Expression in human prostate of drug- and carcinogen-metabolizing enzymes: association with prostate cancer risk

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Summary The role of two common polymorphisms of enzymes involved in the metabolism of drugs and carcinogens was studied in relation to prostate cancer. The gene encoding one of these enzymes (NAT2) is located in an area where frequent allelic loss occurs in prostate cancer. Mutations at the genes CYP2D6 and NAT2 were analysed by allele-specific polymerase chain reaction and restriction mapping in DNA from 94 subjects with prostate cancer and 160 male healthy control subjects. Eleven prostate specimens were analysed for genotype and enzymatic activities NAT2, CYP2D6 and CYP3A by using the enzyme-specific substrates sulphamethazine and dextromethorphan. Enzyme activities with substrate specificities corresponding to NAT2, CYP2D6 and CYP3A are present in human prostate tissue, with mean ± s.d. activities of 4.8 ± 4.4 pmol min⁻¹ mg⁻¹ protein, 156 ± 91 and 112 ± 72 nmol min⁻¹ mg⁻¹ protein respectively. The Kᵣ values for the prostate CYP2D6 and CYP3A enzyme activities corresponded to that of liver CYP2D6 and CYP3A activities, and the CYP2D6 enzyme activity is related to the CYP2D6 genotype. The N-acetyltransferase, in contrast, had a higher Kᵣ than NAT2 and was independent of the NAT2 genotype. The CYP2D6 and CYP3A enzymes, and an N-acetyltransferase activity that is independent of the regulation of the NAT2 gene, are expressed in human prostate tissue. The presence of carcinogen-metabolizing enzymes in human prostate with a high interindividual variability may be involved in the regulation of local levels of carcinogens and mutagens and may underlie interindividual differences in cancer susceptibility.

Keywords: prostate; cancer; polymorphism; NAT2; CYP2D6

Prostate cancer is one of the most common cancers throughout the world, and it is one of the major causes of cancer-related deaths in men in North America (Mahler, 1994) and Europe (Moller-Jensen et al. 1990). Patient survival is higher when the cancer affects the gland only. It has been shown that screen-detected prostate cancers are more frequently located in the gland only than clinically detected cancers (Catalona et al. 1993). Therefore, early detection of prostate cancer has become a topic of major interest in the fields of public health and preventive medicine.

The aetiology of prostate cancer is unknown and to date no unequivocal biomarkers of susceptibility to prostate cancer have been identified. Evidence for a genetic predisposition has been found (Cannon et al. 1982), and family history appears to be a major risk factor to be considered (Narod et al. 1995). Segregation analyses suggest that familial clustering of prostate cancer may be caused by a high penetrance predisposition gene (Carter et al. 1992) the most likely candidate genes are those located in regions in which allele loss occurs in prostate cancer. These regions are on chromosomes 16q, 10q and 8p (Carter et al. 1990; Bergenheim et al. 1991; Kunimi et al. 1991).

Here we have studied the relationship between two polymorphisms of enzymes that metabolize drugs and carcinogens, and prostate cancer risk. The polymorphisms studied have been proposed as genetic biomarkers for susceptibility to some forms of cancer. It should be pointed out that the role of such polymorphisms on cancer risk should be considered as preliminary and controversial. However, the polymorphic CYP2D6 gene seems to be involved in susceptibility to lung and liver cancer (Agúndez et al. 1995a; Bouchard et al. 1996). NAT2 is a gene of likely importance in bladder and liver carcinogenesis, as subjects with enzyme-inactivating mutations are at increased risk of developing bladder and liver cancer (Caporaso et al. 1991; Agúndez et al. 1996a). The gene coding for the NAT2 enzyme is located in the short arm of chromosome 8, in the same area in which the most frequent allelic loss occurs in prostate cancer tissue (Bergenheim et al. 1991; Franke et al. 1994; Vatsis et al. 1995). The loss of a gene coding for an enzyme involved in the detoxification of carcinogens or mutagens could constitute a risk factor for carcinogenesis.

To date, no studies involving a possible association of prostate cancer and the polymorphisms studied here have been published. Another point that has not been elucidated is whether the CYP2D6 and NAT2 enzymes are functionally expressed in human prostate tissue. Expression of an N-acetyltransferase activity in rat and dog prostate has been shown (Hein et al. 1991; Sone et al. 1994), but the identity of such an enzyme remains unclear. Recent studies also indicate the presence of cytochrome P450 enzymes in human prostate cancer tissues (Murray et al. 1995). So far, most associations between polymorphic drug metabolizing enzymes and carcinogenesis have been demonstrated in tissues in which the enzymes are expressed (Caporaso et al. 1991). This is probably
due to a strong local effect on the activation or deactivation of carcinogens in situ. For instance, it has been shown that N-acetyltransferase polymorphism plays a relevant role in the formation of 2-aminofluorene-DNA adducts in tumour target organs (Feng et al. 1996). In an attempt to elucidate the events that occur as previous steps to prostate carcinogenesis, we have studied whether the polymorphic enzymes CYP2D6 and NAT2 are actively expressed in human prostate tissue, as well as the impact of such genetic polymorphisms in prostate cancer susceptibility. In order to evaluate whether allelic loss of these genes occurs in adenomatous prostate tissue, the occurrence of allelic losses at the CYP2D6 and NAT2 gene loci in prostate tissues was also studied. If the CYP2D6 enzyme is functionally expressed in prostate, allelic loss of CYP2D6 could cause changes in local enzyme activity, modifying the local metabolism of carcinogens and mutagens. To our knowledge, no studies involving allelic loss of the polymorphic gene CYP2D6 have been performed. This is also the first study involving allelic loss of the NAT2 gene in prostate. Indeed only one study involving allelic loss of NAT2 has been published, and it was performed in colon cancer (Hubbard et al. 1997).

**METHODS**

**Patients and controls**

All the subjects included in this study were unrelated white Spanish men. Ninety-four patients with prostate carcinoma, with ages ranging from 56 to 93 years (mean ± s.d. 74.8 ± 7.6), and a group of healthy subjects, composed of 160 men, aged 18–95 (mean ± s.d. 45.4 ± 12.9) were included in the study. All the cases were patients attending the Urology Service, San Carlos University Hospital, Madrid. All patients attending the Hospital between March and December 1994 were included in the study. As all prostate cancer patients undergo a periodic health evaluation every 6 months, virtually all patients diagnosed with prostate cancer in the last years prior to the end of sample collection (December, 1994) in the University Hospital were included in the study group. The eligibility criteria included patients with positive histological identification of prostate carcinoma and no evidence of any other malignant disease. These included all ages and all stages of disease. All patients requested agreed to participate in the study.

The control subjects were recruited in the same area as the cases (the centre of Spain). Over 98% of healthy subjects requested agreed to participate in the study. Most of them were medical students and staff from the University Hospitals and participating Universities. All the control subjects were unrelated, in good health and with no antecedents of disease. Informed consent was obtained from all the participants, patients and control subjects before their inclusion in the study. The protocol of this study was approved by the Ethics Committees of the University Hospital Infanta Cristina (Badajoz, Spain) and the San Carlos University Hospital (Madrid, Spain).

Venous blood samples (10–20 ml) were obtained from each subject and collected in heparinized (sodium heparin 143 u.s.p. units) sterile glass tubes (Vacutainer®. Becton Dickinson Systems Europe. B.P. no. 37-38241 Meylan Cedex-France), and stored at −80°C until DNA isolation. Samples of adenomatous prostate tissue were obtained during surgery from patients attending the same hospitals as the prostate cancer patients. The tissue samples were immediately frozen and stored at −80°C until analysis. Blood samples from the same patients were obtained for comparison and for the study of genetic changes in the prostate tissue. Human liver samples were used for comparison with the enzyme activities identified in prostate tissue. These samples were biopsies obtained from patients undergoing surgery, as described elsewhere (Agundez et al. 1990). The cytosolic and microsomal fractions from prostate and liver tissues were performed as described elsewhere (Agundez et al. 1990; Grant et al. 1990).

**Enzymatic assays**

The analyses of enzymatic activities were carried out by the use of substrates specific for the NAT2 and the CYP2D6 enzymes. N-acetyltransferase activity was analysed by using sulphamethazine as described elsewhere (Grant et al. 1990). The amount of parent drug and the acetylated metabolite were determined by high-performance liquid chromatography (HPLC) analysis (Grant et al. 1990). The CYP2D6 activity was analysed by the use of dextromethorphan (Kerry et al. 1994). The standard reaction mixture consisted of an NADPH regenerating system (0.5 mM

| Sample identification | CYP2D6 genotype | Predicted phenotype | Dextromethorphan O-demethylehy activity (nmol min⁻¹ mg⁻¹) |
|-----------------------|-----------------|---------------------|---------------------------------------------------|
| P01                   | CYP2D6¹/CYP2D6¹ | Rapid               | 297 ± 16                                          |
| P02                   | CYP2D6¹/CYP2D6¹ | Rapid               | 90 ± 7                                           |
| P03                   | CYP2D6¹/CYP2D6¹ | Rapid               | 145 ± 13                                         |
| P04                   | CYP2D6¹/CYP2D6¹ | Rapid               | 242 ± 21                                         |
| P05                   | CYP2D6¹/CYP2D6¹ | Rapid               | 107 ± 32                                         |
| P06                   | CYP2D6¹/CYP2D6¹ | Rapid               | 190 ± 7                                          |
| P07                   | CYP2D6¹/CYP2D6¹ | Intermediate        | 79 ± 12                                          |
| P08                   | CYP2D6¹/CYP2D6¹ | Rapid               | 297 ± 18                                         |
| P09                   | CYP2D6¹/CYP2D6¹ | Rapid               | 59 ± 14                                          |
| P11                   | CYP2D6¹/CYP2D6¹ | Intermediate        | 48 ± 8                                           |
| P12                   | CYP2D6¹/CYP2D6¹ | Intermediate        | 159 ± 33                                         |

The results are mean ± s.d. of at least three independent measurements. These values were averaged in subgroups according to the CYP2D6 genotype and are shown in the text.
NADPH, 50 mM glucose-6-phosphate and four enzyme units of glucose-6-phosphate dehydrogenase, 5 mM magnesium chloride, 50 \( \mu \)M dextromethorphan and 50–100 \( \mu \)g of microsomal protein in 10 mM Tris-HCl buffer, pH 7.5, in a final volume of 250 \( \mu \)l. The reaction was started with the addition of micromoles and was carried out at 37°C for 30 min, then stopped by the addition of 20 \( \mu \)l of 15% perchloric acid. The mixture was frozen for 30 min, and centrifuged at 12,000 \( \times \)g for 10 min. An aliquot of 20 \( \mu \)l of the supernatant was analysed for parent drug and metabolites by HPLC analysis and fluorescence detection (Chen et al. 1990). Besides the CYP2D6 activity, as calculated from the rate of production of dextromorphan, the analysis of the dextromethorphan metabolite 3-methoxymorphinan permits the determination of the CYP3A activity (Kerry et al. 1994). Therefore, the measurements of CYP3A activity in every prostate specimen were carried out under identical conditions to those of CYP2D6 activity except that the concentration of dextromethorphan was 4 mM. For the \( K_c \) analysis of the CYP3A activity, dextroin instead of dextromethorphan was used (Kerry et al. 1994). All the measurements were performed at least in triplicate and in incubation time and enzyme quantity linear conditions.

DNA isolation and analyses

Genomic DNA was purified from peripheral leucocytes and from prostate tissues using standard protocols (Neitzel. 1986) and kept in sterile plastic vials at 4°C until analysis.

The NAT2 polymorphism was studied by the use of a PCR-based analysis, which was carried out in two steps. First, a fragment containing the coding region and a part of the 5' and 3' flanking regions of the NAT2 gene was amplified (Agúndez et al. 1994). The 1213-bp product was used as a template for a set of seven pairs of secondary mutation-specific PCR reactions (one for every mutation studied). The association of several mutations in the same allele was studied by mutation-specific PCR and/or restriction mapping of the PCR products. The mutations studied were: 191A, 282T, 341C, 481T, 590A, 803G and 857A. All these mutations are within the coding region of the NAT2 gene. These mutations, isolated or combined, have been reported as being present in several allelic variants, all of which were identifiable by the methods used in this study (Agúndez et al. 1996b). Details about the method used are described elsewhere (Martínez et al. 1995). The CYP2D6 genotyping was carried out by the combined use of mutation-specific PCR and restriction mapping with the enzymes EcoRI and Xbal as described elsewhere (Skoda et al. 1988; Gaedigk et al. 1991; Tyndale et al. 1991; Heim and Meyer. 1991; Johansson et al. 1993). The analyses performed permitted the identification of the allelic variants CYP2D6*1 (wild type), two active allelic variants (CYP2D6*2 and CYP2D6*9), two defective allelic variants (CYP2D6*3 and CYP2D6*4) and the occurrence of complete gene deletion (CYP2D6*5) as well as gene duplications or amplifications (CYP2D6*x2 and CYP2D6*xn).

Sequence analysis of the NAT1 gene in prostate specimens

A fragment spanning the whole coding region of the human NAT1 gene, as well as 5' and 3' flanking regions, was amplified and sequenced as follows. Genomic DNA obtained from all prostate specimens was subject to a PCR amplification by using the primers TCAATCCAGTGAAAAGT (position -62 to -43) and GATACATGATAGGTGC (position 946 to 929 of the NAT1 gene according to Blum et al. 1990). PCR amplification was carried out for 35 cycles of 1 min at 94°C, 1 min at 45°C and 1 min at 72°C and a final extension period of 7 min at 72°C. The amplified fragment contains the mutations present in most allelic variants of the NAT1 gene, including the allelic variants NAT1*5, NAT1*11, NAT1*14, NAT1*15 and NAT1*17. The allelic variant NAT1*4 (wild type) is defined by the absence of mutations (for a review, see Grant et al. 1997). Automated sequencing of the amplified fragments was carried out in an ABI Prism Mod. 310, using a DRLodamer terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). Sequencing reactions were carried out with primers corresponding to the positions -62 to -43.

The results are mean ± s.d. of at least three independent measurements. In the evaluation of the results no subgroups were made as no genetic polymorphisms of the CYP3A activity have been shown. The results are summarized in the text.

### Table 3 Individual values of CYP3A activities in prostate tissue

| Sample identification | Dextromethorphan \( \alpha \)-demethylase activity (nmol min\(^{-1}\) mg\(^{-1}\) protein) |
|-----------------------|-------------------------------------------------|
| P01                   | 278 ± 12                                        |
| P02                   | 98 ± 3                                         |
| P03                   | 78 ± 12                                        |
| P04                   | 118 ± 23                                       |
| P05                   | 82 ± 17                                        |
| P06                   | 85 ± 24                                        |
| P07                   | 110 ± 5                                        |
| P08                   | 216 ± 11                                       |
| P09                   | 42 ± 3                                         |
| P11                   | 39 ± 5                                         |
| P12                   | 91 ± 7                                         |

### Table 4 NAT2 genotype in 94 patients with prostate cancer and 160 healthy control subjects

| Genotype                  | Prostate cancer (% of subjects) | Healthy subjects (% of subjects) |
|---------------------------|----------------------------------|----------------------------------|
| NAT2*4/NAT2*4             | 10.6                             | 6.9                              |
| NAT2*4/NAT2*5A            | 2.1                              | 1.2                              |
| NAT2*4/NAT2*5B            | 12.8                             | 21.2                             |
| NAT2*4/NAT2*6A            | 14.9                             | 13.1                             |
| NAT2*4/NAT2*7B            | 1.1                              | 2.5                              |
| NAT2*5B/NAT2*5B           | 17.0                             | 18.1                             |
| NAT2*5B/NAT2*6A           | 19.1                             | 23.8                             |
| NAT2*5B/NAT2*7B           | 2.1                              | 0.6                              |
| NAT2*6A/NAT2*6A           | 3.2                              | 1.2                              |
| NAT2*6A/NAT2*7B           | 3.2                              | 1.9                              |
| Rare genotypes            | 6.4                              | 5.6                              |

Summary of genotype categories

| Rapid/rapid               | 11.7                             | 8.6                              |
| Rapid/slow               | 33.0                             | 39.3                             |
| Slow/slow                | 55.3                             | 51.9                             |

Twenty different NAT2 genotypes were identified in this study. Only those present in 2% of subjects or over, either among cases or control subjects, are listed in the table. The rest of the genotypes are included in the group of rare genotypes at the end of the table. The intergroup comparison analyses indicate that no statistically significant differences exist between cases and control subjects. In all cases P-value was > 0.05. The relative risk ratio for slow acetylators is 1.1 (95% CI = 0.7–1.9).
Table 5  CYP2D6 genotype in 94 patients with prostate cancer and 160 healthy control subjects

| Genotype | Prostate cancer (%) of subjects | Healthy subjects (%) of subjects |
|----------|---------------------------------|----------------------------------|
| CYP2D6*1 CYP2D6*1 | 62.8 | 63.1 |
| CYP2D6*1 CYP2D6*3 | 1.1 | 2.5 |
| CYP2D6*1 CYP2D6*4 | 13.8 | 16.9 |
| CYP2D6*1 CYP2D6*9 | 5.3 | 3.7 |
| CYP2D6*1 CYP2D6*5 | 6.4 | 2.5 |
| CYP2D6*1 CYP2D6*2 | 2.1 | 6.2 |
| CYP2D6*4 CYP2D6*4 | 4.3 | 2.5 |

Summary of genotype categories

- Rapid rapid: 62.8
- Rapid slow: 31.9
- Slow slow: 3.3

Twelve different CYP2D6 genotypes were identified in this study. Only those present in 2% of subjects or over, either among cases or control subjects, are listed in the table. The rest of the genotypes are included in the group of rare genotypes at the end of the table. The intergroup comparison analyses indicate that no statistically significant differences exist between cases and control subjects. In all cases P-value was > 0.05. The relative risk ratio for poor metabolizers is 1.4 (95% CI: 0.4–4.6).

The genotypes were identified by the method described elsewhere in the study. These are NAT1*3, NAT1*10 and NAT1*16. None of them induce amino acid changes and their impact on NAT1 enzyme activity is doubtful (Grant et al. 1997).

Statistical analyses

The intergroup comparison values were calculated by applying the Z-test. The 95% confidence intervals were calculated according to Bulpett (1987). Exact tests were used when required.

RESULTS

Enzyme activities in prostate tissue

CYP2D6. CYP3A and NAT2 enzyme activity was investigated in adenomatous prostate tissue from 11 individuals. In these individuals the CYP2D6 and NAT2 genotypes were determined, besides leukocytic DNA, in DNA extracted from the prostate tissue, in order to ensure the absence of any genetic changes in the tissue that could lead to mistyping of the samples. In all cases the genotypes were concordant in blood and prostate DNA.

The mean ± s.e. values for N-acetyltransferase and CYP2D6 activities, as measured with enzyme-specific substrates sulphamethazine and dextromethorphan, were 4.8 ± 4.4 pmol min⁻¹ mg⁻¹ protein (range 1.4–15.7) and 156 ± 91 nmol min⁻¹ mg⁻¹ protein (range 48–297) respectively. N-acetyltransferase activity was not associated with the number of active NAT2 genes, as shown in Table 1. The activity, as expressed in pmol min⁻¹ mg⁻¹ protein, was 2.9 ± 0.3 in a specimen that had two active NAT2 genes. 2.8 ± 1.5 in five specimens with one active gene and 7.1 ± 5.8 in five specimens with no active genes, thus indicating that the activity is independent of the NAT2 genotype. In contrast, the CYP2D6 activity shows a gene-dose effect when compared with the CYP2D6 genotype (Table 2). The mean value for the eight samples with two active genes was twofold higher (178 ± 93 nmol min⁻¹ mg⁻¹ protein) than the activities in the three samples with one single active gene (95 ± 57 nmol min⁻¹ mg⁻¹). This is in accordance with the expression of the CYP2D6 enzyme in liver (Gonzalez et al. 1988). The difference observed was not statistically significant because of the small sample size.

The method used for the determination of CYP2D6 activity also permits the determination of the CYP3A activity, which carries out the N-demethylation of dextromethorphan (Kerry et al. 1994). As no genetic polymorphism for CYP3A activity has been described, all the samples were considered to belong to the same group (Table 3). The mean ± s.e. activity is 112 ± 72 nmol min⁻¹ mg⁻¹ protein (range 39–278). This represents an enzyme activity about ten times lower than that measured in human liver microsomes (Kerry et al. 1994).

Genetic analyses of the CYP2D6 and NAT2 polymorphisms

All the DNA samples analysed were correctly amplified by PCR, giving DNA fragments of identical size. The study of point mutations at the NAT2 gene in blood DNA samples revealed a similar prevalence of mutations between cases and control subjects. The most common genotypes identified are listed in Table 4. Phenotype prediction, according to the NAT2 genotype (for a review of active and defective NAT2 alleles see Vatsis et al. 1995) indicates that 52 out of the 94 patients were slow acetylators (55.3%, 95% CI: 45.2–65.3%). Among control subjects the frequency was almost identical: 83 subjects were classified as slow acetylators (51.9%, 95% CI: 44.1–59.6%).

The restriction mapping analysis of the CYP2D6 locus aimed at identifying complete gene changes. CYP2D6 gene deletions or
P11 and P12, were analysed for CYP2D6 allelic loss. Seven samples with heterozygosity at the NAT2 gene were analysed for allelic loss. These were P02, P03, P04, P05, P08 and P12. For this, Southern blotting of the DNA samples digested with adequate restriction enzymes (see Methods) was performed and the completion of the digestion was evaluated by comparing the band densities obtained in the DNA samples from blood and prostate, which were digested and electrophoresed in parallel. After obtaining semiquantitative Southern blot conditions, the band densities were compared in blood and prostate DNA. The complementary analysis of the leucocyte DNA rules out the possibility that subjects with a complete allelic loss in prostate tissue would be misclassified as heterozygous for the remaining allele. In such a case, a discrepancy between prostate and leucocyte DNA would be apparent. No such discrepancies were observed, and no allelic losses could be evidenced in any adenomatous prostate sample. Figure 1 shows typical analyses of allelic loss at CYP2D6 and NAT2 genes.

**Kinetic analyses of the enzyme activities identified in prostate tissue**

In order to assure the identity of the enzyme activities identified in prostate tissue, a kinetic analysis was performed to compare the $K_m$ of prostate activities with that of the CYP2D6, CYP3A and NAT2 human liver enzymes. For this, microsomes (CYP2D6 and CYP3A) and cytosols (NAT2) from prostate samples and from four human liver biopsies were prepared as described under Methods. Five out of 11 prostate samples with higher activity for every substrate were pooled for the $K_m$ analyses. For CYP2D6 enzyme the samples selected were P01, P04, P06, P08 and P12. For CYP3A the samples were P01, P02, P04, P07 and P08. For NAT2 the samples were P02, P03, P05, P11 and P12. The liver and prostate preparations were analysed with identical substrates and under identical conditions, as described under Methods. The $K_m$ values for the prostate CYP2D6 and the CYP3A enzyme activities (6 ± 3 μM and 3.6 ± 1.2 μM respectively) were identical to those found in human liver (5.5 ± 4 μM and 2.5 ± 1.5 μM), therefore indicating that these enzymes have the same properties in prostate tissue as those described in liver. In accordance with the phenotype–genotype discrepancy, the prostate N-acetyltransferase activity has a different $K_m$ value (8 ± 2 μM) as compared with that found in liver (185 ± 43 μM). This argues against the identity of the prostatic and liver enzyme activities, indicating that the prostatic enzyme is an N-acetyltransferase enzyme different to that encoded by the NAT2 gene.

**Genetic analysis of the NAT1 polymorphism in prostate specimens**

Given the lack of association of the prostate N-acetyltransferase activity with the NAT2 genotype, we analysed the possible association of mutations at the NAT1 gene locus as a possible factor influencing the enzyme activity. For this, the coding region and flanking regions of the NAT1 gene were sequenced in DNA obtained from all prostate specimens whose N-acetyltransferase was known (Table 1). Only one sample contained mutations at the NAT1 locus. The prostate DNA sequence from the sample P05 indicates a multiple heterozygosity at positions 40A/T, 445G/A, 459G/A and 640 T/G. This is consistent with a NAT1*/4/NAT1*/17
genotype (Doll et al. 1997). Such heterozygosity was confirmed by sequencing of the NAT1 gene from leucocyte DNA from the same subject. The rest of the samples analysed had no mutations in the studied DNA fragment. Therefore, the N-acetyltransferase activity present in prostate tissue (Table 1) had no relationship to mutations known to induce amino acid changes, or related to changes of NAT1 activity in vivo or in vitro (Grant et al. 1997).

**DISCUSSION**

The findings obtained in the present study indicate that enzymatic activities that may lead to regulation on the local levels of carcinogens and mutagens, namely CYP2D6 and CYP3A, are present in human prostate tissue. In addition, an N-acetyltransferase enzymatic activity is also present in human prostate.

Whereas this is to our knowledge the first report indicating the presence of CYP2D6 and CYP3A enzyme activities in prostate, the occurrence of an N-acetyltransferase activity in rat prostate has already been demonstrated (Hein et al. 1991). However, whether this enzyme activity was present in human prostate tissue and, a more relevant topic for cancer susceptibility, whether such activity is under the regulation of the NAT2 gene (i.e. subject to genetic polymorphism), remained to be elucidated. The present study shows that the N-acetyltransferase activity present in prostate tissue is not related to the NAT2 or NAT1 genotypes, and therefore seems to be the product of other genes. It is to be noted that such enzymatic activity has a high interindividual variability (the maximum and minimum activities are in a tenfold range). If such enzyme activity is, like NAT2, able to metabolize carcinogens and mutagens, it can be speculated that it may be a cause of interindividual variability in prostate cancer susceptibility, through N-acetylation pathways that are independent of NAT1 and NAT2 regulation. Polymorphisms on genes encoding enzymes involved in the activation and detoxification of drugs and carcinogens have been related to susceptibility to develop several forms of cancer (for reviews see Caporaso et al. 1991; Evans; 1992; Caporaso and Goldstein, 1995). The analyses of these polymorphisms as genetic biomarkers of susceptibility may be used to determine the risks of environmental exposures to susceptible individuals and populations (Hirvonen, 1995). For instance, the association with bladder cancer risk is supported by a decreased clearance of low-dose carcinogens in subjects with the slow acetylator genotype (Vineis et al. 1994). Recently we also described an association of low NAT2 activity with primary liver cancer (Agundez et al. 1996a).

It should be pointed out that the enzyme polymorphisms analysed in the present study seem to be involved in carcinogenesis through the modulation of the levels of active carcinogens and mutagens, although with apparently inverse effect. Cytochrome P450 enzymes are usually involved in the activation of environmental and food carcinogens. These include nitrosamines, aflatoxin B1, as well as polycyclic aromatic hydrocarbons and heterocyclic amines (Aoyama et al. 1990; Crespi et al. 1991; Degawa et al. 1994; Yanagawa et al. 1994). Accordingly, subjects with high CYP2D6 enzyme activity are at increased risk of developing lung and liver cancer (Agundez et al. 1995a; Bouchardy et al. 1996). In contrast, NAT2 enzyme acts as a detoxifying system for aryamines and hydrazines through N-acetylation (Hein, 1988; Guengerich, 1991; Hein et al. 1993). In fact, epidemiological studies showed that the subjects with low NAT2 activity are at increased risk of developing cancer of the bladder and larynx (for a review, see Evans, 1992). In addition, the joint effects of NAT1 and NAT2 should be considered, as this gene–gene effect has been reported for bladder carcinogenesis (Kloth et al. 1994; Badawi et al. 1995; Kadibar and Badawi, 1995). Our findings in prostate specimens do not indicate any association of N-acetyltransferase activity with the NAT1 or the NAT2 genotypes, or a joint effect caused by both of them.

Our findings indicate a lack of relationship between the NAT2 genotype and prostate cancer risk. This, together with the lack of association between the N-acetylation activity and the NAT2 genotype in prostate tissue, virtually rules out any major association between the polymorphism of the NAT2 enzyme and prostate cancer risk. Our findings also indicate that, despite the presence of CYP2D6, CYP3A and N-acetylation enzyme activities in prostate, the polymorphisms of the CYP2D6 and NAT2 enzymes are not linked to a particular susceptibility to develop prostate cancer. However, carcinogen-specificity analyses of the enzyme activities present in human prostate tissue will be required to provide definitive answers to whether these enzymes are involved in processes of activation or detoxification of carcinogens and mutagens, thereby being involved in the aetiology of prostate cancer.

**ACKNOWLEDGEMENTS**

Supported in part by Grants CICYT-SAF96-0006 from Comisión Interministerial de Ciencia y Tecnología (Madrid, Spain), FISs 93/0632 and 94/0326 from Fondo de Investigaciones Sanitarias de la Seguridad Social (Madrid, Spain), BMH1-CT94-1622 from European Union, UE95-0043 from Secretaría de Estado de Universidades, Investigación y Desarrollo, and EIA94-06 from Junta de Extremadura (Mérida, Spain). This study was carried out in coordination with COST B1-phase III (Brussels, Belgium).

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