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Association Between NAT2 Polymorphisms and Prostate Cancer

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Abstract

Background: NAT2 enzyme involved in bioconversion of aromatic amines, heterocyclic arylamines and certain drugs into electrophilic ions that can be important initiators in tumorigenesis process.
Objectives: The aim of this study was to assess the possible association between NAT2 polymorphisms (857 G > A, 481 T > C, and 590 G > A) and risk of prostate cancer (PC).
Methods: Totally, 207 benign prostate hyperplasia (BPH) and 147 PC Iranian patients were evaluated. NAT2 genotypes were detected by restriction fragment length polymorphism (RFLP). Multiple logistic regression models were used to estimate the odds ratios for the association between presence of each genotype and developing PC.
Results: For NAT2 G857A, the frequency of AA and AG genotypes was lower among PC patients compared to those without it (1.01% vs. 0 and 55.88% vs. 54.55%, respectively; P = 0.7). For NAT2 T481c, the odds ratios for the association of TT and CT genotypes with PC were 0.65 and 0.55, respectively, which were not statistically significant (P = 0.5 and P = 0.09, respectively). For NAT2 G590a, both AA (11.11% vs. 12.87%) and AG (45.83% vs. 52.48%) genotypes were significantly more common among PC patients compared to BPC patients (P = 0.008). However, none of the relevant odds ratios were statistically significant (OR = 2.2, P = 0.2 and OR = 1.72, P = 0.1, respectively). Among PC patients, CT genotype of T481C caused more than 4-fold significant increase in the risk of developing advanced stages of PC.
Conclusions: Our study represented credible evidence that carrying G857A, G590A and T481C polymorphisms of NAT2 may not affect developing PC, its grading or invasion, but heterozygote genotype of T481C polymorphism (Rapid acetylator) can be associated with more advanced stages of cancer earlier in life. Further longitudinal studies with larger sample sizes are needed to more precisely assess the genetic risk factors of PC.

Keywords: N-Acetyltransferase, Genetic Polymorphism, Prostate Cancer

1. Background

Prostate cancer is one of the most prevalent cancers in males around the world (1). It is a complex disease and its etiology still remains unclear. There are several well-established risk factors for the occurrence of prostate cancer (PC) such as age, race, diet, environmental factors and genetics. The possible interactions among chemical and dietary factors, and polymorphic genes encoding metabolic enzymes involved in their bio-activation or inactivation are considered as the potential risk factors for PC as well (2). Several studies have investigated the relationship between different metabolic enzymes such as N-acetyltransferase (NAT) and risk of PC. The NAT2 gene encoding N- acetyltransferase 2 is located on the short arm of chromosome 8, 8q21.3-23.1 (3, 4). NAT2 enzyme participates in bioconversion of aromatic amines and heterocyclic arylamines to electrophilic ions that can be important initiators of tumorigenesis process (5). NAT2 is most frequently expressed in the liver and implicates a polymorphism which results in the expression of four mutant alleles (M1, M2, M3 and M4) (6, 7). These mutant alleles produce a wide range of metabolic phenotypes as rapid or slow acetylators. The presence of at least one wild type allele exhibits rapid acetylator phenotype; whereas, presence of two mutant alleles results in slow acetylator phenotype (6, 8, 9).

2. Objectives

The aim of this study was to assess possible association between NAT2 polymorphisms and risk of PC. We also examined the correlation of NAT2 polymorphism with pathological grade and clinical stage of PC.

3. Methods

We studied a total of 354 patients including 147 PC and 207 BPH cases as controls who were recruited from department of Urology of Shahid Labafinejad Medical center Tehran, Iran between February 2010 and February 2014.

Written informed consent was obtained from all cases and a structured questionnaire was filled out to collect information on potential risk factors such as age of onset, Body Mass Index (BMI) and total and free Prostate Specific Antigen (PSA) level.

Diagnosis work up was done based on the PSA level and digital rectal examination (DRE) and prostate biopsy. Tumor size, pathological stage and grade, and perineural and vascular invasion were determined by open laparoscopic or radical prostatectomy.

The control group with BPH had to fulfill the following inclusion criteria for decreasing the likelihood of misdiagnosed PC (10, 11):

1. Either serum PSA < 4.0 ng/mL or negative pathological report of the prostate biopsy if these were a serum PSA > 4.0 ng/mL.

2. Negative pathological report of malignancy in resected prostatic tissues by open surgical prostatectomy.

Family history of PC, a pathological report of malignancy, taking PSA decreasing medications, orchiectomy, hormone therapy and non-adenocarcinoma prostate cancer were the exclusion criteria for the control group.

3.1. NAT2 Genotyping

Genomic DNA was extracted from peripheral leucocytes by salting out method (12).

The NAT genotyping was done using Polymerase Chain Reaction- Restriction Fragment Length Polymorphism (PCR-RFLP) as described previously by Vatsis et al. in 1995 (13).

PCR was carried out using created PCR primers (5'-CTT CTC CTG CAG GTG ACC AT-3'; 5'-AGC ATG AAT CAC TCT GCT TC-3'). Following PCR, 10 μL aliquots were removed and subjected to restriction digest with KpnI (M1 allele) at 65°C for 3 hours, TaqI (M2 allele) at 65°C for 3 hours, and BamHI (M3 allele) at 37°C for 3 hours. The digested products were resolved by electrophoresis on a 4% agarose gel, stained with safe stain.

If the corresponding alleles could not be identified after digestion, the remaining alleles were ascertained as Wild Type polymorphism alleles (WT) that have all of these restriction sites. The genotype of each individual was classified as homozygote and heterozygote forms of M1, M2, M3, and WT alleles. The rapid and slow phenotypes are defined as WT allele homo/heterozygotes and two mutant alleles in homo or hetero respectively.

3.2. Statistical Analysis

Demographic, habitual and genetic factors were compared between patients with BPH and PC using Chi square and Fisher's exact tests (categorical variables) as well as Independent t-test and Mann Whitney U test (continuous variables). Multiple logistic regression models were used to estimate the odds ratios for association between presence of each genotype and developing cancer, stage, grade and invasion of the tumor, controlling for age, cigarette smoking and family history of PC. P values less than 0.05 were considered significant. All analyses were performed using STATA V.11 software.

4. Results

We recruited 354 patients with a mean (standard deviation) age of 66.65 (9.19) years. Their mean (SD) BMI was 24.76 (2.94). Totally, 207 (58.47%) BPH patients and 147 (41.53) PC cases were evaluated. Patients with PC had higher frequency of cigarette smoking than BPH patients (44.64% vs. 18.25% respectively; P < 0.0001). Family history of PC among patients with cancer was significantly higher than that among BPH patients (11.88% vs. 1.57%, respectively; P = 0.001). In addition, patients with BPH were on average older than the other group (mean age of 70.27 years vs. 62.01 years; P < 0.0001). The BPH and PC patients were matched in terms of marital status (P = 0.2) and the mean BMI (P = 0.9) (Tables 1 and 2).

4.1. Association of Different Genotypes of NAT2 Polymorphisms and Prostate Cancer

For 857 G > A polymorphism, the adjusted odds ratios between presence of 857 G > A polymorphism and PC were 0.62 (AA genotype) and 1.03 (AG genotype). None of these associations were significant (P = 0.7 and P = 0.9, respectively). For the second polymorphism 481 T > C polymorphism the odds ratios between TT and CT genotypes, and PC were 0.65 and 0.55, respectively. But none of them were significant (P = 0.5 and P = 0.09, respectively). In 590 G > A polymorphism both AA (11.11% vs. 12.87%) and AG (45.83% vs. 52.48%) genotypes were significantly more common among patients with PC compared to BPH patients (P =

Table 1. Demographic and Habitual Characteristics of Patients with and Without Prostate Cancer[a]

Variables	Benign Prostatic Hyperplasia	Prostate Cancer	P Value
Familial history of prostate cancer[b]			
Negative	125 (98.43)	89 (88.12)	0.001
Positive	2 (1.57)	12 (11.88)	
Cigarette smoking[b]			
Negative	103 (81.75)	62 (55.36)	< 0.0001
Positive	23 (18.25)	50 (44.64)	
Marriage status[b]			
Single	0 (0)	2 (1.94)	0.2
Married	132 (100)	101 (98.06)	
Age			
Mean (SD)	70.26 (8.68)	62.01 (7.63)	< 0.0001
BMI			
Mean (SD)	24.78 (3)	24.72 (2.87)	0.9

[a] Value are expressed as N. (%).
[b] NAT polymorphisms and developing prostate cancer.

0.008). However, none of the relevant odds ratios were statistically significant (OR = 2.2, P = 0.2 and OR = 1.72, P = 0.1, respectively) (Table 3).

4.2. NAT2 Polymorphisms Association with the Stage and Grade of PC

There are no significant association between different genotypes of 857 G > A and 590 G > A NAT2 polymorphism with the stage and grade of PC but the odds ratio between CT and stage of cancer was 4.07, which was statistically significant (P = 0.03) and the CT genotype of this polymorphism demonstrated a significant association (Tables 4 and 5).

4.3. NAT Polymorphisms and Vascular and Perineural Invasion of the Tumor

There is no significant association between the corresponding polymorphisms and perineural invasion, and for vascular invasion heterozygote genotypes of M1 cancer developed significantly earlier than wild type individuals (Table 5).

4.4. Allelic Frequencies

4.4.1. 857 G > A Polymorphism

Frequency of A allele among PC patients was less than that in BPH patients (28% vs. 28.29%, respectively; P = 0.9). The odds ratio between presence of A allele and developing cancer was 0.98 which was not significant (P = 0.9).

4.4.2. 481 T > C Polymorphism

Patients with PC had significantly lower rates of T allele compared with BPH patients (34.03% vs. 52%, respectively; P < 0.0001). The corresponding odds ratio was significant (OR = 0.47; P < 0.0001).

4.4.3. 590 G > A Polymorphism

An allele was more common among patients with PC than BPH patients (41% vs. 29.43%, respectively; P = 0.002). The odds ratio between this allele and developing prostate cancer was 1.66, which was statistically significant (P = 0.003) (Table 6).

4.5. NAT Acetylation Phenotypes and Prostate Cancer

Frequency of slow acetylation phenotype of 481 T > C among cases and controls were 43.6% and 34.65% respectively (P = 0.1). The corresponding figures for 857 G > A and 590 G > A slow acetylation phenotypes were zero and 1.01% (P = 0.2) and 17.21% and 7.29% (P = 0.006) respectively. The odds ratios between these three phenotypes and developing cancer were 1.77 (P = 0.09), 1.46 (P = 1) and 1.63 (P = 0.4) respectively (Table 7).

5. Discussion

Prostate cancer is a heterogeneous disease and many factors affect its etiology. Several studies have shown the relationships between gene polymorphisms and PC. The NAT2 enzyme has been shown to be polymorphic and the involvement of NAT2 gene polymorphism in PC has been investigated by a small number of studies.

In the present study, we tested NAT2 rs 1799929, rs1799930 and rs 1799931 polymorphisms with PC susceptibility both in controls and PC patients.

In a meta-analysis of 20 studies on the relationship between NAT2 rs1799930 and rs1799931 polymorphism with the risk of cancer, a significant association was observed between NAT2 rs1799930 polymorphism and cancer risk in Asians (GA vs. GG: OR = 1.22, 95%CI = 1.03 - 1.45) and population based controls (GA vs. GG: OR = 1.10, 95%CI = 1.01 - 1.1.19). Also a significant association was found between the NAT2 857 G > A polymorphism and decreased cancer susceptibility in the Asian but not in the Caucasian population (AA vs. GG: OR = 0.55,95% CI = 0.33 - 0.93, GA vs. GG: OR = 1.00, 95% CI =0.880 - 1.14) (14).

In previous studies, no association was found between NAT2 genotype and PC (15, 16). Several studies have explored the association between acetylation phenotype and cancer susceptibility.

DS Steivastava et al. (17) in a study of 130 patients and 140 controls of an Indian population found a relationship

Table 2. Crude and Adjusted Mean Differences Between Carriers of Different Genotypes of NAT Polymorphisms

Genotypes	Number	Mean (SD)	P Value	Adjusted[a] Mean Difference (P Value)	95% Confidence Interval
G857a					
GG	58	62.69 (6.83)			
AG	71	61.37 (8.41)	0.3	-1.15 (0.5)	-4.67 - 2.37
AA	0	-		-	-
T481c					
CC	62	63.62 (7.03)			
CT	59	60.19 (8.17)	0.03	-4.34 (0.01)	-7.79 - 0.88
TT	15	61.47 (6.92)		-2.51 (0.5)	-9.38 - 4.35
G590a					
GG	40	60.27 (8.79)			
AG	54	63.07 (7.31)	0.2	2.78 (0.2)	-1.24 - 6.81
AA	20	61.55 (6.63)		2.66 (0.3)	-3 - 8.32

[a] Adjusted for age, cigarette smoking habit and familial history of cancer.

Table 3. Crude and Adjusted Associations Between Different Genotypes of NAT Polymorphisms and Prostate Cancer

Genotypes	BPH	Prostate Cancer	P Value	Adjusted[a] OR (P Value)	95% Confidence Interval
G857a					
GG	88 (44.44)	60 (44.12)		1	
AG	108 (54.55)	76 (55.88)	0.7	1.03 (0.9)	0.65 - 1.64
AA	2 (1.01)	0 (0)		0.62 (0.7)	0 - 8
T481c					
CC	70 (34.65)	62 (43.06)		1	
CT	106 (52.48)	66 (45.83)	0.3	0.55 (0.09)	0.28 - 1.1
TT	26 (12.87)	16 (11.11)		0.65 (0.5)	0.18 - 2.39
G590a					
GG	93 (48.44)	43 (35.25)		1	
AG	85 (44.27)	58 (47.54)	0.008	1.72 (0.1)	0.82 - 3.57
AA	14 (7.29)	21 (17.21)		2.2 (0.2)	0.69 - 7

[a] Adjusted for age, cigarette smoking habit and familial history of cancer.

Table 4. Crude and Adjusted Associations Between Different Genotypes of NAT Polymorphisms and Staging and Grade of Prostatic Tumor

Variables	G857a			T481c			G590a		
Genotype	GG	AG	AA	CC	CT	TT	GG	AG	AA
Low stages	14 (40)	21 (60)	0	23 (65.71)	10 (28.57)	2 (5.71)	12 (38.71)	14 (45.16)	5 (16.13)
Advanced stages (TNM > PT2C)	7 (31.82)	15 (68.18)	0	7 (30.43)	16 (69.57)	0 (0)	6 (31.58)	12 (63.16)	1 (5.26)
Overall P value		0.4			0.005			0.4	
Adjusteda OR[a] (P value)	1	1.18 (0.8)	-	1	4.07 (0.03)	1.02 (1)	1	2.06 (0.3)	0.45 (0.5)
95% confidence interval		0.36 - 3.83	-		1.11 - 16.24	0 - 14		0.48 - 8.79	0.04 - 5.29
Gleason < 7	22 (50)	22 (50)	0	25 (55.56)	12 (26.67)	8 (17.78)	14 (35)	16 (40)	10 (25)
Gleason ≥ 7	38 (41.30)	54 (58.7)	0	37 (37.37)	54 (54.55)	8 (8.08)	29 (35.37)	42 (51.22)	11 (13.41)
Overall P value		0.3			0.006			0.2	
Adjusteda OR[a] (P value)	1	1.57 (0.3)	-	1	2.19 (0.1)	1.13 (0.9)	1	1.18 (0.8)	0.98 (1)
95% confidence interval		0.63 - 3.95	-		0.83 - 6.35	0.18 - 6.92		0.40 - 3.42	0.23 - 4.27

[a] Adjusted for age, cigarette smoking habit and familial history of cancer.

between NAT2 genotype and PC risk (OR = 1.452, 95%CI: 0.54 - 1.87, P = 0.13) which was not significant and there was no association between NAT2 genotype, grade or level of PSA. In our study, except for AA genotype of 590 G > A, all of the other genotypes increased the risk of tumor progression (Gleason grade of more than 7) but none were significant.

Table 5. Crude and Adjusted Associations Between Different Genotypes of NAT Polymorphisms and Vascular Invasion and Perineural Invasion of the Tumor

Variables	G857a			T481c			G590a		
Genotype	GG	AG	AA	CC	CT	TT	GG	AG	AA
Without vascular invasion	11 (34.38)	21 (65.63)	0	15 (46.88)	15 (46.88)	2 (6.25)	15 (50)	12 (40)	3 (10)
With vascular invasion	4 (50)	4 (50)	0	4 (50)	3 (37.50)	1 (12.50)	1 (20)	3 (60)	1 (20)
Overall P value		0.3			0.8			0.4	
Adjusted[a] OR (P value)	1	0.29 (0.2)	-	1	1.17 (0.9)	5.30 (0.3)	1	3.82 (0.3)	5.02 (0.3)
95% confidence interval		0.04- 2.27	-		0.12 - 11.15	0.21 - 133		0.31 - 46	0.24 - 105
Without perineural invasion	5 (55.56)	4 (44.44)	0	4 (44.44)	4 (44.44)	1 (11.11)	4 (50)	3 (37.50)	1 (12.50)
With perineural invasion	22 (34.92)	41 (65.08)	0	36 (57.14)	23 (36.51)	4 (6.35)	20 (37.74)	26 (49.06)	7 (13.21)
Overall P-value		0.2			0.6			0.9	
Adjusted[a] OR (P value)		1.85 (0.5)			0.87 (0.9)	0.37 (0.5)		2.39 (0.4)	1.70 (0.7)
95% confidence interval		0.35 - 9.79			0.12 - 6.05	0.03 - 5.16		0.35 - 16.27	0.10 - 28.12

[a] Adjusted for age, cigarette smoking habit and familial history of cancer.

Table 6. Allelic Frequencies and Odds Ratios for Patients with BPH and Prostate Cancer[a]

Genotypes	BPH	Prostate Cancer	P Value	OR (P Value)	95% Confidence Interval
G857a					
G	71.71	72	0.9	0.98 (0.9)	0.69 - 1.40
A	28.29	28			
T481c					
C	48	65.97	< 0.0001	0.47 (< 0.0001)	0.34 - 0.67
T	52	34.03			
G590a					
G	70.57	59	0.002	1.66 (0.003)	1.17 - 2.36
A	29.43	41			

[a] Value are expressed as number percent.

Table 7. Crude and Adjusted[a] Associations Between NAT Acetylation Phenotypes and Prostate Cancer

Phenotypes	Healthy	Cancer	P Value	crude OR	P Value	95% CI[b]		adjusted OR	P Value	95% CI	
t481c											
Rapid	132 (65.35)	82 (56.94)									
Slow	70 (34.65)	62 (43.06)	0.1	1.42	0.1	0.92	2.21	1.77	0.09	0.91	3.42
g857a											
Rapid	196(98.99)	136 (100)									
Slow	2 (1.01)	0 (0)	0.2	0.6	0.7	0	7.75	1.46	1	0	58
g590a											
Rapid	178 (92.71)	101 (82.79)									
Slow	14 (7.29)	21 (17.21)	0.006	2.64	0.008	1.29	5.42	1.63	0.4	0.55	4.79

[a] Adjusted for age, cigarette smoking and familial history of cancer.
[b] Confidence interval.

Wadelius et al. (16) reported no association between PC and NAT2 genotypes in Swedish and Danish populations that was in agreement with the findings of Agundez et al. (15), Hooker et al. (18), Igucli et al. (19) and Rovito et al. (20) in Spanish, African-American and Caucasian North-American populations, respectively but in a study by Hamaeski et al. in 2003 (21), it was found that slow acetylation genotype increased the risk of PC by 24 times in a Brazilian population, which was significant. Our study, similar to that of Hamasaki et al. (21), showed that the NAT2 slow acetylator genotype was associated with advanced stage and higher grade of PC (17, 22, 23). In another meta-analysis on the results of 10 studies on the role of NAT2 phenotype in PC a statistically significant association was found between NAT2 polymorphism and PC in an Asian but not in a Caucasian populations (24).

Another study by Costa S et al. (25) showed the role of NAT2 polymorphisms in the carcinogenic pathway of prostate cancer, specifically in a population of Southern. Hein DW et al. (26) in a pilot study demonstrated that combination of two genotypes (NAT1*10 and slow NAT2) increased susceptibility to prostate cancer.

In conclusion, our study presented credible evidence that carrying 857 G > A, 590G > A and 481 T > C polymorphisms may not affect developing PC, its grading or invasion, but heterozygote genotype of T481c polymorphism can be associated with more advanced stages of cancer earlier in life. Further longitudinal studies with larger sample sizes are needed to more precisely assess the genetic risk factors of PC.

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Footnotes

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