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ORIGINAL ARTICLE

Association between HOXB13 G84E Mutation and Risk of Prostate Cancer in Iranian Patients

Running Title: HOXB13 G84E Mutation and Risk of Prostate Cancer

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ABSTRACT

Introduction: Prostate cancer is the second most lethal cancer in men after lung cancer in world. Genetic predisposition is fast emerging as risk factor for prostate cancer. The HOXB13 gene is a tumor suppressor gene for prostate cancer and other cancers. The present study was conducted to evaluate G84E mutation of HOXB13 in patients with prostate cancer in Kurdistan and Kermanshah provinces of Iran. **Materials and Methods:** DNA was extracted from blood samples of cases of Benign Prostatic Hyperplasia and 75 healthy control. The G84E mutation of HOXB13 was determined by direct sequencing. All statistical calculations were performed using SPSS version17. **Results:** DNA sequencing results showed that the genotype of this site was GGA in all cases and controls. **Conclusion:** The results of this study showed that the GOXE position of the HOXB13 gene was not associated with prostate cancer in selected patients of west Iran. This finding showed doesn't a statistically significant difference in carrier frequency highlights the genetic predispositions to prostate cancer with respect to G84E mutation of HOXB13 gene.

INTRODUCTION

Prostate cancer is the second most common male malignancy worldwide (1). Investigating the relationship between carriers and high risk individuals with prostate cancer suggests that there are hereditary components for the disease (2-8). Research (GWAS) has identified more than 75 common alleles that are associated with an increased risk of prostate cancer (9-15). Epidemiological studies have shown that hereditary factors contribute to the incidence of this disease in 10% of cases, so that men with familial history of prostate cancer are more at risk than the general population and six to seven years earlier (16). Several studies have shown a family history of prostate cancer. The main reason for this is the inheritance of the involved genes. The first gene site known for prostate cancer is the location of a hereditary prostate cancer (Hpc1). After this discovery, several other genes are also known, although most of them are less important because of their low abundance (16,17).

In men, increased levels of androgen are associated with an increased risk of prostate cancer. The androgen receptor gene plays an important role in the incidence and progression of prostate cancer. Also, the H2D3BI and SRD5A2 genes, and cyp17, and AR and H2D3B2, have a special place in the metabolism of androgens and cell proliferation in the prostate. Some polymorphisms in these genes are associated with an increased risk of prostate cancer. The mutation in the androgen receptor is seen in almost all cases of prostate cancer, and the treatment options for this cancer have been shown to reduce or eliminate testosterone binding on the androgen receptor (17,18). The sequence of exons in 202 genes in the chromosomal region of the sequence of 17q22-22 was associated with a more susceptible prostate cancer, resulting in a new discovery in HOXB13 (19). The HOXB13 gene is a transcription factor, homobox involved in the development of normal prostate and the main factor in response to androgen. A single-nucleotide change in the G84E has been found in the HOXB13 gene, which is associated with a 3 to 5-fold increase in the risk of prostate cancer (20). A change in the single-nucleotide polymorphism of G to A in codon 84 from the HOXB13 gene has led to a change in the character of this gene as a tumor suppressor, according to previous studies (20-22).

The purpose of the current study is investigating the prev-

alence of this single-nucleotide change in a number of prostate cancer Kurdish patients in order to evaluate their role in the incidence of prostate cancer.

MATERIALS AND METHODS

Mutation screening

Subjects

In a cross-sectional study conducted from August 2015 to June 2017, enrolled 70 men with Benign Prostatic Hyperplasia (BPH) and 75 healthy control (ages ranged from 45 to 78, with an average age of 67 years). The control group was selected from men over 60 who had no prostate problem. They refer to surgical resection in the Department of Surgery, the Hospitals of Kurdistan and Kermanshah Medical University. The study protocol was approved by research committee and all participants signed a written consent form.

DNA extraction

Genomic DNA extraction from peripheral blood samples, were carried out using standard extraction protocols (Using a Promega DNA purification kit (catalogue no. LA1620). The purity of DNA was checked by calculating the ratio of OD at A260 to A280 and the integrity was assessed by gel electrophoresis on 0.8% agarose gel. DNA concentration (µg/ml) = A260 × 50 × dilution factor/1000.

Polymerase Chain Reaction (PCR)

The forward and reverse primers were designed using the ABI Primer Express 3.0 Software (Applied Biosystems, Foster City, CA, USA). The sequence of the primers used were 5'-CGAGCTGGGAGCGATTTA-3(HOXB13-forward) and 5'-AGCTCCAAGTCTCCCTCCTC-3' (HOXB13-reveres). The oligonucleotides of primers were synthesized by CinnaGen biotechnology company (Iran). Each 25 ul (microliters) PCR reaction contained ~30 ng of genomic DNA, 2 ul 10X buffer, 2.5 ul dNTPs, 2.5ul Mgcl2, 0.2 ul forward primer, 0.2 ul reverse primer, 0.2 ul Taq polymerase and 20 ul of deionized dH2O in 25 ul reaction mix. The PCR mixture was denatured at 93° C for 1 min (except the initial denaturing for 3min), annealed for 1 min at a temperature 51° C and extended at 72° C for1 min (except the extension for the final cycle for 2min) for 3o cycles. The presence of the PCR product was investigated by agarose-gel electrophoresis (1%). DNA marker were used for the detection of size of the amplified products. The amplified PCR products were purified using KBC pure kit (KBCco., Tehran, Iran) according to the manufacturer's instructions, and eluted in 70-140 ul ddH2O prior to sequencing.

Direct sequencing

The PCR products sequencing were performed with an ABI PRISM Dye Deoxy Terminator Cycle Sequencing Kit and an ABI 3100 Genetic Analyzer (Applied Biosystems Inc., Warrington, UK) using 5-100 ng of purified PCR products, 10.4 pmoles of forward or reverse primers, Ready Reaction Premix and 1× reaction buffer in a total volume of 20 µl. Cycle sequencing reactions were performed in a Primus 96 cycler (ABI, UK) at 96°C for 60 s ,96°C for 10 s, 50°C for 5 s, and 60°C for 4 min for 28 cycles. The prior capillary electrophoresis, unincorporated dye terminators were removed from the extension product by using KBC pure kit and DNA was precipitated by using ethanol precipitation. The purified extension products were denatured at 95°C for 5 min and placed on ice for 3 min. The sequencing was performed on ABI Genetic Analyzer 3130 and 3130xl machines (Applied Biosystems Inc., USA) by KBC Co. (KBC Co., Tehran, Iran).

We processed the collected data from the ABI detection system using the Sequencing Analyzer version 5.2 software. Sequence alignments for the polymorph position in the HOXB13 gene read were viewed in the Consed viewer and sequence variations was annotated and recorded. The negative controls were checked for the absence of analyzable sequence. Electropherograms were checked for the quality of sequencing reactions results were therefore checked against normal sequence in the Genbank (NCBI) using Gene Runner software.

STATISTICAL ANALYSIS

Genotype distribution and allele frequencies in different groups were compared by chi-square analysis. Pearson's correlation and linear regression analysis were carried out to determine the correlation between different parameters. A P value less than 0.05 was significant. All statistical calculations were performed using SPSS for windows, version 17(SPSS Inc., Chicago, IL, USA).

RESULTS

In this study, 70 blood samples from prostate cancer patients ages ranged from 45 to 78, with an average age of 67 years and 75 blood samples from control group (men over 60 years old with an average age of 71.20) were collected. The findings of polymorphism of GOXE locus of HOXB13 gene showed that the frequency of healthy controls and patients according to the status based on the results of DNA sequencing of patients and healthy samples, the P-value calculation showed that the difference in the frequency of healthy and healthy individuals for the polymorph position in the HOXB13 gene is not statistically significant at 5% level. The number of GGA genotypes in patients and healthy individuals is the same. That is, all patients and all healthy people showed the GGA genotype. PCR products for G84E mutation in HOXB13was shown in figure1.

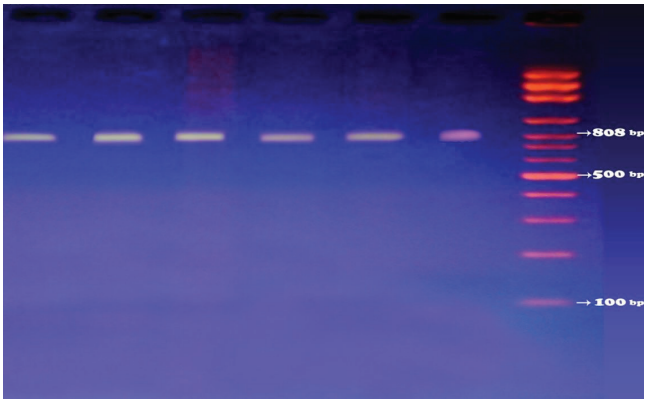

Fig 1. Electrophoresis of PCR products on agarose (2%).

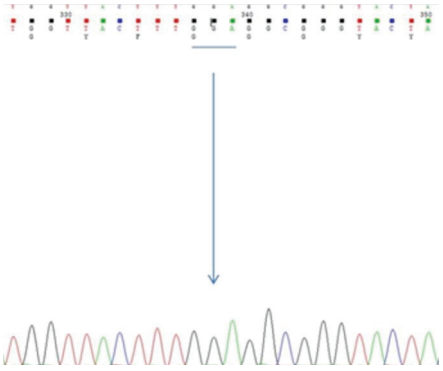

Fig 2. DNA sequencing was performed with forwarding primer.

(First row: normal sequence, Tier 2: Quality of Stacking, Third row: Sequence of samples for Stack sinks, Fourth row: sequence of amino acids)
The position of the polymorph in the nucleotide G of the GGA sequence, which has been identified on an offline basis. The amino acid 84 in the exon has a HOXB13 gene polymorphism (G84E).

DISCUSSION

The hereditary history of prostate cancer is a major contributor to this cancer. The history of prostate cancer in the near-first-degree family increases the likelihood of it. Hereditary factors are involved in a small percentage (10%) of cases of prostate cancer (22). Studies show that molecular changes in HOXB13 gene polymorphism that lead to creating a difference in main protein is associated with the development of prostate cancer (20). Studies have suggested that factors such as genetic polymorphism may be indicative of individual differences in the incidence of cancer. Molecular changes, including the HOXB13 gene polymorphism, are associated with the growth and development of prostate cancer. Polymorphism means the occurrence of different types of alleles in a gene that has different types. One of the forms of this change is codon, followed by a change in the protein. This change causes the difference in the main protein function (20 - 22). In the present study, the frequency of healthy and healthy subjects according to the status of polymorphism of the position of GOXE of HOXB13 gene based on DNA sequencing results of the patient and healthy samples was obtained by using the P-value test. The difference was found to be abundant. The patient and healthy subjects for this polymorph position in HOXB13 gene are not statistically significant at 5% level. The genotype of all subjects in the control and case group was the same as the GGA genotype. Prostate cancer is the second most common cancer after skin cancer, and the second most lethal cancer after lung cancer in men. One in six people suffers from this cancer (21). The past investigations had showed that prostate cancer seen the lowest level in Asian population alike Iranian (22). In several studies have been conducted on G84E polymorphism HOXB13 gene and in prostate cancer. For example, in a study in 2013, the association of G84E mutations and the risk of prostate cancer was studied. In this study, G84E mutation in affected patients was 42.2% (24). In another study in 2014, the G84E mutation was screened on healthy and ill patients in Sweden. The results showed that G84E mutation was more common in people with prostate cancer who had a familial family history and suffered from 35 to 55 years of age. So mutation in infected people was 4.6% and in healthy subjects was 1.3% (25). In another study in 2013, the association of the G84E mutation of the HOXB13 gene with the risk of prostate, breast, and colorectal cancer in Finland was investigated. In this study, the G84E mutation was significantly higher among prostate cancer patients with family history (4 / 8% versus 1%). In this study, there was no significant difference in G84E mutation in breast cancers and family colorectal (26). In another study in 2013, the mutation of the G84E of the HOXB13 gene and its association with the increased risk of prostate cancer were studied with respect to different geographical regions and race. The results showed that the G84E mutation in HOXB13 differed according to different geographic regions and races, so that in North Europe the highest percentage of mutation was 60.1% followed by Western Europe 60.0% and in North America, 0.31% G84E mutation among individuals Observed (27). Considering the result observed in the current study and comparing it with other studies, it seems that a greater percentage of patients need to be considered to achieve a risk percentage for this locus.

CONCLUSION

The results of this study showed that the GOXE position of the HOXB13 gene was not associated with prostate cancer in selected patients of west Iran. This finding showed doesn't a statistically significant difference in carrier frequency highlights the genetic predispositions to prostate cancer with respect to G84E mutation of HOXB13 gene.

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AUTHOR CONTRIBUTIONS

Fatemeh keshavarzi and Nader Rashahmadi designed the study; Fatemeh keshavarzi wrote the paper; Negin Vermzyar conceived the experiments, prepared the figures; collected the samples. All authors gave final approval for the manuscript to be submitted for publication.

CONFLICT OF INTERESTS

None

ETHICAL STANDARDS

The study was ethically approved by the Council Research Committee of Sanandaj Branch, Islamic Azad University Sanandaj, Iran.

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