family, conjugate metabolic intermediates to soluble forms which are then easily excreted [42, 43]. Therefore, it can be assumed that individuals with elevated metabolic activity and low detoxifying activity in the presence of environment factors (xenobiotic compounds) are at higher risk of PCa development.

First of all, we analyzed the relationship between multiple combinations of gene–gene and gene–environment interactions using ORs as measured effect and PCa risk. Later, we examined gene–gene and gene–environment effect using IORs in a multiplicative scale and PCa risk by case–control and case-only design, respectively. When these polymorphisms were analyzed in combination, the *GSTM1 null* and *CYP1A1-M1** genotype combinations was statistically associated with PCa risk. In contrast, when we analyzed multiple combinations for *GSTT1 null* genotype and the other genetic polymorphisms studied, the associations studied were not significant and most of cases showed an inverse relation with PCa risk indicating a probably protector effect of *GSTT1*. The positive trend was only observed when the *CYP1A1-M1** genotype was present. When we assessed gene–gene through SIM, more than multiplicative interactions were observed within *GSTM1-GSTT1* and *GSTT1-CYP1A1* susceptibility genotypes in both epidemiologic designs indicating a modifier effect of these genes on PCa risk. These increased interactions may be in a great percentage explained by the positive effect of *GSTM1 null* and *CYP1A1-M1** variant due to the possibility that *GSTT1 null* variant would have a protective effect on PCa risk in both cases. IORs for *GSTM1-CYP1A1* were almost unity indicating a multiplicative effect between these genes on PCa risk.

Few studies have evaluated gene–gene combinations between GST and *CYP1A1* susceptibility polymorphisms. Murata et al. [44, 45] ffound increased association with PCa risk in Japanese people

Table 3. Gene-environment interactions for combinations of *GST*, *CYP1A1* genotypes and smoking and alcohol drinking for prostate cancer risk

Genotype	Smoking	Cases	Controls	OR		95% CI	Alcohol	Cases	Controls	OR		95% CI
GSTM1												
Null	+	6	9	OR_{ge}	1.25	0.39–4.01	+	13	11	OR_{ge}	1.45	0.57–3.69
Null	–	31	21	OR_{g}	2.16	1.07–4.31	–	25	18	OR_{g}	1.72	0.81–3.65
Present	+	15	23	OR_{e}	1.23	0.55–2.74	+	16	31	OR_{e}	0.65	0.30–1.38
Present	–	50	79	Ref	1.00	–	–	49	71	Ref	1.00	–
Multiplicative Interaction (SIM)												
Case-control design				IOR_{cc}	0.47	0.12–1.91				IOR_{cc}	1.30	0.39–4.32
Case-only design				IOR_{co}	0.65	0.23–1.87				IOR_{co}	1.55	0.63–3.81
GSTT1												
Null	+	2	3	OR_{ge}	0.87	0.47–2.02	+	1	8	OR_{ge}	0.13	0.01–1.10
Null	–	4	11	OR_{g}	0.36	0.10–1.22	–	5	6	OR_{g}	0.87	0.23–3.25
Present	+	19	28	OR_{e}	0.98	0.13–6.10	+	27	34	OR_{e}	0.91	0.42–1.73
Present	–	75	87	Ref	1.00	–	–	67	81	Ref	1.00	–
Multiplicative Interaction (SIM)												
Case–control design				IOR_{cc}	2.47	0.27–22.30				IOR_{cc}	0.16	0.01–1.95
Case-only design				IOR_{co}	2.00	0.34–11.84				IOR_{co}	0.52	0.06–4.70
CYP1A1 (Msp1)												
*M1**	+	12	13	OR_{ge}	1.82	0.69–4.84	+	17	16	OR_{ge}	1.54	0.65–3.67
*M1**	–	51	44	OR_{g}	2.07	1.10–3.90	–	46	41	OR_{g}	1.67	0.86–3.27
*Wt**	+	9	17	OR_{e}	1.27	0.48–3.41	+	11	27	OR_{e}	0.61	0.25–1.47
*Wt**	–	30	56	Ref	1.00	–	–	28	46	Ref	1.00	–
Multiplicative Interaction (SIM)												
Case-control design				IOR_{cc}	0.69	0.19–2.53				IOR_{cc}	1.51	0.45–5.01
Case-only design				IOR_{co}	0.82	0.30–2.32				IOR_{co}	1.04	0.42–2.61

*Null and M1** = Susceptibility genotypes; *M1** = *Wt/M1 and M1/M1*; *Wt** = *Wt/Wt*; OR = Odds Ratio adjusted by age; 95% CI = Confidence interval; ORge = Odds Ratio gene-environment interaction; ORg = Odds Ratio gene effect; ORe = Odds Ratio environment effect; IOR_{cc} and IOR_{co} = Interaction Odds Ratio case–control and case-only, respectively; SIM = Synergy Index; Ref = Reference group.

(OR: 2.3; 95% CI: 1.18–4.48; OR: 2.2; 95% CI: 1.12–4.20) when *GSTM1 null* and *CYP1A1 M2* genotypes were present. On the other hand, diverse authors have evaluated *GSTM1* and *GSTT1* polymorphic combination and PCa risk. In general, in combinations for *GSTM1* and *GSTT1 null* genotypes only non-significant associations have been found. Kote-jarai et al. [46] found a higher risk of PCa when they evaluated three of the putative high-risk genotypes (*GSTM1* and *GSTT1 null* and *GSTP1* val/val), however, this risk was not significantly different when *GSTP1* was considered alone, probably indicating that the *GSTM1* and *GSTT1* combination is not associated with PCa risk. Neither Rebbeck et al. [28] nor Beer et al. [47] found an association between PCa and these two *null* genotypes. In contrast, Steinhoff et al. [27] reported a significant association with PCa risk when they evaluated the same genotypes. However, their findings must be evaluated with caution due to small sample size.

When we evaluated gene–environment interactions, a numerically elevated PCa risk was observed for *GSTM1 null*, *CYP1A1-M1** and smoking. However, this elevation was not significant. Also, alcohol use and susceptibility genotype factors were not associated with PCa risk. On the other hand, when these factors were evaluated alone, only genotypic effect was clearly associated with PCa risk. Similarly, *GSTT1 null* genotype risk factor was slightly but inversely associated with PCa, however, this trend was not significant. On the other hand, when we assessed departures of multiplicative interaction using both design the IOR were close to nullity, indicating an independent effect between these polymorphisms and the environmental factors analyzed on PCa risk. Also, in the case of *GSTT1* genetic polymorphisms and environmental factors, the relationship might be unclear due to the low frequency of this allelic variant in the analyzed sample.

Not many studies have evaluated these gene–environmental interactions directly. Kelada et al. [48] found a significant increase in the probability of having PCa in men who were both smokers and carried a *non-null GSTT1* genotype compared with men who had neither or only one of these risk factors. In contrast, they did not find an effect between smoking and *GSTM1 non-null* genotype [48]. Similar findings reported by Murata et al. did not describe any modification effect of PCa risk when smoking was considered [45]. In general, epidemiologic studies did not support a clear causal relationship between smoking and PCa risk [49–54]. However, a recent study indicated that men who are heavy smokers would have a significant impact on the risk of developing PCa, especially the more aggressive forms of the disease [55]. In the case of alcohol use, a clear non-relationship between the genetic polymorphisms studied, alcohol consumption and PCa risk was observed in this study. No reports have been described about this genetic–environment interaction and PCa risk to date. However, some studies in breast cancer and these polymorphisms in people using alcohol have been reported. Park et al. [56] found a positive association between GST (*M1*,*T1*) *null* polymorphisms, alcohol use interaction and breast cancer in premenopausal women (OR: 5.3; 95% CI: 1.0–27.8). Similar findings were described by Zheng et al. [57]. On the other hand, Rundle et al. [58] found increased levels of PAH–DNA adducts in breast tissue among subjects with the *GSTM1 null* genotype who used alcohol. The probable mechanism involved would be that the lack of GST genes could reduce the capacity to conjugate lipid peroxidation products, cytotoxic compounds and free radicals generated during alcohol metabolism [56]. Toxicological studies *in vivo* and *in vitro* have shown inconsistent results on whether or not alcohol alters GST expression [58]. Most of epidemiologic studies indicate that neither amount nor type of alcohol seems to be clearly associated with a risk of developing PCa [54, 59–61]. Bagnardi et al. [62] in a meta-analysis study evaluated the association between alcohol consumption and the risk of 18 kinds of neoplasms, no significant or consistent relation was observed for cancers of the pancreas, lung, prostate or bladder.

We also considered a number of potential limitations to this study. The main limitation in this study is sample size, especially after stratification by susceptibility genotypes and environmental status. In the literature of genetic polymorphisms and cancer there is little consensus as to which positive associations is likely to be true. In general, these inconsistencies are due to small sample size, publication bias and population stratification among others causes. Having presented these antecedents, we evaluate interactions in a case-only model whose sample sizes are significantly smaller than those needs in the design of case–control studies, however with the objective to compare we also presented the results for case–control design. Case-only study has been promoted as a more precise method to evaluate gene–gene or gene–environment interactions on a multiplicative scale [37, 63]. Well-known is that obtaining a representative control group is one of the most difficult tasks in many case–control studies because factors related to inclusion, such as social class, education, ethnicity and lifestyles, may be related to the exposure variables under study [64]. On the other hand, Botto and Khoury [65] recommend using both designs as complementary rather than alternative approaches to assess interactions; together these two calculations can contribute to the efficient and systematic assessment of the role of multiple factors, complex genotypes, and their interaction in disease etiology. Other confounding factors not considered in this study were diet and occupation. Both have been associated with PCa risk in many studies [2, 66]. Also, a population stratification bias could exist because subjects' ethnicity was not

identified in this study. However, similar frequencies of *GSTM1* and *CYP1A1* genetic polymorphisms were described in preliminary studies made in Chilean populations and therefore the sample analyzed is likely to be homogeneous in terms of genetic background [67]. In respect to the population stratification bias factor Wacholder et al. [68] indicates that self-reported ethnicity may be a better and more appropriate tool to reduce confounding factors in cancer studies whereas population stratification may be a great concern. Another source of bias could be the manner in which subjects were selected. Because they were selected through a screening program, and were not a random sample of the target population, it is probable that the participants are healthier than the rest of the population and therefore the risk of PCa is underestimated. Also, it is probable that there are different evolution grades among the cases and therefore survival effect could influence the results. Finally, no departure of linkage disequilibrium between any of the loci examined was uncovered when we compared the observed frequencies of heterozygous and homozygous combination of *GSTM1*, *GSTT1* and *CYP1A1*2A* with those expected from their population frequencies. This suggests that for these alleles there is no linkage between any of the polymorphic alleles at these loci (data not shown).

In conclusion, our results suggest that the interaction between genetic polymorphisms in *GST (T1; M1)* and *CYP1A1-M1** would plays a significant role as a modifier factor on PCa risk in Chilean people. In contrast, no clear association was observed when smoking, alcohol use and genetic polymorphisms were evaluated together. The absence of a clear association between *GST*s, *CYP1A1* and the environmental factors suggests that other genetic or environmental exposures may modify the risk to PCa in the population studied. However, these preliminary exploratory results should be confirmed in a larger study, using this as hypothesis that could be investigated further in family-based association studies.

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