Acetylation Genotype and the Genetic Susceptibility to Prostate Cancer in a Southern European Population

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BACKGROUND. Epidemiologic studies have suggested that environmental factors and diet are important risk factors in the pathogenesis of prostate cancer. The *N*-acetyltransferases (NAT) are important enzymes in activation and inactivation of various carcinogens, including those found in well-cooked meat and cigarette smoke.
METHODS. We analyzed DNA samples from 146 prostate cancer patients and 174 healthy men. We used PCR–RFLP method to analyze *NAT1* and *NAT2* polymorphisms.
RESULTS. We did not find statistically significant differences in *NAT1* genotypes frequencies between prostate cancer patients and control group. We observed an association of the slow acetylator genotype, *NAT2*6/NAT2*6* with prostate cancer protection ($P = 0.017$; OR $= 0.31$, 95% CI 0.11–0.84). Multivariate logistic regression analysis confirmed this association (0.030; OR $= 0.32$, 95% CI 0.12–0.89).
CONCLUSIONS. Our results indicate a role of *NAT2* polymorphisms in the carcinogenic pathway of prostate cancer, specifically in a population of Southern Europe. *Prostate 64: 246–252, 2005.* © 2005 Wiley-Liss, Inc.

KEY WORDS: **NAT; polymorphisms; prostate cancer; acetylation; heterocyclic aromatic amines**

INTRODUCTION

Prostate cancer is one of the most common malignancies in Western countries, being the second in cancer incidence and the third in cancer mortality among men in Portugal [1]. Several factors are associated with an increased risk for prostate cancer. Epidemiologic studies have suggested that environmental factors, including ultra-violet radiation [2], smoking [3] and diet, including meat and fat intake [4], are involved in the development of prostate cancer. Many chemical and dietary carcinogens, especially heterocyclic amines derived from well-cooked meat, seem to be involved in the pathogenesis of prostate cancer [5].

The *N*-acetyltransferase (NAT) activity enrolls an important step in both activation and inactivation of numerous carcinogens, found for example in well-cooked meat and cigarette smoke, respectively [6]. Therefore, the acetylator status may modify individual response to various chemicals and thus individual cancer susceptibility.

NATs are encoded by two genes, *NAT1* and *NAT2*, located in chromosome 8p 21.3-23.1 [7]. Both genes are highly polymorphic. To date, 29 *NAT2* alleles have been identified [8]. Individuals who have two or more *NAT2* polymorphisms have a slow acetylator phenotype, individuals heterozygous for *NAT2* polymorphisms have a rapid/intermediate acetylator phenotype, and those who lacked *NAT2* polymorphisms have a rapid acetylator phenotype. Among all the *NAT2* allelic

variants, two (*NAT2*5* and *NAT2*6*) were shown to account for most of slow *NAT2* acetylator genotypes in Caucasian populations [9]. Previous studies have found associations between *NAT2* polymorphisms and cancer risk [10–15].

Polymorphisms in *NAT1* yield over 25 variant alleles [8]. A common *NAT1* allelic variant (*NAT1*10*) is associated with increased catalytic activity (rapid acetylator phenotype) [16]. This allelic variant has been associated with increased risk to colorectal cancer, compared with *NAT1*4* homozygotes [17].

It has been reported that human prostate epithelial cells metabolize potential carcinogens [17]. Furthermore, Wang et al. demonstrated the expression of *NAT1* and *NAT2* transcripts in prostate cells [5]. Therefore, individual susceptibility to prostate cancer may be modified by genetic polymorphisms in NAT1 and NAT2 enzymes. The aim of this study was to assess the association between *NAT1* and *NAT2* polymorphisms and prostate cancer in a Portuguese population.

MATERIALS AND METHODS

Patients

Consecutive patients (n = 146) with histologically confirmed prostate cancer (median age 66 years) were enrolled in this study between 1999 and 2000 from the Department of Urology of the Portuguese Institute of Oncology, Porto. Clinical characterization including Gleason grade, disease status, age at diagnosis, and prostate specific antigen (PSA) was obtained from medical records. The control group consisted of 174 healthy individuals with no evidence of neoplastic disease and a median age of 64 years. All participants were Caucasian living in Porto district.

Approximately, 8 ml of venous blood was obtained with a standard venipuncture technique using EDTA tubes. DNA was extracted from the white blood cell fraction from each study subject using a salting-out protocol [18].

Genotyping of *NAT1* and *NAT2* Polymorphisms

For *NAT1* genotype analysis we used a PCR–RFLP method already described [19]. PCR conditions was performed as follows: 100 ng of genomic DNA was added to 0.25 μM of each primer, 0.2 mM of each dNTP, 1.5 mM MgCl$_2$, 1× *Taq* buffer and 1U of *Taq* DNA-polymerase to a final volume of 50 μl. Thirty five cycles were performed, consisting of 94°C (30 sec) for denaturation, 45°C (30 sec) for primer annealing, and 72°C (45 sec) for primer extension. To differentiate *NAT1*4*, *NAT1*10*, and *NAT1*11* alleles, PCR product was subjected to enzymatic digestion with *Mbo* II enzyme. Restriction products were submitted to electrophoresis in 4% Metaphor high resolution agarose
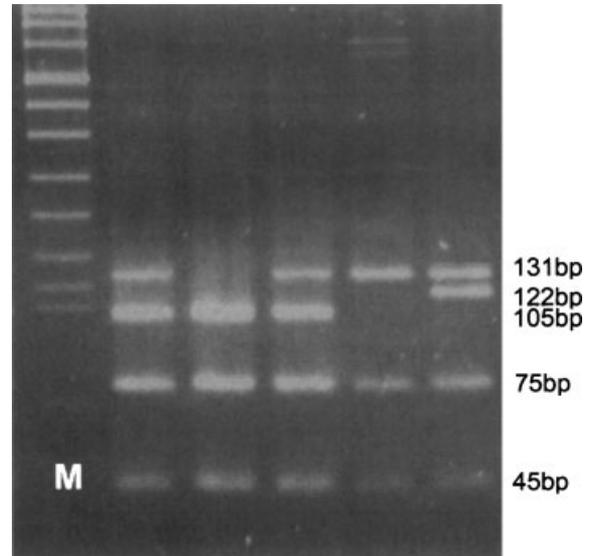

Fig. I. *Mbo* II RFLPs of the PCR products of the *NAT1* alleles: *NAT1*4* alleles—105, 71, 45, and 26 bp fragments, *NAT1*10* allele—131, 75, 45, and 26 bp fragments and *NAT1*11* allele—122, 75, 45, and 26 bp fragments (M-DNA Molecular Weight Marker VIII (Roche^R)).

gel (Fig. 1). Digestion of *NAT1*4* resulted in fragments of 105, 71, 45, and 26 base pairs (bp), *NAT1*10* in fragments of 131, 75, 45, and 26 bp. The *NAT1*11* allele can be distinguished by observation of a 9 bp mobility ship of the 131 bp band to a 122 bp band.

Genotyping for *NAT2* was carried out using the PCR–RFLP method [20]. The reactions of PCR consisted of nearly 100 ng of genomic DNA, 1 μM of each primer, 0.2 mM of each dNTP, 1.5 mM MgCl$_2$, 1× *Taq* buffer, and 1 U of *Taq* DNA-polymerase to a final volume of 50 μl. Thirty cycles were performed, consisting of 98°C (30 sec) for denaturation, 62°C (1 min) for primer annealing, and 72°C (1 min) for primer extension. After amplification, 15 μl of PCR was digested with 20 U of the *Kpn* I and *Taq* I restriction enzymes (positions 480 and 590) specific for the two different NAT2 allelic variants to be screened (*NAT2*5* and *NAT2*6*, respectively). Restriction products were submitted to electrophoresis in 2% agarose gels. After digestion with *Kpn* I, the 481-T (*NAT2*5*) and 481-C (*NAT2*4*) alleles were visualized as fragments of 290 bp and 170 plus 120 bp, respectively (Fig. 2A). The 590-A (*NAT2*6*) and 590-G (*NAT2*4*) alleles were visualized as fragments of 290 and 230 plus 60 bp, respectively, after digestion with *Taq* I (Fig. 2B).

Statistical Analysis

Analysis of data was performed using the program SPSS for Windows (version 11.0). Chi-square analysis was used to compare categorical variables. A 5% level significance was used in the analysis. The odds ratio
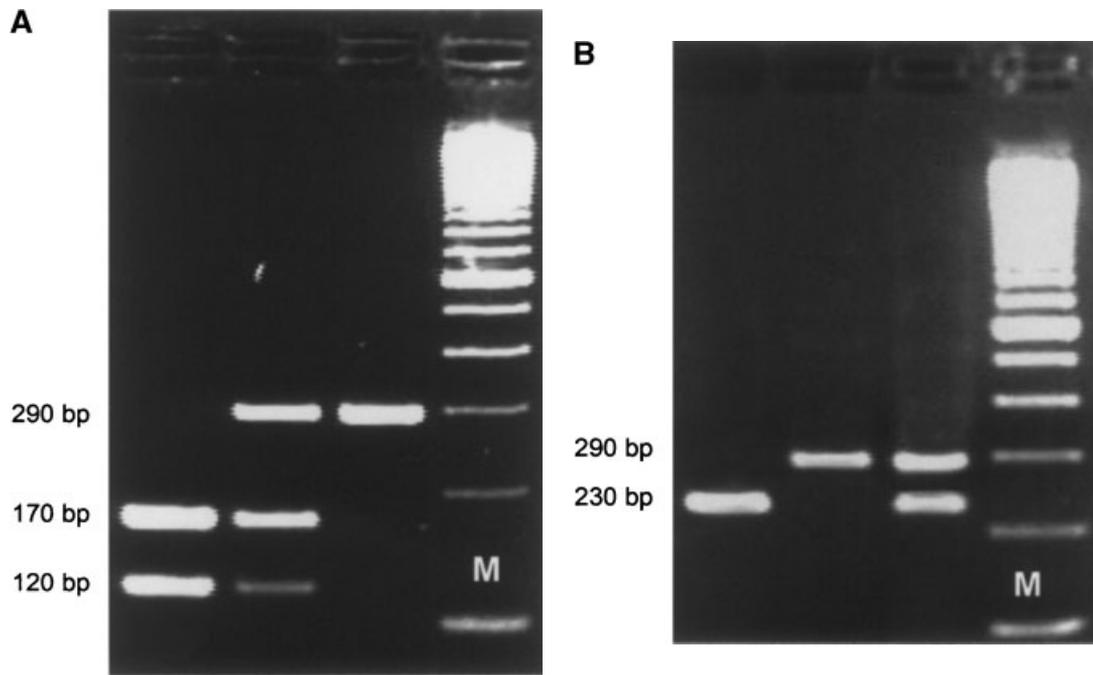
Fig. 2. RFLP of the PCR products of the *NAT2* alleles. *Kpn* I restriction (**A**) 290 bp fragment—*NAT2*5* allele; 170 plus 120 bp fragments—*NAT2*4* allele. *Taq* I restriction (**B**) 290 bp fragment—*NAT2*6* allele; 230 bp plus 60 bp fragments (not visualized)—*NAT2*4* allele. (M—100 bp DNA ladder (Gibco BRL[R])).

(OR) and its 95% confidence interval (CI) was calculated as a measure of the association between *NAT1* and *NAT2* genotypes and prostate cancer risk. Multivariate logistic regression analysis was used to calculate the adjusted OR and 95% CI for the influence of *NAT* genotypes in the risk of prostate cancer, with adjustment for age. We stratified the analysis according to Gleason grade, disease status, age at diagnosis (median age), and risk of disease progression (PSA higher than 10 mg/ml).

RESULTS

The frequencies of *NAT1* genotypes in prostate cancer cases and controls are shown in Table I. The most common genotype was *NAT1*4/NAT1*4* both in cases (73.6%) and controls (75.2%). The *NAT1*11* allele was rare in controls (0.7%) and was not found in the case groups. The frequency of *NAT1*10* genotypes was similar between cases and controls. There were not statistically significant differences in *NAT1* genotypes between prostate cancer patients and healthy individuals.

The frequency of *NAT2* genotypes in cases and controls is shown in Table II. A statistically significant difference was observed in the frequency of a *NAT2* slow acetylator genotype, *NAT2*6/NAT2*6*, in prostate cancer patients (3.4%) when compared with the control group (10.3%) ($P = 0.017$; OR = 0.31, 95% CI 0.11–0.84).

TABLE I. *NAT1* Genotype Frequencies in Prostate Cancer Cases and Controls With Odds Ratio (OR)

	Cases		Controls			
NAT1 genotype	n	%	n	%	OR (95% CI)[a]	*P*
*NAT1*4/NAT1*4*	95	73.6	109	75.2	0.92 (0.53–1.58)	0.772
*NAT1*4/NAT1*10*	26	20.2	29	20.0	1.01 (0.55–1.82)	0.974
*NAT1*10/NAT1*11*	0	0	1	0.7	—	0.345
*NAT1*10/NAT1*10*	6	4.7	6	4.1	1.13 (0.35–3.59)	0.836

[a]OR were calculated from the ratio of the number of genotypes in interest versus all the other genotypes.

TABLE II. NAT2 Genotype Frequencies in Prostate Cancer Cases and Controls With OR

NAT2 genotype	Cases		Controls		OR (95% CI)[a]	P
	n	%	n	%		
NAT2*4/NAT2*4	9	6.2	9	5.2	1.20 (0.46–3.11)	0.701
NAT2*4/NAT2*5	39	26.7	54	31.0	0.81 (0.49–1.31)	0.396
NAT2*4/NAT2*6	38	26.0	32	18.4	1.56 (0.091–2.66)	0.100
NAT2*5/NAT2*6	31	21.1	37	21.3	0.99 (0.058–1.70)	0.995
NAT2*5/NAT2*5	24	16.4	24	13.8	1.23 (0.66–2.27)	0.509
NAT2*6/NAT2*6	5	3.4	18	10.3	0.31 (0.11–0.84)[b]	0.017

[a]OR was calculated from the ratio of the number of genotypes in interest versus all the other genotypes.
[b]Adjusted OR for age (logistic regression analysis): OR = 0.32, 95% CI 0.12–0.89; P = 0.03.

Multivariate logistic regression analysis confirmed this association of NAT2*6/NAT2*6 genotype with prostate cancer protection (P = 0.030; OR = 0.32, 95% CI 0.12–0.89).

The association of NAT1 genotypes and clinico-pathologic features of prostate cancer cases studied is shown in Table III. No differences were found in the frequencies of NAT1 genotypes regarding median age of diagnosis, Gleason grade, disease status (advanced or localized), and PSA levels. The same results were obtained considering NAT2 genotypes (Table IV). However, NAT2*6/NAT2*6 genotype frequency was higher in prostate cancer patients with PSA levels lower than 10 mg/ml, with suggestive statistical significance (P = 0.054).

DISCUSSION

Prostate cancer appears to be dependent on the interaction between environmental and genetics factors, particularly dietary [21]. Several studies reported that diet could alter steroid hormonal profile and modify prostate cancer risk [22]. It has been proposed that heterocyclic amines and polycyclic hydrocarbons, which are produced by cooking meat at high temperature, act as carcinogens in prostate cancer carcinogenesis [5,17].

TABLE III. Association of NAT1 Genotypes With the Clinical and Pathological Features of Prostate Cancer Cases Studied

	NAT1 genotypes							
	NAT1*4/NAT1*4		NAT1*4/NAT1*10		NAT1*4/NAT1*11		NAT1*10/NAT1*10	
	n (%)	P value	n (%)	P value	n (%)	P value	n (%)	P value
Median age								
Age >66	40 (71.4)		13 (23.2)		1 (1.8)		2 (3.6)	
		0.607		0.448		0.682		0.472
Age ≤66	55 (75.3)		13 (17.8)		1 (1.4)		4 (5.5)	
Gleason grade								
Gleason >7	15 (78.9)		2 (10.5)		1 (5.3)		1 (5.3)	
		0.422		0.209		0.284		0.571
Gleason ≤7	77 (73.3)		23 (21.9)		1 (1.0)		4 (3.8)	
PSA levels								
PSA >10	64 (72.7)		19 (21.6)		2 (2.3)		3 (3.4)	
		0.741		0.964		0.527		0.202
PSA ≤10	27 (69.7)		3 (21.2)		0 (0.0)		3 (9.1)	
Disease status								
Advanced	44 (69.8)		13 (20.6)		1 (1.6)		5 (7.9)	
		0.338		0.894		0.740		0.094
Localized	51 (77.3)		13 (19.7)		1 (1.5)		1 (1.5)	

TABLE IV. Association of NAT2 Genotypes With the Clinical and Pathological Features of Prostate Cancer Cases Studied

	NAT2 genotypes											
	NAT2*4/NAT2*4		NAT2*4/NAT2*5		NAT2*4/NAT2*6		NAT2*5/NAT2*6		NAT2*5/NAT2*5		NAT2*6/NAT2*6	
	n (%)	P value	n (%)	P value	n (%)	P value	n (%)	P value	n (%)	P value	n (%)	P value
Median age												
Age >66	4 (5.6)	0.535	16 (22.5)	0.267	18 (25.4)	0.856	19 (26.8)	0.112	12 (16.9)	0.883	2 (2.8)	0.526
Age ≤66	5 (6.7)		23 (30.7)		20 (26.7)		12 (16.0)		12 (16.0)		3 (4.0)	
Gleason Grade												
Gleason >7	1 (4.0)	0.494	9 (36.0)	0.241	7 (28.0)	0.935	5 (20.0)	0.832	3 (12.0)	0.496	0 (0.0)	0.365
Gleason ≤7	8 (7.0)		28 (24.6)		31 (27.2)		25 (21.9)		17 (14.9)		5 (4.4)	
PSA levels												
PSA >10	8 (8.5)	0.267	25 (26.6)	0.460	25 (26.6)	0.791	23 (24.5)	0.136	12 (12.8)	0.308	1 (1.1)	0.054
PSA ≤10	1 (3.0)		11 (33.3)		8 (24.2)		4 (12.1)		6 (18.2)		3 (9.1)	
Disease status												
Advanced	2 (2.7)	0.083	23 (31.5)	0.190	18 (24.7)	0.706	17 (23.3)	0.544	12 (16.4)	1.000	1 (1.4)	0.183
Localized	7 (9.6)		16 (21.9)		20 (27.4)		14 (19.2)		12 (16.4)		4 (5.5)	

NAT enzymes are involved in the metabolism of many carcinogens, including heterocyclic amines present in cooking meat at high temperature [4,17]. Both NAT1 and NAT2 are responsible for both N-acetylation (usually deactivation) and O-acetylation (usually activation) activities of aromatic and heterocyclic amines carcinogens [23]. Another fact that should be taken in consideration is that prostate cancer has been associated with genetic alterations that include regions of deletions on different chromosomal regions, such as 8p 22-23 [24], which is the region of NAT genes [7]. Therefore, we hypothesise that NAT1 and NAT2 acetylator genotypes could be associated with susceptibility to prostate cancer.

We observed no association between NAT1 polymorphism and prostate cancer susceptibility. Controversial results have been reported regarding the role of NAT1 and NAT2 in the susceptibility to prostate cancer [10,25,26]. Previous reports presenting different results [10,27], could be explained by the geographic difference between populations, since the frequencies of NAT1 and NAT2 polymorphisms differ greatly with ethnical characteristics [8]. In our results, the NAT2 slow acetylator genotypes, NAT2*6NAT2*6, was significantly associated with prostate cancer ($P = 0.030$; OR = 0.32, 95% CI 0.12−0.89). It is well known that this slow acetylator genotype presents a lower enzyme activity than rapid genotypes [28]. Individuals that present this genotype show a lower capacity of metabolizing carcinogens. Many N-hydroxy heterocyclic amines, carcinogens are metabolically activated to a great extent by NAT2 [6,29], and these carcinogens seem to be of potential carcinogenicity to human prostate epithelial cells [5,17]. Therefore, carriers of NAT2*6NAT2*6 genotype have a lower capacity to activate carcinogens. This is consistent with the protective effect of NAT2*6/NAT2*6 genotype to prostate cancer that we reported in our study. Furthermore, we found that NAT2*6/NAT2*6 genotype is over-represented in the group of prostate cancer patients with PSA levels lower than 10 mg/ml in comparison with patients with PSA levels higher than 10 mg/ml. This reinforces a role for NAT2*6NAT2*6 genotype in the prostate cancer biology.

Several reports have shown the contribution of genetic polymorphisms to the risk of prostate cancer [30−35]. Our study brings new reports that may help to clarify the function of NAT polymorphisms in prostate cancer development. Our results suggest a role of NAT2 polymorphisms in the carcinogenic pathway of prostate cancer in a population of Southern Europe. Future studies concerning the association of NAT genotypes and environmental or lifestyle factors (e.g., diet) will be important to elucidate the real meaning of NAT polymorphisms in the susceptibility to prostate cancer.

ACKNOWLEDGMENTS

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