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The Correlation between CYP450 Ile462Val Polymorphism and Prostate Cancer in a Group of Iranian Men

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Abstract

Background: Cytochrome P450 plays an important role in pharmaceutical metabolism, steroidal hormones and procarcinogens. CYP1A1 is an enzyme, which is very active in the formation of reactive mediators or injurious agents for DNA. The aim of this study is to evaluate the prevalence rate of genetic polymorphism RS 1048943 gene CYP1A1 in men diagnosed with prostate cancer compared to a control group of healthy men.

Methods: This case-control study analysed blood samples from 79 patients with prostate cancer as well as 79 healthy men. Genomic DNA was extracted by the salting out method. After selecting the suitable primers from the papers, the samples were amplified for the considered segment and genotypes of the participants were determined by PCR-RFLP.

Results: Individuals with prostate cancer had the following genotypes: AA (31.64%), GG (59.49%) and AG/GA (8.86%) compared to the control group that had genotypes AA (55.69%), GG (29.11%) and AG/GA (15.18%). According to the Hardy–Weinberg equilibrium, the frequency of allele A was 36.7% in the cancer group and 63.29% in the control group. The frequency of allele G was 63.92% in the cancer group and 36.70% in the control group. There were meaningful differences in the frequencies of homozygotes GG (P<0.001) and AA (P=0.002) between patients and controls.

Conclusion: Polymorphism RS 1048943 in gene CYP1A1 is related to the risk of developing prostate cancer and it is likely one of the major factors in its occurrence.

Keywords: Polymorphism RS 1048943, Cytochrome 1A1 (CYP1A1), Prostate cancer

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Introduction

Prostate cancer is the most common cancer in males.[1] Some studies have shown that prostate cancer is not common in Iran in comparison to the US and Europe, but it has increased in recent years.[2]

Androgens are male sexual hormones produced by the testis and adrenal glands which have a vital role in the reproductive system of males and in their sexual behavior.[3]

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Androgens are essential for development, function and maintenance of the prostate. In addition to androgens, estrogens and female sexual hormones have an important role in the male reproductive tract through special receptors of estrogen.[4] The estrogen hormone is essential for either stimulating DNA synthesis or inducing the morphology of metaplastic epithelial in the prostates of humans and mice.[5] It appears that the development of the prostate is particularly sensitive to the amount of estrogen available. In the whelps of mice that are not exposed to estrogen, it results in the inhibition of the growth of the prostate and its function.[6]

Cytochrome P450 (CYP) is a large family of hemoproteins (proteins with Fe),[7] of which more than 15 various enzymes have been identified in humans.[8] These enzymes are facilitators and catalysers for different reactions. They are considered to be elements of the electron transportation system in the detoxification of the complex endoplasmic network. Their important role is to remove the toxic materials in the cells.[9] These proteins are mono-oxygenases, which have a vital role in many reactions, including the metabolism of medicine and the synthesis of cholesterol, steroids and other lipids.[10] Studies have demonstrated that these enzymes play a role in other cellular functions such as the metabolism of icozanoids, the biosynthesis of bile acids and vitamin D3, the biological destruction of amines, and the hydroxylation of retinoic acid.[11,12]

One of the members of this family is the CYP1A1 enzyme, which is involved in the activation of polycyclic aromatic hydrocarbons (PAH) such as benzopyrene. Benzopyrene is considered an important carcinogen[8] because many products of the reactions are formed by hydrolysis of the aromatic cycle,[13] which has a mutagenic feature, and it initiates the characteristic of carcinogenesis by binding to DNA[14]. In addition, this enzyme has key role in the oxidative metabolism of estrogen.[15] The stages of the metabolism of estrogen include converting the estrogens to various metabolites of catechol estrogen (2 and 4 hydroxy estrogen), which is performed by enzymes of CYP1A2, CYP1A1 and CYP1B1.[16, 17] CYP1A1 undertakes the first stage of oxidation of estrogen to 2-OH hydroxylation.[18] It results in the development of tumors in rodents[15] by hydroxylating two major estrogens of E1 and E2 in the position of C2.[12]

Gene CYP1A1 is located at position 15q22-q24. This gene contains seven exons and six introns that are 5810 bp in length.[15] When the open proofreading framework (ORF) is initiated from the second exon, a protein with 512 amino acids is encoded.[19] CYP1A1 mRNA is expressed in most human tissue such as the lungs, esophagus, stomach, small intestine, colon, prostate and breasts.[20,21]

Several polymorphic regions have been reported in the gene CYP1A122 of which the most common is the displacement of amino acids in position 462 in the CYP1A1*2 allele. This condition is related to an increased risk of developing various cancers.[23] Due to the important role of this gene in the carcinogenesis of different cancers, carcinogens of prostate cancer may occur by variation in the geno-toxicity balance and hormones.[24] The relationship between this polymorphism and cancer of the prostate in Iranian men has been evaluated in this study.

Materials and Methods

This case-control study enrolled a number of Iranian men diagnosed with primary adenocarcinoma of the prostate as the case group. Males from the case group underwent digital rectal examinations, serum PSA levels, fine needle aspiration biopsy of the prostate, radical prostatectomies and confirmation of the histopathology report. The control group was selected from healthy men who did not use any medications and had no familial background or acquired cancers, including cancer of the prostate. This study was performed by compliance with all measures and criteria following approval from the Ethics Committee. Written consent was obtained from each participant.

Results

The cancer patients were selected from an age

group of 43–70 years, with an average age of 61.34. Control group participants were selected from the same age range, with an average of 54.16 years. Among patients, 47 (59.49%) were in stage 1, 28 (35.44%) in stage II, 3 (3.79%) in stage III, and one person (1.26%) was stage IV adenocarcinoma of the prostate.

Polymerase chain reaction (PCR)

Approximately 5–7 ml of blood was collected from all participants, mixed with EDTA and kept at -20°C. We used the salting out method[25] to extract available DNA from the white blood cells. Their concentrations were determined by a spectrophotometer. Specific primers were used for amplifying the segment that contained the I1e462Val polymorphism. The following primer sequences were used for forward: 5'CTGTCTCCCTCTGGTTACAGGAAGC3′ and reverse: 5'-TTCCACCCGTTGCAGCAGGATAGCC3′.[26,27]

Water (10 µl) and a commercial master mix (10 µl; Master Mix Amplicon) of 2X were added to each 0.2 ml microtube. This Master Mix Amplicon contained MgCl2 (1.5 mM), buffer, Taq enzyme, dNTP, and other necessary materials for the test. We added 1 µl of each primer and 50 ng (1 µl) of genomic DNA from each participant to the mixture. After a rapid, short centrifugation period, the samples were placed in the Thermal cycler system. The system proliferated the 204 bp segment from the CYP1A1 gene through the program at 95°C for 7 min in one cycle; 95°C for 3 min, 64°C for 40 s, 72°C for 1 min for 37 cycles; and a final cycle of 72°C for 7 min. The proliferated products were electrophoresed on a 1.5% agarose gel associated with DNA Safe 6x along with a 100 bp size marker (Fermentase) at a fixed voltage of 100 v for 30-40 min, with subsequent observation of the bands under consideration.

Genotypic determination

The polymerase chain reaction (PCR) product with the 204 bp band was incubated with the restrictive enzyme of BsrDI for 16-18 h at a temperature of 37°C. The cut-off site of the enzyme will be complete if the proliferated segment of the CYP1A1 gene is in the polymorphism region that contains adenine, and it can cut the proliferated segment into 150 bp and 54 bp (genotype AA). However the segment does not cut if there is guanine instead of adenine (A→G), and a 204 bp segment (genotype GG) will appear on the gel. The heterozygote genotype (AG/GA) is identified by 204 bp, 150 bp and 54 bp bands. In order to evaluate the changes or lack of changes in the cut-off sites of the enzyme, the digested products along with loading buffer have been electrophoresed on a 2% agarose gel at a voltage of 100 v for 40 min (Figure 1).

Statistical analysis

The frequency of genotypes was determined by counting and calculating their percent in the control and cancer groups. Next, the frequency of alleles was obtained in both groups according to the Hardy–Weinberg equilibrium. Genotypes in the two groups were compared by the chi-square test and SPSS21 software. The summary of the results is listed in Table 1.

Discussion

Identifying the pathophysiology of prostate cancer and understanding its causal factors is difficult and troublesome. The evidence has indicated that both environmental and genetic factors are involved in producing prostate cancer.[28,29] The major risk factors for this cancer include smoking, diet, obesity, increased age, familial background and genetic factors. Cancer of the prostate is rare before the age of 45, but its

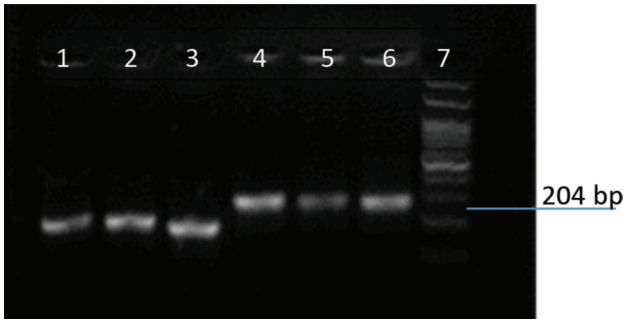

Figure 1. The results of the digestion samples where wells 1-3 represent samples of the AA genotype; wells 4-6 are samples of the GG genotype and well 7 is the 100 bp ladder.

Table 1. The *P*-value, odds ratio and 95% CI for the genotypes determined in the case and control groups.

Genotype	Prostate cancer	Control	*P*-value	Odds ratio	95% CI
AA	25 (31.65%)	44 (55.70%)	0.002	0.37	0.19-0.7
GG	47 (59.49%)	23 (29.11%)	0.000	3.58	1.85-6.94
AG/GA	7 (8.86%)	12 (15.19%)	0.164	0.54	0.2-1.45

prevalence increases in older men.[30] The men who have first-degree relatives with prostate cancer have a twofold risk of developing cancer compared to those with no familial history;[31] people with two first-degree relatives with cancer of the prostate have a five-fold risk.[32]

Estrogen is considered one of the hormonal risk factors for prostate cancer. The balance between androgens and estrogen changes with increased age, where the level of androgen in plasma decreases and the level of estrogen is relatively stable.[33] Two genes of the estrogen receptor (ER) encoding two proteins, ER β and ER α that have various functions, are found in the prostate. This shows that estrogen likely acts on the prostate directly. As the rate of ER expression in the healthy tissue of the prostate is lower than the tissue of breast,[34] its level clearly decreases in the tissue of the prostate with cancer. It reaches its lowest level in patients who are resistant to treatment.[35]

Prostate cancer strongly depends on the metabolism of hormones; therefore, it is reasonable to consider that the genetic variations in active genes can impact hormones and may affect the risk of developing cancer.

CYP1A1 is one of the genes involved in the metabolism of estrogen, which undertakes the first stage of oxidation of estrogen by hydroxylation of 2-OH.[17] Generally, CYP1A1 is expressed in the epithelial tissues, and it has a key role in the metabolism of endogenous and exogenous substrates of carcinogenic material. This enzyme is very active in forming reactive mediators, and it not only damages DNA, but also initiates or precipitates carcinogenesis.[13]

The most important polymorphisms of CYP1A1 includes M1 in which transposition of T to C occurs in nucleotide 3801 in the non-coding 3'region and M2 in which transposition of A to G occurs in nucleotide 2455 (to change isoleucine to valine in codon 462) or the same polymorphism as rs1048943 (462ile > val).[36-39]

In this study we evaluated polymorphism M2 or RS1048943 in men with prostate cancer. The results demonstrated a meaningful correlation between this polymorphism and cancer of the prostate in Iranian men. Our results agreed with other studies performed by Murata,40 Chang,41 Suzuki,[42] Aktas,[43] Yang,[44] Mittal,[45] and Li[46].

Conclusion

Our results indicated that the G allele with a frequency of 63.92% represented the highest prevalence in prostate cancer patients compared to the A allele with a frequency of 36.07%.

The proportion of calculated chance for genotype GG was 3.58, which showed the significant effect of this genotype in producing cancer. However, genotype AA with a proportion of chance of 0.37 indicated that this genotype did not affect production of prostate cancer.

This study has been undertaken for the first time in Iranian men diagnosed with cancer of the prostate. In the future, it is hoped that a variety of genetic polymorphisms can be determined which may have an etiologic role in prostate cancer.

Conflict of Interest

No conflict of interest is declared.

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