

## RESEARCH ARTICLE

# A2 Allele Polymorphism of the CYP17 Gene and Prostate Cancer Risk in an Iranian Population

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### Abstract

**Background:** Studies have shown that alterations of steroid hormone metabolism, particularly involving testosterone, affect the risk of prostate cancer. Therefore, genetic variation in genes of enzymes which are involved could be of importance. The gene most interest is CYP17, whose enzyme product has an essential role in testosterone hormone synthesis. Some studies have indicated that the A2 allele polymorphism of CYP17 associated with increased risk of prostate cancer that could be affected by ethnicity. Therefore, the aim of this study was determination of presence or absence of the A2 allele in patients with prostate cancer. **Materials and Methods:** We studied the association of A2 allele and prostate cancer among 74 patients with prostate cancer and 128 healthy men which were referred to hospitals of SBMU. **Results:** This study revealed a significant association between prostate cancer risk and the A2 allele in an Iranian population so that A1A2 and A2A2 genotypes were more common in cases than controls with P-values of 0.029 and 0.010, respectively. **Conclusions:** Results of our study support a possible role of the A2 allele in sporadic prostate cancer development in Iran, in line with findings elsewhere.

**Keywords:** Iranian population - CYP17 - A2 allele polymorphism - prostate cancer

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### Introduction

Prostate cancer is the second most common cancer all of the world and Iran (Sadjadi et al., 2007). It may appear in familial or sporadic version. In familial cases, usually, there are mutations in genes with high penetrance such as ElaC2, RNase L, MSR1 (Camp and Tavtigian, 2002; Chen et al., 2003; Gsur et al., 2004; Alberti, 2010). But, hereditary prostate cancer has low prevalence. So, mutations and genetic variation in genes with low penetrance must be considered. Association of CYP17 gene polymorphism, as a member of CYP450 gene super family, has been studied in many populations and many of them have revealed a significant association between A2 allele (rs743572) and prostate cancer (Lunn et al., 1999; Gsur et al., 2000; 2004; Habuchi et al., 2000; Haiman et al., 2001; Yamada et al., 2001; Stanford et al., 2002; Ntais et al., 2003; Antognelli et al., 2005; Yang et al., 2006; Sobti et al., 2006; 2009; Sarma et al., 2008; Souiden et al., 2010). Also, some studies have shown significant association between MspAI polymorphism of CYP17 and increased risk of breast cancer (Feigelson et al., 1997; Bergman-Jungeström et al., 1999; Haiman et al., 1999). CYP17 gene is located 10q24.32 (Fan et al., 1992). This

gene has 6569 base pair and 8 exons (Picado-Leonard and Miller, 1987) and codes for P450c17 that catalyzes 17 $\alpha$ -hydroxylase and 17,20-lyase reaction in metabolism pathway of testosterone (Barnes et al., 1991). A2 allele is known with T/C transition in -34 5'UTR of transcript and it is considered that creates an additional SP1-type (CCACC box) promoter site, which may cause increased expression of CYP17 gene (Carey et al., 1994) but by the now, role of this polymorphism in vivo has not been clearly known.

### Materials and Methods

#### Patients

In this research, we studied 202 unrelated Iranian referred men to the hospitals of SBMU, Tehran, Iran, consisting of 74 men with prostate cancer and 128 healthy men as control group, which had been matched for age. Patient sample selection was done based on positive pathology result. Control samples were selected based on normal result of Digital Rectal Examination and PSA levels <4 ngr/mlit (Hong et al., 2010). Age range in two groups was 50-75 years old (Sadjadi et al., 2007). Participated men in this study had not family history of

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Prostate cancer. Also, as regard pathology reports sheet we gathered information about pathologic features of tumors in patient, such as Gleason score. In this research all subjects participated with informed consent.

**Genotyping**

Peripheral blood samples, which had been gathered in K3 EDTA tube, were used for DNA Extraction. DNA extraction was done with two methods; with using "Diatom TMDNA Prep kit" (Diatom, Russia) and "Salting out". Then PCR was carried out with 25 µlit as a total volume and components including; 1.5 mmol 10X PCR buffer with 160 mM (NH4)<sub>2</sub>SO<sub>4</sub> -670 mM Tris-HCl -0.1% tween-20 contain (KBC, Iran), 1.5 mmol Mgcl<sub>2</sub> (KBC, Iran), 200 µmol dNTPs (Bioscience, England), 8pmol from each primers (Bioneer, South Korea), 2.5IU Taq pol (KBC, Iran) and 200ngr of genomic DNA. Sequences of Primers described by Careyet al. (1994) have been shown in Table 1.

PCR condition was an initial denaturation 94°C with 5 min duration, and 30 cycles including; denaturation step 94°C 30s, annealing step 48.2°C 30s, replication step 72°C 35s and final replication 72°C 5min. PCR products were digested with using 5IU MspAI1 restriction enzyme (Promega, USA), BSA and reaction buffer according to the manufacturer's instructions for 16 hours at 37°C. 8µlit

**Table 1. Sequences of Forward and Reverse Primer which were used in PCR**

Forward primer	5'-CATTCGCACCTCTGGAGTC-3'
Reverse primer	5'- GGCTCTGGGGTACTTG-3'

**Table 2. Genotypes of CYP17 among Cases and Controls and Allele Frequency for A1 and A2**

Genotypes	Controls (N=128)	Cases (N=74)	P-value (CI=95%)	Allele frequency in population	X <sup>2</sup>
A1A1	51(39.8%)	17(23.0%)	0.032	A1 A2	
A1A2	62(48.4%)	42(56.8%)	0.029	0.59 0.41	0.92
A2A2	15(11.7%)	15(20.3%)	0.01		

\*P-value <0.05 is significant, 95%CI indicates 95% confidence interval confidence interval

**Table 3. Statistical Result of Relation between Gleason Score and Types of Genotype**

Genotype	Gleason Score			Stage of disease	
	Low	Moderate	High	Local	Advanced
A1A1	11.80%	23.50%	64.70%	50.00%	50.00%
A1A2	2.50%	37.50%	60.00%	50.00%	50.00%
A2A2	0.00%	60.00%	40.00%	61.50%	38.50%
P-value (CI=95%)	0.13	0.14	0.55		

\*Pathological character (Gleason score, stage) and CYP17 high risk genotypes (A1A2 and A2A2) in comparison with CYP17 wild-type genotype (A1A1). 95% CI indicates 95% confidence interval. \*P-value <0.05 was significant

**Table 4. Relation between Addiction and Prostate Cancer Risk**

Groups	Smoking	Smoking and opiate addiction	No smoking
Case	44(59.5%)	1(1.4%)	29(39.2%)
Control	27(26.0%)	1(1.0%)	76(73.1%)
P-value (CI=95%)	≤0.0001	≤0.0001	≤0.0001

\*P-value <0.05 is significant

of digested PCR products were loaded onto 2% ethidium bromide stained agarose gel and then were examined with UV TEC system (Cambridge, USA). The PCR product of 459bp fragment generated one band at 459bp for A1A1 genotype, three bands including 459,335 and 124bp for A1A2 genotype and two bands (335 and 124bp) for A2A2. Electrophorogram pattern has been shown in Figure 1.

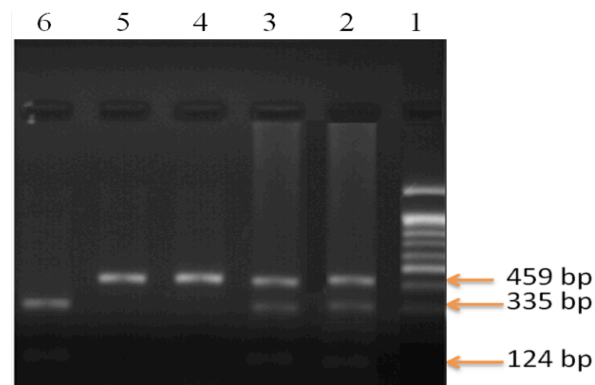
**Statistical analysis**

Unprepared data were analyzed with T-test and Chi square test program of SPSS software. Population was studied about Hardy- Weinberg equilibrium. Allele frequency was calculated for each allele. Association between genotypes and risk of prostate cancer was assessed by calculating P-value and their 95% confidence intervals. P-value<0.05 was considered significant.

**Results**

In this study, mean age of cases and controls was 68.46 and 68.16, respectively (rang: 50-75) therefore, which was not significantly different between case and control groups (P-vau=0.830). Association of prostate cancer risk and genotypes has been shown in Table 2. Analysis of genotype frequency showed; A1A1 genotype rate was higher in control group (39.8%) than case group (23%) vice verse; A1A2 and A2A2 genotypes were high in cases (56.8% and 20.3% respectively) in comparison with controls (48.4% and 11.7% respectively).Therefore, there was a tendency towards higher frequency of A1A2 and A2A2 genotypes amongst patients when compared with healthy controls and further evaluation of these results with statistical analysis revealed a significant association between A2 allele and risk of prostate cancer (P-value=0.029, 0.010, respectively for A1A2 and A2A2 as high risk genotypes). With regard to that, A1A1 as a low risk genotype was high in control group (P-value=0.032). As expected, mean of PSA levels in cases (15.43ngr/mlit) was significantly higher than controls (1.92ngr/mlit) (P-value<0.0001).

Also, we analyzed relation between Gleason score [at three group including low (≤4) and moderate (4-6) and high score (≥7),] and types of CYP17 genotypes. Also, we considered relation between types of genotype and stage



**Figure 1. 2% Agarose Gel Electrophoresis of Restricted Fragments by MspAI.** Lane 1: 100bp DNA ladder. Lane 4, 5: A1/A1 (wild type homozygote). Lane 6: A2/A2 (mutant type homozygote). Lane 2, 3: A1/A2 (heterozygote)

of disease. There was no significant difference between three types of genotype and advancement of Gleason score (P-value>0.05) also, local and advanced disease, (Table 3).

Probable risk factors, such as smoking and opiate addiction was studied based on information which had been gathered with questionnaire. Statistics analysis revealed these factors have associated with increased risk of prostate cancer (P-value<0.0001) (Table 4). There was no deviation from Hardy-Weinbergs' equilibrium in this population ( $X^2=0.92$ , P-value=0.01)

## Discussion

Genetic variation at genes which have important role in steroid hormone metabolism, especially polymorphism, has been considered as an important genetic causation of sporadic prostate cancer as a multifactorial disease. Among these genes, CYP17 has been as an interested gene. Similar to our study more studies have shown a significant association between A2 allele and risk of prostate cancer (Lunn et al., 1999; Habuchi et al., 2000; Stanford et al., 2002; Ntais et al., 2003; Cicek et al., 2003; Madigan et al., 2003; Antognelli et al., 2005). For example, our results are in agreement with the results of Lunn et al. (1999) and Gsur et al. (2000), as we have found that the A2 allele occurs more recurrently in the prostate cancer group than in the controls. But Lunn et al. was able to show an increased risk only when A1/A2 and A2/A2 genotypes were combined (P value=0.05). Also, Gsur et al. showed an association between the risk allele and prostate cancer in men with the A2/A2 genotype (P value=0.03) and no A1/A2 genotype. Yamada et al. (2001) indicated relationship between increased risk of prostate cancer and A1/A2 and A2/A2 genotype (P value=0.03, 0.04 respectively). Yousra Souiden et al. study's (2010) revealed genotypes containing A2 allele (A1/A2 and A1/A2 combined with A2/A2) associate with increased risk of prostate cancer (P value=0.029, 0.029).

Finally, we found a significant association between A1/A2 and A2/A2 genotype and prostate cancer risk (CI=95%, P value=0.029, 0.010, respectively) in an Iranian population. Confirmation of these results will help us to know this gene as an important genetic causation of sporadic prostate cancer and make use of this variation as a marker in screening tests. But, prior to, we must find pathophysiology mechanism resulted from polymorph allele (A2) e.g. effects of A2 about steroid hormones levels of plasma or CYP17 gene expression and so. For that, we can administer researches about expression of CYP17 gene in normal and neoplastic prostate tissue, determination of enzyme product levels in plasma, etc. If we can determine affected pathway, we could use drugs to modifying that in patients.

In conclusion, our study result supports from a significant association between A2 allele of CYP17 and prostate cancer. Therefore, gene polymorphism of CYP17 (A2) is possibly contributed to the prostate cancer pathogenesis but not to stage of disease or score of Gleason grade. As regards, smoking and opiate addiction could be considered as a possible environmental risk factor of

sporadic prostate cancer as a multifactorial disease. But, our study is a pilot study with small sample size. Therefore these results must be confirmed with further researches and meta-analysis studies.

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