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ORIGINAL PAPER

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Kinetics of acetyl coenzyme A: arylamine N-acetyltransferase from rapid and slow acetylator human benign prostatic hyperplasia tissues

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Abstract N-acetyltransferase (NAT) activity was determined in 40 human benign prostatic hyperplasia tissues using 2-aminofluorene (AF) and *p*-aminobenzoic acid (PABA) as substrates. These were then assayed by high performance liquid chromatography for determining the amounts of acetylated AF and PABA and non-acetylated AF and PABA. The activities (mean ± SD) of AF-NAT from human benign prostatic hyperplasia tissues were divided into rapid (2.06 ± 0.08 nmol/min per milligram protein), intermediate (1.25 ± 0.26 nmol/min per milligram protein), and slow (0.58 ± 0.30 nmol/min per milligram protein) acetylator groups. The activities (mean ± SD) of PABA-NAT from human benign prostatic hyperplasia tissues were also divided into rapid (2.00 ± 0.00 nmol/min per milligram protein), intermediate (1.25 ± 0.18 nmol/min per milligram protein), and slow (0.48 ± 0.29 nmol/min per milligram protein) acetylator groups. Kinetic constants for arylamine NAT activities were determined for each of these acetylator groups. Apparent differences in K_m and V_{max} for AF were found. Therefore, there seems to be a polymorphism in NAT activity with two rapid, five intermediate, and 33 slow acetylators among the 40 samples assayed. This is the first demonstation of acetyl CoA – arylamine NAT activity in human benign prostatic hyperplasia tissues.

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Introduction

Species differences in the bioactivation and detoxification of many arylamines, drugs and chemical carcinogens have been demonstrated [11]. Variations in the capacity to acetylate arylamines, drugs and chemical carcinogens are well known. N-acetylation is a major metabolic pathway for arylamine carcinogens which is catalyzed by host cytosolic arylamine N-acetyltransferase (NAT) using acetyl coenzyme A (CoA) as a cofactor [29]. N-acetylation capacity exhibits well defined genetic variation in humans and some mammalian species. Polymorphism in the activity of NAT has been extensively studied in a number of species, including humans [1, 29, 30]. Inbred strains of mice [27], rabbits [14], hamsters [15], and frogs [17] can be classified into either rapid or slow acetylating strains. Our earlier studies also demonstrated that fish could be classified as either rapid or slow acetylators [18].

Humans can also divided into rapid and slow acetylator phenotypes. After exposure to arylamine carcinogens, the rapid acetylator phenotype has been shown to predispose humans to colorectal and breast cancer, whereas the slow acetylator phenotype is related to arylamine-induced bladder cancer [6, 20, 25, 29]. Thus, the genetic variation in NAT activities may indicate different risks for arylamine carcinogen-induced tumors in human populations.

NAT has been found in birds (pigeons [2] and chickens [9]) and in several species of laboratory animals as well as humans [2, 7, 9, 21, 22]. The tissue distribution of NAT activity correlates with susceptibility to tumor induction by arylamine carcinogens [23]. The carcinogenic potential of the arylamine carcinogens corresponds to their efficacy as precursors for the synthesis of N-acyloxyarylamine by NAT activity [23]. Our

earlier studies demonstrated that most tissues of mice contained NAT activity based on the acetylation of the substrate, sodium dodecyl sulfate (SDS) page gel electrophoresis and NAT antibody stains [7]. Other investigators have also demonstrated that in situ RNA hybridization with NAT1* or NAT2* specific probes indicates that NAT transcripts which are present in nearly all tissues analyzed are predominantly NAT1 [31]. However, there is no available information on NAT activity or kinetic data on NAT from human benign prostatic hyperplasia tissues. Thus, our initial choice of 2-aminofluorene (AF) and *p*-aminobenzoic acid (PABA) as test substrates was based on previous studies in mice [7], frogs [17] and fish [18] and on our interest in comparing the metabolism of a carcinogen (AF) to a non-carcinogen (PABA).

Materials and methods

Chemicals and reagents

Ethylenediaminetetraacetic acid (EDTA), PABA, N-acetyl-*p*-aminobenzoic acid (N-Ac-PABA), AF, N-acetyl-2-aminofluorene (AAF), acetylcarnitine, Tris, leupeptin, bovine serum albumin (BSA), phenylmethylsulfonylfluoride (PMSF), dimethyl sulfoxide (DMSO), dithiothreitol (DTT), acetyl CoA, and carnitine acetyltransferase were obtained from Sigma (St. Louis, Mo.). All of the chemicals used were reagent grade.

Human benign prostatic hyperplasia tissues

With the approval by the China Medical College Hospital, 40 benign prostatic hyperplasia tissues were obtained from the urological department from 40 male patients with a diagnosis of tissues having surgical intervention . The age range was 52–85 years with a mean of 66.4 years. All samples were immediately frozen in liquid nitrogen and stored at –80°C until kinetic studies were carried out. Under these conditions the stability of the enzyme was optimized [13].

Preparation of tissue cytosols

The individual tissues were removed, trimmed, and placed in two volumes of the lysis buffer previously described [9]. Tissues were homogenized on ice twice with a polytron homogenizer set at 25,000 rpm for 20 s. The homogenates were centrifuged for 10 min (9000*g*) and the supernatant kept on ice prior to NAT activity and protein determinations. In most cases, the tissues were assayed immediately after preparation. When this was not possible, tissues samples were stored at –80°C until assayed.

NAT activity determination

The determination of acetyl CoA-dependent N-acetylation of PABA and AF were performed as described by Chung et al. [7]. Briefly, the incubation mixture in the assay system consisted of a total volume of 90 µl: tissue cytosol, diluted as required, in 50 µl of the lysis buffer, 20 µl of an acetyl CoA recycling mixture of 50 mM Tris-HCl (pH 7.5), 0.2 mM EDTA, 15 mM acetylcarnitine, 2 units/ml carnitine acetyltransferase, and AF or PABA at the required concentration. Reactions were started by adding 20 µl of acetyl CoA. The control reaction had 20 µl water in place of the acetyl CoA. For the single point activity measurements, the final concentration of AF or PABA was 0.1 mM and acetyl CoA was 0.5 mM. Reaction mixtures were incubated at 37°C for 10 min and stopped with 50 µl of 20% trichloroacetic acid for PABA reactions and 100 µl of acetonitrile for AF reactions. All reactions were run in triplicate. The amounts of acetylated product and remaining non-acetylated substrates were determined by HPLC. An aliquot of the NAT incubation was injected onto a C18 reversed-phase column (Spherisorb 4.6×250 mm) of a Beckman HPLC (pump 168 and detector 126) and eluted at a flow rate of 1.2 ml/min. For PABA and N-acetyl-PABA, the solvent system was 50 mM acetic acid/CH_3CN (86:14) with detection at 266 nm. The retention time of PABA was 8 min and that of N-acetyl-PABA was 11 min. For AF and AAF, the solvent system was 20 mM KH_2PO_4 (pH 4.5)/CH_3CN (53:47), with detection at 280 nm. The retention time was 6.5 min for AAF and 9 min for AF. All compounds were quantitated by comparison of the integrated area of the elution peak with that of known amounts of standards. NAT activity is expressed as nanomoles acetylated per minute per milligram of cytosolic protein [7].

Protein determination

Protein concentration of the cytosol in the examined tissues from individual tissues were determined by the method of Bradford as described previously [7] All samples were assayed in triplicate.

Statistical analysis

Statistical analysis of the data was performed with an unpaired Student's *t*-test. The kinetic constants were calculated with the Cleland HYPER program [8] that performs linear regression using a least squares method.

Results

NAT activity

All examined human benign prostatic hyperplasia tissues had detectable arylamine NAT activity toward both AF and PABA. The means ± SD of NAT activity with both substrates is shown in Table 1. The individual values for AF- and PABA-NAT activities in the tissues examined which seemed to be divided into high-, intermediate-, and low-activity groups, based on the results with AF (Table 1). A plot of the activity distribution is shown in Fig. 1a, b. Based on Fig. 1, we chose 1.0 and 2.0 nmol/min per milligram protein as the dividing line

Table 1 N-acetyltransferase (*NAT*) activity of tissues homogenates from human benign prostatic hyperplasia tissues. Values are mean ± SD of the activity (nmol/min per milligram protein) in human benign prostatic hyperplasia tissues. Acetylator groups were determined based on aminofluorene (*AF-NAT*) activity: slow = 0–0.99, intermediate = 1.0–1.99, and rapid ≥2.0 nmol/min per milligram protein. *PABA p*-aminobenzoic acid

AF-NAT activity	*n*	Substrate	
		AF	PABA
Rapid	2	2.06 ± 0.08	2.00 ± 0.00
Intermediate	5	1.25 ± 0.26[a]	1.25 ± 0.18[a]
Slow	33	0.58 ± 0.30[b]	0.48 ± 0.29[b]

[a] Differs from rapid, $P < 0.05$
[b] Differs from intermediate, $P < 0.01$

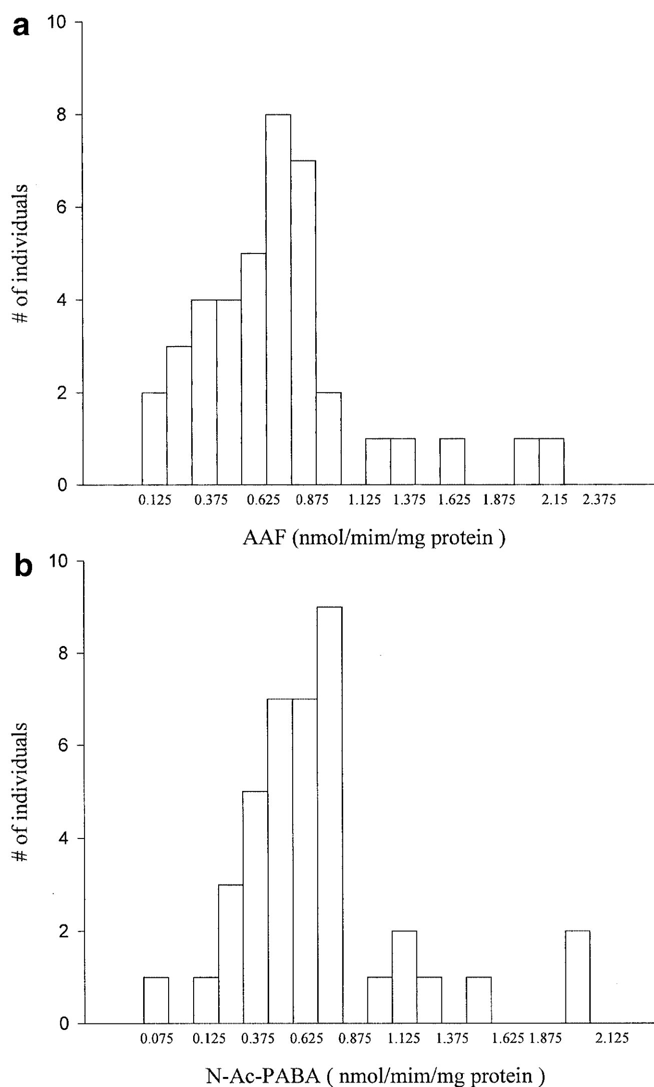

Fig. 1a, b Distribution of 2-aminofluorene (AF) and *p*-aminobenzoic acid N-acetyltransferase (NAT) activity in human benign prostatic hyperplasia tissues. NAT activities were determined by incubation of tissues homogenates with **a** 60 μM AF or **b** PABA and 500 μM acetyl CoA for 10 min at 37°C. The amounts of acetylated product were determined by high performance liquid chromatography. Numbers on the *abscissa* represent the ranges 0–0.125, 0.126–0.375, 0.376–0.625, etc. *AAF* N-acetyl-2-aminofluorene

between low- and intermediate- and between intermediate- and high-activity acetylators, respectively (Tables 1and 2). A similar plot based on PABA-NAT activity did indicate a tri- or bimodal distribution of activity (Fig. 1b).

Kinetic constant

K_m and V_{max} values of NAT from human benign prostatic hyperplasia were determined using representatives of the rapid-, intermediate-, and low-activity groups. Examples of the double-reciprocal plots are shown in Figs. 2a–c and 3a–c. Tissues from benign prostatic hyperplasia with high-, intermediate-, and low AF-NAT activities gave K_m and V_{max} values that correlated with AF-NAT activity levels (Table 1). The differences of K_m and V_{max} among the rapid-, intermediate- and slow-groups were significant ($P < 0.05$ and $P < 0.01$, repectively) (Table 2).

Discussion

The data demonstrated that arylamine NAT activity is present in human benign prostatic hyperplasia tissues. The distribution of specific activities of this NAT enzyme in these tissues supports the probability that the enzyme NAT is polymorphous, with rapid-, intermediate-, and slow-acetylators being evident. Of course, confirmation of the existence of a genetic polymorphism in NAT activity will require inheritance studies in order to determine whether the NAT activity level is indeed inherited and that crosses of rapid- with slow-acetylator human benign prostatic hyperplasia yield Mendelian inheritance patterns.

Our previous studies demonstrated that human prostate tumor cells (PC-3) contain NAT activity [32]. More interestingly, there are no reports of NAT activity from human benign prostatic hyperplasia tissues. Further studies are needed, such as purifying NAT enzyme from and determining NAT DNA sequences in the examined tissues as a small change in the primary structure accounts for the catalytic differences which are reported

Table 2 Kinetic data for acetylation of 2-aminofluorene (*AF*) and *p*-aminobenzoic acid (*PABA*) in human benign prostatic hyperplasia tissues. Values are means ± SD for rapid, intermediate, or slow acetylator phenotypes. The acetyl coenzyme A concentration was 0.1 mM, and the kinetic constants were calculated from the modified HYPER program of Cleland [8]

	AF		PABA	
	K_m (mM)	V_{max} (nmol/10^6 cells)	K_m (mM)	V_{max} (nmol/10^6 cells)
Rapid	4.09 ± 0.42	25.06 ± 3.24	3.29 ± 0.24	16.67 ± 2.16
Intermediate	3.13 ± 0.34[a]	17.62 ± 2.28[b]	2.58 ± 0.16[a]	14.26 ± 1.86
Slow	2.40 ± 0.24[a]	13.02 ± 1.02[b]	1.85 ± 0.12[a]	12.58 ± 1.12[b]

[a] Differences from rapid, intermediate $P < 0.05$
[b] Differences from rapid, intermediate $P < 0.01$

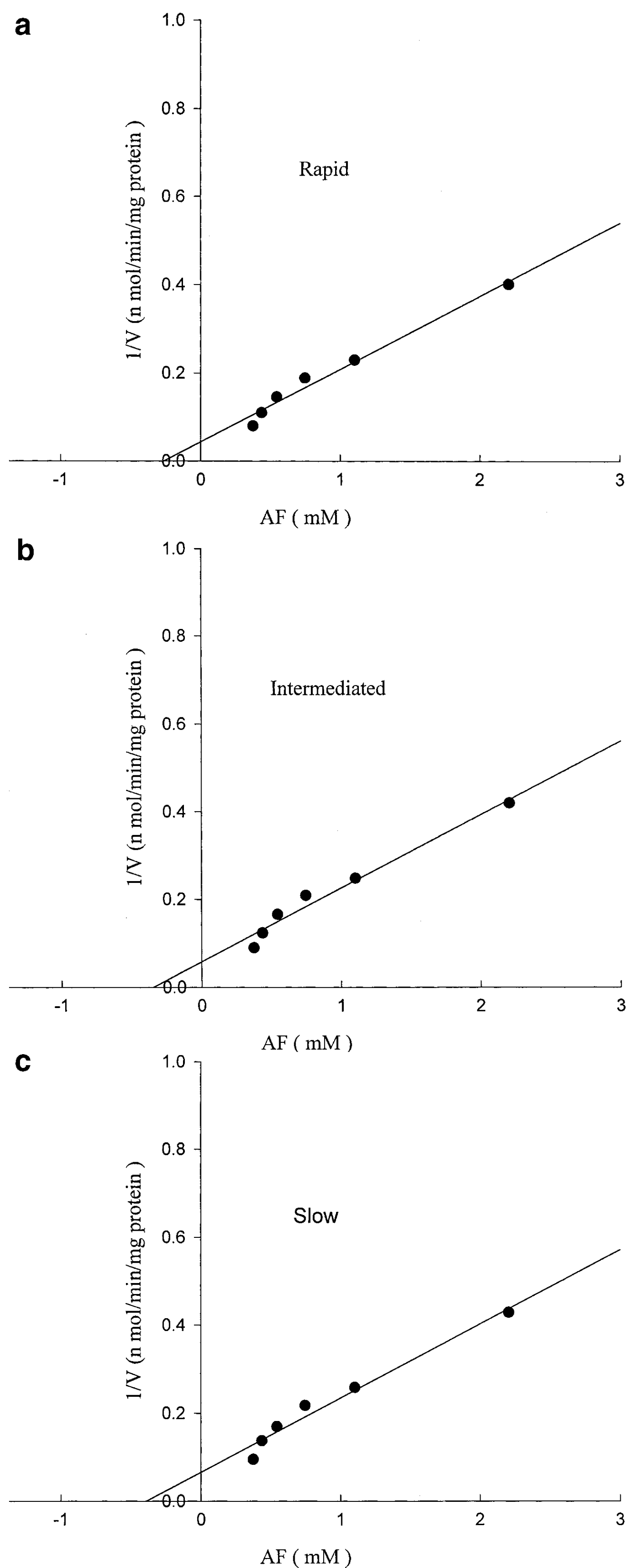

Fig. 2a–c Lineweaver-Burk double-reciprocal plot of N-acetyltransferase (NAT) activity for 2-aminofluorene (*AF*) in human benign prostatic hyperplasia tissues. **a** The rapid acetylator, **b** the intermediate acetylator and **c** the slow acetylator

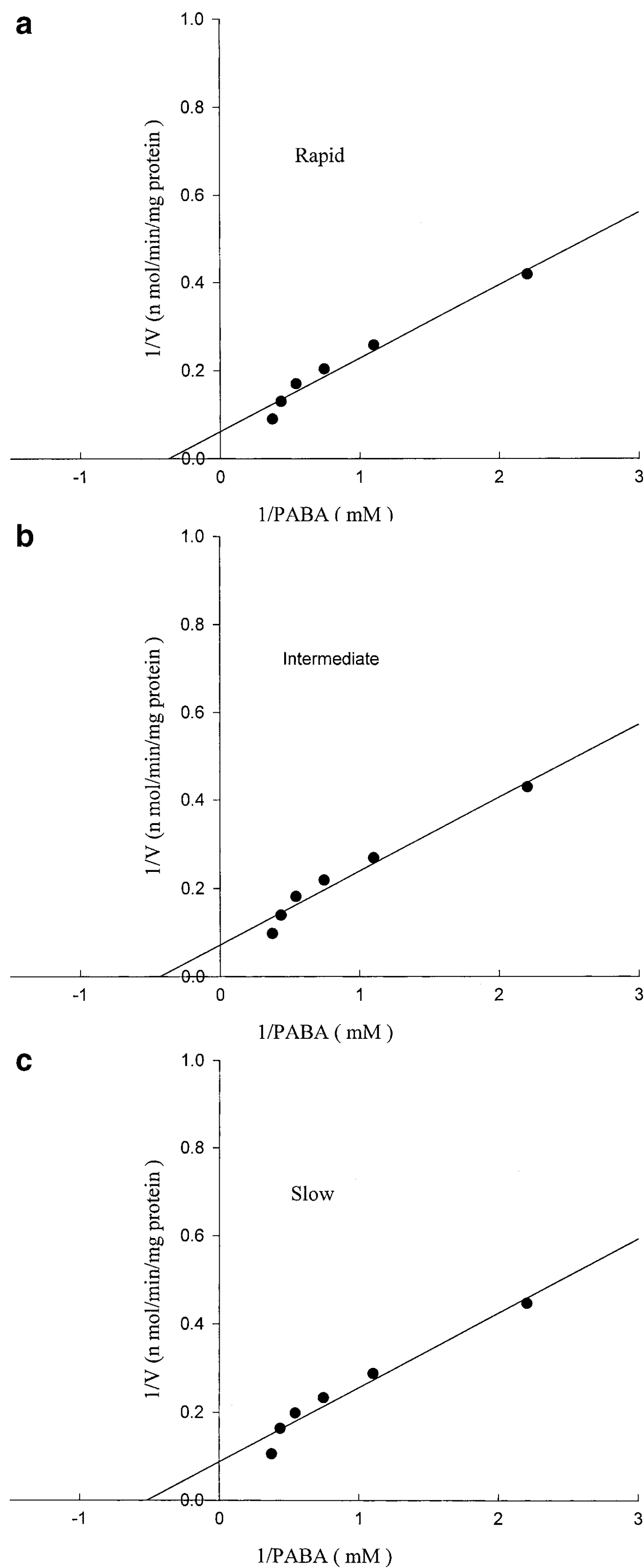

Fig. 3a–c Lineweaver-Burk double-reciprocal plot of N-acetyltransferase (*NAT*) activity for *p*-aminobenzoic acid (*PABA*) in human benign prostatic hyperplasia tissues. **a** shows the rapid acetylator, **b** the intermediate acetylator and **c** the slow acetylator

for liver NAT from rapid- and slow-acetylator rabbits [15]. Although rapid and slow acetylations have been shown to be a predisposing factor for the sensitivity of individuals to toxicity during exposure to many arylamine drugs and carcinogens [29], this N-acetylation is a primary determinant in the elimination of several therapeutic compounds and arylamines.

The effect of heredity on the metabolic activation of arylamine carcinogens in human tissues has been studied for the past 20 years or more. Although the original genetic and enzymologic studies of acetylation polymorphism concentrated mainly on the liver, it is now appreciated that the expression of this trait occurs in extra-hepatic tissues, such as human colon tissues [10, 24] and the human urinary bladder [19]. An association between acetylator phenotype and breast cancer has been reported [5]. It is still controversial as to whether acetylator phenotypes are related to bladder cancer in human populations. Some reports have raised the possibility that slow-acetylators raise the risk for bladder cancer [26]. On the another hand, Hayes et al. [12] demonstrated that the NAT2 related slow acetylator might not be associated with an increased risk of bladder cancer in workers exposed to benzedrine but may have a protective effect. A factor which complicates studies of the role of NAT activity in mammals is that there is more than one NAT enzyme. For example, two NATs have been demonstrated for rabbits [3], hamsters [16], humans [4], and also frogs [17]. Furthermore, three NATs have been found in mice [22].

The substrate specificity of the NAT enzymes is different, although there is overlap. In humans, NAT1 acetylates PABA and *p*-aminosalicylic acid, wheras NAT2 does not. However, NAT2 acetylates sulfamethazine, whereas NAT1 does not. Interestingly, both NAT1 and NAT2 have significant activity with AF. Therefore, AF was chosen in the present study because it is a common substrate for both NAT enzymes [2]. The reason for selecting AF and PABA for determining NAT activity was to allow a comparison of the metabolism of a carcinogen (AF) and a non-carcinogen (PABA) in human benign prostatic hyperplasia tissues. Based on the data in this study, more than one NAT may exist in some of the human benign prostatic hyperplasia tissues examined. First, the mean values for AF-NAT specific activity vary among the 40 examined samples. This also occurred for PABA-NAT activity. Thus, it is probable that AF is acetylated by a separate NAT isoform than PABA (Table 2). There may be some overlap in specificity between these enzyme isoforms. Second, the values of K_m and V_{max} for AF differ among the examined samples. The higher K_m and V_{max} values could indicate a second NAT isoform with different kinetic properties. Whether the NAT from human benign prostatic hyperplasia tissues belongs to NAT1 or NAT2 is still not known. Further investigations are needed to elucidate this. In this regard, it will be interesting to determine if NAT1 or NAT2 probes of humans will recognize benign prostatic hyperplasia NAT DNA. Differential display, polymerase chain reaction, and southern blotting of human genomic DNA with NAT probes under a variety of conditions will help answer this question.

In conclusion, our results indicate that human benign prostatic hyperplasia tissue is similar to human liver and breast tissues, in that there seems to be a polymorphism in NAT activity (N-acetylation of substrates). More importantly, this study is the first to show that the NAT exzyme is present in human benign prostatic hyperplasia tissues.

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