Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 1604-1616
Received 25 April 2021; Accepted 08 May 2021.

Association Between HOXB13 Gene Genotypes and Prostate Cancer in Basrah, Iraq

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Abstract

Prostate cancer is the sixth most common cancer in the world, the second most common cancer in men in developed countries. Development of prostate cancer as any other cancer could be provoked by accumulation of mutations in various genes: oncogenes, tumor suppressor genes, DNA repair genes, and epigenetic alterations. The mammalian Hoxhomeobox genes are transcription factors that regulate axial regional specialization during embryonic development. The present study including 67 patients and 70healthy control, genomic DNA was extracted from all blood samples (patients and controls) and the HOXB13 gene was amplified using PCR technique and sequencing in order to detect the genetic variation. Three variation sites were detected in exon one of HOX B13 gene, A1098G (rs9900627), G951A (rs8556), C773T (rs199813155). As a result of changing the base A by G in (A1098G) rs9900627, three polymorphisms were detected, the statistical analysis found that AG polymorphism increases the risk of disease about one and a half fold (OR=1.4) while AA and GG were not significant.Our study detected ten genotypes in exon 1, AGC genotype was the wild type while the other nine were novel and recorded in DDBJ as a new genotype in the world RGC (LC483165.1), GGC (LC483166.1), RRC (LC483167.1), ARC (LC483168.1), AAY (LC483170.1), ARY (LC483172.1), AAC (LC483173.1), AGY (LC483171.1) and RGY (LC483169.2).

Keywords: HOXB13 gene; Genotypes; Prostate Cancer; Basrah.

Introduction

Prostate cancer is the sixth most common cancer in the world, the second most common cancer in men in developed Western countries, including European countries, the United States, Australia, New Zealand, and parts of Africa.Prostate cancer is strongly associated with the advanced age, the majority of the cases diagnosed at age more than 60 years, the mean age about

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(65-70) years (1, 2).Classical risk factors including family history, advanced age, genetic variation, race and environmental factors such as diet, type of work, cigarette smoking, physical activity as well as inflammation of the prostate gland (3, 4). In adult human, the prostate gland is small corn-shaped tissue divided into three distinct morphological regions: central zone, transitional zone, and peripheral zone. Prostate carcinoma arises from peripheral zone while Benign Prostatic Hyperplasia(BPH) occur mainly in transitional zone (5).Development of prostate cancer as any other cancer could be provoked by accumulation of mutations in various genes: oncogenes, tumorsuppressor genes, DNA repair genes, and epigenetic alterations. The mammalian Hoxhomeobox genes are a transcription factors that regulate axial regional specialization during embryonic development. TheHox genes are separates to four unlinked genetic clusters (a-d), each clustercontains 9 to 11 genes.HOXB13 gene located on chromosome 17q21 (6), consists of two exons and essential for prostate organogenesis, as well asoverexpressed in prostate cancer (7-9). The association between the HOXB13 gene and prostate cancer shown tobe caused by the c.215G>A, p. G84E variant (rs138213197) in the HOXB13 gene (8, 10, 11).

Material and methods

In the present study sixty-seven blood samples were collected from prostate cancer patients in Al-Sadder Teaching Hospital – Basrah Center for Oncology and Hematological Disease, their ages range between (45-90) years old. On the other hand, seventy blood samples of males without cancer were collected as a control group their ages range between (45-90) years old. Two ml of peripheral blood was drawn by sterilized syringe from the two groups in sterilized EDTA tubes. The genomic DNA was extracted by using genomic DNA Mini Kit (Geneaid, Taiwan) after that detected by 0.8% Agarose Gel Electrophoresis, containing Ethidium bromide, and viewed under UV transilluminator (300nm). HOXB13 gene amplification done by PCR technique,using a pair of primers, F- **5'-** CGAGCTGGGAGCGATTTA **-3'**and R- **5'-**

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AGCACCAAGCTCATCCTCAC **-3'.** The PCR program includes initial denaturation at 95ºC for five minutes, subsequently 30 cycles of denaturation at 95ºC for 30 second, annealing at 60ºC for 30 second and extension at 72ºC for 1 minute. The final extension was done at 72ºC for 5 minutes. Thirty-five µl of PCR products were transmitted to Macrogen Company "http://dana.macrogen.com" for sequencing. The sequencing results were analyzed and processed by using Basic Local Alignment Search Tool "BLAST" to search for the homologous sequence in the National Center for Biotechnology Information database(NCBI)http://www.blast.ncbi.nlm.nih.gov.Descriptive statistics were applied to describe patients and controls characteristic according to use percentage. ORs and 95%CLs were calculated by using the SPSS program. OR were considered significant if OR ≥1.5.

Result

The extracted genomic DNA was electrophoresis on 0.8% agarose gel as shown in fig.1.

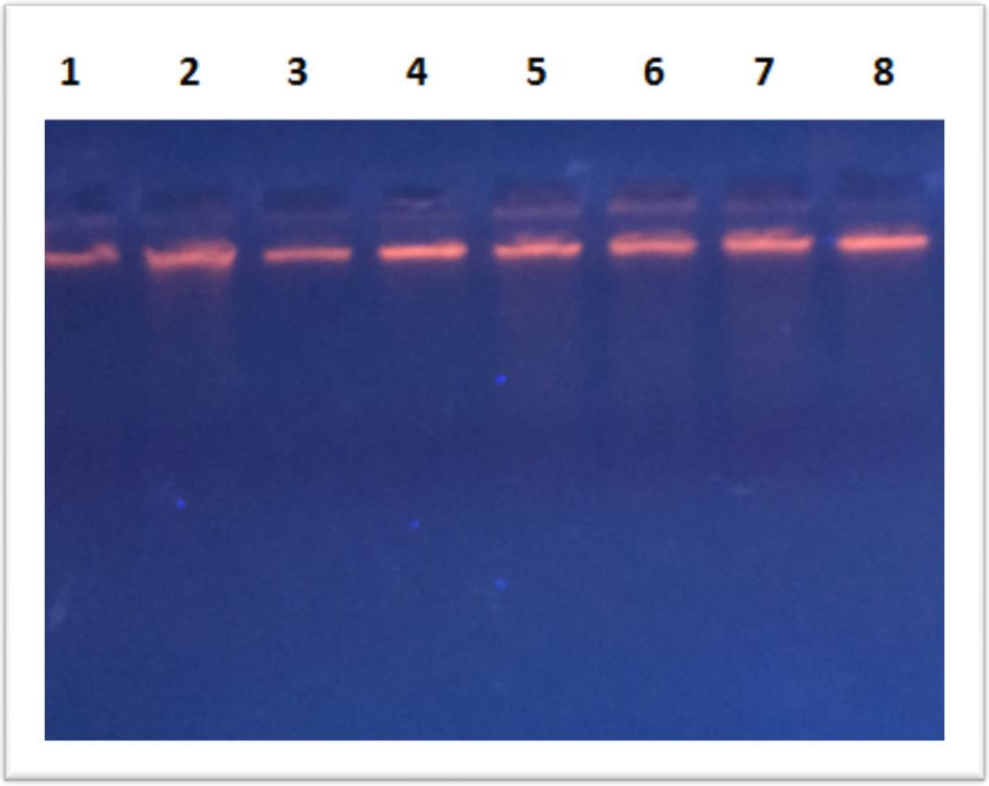

Figure 1. Total genomic DNA on agarose gel electrophoresis (0.8%, 60Vfor 30 minutes). (1-8) patients and control groups.

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The PCR products of HOXB13 gene were detected by using 2% agarose gel electrophoresis as shown in (fig.2), the product size is 900 bps.

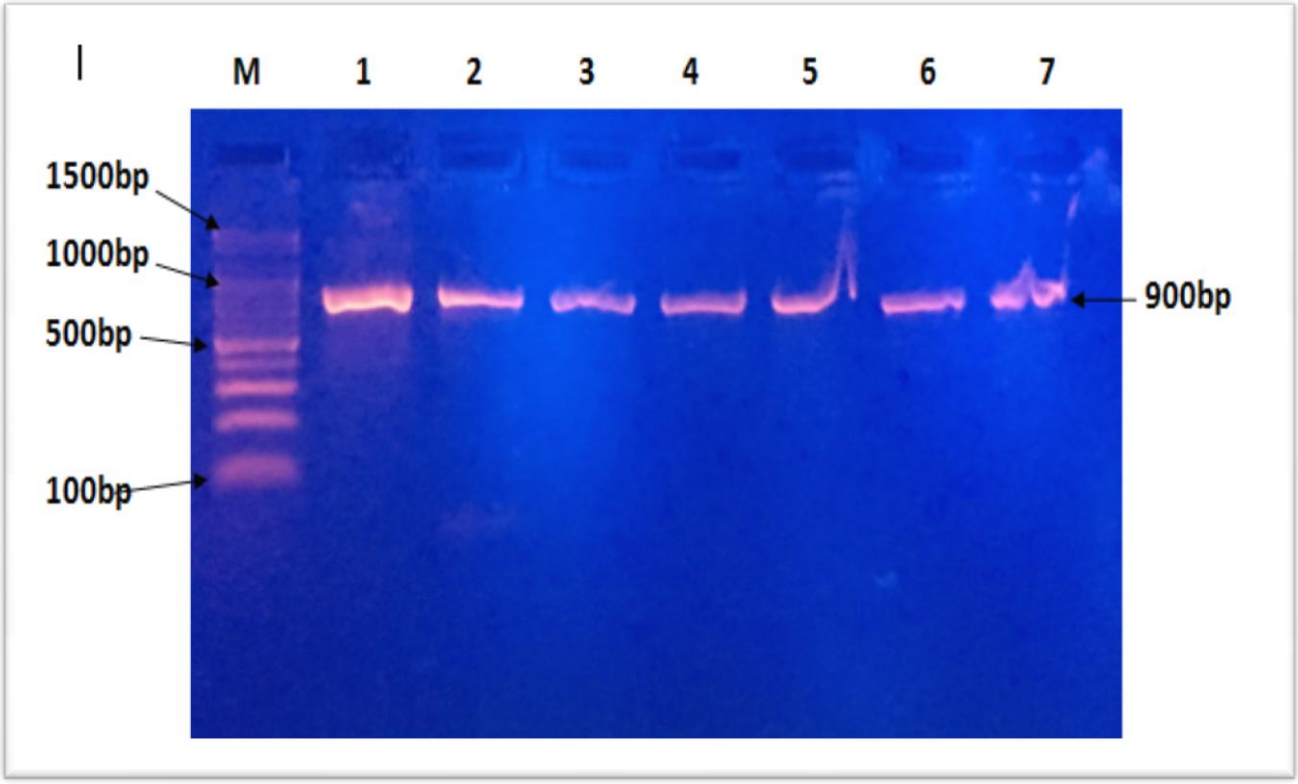

Figure 2: Agarose gel electrophoresis (2%, 60Vfor 90 minutes) for PCR Products of HOXB13 gene (900 bps).

The result found that AG polymorphism of Rs9900627 SNPs was slightly increase the risk of prostate cancer (OR=1.4) while other polymorphisms were not significant as shown in table 1.

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Table (1) Distribution of polymorphisms among patients and control groups

	Polymorphism		Controls	Patients	OR	95% CI
1	Rs9900627 c.A1098G	AA	44 (63%)	38 (57%)	1.0	--------
		AG	23 (33%)	28 (42%)	1.4	0.699-2.849
		GG	3 (4%)	1 (1%)	0.38	0.03-3.867
2	Rs8556 c.G951A	GG	44 (63%)	44 (63%)	1.0	---------
		AG	24 (34%)	21 (31%)	0.875	0.426-1.787
		AA	2 (3%)	2 (3%)	1.0	0.135-7.419
3	Rs199813155 c.C773T	CC	68 (97%)	65 (97%)	1.0	----------
		CT	2 (3%)	2 (3%)	1.046	0.143-7.647

OR= Odds ratio, 95%CI=95% confidence interval

Three SNPs have been detected in 67 Patients samples (figure 3 a, b and c).

The study subjects were classified into nine genotypes as in (figure 4).

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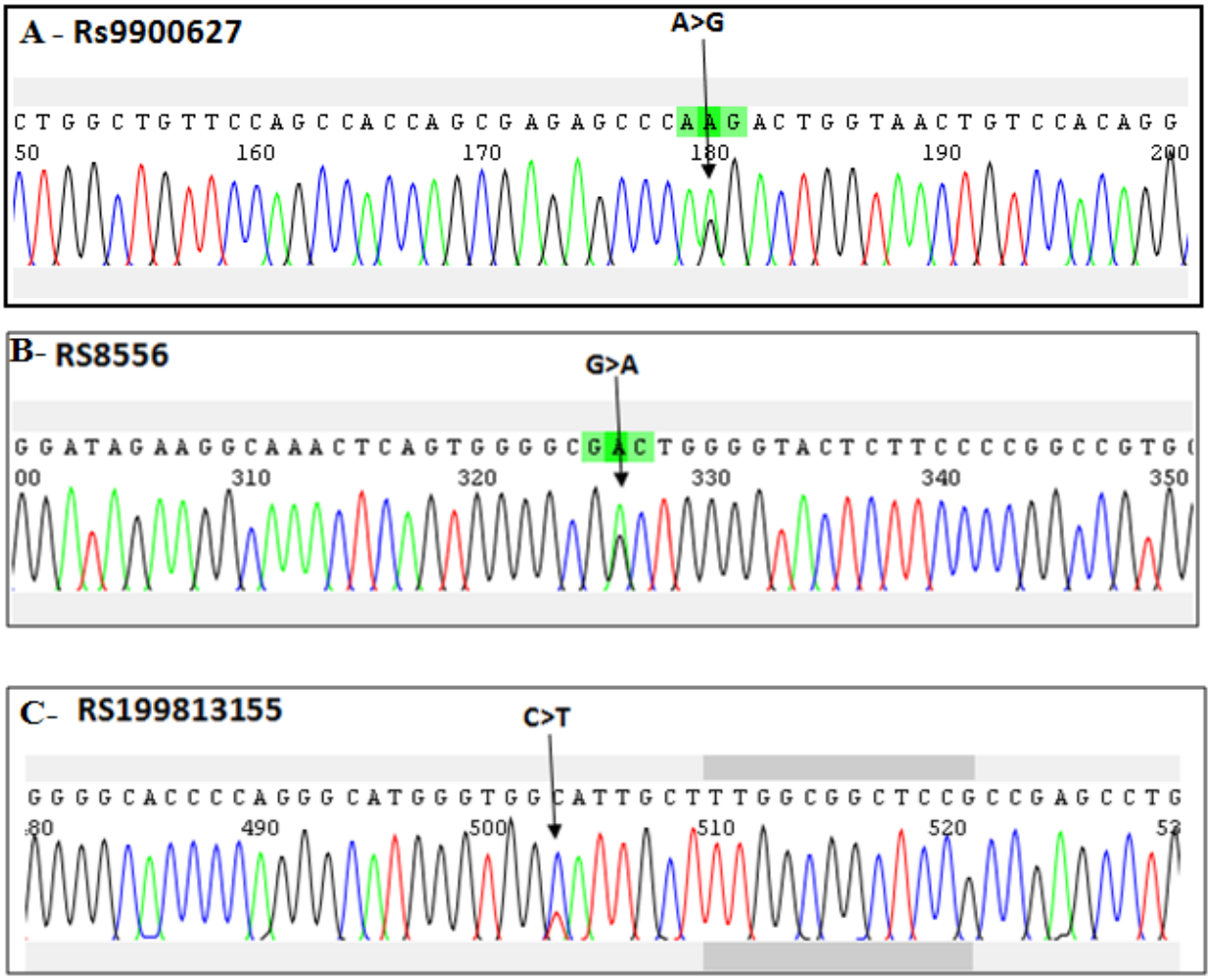

Figure (3) A-Sequences of detected polymorphisms, Synonymous variant rs 9900627(A>G), position in cDNA 1098. B- Sequences of detected polymorphisms, Synonymous variant rs 8556 (G>A), position in cDNA 951. C- Sequences of detected polymorphisms, missense variant rs199813155 (C>T), was change amino acid C/Y, it is position in cDNA 773

Twenty-one from patients were carrying the wild type of HOXB13 gene against (29) from the control group shown in table (2). On the other hand, (10) patients were carrying two SNPs against (12) from control group while patients who carrying one SNPs were (37), control (29). Patients with one SNPs were significant to develop prostate cancer about two-fold (OR=1.76, 95%CI=0.838-3.703, comparing with wild type.

Table (2) Distribution of SNPs between Patients and Controls individuals

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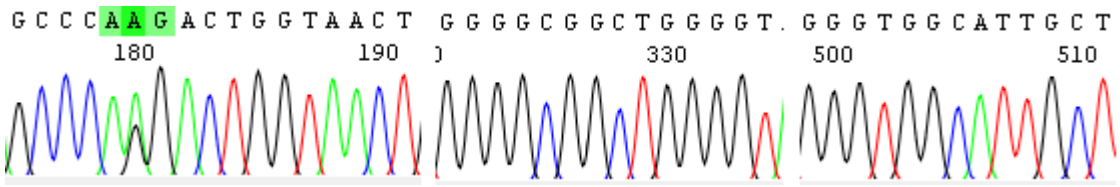
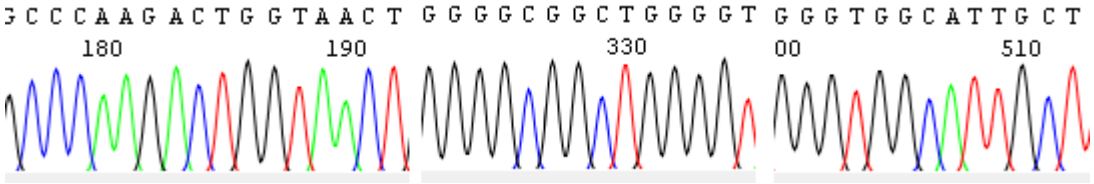
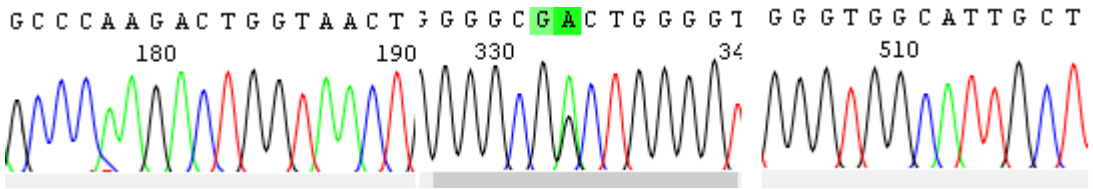
SNPs	control	patient	OR	95%CI
wild	29	21	1	---------
Three SNPs	0	0	--------	---------
Two SNPs	12	10	1.15	0.419-3.159
One SNPs	29	37	1.76	0.838-3.703

OR= Odds ratio, 95%CI=95% confidence interval

We retested 10 genotypes in DDJB and NCBI, AGC was wiled genotype and nine were novel genotypes (RGC, ARC, RRC, GGC, AAY, AAC, ARY, AGY, and RGY) as shown in figure (4).

Genotype sequence	Genotype & Accession number
	RGC (LC483165.1)
	AGC (LC483174.1)
	ARC (LC483168.1)
	RRC (LC483167.1)

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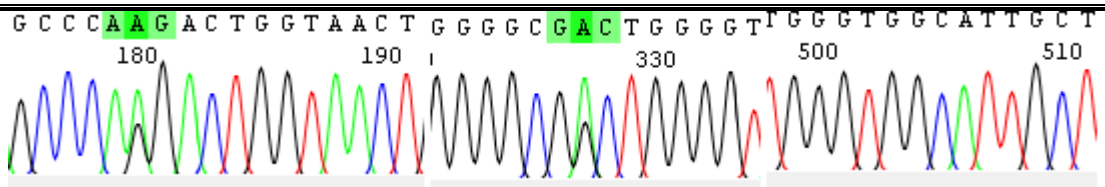
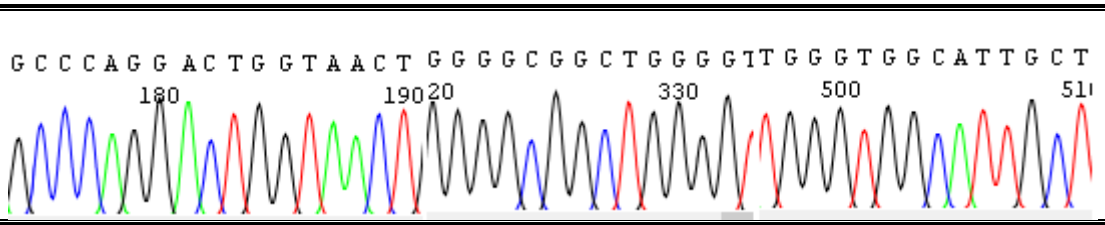
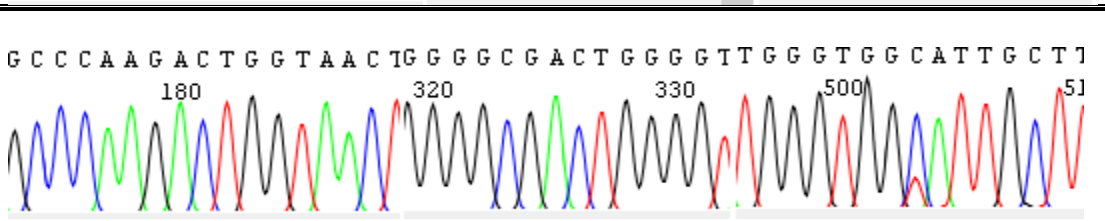
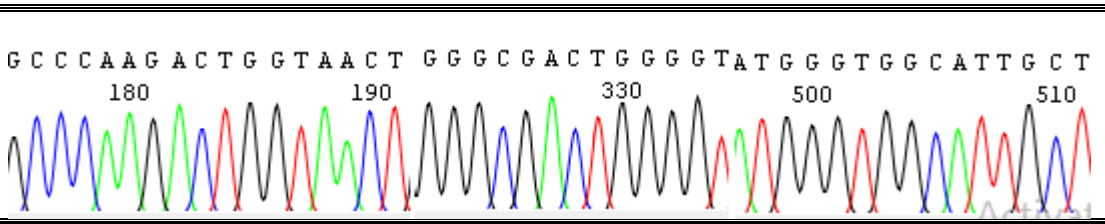
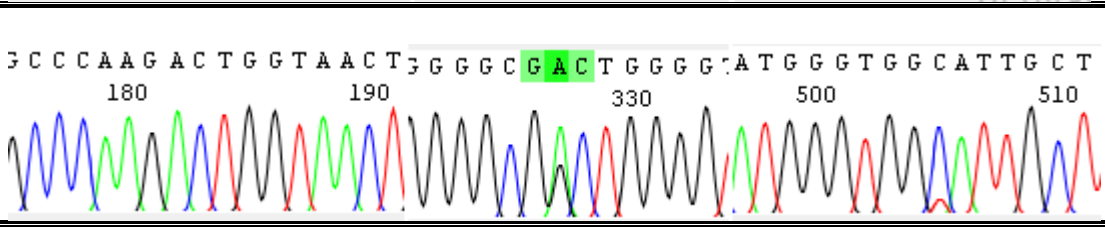
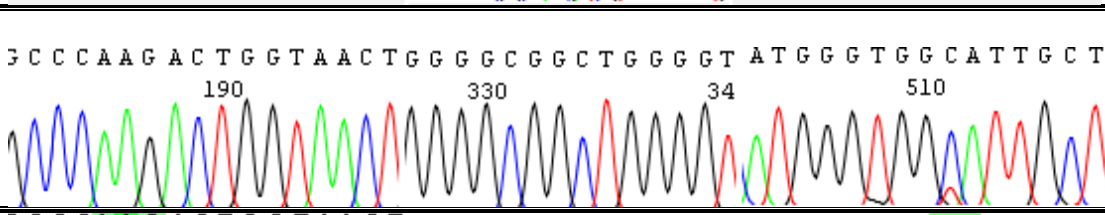
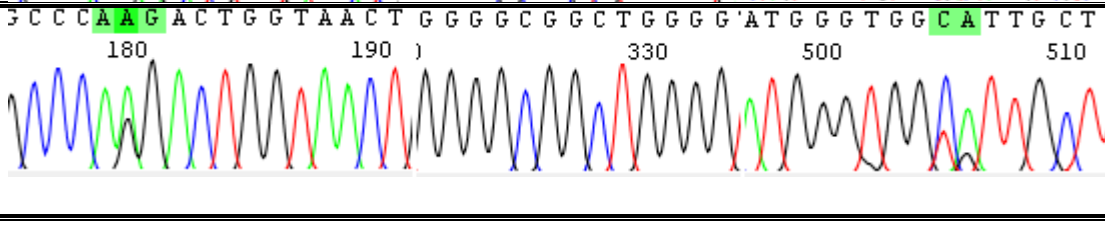
	
	GGC (LC483166.1)
	AAY (LC483170.1)
	AAC (LC483173.1)
	ARY (LC438172.1)
	AGY (LC483171.1)
	RGY (LC483169.2)

Figure 4 Sequences of HOXB13 novel genotype which submitted to(DDBJ)

R= **A or G, Y = C or T**

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Table (4) explained the distribution of genotype among patients and control groups. The most frequent genotypes were AGC in both patients and control groups which is the wild genotype according to NCBI.

The RGC genotype was significantly higher in patient then ARC genotype

Table 4 Distribution of HOXB13 genotypes among patients and control group

	genotype	**Control**	**patient**	**O.R.**	**95/CI**
1	AGC	29 (41.4%)	21 (31.3%)	1.0	----------
2	RGC	10 (14.3%)	21 (31.3%)	3.0	1.141-7.89
3	GGC	3 (4.2%)	1 (1.5%)	0.428	0.042-4.422
4	RRC	10 (14.3%)	6 (8.9%)	0.857	0.263-2.789
5	ARC	14 (20%)	14 (20.9%)	1.384	0.538-3.566
6	AAY	0	1 (1.5%)	---------	----------
7	AGY	1 (1.4%)	1 (1.5%)	1.285	0.076-21.784
8	ARY	0	1 (1.5%)	---------	----------
9	AAC	2 (3%)	1 (1.5%)	0.642	0.055-7.579
10	RGY	1 (1.4%)	0	---------	----------

OR= Odds ratio, 95%CI=95% confidence interval

Discussion

Homeobox B13(HOXB13) gene belong to evolutionary conserved HOXB gene cluster located on chromosome 17q21 coding for transcriptional factor (TF) and together with androgen receptorplay a crucial role in development and maintenance of prostate gland, mutations in this gene causing the formation of tumor and prostate cancer (12-14).

In our study, exon 1 from the HOXB13 gene was screened in the 17q21-22 region by sequencing gremlin DNA from 67 patients with prostate cancer and 70 persons as the control group, to characterize the frequency of the identified mutations.Three SNPs diagnosed, rs9900627 synonymous variant, c.A1098G detected in 26 patients and 29 of the control group. Another mutation was rs8556 synonymous variant, c.G951A detected in 26 patients and 23 control group while rs199813155 missense variant, c.C199813155T detected in 2 patients and 2 control.
HOXB13 has several forms called polymorphisms as shown in table (1), polymorphisms occurred due to rs9900627 changing the site of A to G (AA, AG, GG). AG polymorphism slightly associated with increased the risk of prostate cancer about one and half fold (OR=1.4, 95%CI=0.699-2.849,P value=0.337) while GG were not showing a significant association.
The rs8556 cause (GG, AG, AA) according to changing the base of G by A, GG polymorphism as well as AG, AA were not significant for causing prostate cancer. Two polymorphism CC, CT of rs199813155 occurred due to replace the base of C for T, these polymorphisms also were not significant for prostate cancer. The benefits of the variants of the gene are increased HOXB13 copies (15).Table (3) explained the distribution of these ten genotype among patients and control groups, the wild type AGC was the most frequent genotype in both patients 21(31.3%) and control 29(41.4%), AGC was the wild type while the other nine genotypes were novel and recorded for the first time in DDBJ and NCBI as anew genotype (RGC, GGC, RRC, ARC, AAY, AGY, ARY, AAC, RGY).The prostate cancer risk was increased to threefold in individuals having the RGC genotype (OR=3, 95%CI=1,141-7.89, P value=0.0236).
In normal prostate, the highly expressed HOXB13 transcription factor plays a key role in the development of the prostate. Notably, HOXB13 has been shown to interact with the androgen receptor (AR), A protein essential for prostate development and necessary for the growth of all stages of prostate cancer. Norris demonstrated that HOXB13 acts as both a repressor and coactivator of AR target genes; in target genes with an androgen-response element (ARE) the HOXB13: AR complex inhibits transcription, but in genes with a HOX element, the complex enhances
transcription.HOXB13 was reported to act as a growth promoter and growth suppressor inmodels of prostate cancer, depending on factors such as tumor androgen sensitivity status and cellular localization of the protein. The G84E variant results in an increase or loss of gene function or increases the risk of prostate cancer through other mechanisms (12, 16).Table (2) shows the

distribution of SNPs among patients and control groups.We found that some patients and control have more than one SNPs, the statistical analysis indicate that prostate cancer has increased about two-fold in individuals having one SNP (OR=1.7, 95%CI=0.838-3.703). The result has not found any patient with three SNPs.These results may be attributed to the interaction between environmental and genetic factors as common caused for prostate cancer (17-21). Our study agreed with (8, 17) in these studies screened many genes in the 17q21-22 region by sequencing gremlin DNA from 94 unrelated patients with prostate cancer from families chosen to link to the applicant region. The members of the family were tested, additional case subjects and control subjects to characterize the frequency of the identified mutations. Probands from four families were discovered to have a rare but recurrent mutation (G84E) in HOXB13 (rs138213197), The novel HOXB13 G84E variant is associated with a significantly increased risk of hereditary prostate cancer (8).Many studies agreed with our result such as (6, 21-23) about the relationship between the mutations in this gene with prostate cancer disease.The study of (24) indicates that the variants in axon 2 of HOXB13 may influence the risk of prostate cancer. Also, evaluation of HOXB13 mutation may be considered as a novel marker for screening prostate cancer. by screening 51 samples, including 21 blood and tissue of prostate cancer cases, and compared to 30 cases affected by BPH using PCR/sequencing. Then, the existence of potential association was investigated between genomic DNA alterations in blood and tissue prostate cancer specimens.Given the recent evidence that the G84E mutation confers a more aggressive clinical and pathological (25, 26), evaluation of the HOXB13 could be of a significant prognostic outcome.

Conflict of Interest

We clarify that there is no conflict of Interest between authors

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