

Positive Correlation Between Single or Combined Genotypes of CYP1A1 and GSTM1 in Relation to Prostate Cancer in Chilean People

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BACKGROUND. The prostate cancer is a slowly progressing disease that begins decades prior to diagnosis. It has been suggested that there might be differences in susceptibility due to genetic polymorphisms in biotransformation enzyme genes. In the present work, associations between CYP1A1(*Msp1*), GSTM1(–/–) polymorphisms, and prostate cancer were analyzed in a case-control study.

METHODS. Genomic DNA was isolated from peripheral blood samples, collected on EDTA. PCR-RFLP was used to determine simultaneously *Msp1* and GSTM1(–/–) polymorphisms.

RESULTS. In cancer patients, frequency of m2 variant allele (0.377) and GSTM1(–/–) (0.362) showed statistically significant increases compared to the control group (0.262 and 0.227, respectively). The estimate relative risks (OR) were higher for individuals carrying combined CYP1A1 and GSTM1 rare genotypes, in relation to individuals carrying CYP1A1 or GSTM1 alone. Multivariate logistic regression analysis including confounding factors (age, digital examination, and PSA antigen) showed even higher risk for individuals carrying m2m2 genotype (OR = 3.99; 95% CI, 1.27–12.54), GST(–/–) genotype (OR = 2.75; 95% CI, 1.31–5.79), and m2m2/GST(–) genotype (OR = 16.63; 95% CI, 1.67–165.48).

CONCLUSIONS. Taken together, these findings suggest that Chilean people carrying single or combined GSTM1 and CYP1A1 polymorphisms are more susceptible to prostate cancer. *Prostate* 57: 111–117, 2003. © 2003 Wiley-Liss, Inc.

KEY WORDS: CYP1A1; *Msp1*; GSTM1; polymorphisms; prostate cancer

INTRODUCTION

Prostate cancer will account for 40% of all new diagnosed cancer cases (excluding skin cancer) and cause over 31,900 deaths in USA [1,2]. Prostate cancer deaths in Chile ranks fourth, following gastric cancer, lung cancer, and breast cancer in the general population and is the second cause among males. Opportunities for prevention must be identified, cost-effective preventive strategies tested, and programs established for the dissemination of knowledge to the medical community and the public.

Prostate cancer is rarely detected prior to the age of 40 years, and the incidence rate increases rapidly for each subsequent decade of life. It shows heterogeneity

in presentation and unpredictable rates of progression. A hereditary component of prostate cancer has been demonstrated, although the role of specific gene

Abbreviations: PIN, prostatic intraepithelial neoplasia; CYP, cytochrome P450; PCR, polymerase chain reaction; OR, odds ratio; CI, confidence interval.

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products remains poorly understood [3]. The vast majority of prostate cancer cases, perhaps 90%, cannot be explained by rare and highly penetrant hereditary factors.

Scientists are beginning to appreciate the presence of genes exhibiting common polymorphisms that may modulate physiologic and biochemical processes related to prostate cancer, such as androgen sensitivity [4,5], xenobiotic and endobiotic metabolism. It is probable that many genetic polymorphisms modulating hormone activity, androgen receptor, and nutrient metabolism contribute to the risk of prostate cancer.

An association between prostate cancer incidence and tobacco use has been inconsistent, and most literature indicates that tobacco is not likely to be a major determinant of overall risk [6,7]. Nonetheless, a series of studies have reported higher incidence or mortality rates from prostate cancer among smokers and has been related to more aggressive and lethal prostate cancer [8–12]. The effects of smoking on prostate cancer risk could be mediated by carcinogens found in tobacco smoke. In this way, the etiology may be the key factor associated with the higher risk of prostate cancer in different populations [13]. However, more work is necessary in order to understand how environmental factors, as well as genetic polymorphisms may ultimately modulate the risk of prostate cancer.

On the other hand, the phase I cytochrome P450A1 (CYP1A1) and phase II enzymes, including glutathione S-transferase (GSTM1) have been implicated in risk of prostate cancer [14,15].

It is possible that individual variations in metabolic activities in each phase or in the coordination of these two phases regulate the clearance of DNA toxic metabolites and might be partially responsible for individual host susceptibility to chemical exposure-related prostate cancer.

CYP1A1 is an enzyme with aryl hydrocarbon hydroxylase activity (AHH) that exhibits trimodal distribution in human populations with its higher activity shown to be associated with high lung cancer risks [16]. The entire *CYP1A1* gene has been sequenced [17] and four polymorphisms have been reported, namely, two in the 3'-flanking region called *Msp1* [18] and AA-RFLP (specific to Afro-American people) [19] and two others within the exon 7, called ile/val [20] and m4 (thr/aspn) [21,22]. Though the relationship of these mutations with AHH inducibility has not been fully established, it has been suggested that homozygotes of variant allele of each mutation are correlated with an enhanced susceptibility to several types of cancer, specially to lung cancer in Japanese smokers [18].

GSTM1 is one of the enzymes belonging to a family of glutathione transferases that is polymorphic with a

deficient activity in approximately 50% of the Caucasian population [23]. Lack of this detoxification activity has been demonstrated to be caused by an inherited homozygous deletion of the gene [24]. Polymorphisms in CYP1A1 and GSTM1 have been associated to increase in: lung, bladder, liver, pharynx, larynx, skin, rectum, breast, and colon cancer risk [25–32].

In this work, we have investigated the distribution of single and combined genotypes of CYP1A1 (*Msp1*) and GSTM1 in two Chilean groups (control and prostate cancer).

MATERIALS AND METHODS

Subjects

Blood samples were obtained from 128 Chilean control patients with benign prostatic hyperplasia (BPH) and 102 unrelated prostate cancer patients living in Santiago. For selection of patients we realized a voluntary screening on Santiago population based on PSA and DRE examinations. All suspicious people (altered PSA or DRE, or both) were biopsied and those with no cancer were considered as controls. All controls were diagnosed as BPH patients. The samples were obtained following informed written consent previously approved by the ethic committee; after DNA extraction, they were analyzed for CYP1A1 and GSTM1 genetic polymorphisms. Both control and cancer patients were interviewed regarding smoking habits, alcohol drinking, use of oral contraceptives or hormones, incidence of past records of cancer in related family members, and exposure to occupational, outdoor, and indoor carcinogenic pollutants. Prostate cancer patients were all previously diagnosed histologically and their medical records were available from the CONAC (Corporación Nacional del Cancer), Santiago. Extent of tobacco smoke exposure was assessed by smoking index (SI) (cigarettes \times day \times year). A smoker was defined as a person with an SI of 800 or higher. Both, present smokers and former smokers at the time of the analysis were considered as smokers. All individuals were tested for PSA antigen (INMULITE™) and digital examination to detect a possible prostate carcinoma. The suspicious people (PSA \geq 4.0 ng/ml and/or DRE positive) were biopsied and divided in control and cancer cases depending on the result. The Gleason score was determined in all cancer cases. The biopsy was obtained by transrectal ultrasound-guided procedure and analyzed by a unique pathologist. Six randomized cores were taken according to the procedure described by Hodge et al., 1989, using an ultrasound machine. Additional cores were analyzed from the suspicious hypoecogenic lesions in the peripheral zone of the prostate.

Genotyping

PCR based restriction fragment length polymorphism (RFLP) was used to examine the polymorphisms of interest. DNA was isolated from peripheral blood samples, collected on EDTA [33]. All samples were submitted to separate amplifications followed by digestion with appropriate restriction enzymes.

PCR Amplification

CYP1A1: For the *Msp1* site, PCR amplification carried out using primers C44 and C47 [18] yielded a fragment of 340 bp. *GSTM1*: *GSTM1* genetic polymorphism was determined using primers described by Ambrosone et al. [32], simultaneously with *Msp1* primers as internal control for amplification. The presence of the *GSTM1* null genotype was determined by the absence of a 273 bp fragment, using 2% agarose electrophoresis.

Digestion by Restriction Enzymes

The PCR products were subjected to restriction enzyme digestion at 37°C for 1 hr with *Msp1* for *CYP1A1* (GIBCO BRL, Life Technologies, Inc., Gaithersburg, MD). The samples were then analyzed by electrophoresis in agarose 2% or polyacrylamide (6%) gels (Biorad Lab., Richmond, CA).

Statistics: Study Design and Sample Size

In order to evaluate the relationship between *CYP1A1* and *GSTM1* genetic polymorphisms and prostate cancer risk, we design a case-control statistical analysis, performed by the Epi Info 6.0 and STATA 5.0 programs. To calculate sample size we considered the following criteria:

- 21% as expected frequency for the *GSTM1*(-) polymorphism (less frequent genotype) in the Chilean population [23,28].
- CI 95% ($1 - \alpha$).
- A β value of 20%.

This gives a size of 102 controls and 102 cases.

The association of prostate cancer with any single or combined genotypes was estimated by the Chi-square test and a multivariate logistic regression analysis was done in order to control confounding factors.

RESULTS

The *Msp1* polymorphism of the *CYP1A1* gene was studied in each subject using PCR based methods. The *CYP1A1* analyzed region consisted of a 340 bp region containing the *Msp1* restriction site in the 3'-flanking region followed by digestion with the restriction enzyme [18]. The *GSTM1* gene deletion was also

determined by PCR analysis of the 273 bp region using *Msp1* primers as an internal control [28]. Figure 1 represents the simultaneous genotyping detection of *Msp1* and *GSTM1* genotypes to case and control individuals. Lanes a-f show different obtained genotypes to prostate cancer patients. The appearance of 200 and 140 bp fragments represent a digestion by *Msp1* of the *CYP1A1* 340 bp amplified fragment in individuals carrying the m2 allele. The *GSTM1* null genotype was assessed by the absence of the 273 bp fragment.

The characteristics of the studied group's are illustrated in Table I. Controls were significantly younger than the cancer group. The smoking and drinking status were similar for both groups. Digital examination and PSA antigen showed a good correlation with prostate cancer as expected (data not shown). Finally, no relationship was established between the pathology and family history of cancer.

Table II shows allele distributions and frequencies for the *Msp1* polymorphism on *CYP1A1* gene and the *GSTM1* genotypes in control and prostate cancer patients. For *Msp1* *CYP1A1* genotypes, the presence of variant allele was more frequent in cancer cases than in controls (0.377 vs. 0.262, respectively). Similarly, the frequency of homozygous deletions of *GSTM1* loci was higher in prostate cancer patients (0.363 vs. 0.227, respectively).

Prostate cancer risk associated with polymorphisms in *GSTM1* and *CYP1A1* genes compared to the wild-type genotype is shown in Table III. For the *Msp1* polymorphism, the heterozygous genotype was present in 48.1% of cancer patients and in 35% of controls, with an OR of 2.01, whereas the homozygous mutant genotype was present in 13.7% of the cancer patients and in 8.6% of controls with an OR of 2.35. On the other hand, the null *GSTM1* genotype was found in 36.3% of cases and 22.7% of controls; the OR was 1.94. This table also shows the estimated relative risk for prostate cancer to combined *CYP1A1* and *GSTM1* genotypes.

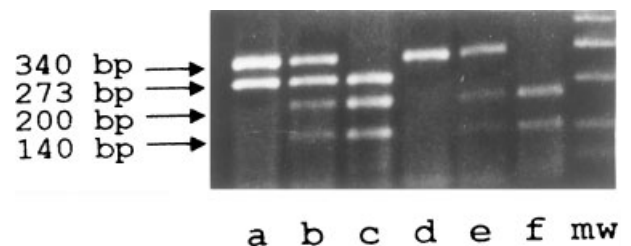


Fig. 1. Representative profiles of RFLP-PCR analysis for *CYP1A1* and *GSTM1* in Chileans. Different genotypes for *Msp1* and *GSTM1* polymorphisms. **a:** m1m1/*GSTM1*(+/+), **(b)** m1m2/*GSTM1*(+/+), **(c)** m2m2/*GSTM1*(+/+), **(d)** m1m1/*GSTM1*(-/-), **(e)** m1m1/*GSTM1*(-/-), and **(f)** m2m2/*GSTM1*(-/-). The 273 bp fragment and 340 bp represent the normal alleles for *GSTM1* and *CYP1A1* *Msp1* polymorphisms.

TABLE I. General Characteristics of the Chilean Control and Prostate Cancer Patient Groups

	Controls N = 128	Prostate cancer N = 102
Age (years)	63.4 ± 7.4	68.6 ± 7.3
Smoking status		
Smokers	29 (22.7%)	21 (20.6%)
Non-smokers	95 (77.3%)	81 (79.4%)
Alcohol status		
Drinkers	42 (32.8%)	28 (27.5%)
Non-drinkers	82 (67.2%)	73 (72.5%)
Digital examination		
Positive	29 (22.7%)	55 (53.9%)
Negative	93 (77.3%)	45 (44.1%)
PSA antigen (average)	6.3 ± 5.4	34.2 ± 83.9
Family cancer		
Yes	19 (14.8%)	17 (16.7%)
No	97 (85.2%)	51 (83.3%)

The OR values were 2.46 and 8.54 for m1m2/GSTM1(-/-) and m2m2/GSTM1(-/-), respectively, showing a potentiated effect of both polymorphisms.

In order to control the confounding factors we applied a multivariate logistic regression risk analysis considering only the factors, which individually had an association (m2, GSTM1(-/-), age, digital examination, and PSA) to establish an association between single or combined CYP1A1 and GSTM1 genotypes and prostate cancer in Chilean patients. The results are shown in Table IV. The OR values, related to the presence of rare genotypes, were 2.75 for GSTM1(-/-), 3.99 for m2m2, and 16.6 for m2m2/GSTM1(-/-) showing higher risk for the combination than each separate genotype.

We have found no association between smoking habits and prostate cancer (OR = 0.85, $P = 0.614$), and between smoking habits and single or combined genotypes in relation to prostate cancer (data no shown).

On the other hand, we have analyzed a possible association between GSTM1 and *Msp1* genotypes and the differentiation grades (Gleason scores). We have found no correlation between GSTM1 genotypes and Gleason scores (data not shown), however, a slight correlation between m2* genotypes (m1m2 or m2m2) and the Gleason scores was observed as is shown in Table V. Interestingly, we notice that all m2m2 patients had a poorly differentiated tumor, giving an indication of a possible role of this genotype as prognostic marker for prostate cancer.

DISCUSSION

In this work CYP1A1 and GSTM1 genotypes have been analyzed in 102 Chilean prostate cancer patients and 128 controls. The frequencies of alleles m2 in CYP1A1 and the null genotype for GSTM1 were significantly higher in prostate cancer patients. The most associated genotypes to the disease were those of *Msp1* polymorphism and the presence of the null genotype of the *GSTM1* gene. The higher frequency of the null genotype for the *GSTM1* gene in prostate cancer patients than in controls suggests that deletion of the *GSTM1* gene causes the loss of detoxification of the ultimate carcinogen, resulting also in higher risk for prostate cancer.

The estimated relative risk for combined genotypes was particularly high for combined *Msp1* and GSTM1, particularly for m2m2/GSTM1(-/-) (8.54, $P = 0.023$), suggesting that the presence of m2 allele together with lack of detoxification due to GSTM1 deletion increases the risk to prostate cancer. This high risk to individuals carrying m2 and GSTM1(-/-) genotype might be explained both by the increased production of activated metabolites (CYP1A1) and the deficient detoxification of the final carcinogen (GSTM1). Therefore, m2 and GSTM1 null genotype could be used as a remarkable risk assessment tools for prostate carcinomas.

TABLE II. Distribution and Allele Frequencies of CYP1A1 and GSTM1 Polymorphisms in Chilean Control and Prostate Cancer Patients

Group	n	Genotype			Allele frequencies
CYP1A1 (<i>Msp1</i>)		m1m1	m1m2	m2m2	
Control	128	72	45	11	m1 = 0.738 m2 = 0.262
Cancer	102	39	49	14	m1 = 0.623 m2 = 0.377
GSTM1		GSTM1(+/+)		GSTM1(-/-)	
Control	128	99		29	+/+ = 0.773 -/- = 0.227
Cancer	102	65		37	+/+ = 0.637 -/- = 0.363

TABLE III. Prostate Cancer Risk Associated With Single or Combined Genotypes for GSTM1 and CYP1A1 Genes in Reference to the Wild-Type Genotype

Gene	Genotype	OR	95% CI	P (value)
<i>CYP1A1</i> (<i>Msp1</i>)	m1/m1	1.00	—	—
	m1/m2	2.01	1.10–3.69	0.014
	m2/m2	2.35	0.89–6.26	0.053
<i>GSTM1</i>	GSTM1 (+/+) ^a	1.00	—	—
	GSTM1 (-/-) ^b	1.94	1.04–3.63	0.023
	Combined			
	m1m2/GSTM1(-/-)	2.46	1.05–5.83	0.022
	m2m2/GSTM1(-/-)	8.54	0.91–22.5	0.023

^aGSTM1 present.

^bGSTM1 null.

In previous work, we have reported that frequencies of m2 and val alleles were three and five times higher in a Chilean control group than in a healthy French control group, whereas the frequency for the null genotype for GSTM1 was almost twice as high as in the French group [23]. These results confirm studies by Kihara et al. [34] showing that CYP1A1 and GSTM1 mutations vary among ethnic groups and that the risk to cancer will depend on the balance of unfavorable genotypes, including other polymorphic enzymes found in each

ethnia. Moreover, we have also shown a positive association between the CYP1A1 and GSTM1 polymorphisms and lung cancer, showing that these polymorphism could be responsible, at least in part, for the susceptibility to several types of cancers [28]. This ethnic difference could explain, at least in part, differences in susceptibility to cancer (including prostate cancer) among populations, and the potential usefulness of these polymorphisms as cancer markers.

In this study, we have not found an environmental induced genetic risk, either with alcohol or smoking, opposite to that observed by Kelada et al. [15] and in agreement with other researchers [7]. Thus, we believe that more research has to be performed to help to understand the role of environmental or dietary factors and the involved mechanisms, as well as to suggest the use of GSTM1 and *Msp1* genotypes as prognostic markers, based on the slight correlation observed in this work, specially for patients carrying m2 allele which have poorly differentiated tumors.

Taken together, our results suggest that the balance of altered genotypes in CYP1A1 and GSTM1 may determine the susceptibility to prostate cancer in the Chilean population as also observed for lung cancer [28].

In addition to evaluating prostate cancer risk in the Chilean population our data might help to understand inter-ethnic differences in the distribution of polymorphic enzymes, as well as the function of single and simultaneous rare alleles in metabolic genes and prostate cancer susceptibility.

This is the first study relating CYP1A1, and GSTM1 gene polymorphisms with prostate cancer risk in a Chilean population, a region where environmental and ethnical factors might play an important role in the etiology of this and other diseases.

TABLE IV. Multivariate Logistic Regression Risk Analysis for Single or Combined CYP1A1 and GSTM1 Genotypes in Chilean Controls and Prostate Cancer Patients

Genotype	OR ^a	95% CI	P (value)
(a)			
Age	1.09	1.03–1.14	0.001
Digital examination	4.56	2.17–9.59	<0.0001
PSA	1.16	1.09–1.23	<0.0001
GSTM1(-/-)	2.75	1.31–5.79	0.008
(b)			
Age	1.08	1.01–1.15	0.032
Digital examination	3.00	1.07–8.37	0.037
PSA	1.21	1.10–1.32	<0.0001
m2m2	3.99	1.27–12.54	0.018
(c)			
Age	1.07	1.00–1.15	0.041
Digital examination	3.40	1.21–9.58	0.021
PSA	1.21	1.10–1.32	<0.0001
GSTM1(-/-)/m2m2	16.63	1.67–165.48	0.016

Model P value <0.0001. Some combinations were not analyzed because of the small number of individuals carrying both mutated alleles.

^aOdds ratios were calculated including only the individually significant factors in control and prostate cancer groups.

TABLE V. Gleason Scores for Prostatic Tumors in Relation to m2 Genotypes for CYP1A1 Gene

Genotype	Well differentiated tumors (Gleason score 2–4)	Moderately differentiated tumors (Gleason score 5–7)	Poorly differentiated tumors (Gleason score 8–10)	Total n (%)
m1m1	14 (35.9)	25 (64.1)	0 (0)	39 (100)
m2*	15 (23.8)	41 (65.1)	7 (11.1)	63 (100)
Total	29 (28.4)	66 (64.7)	7 (6.9)	102 (100)

$P = 0.062$ (Chi²).

m2*, m1m2 or m2m2 genotypes.

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