# GENETIC VARIABILITY IN THE PRNCR1 GENE AMONG A SAMPLE OF IRAQI PROSTATE CANCER PATIENTS

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**ABSTRACT**
The *PRNCR1* gene variant among sample of Prostate Cancer (PCa) Iraqi patients case-control study was used, with PCa patients (n=32) and healthy individual control (n=33). It was successfully identified two SNPs in prostate cancer-associated noncoding RNA-1 (*PRNCR-1*) gene by using PCR technique and direct sequencing . The first SNP is rs13252298 (A>G) have three genotypes (AA,AG,GG )and the second SNP is rs5013678 (T> C) have three genotypes TT,TC,CC),these genotype frequencies were not in agreement with Hardy-Weinberg Equilibrium (HWE) in patients and controls .It was observed that AG genotype  was more frequent in patients than controls(68.7% *vs.* 36.4%) with significant difference $p<0.01$   ,and the estimated OR of such a difference was 3.85 , this value was positive  associated with PCa patients that mean this genotype AG was risk factor for PCa patients, it was also noticed that *G*  allele in PCa patients compared with controls(40.6 *vs.*33.3%)   show not significant difference $p>0.05$ . It was revealed  that *G*  allele have  OR=1.16 that mean this allele have positive risk factor for PCa patients .  In conclusion  AG genotype for SNP rs13252298  was associated with PCa patients.
**Key words: cancer, pca patients, case control study, risk factor, PCR technique**

**التغاير الوراثي لجين *PRNCR1* لدى  عينة من مرضى عراقيين مصابين بسرطان البروستات**

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**المستخلص**

المتغير الجيني *PRNCR1* بين عينة من مرضى سرطان البروستات العراقيين تم استخدام دراسة الحالة مع مرضى سرطان البروستات وعددهم (32) وعينات ناس اصحاء عددهم (33) تم التعرف بنجاح على اثنين من SNPS في جين (*PRNCR1*) باستخدام تقنية PCR الطفرة الاولى rs13252298 (A>G) وله ثلاثة انماط وراثية (AA, AG, GG) والطفرة الثانية هو rs5013678 (T>C) وله ثلاث انماط وراثية أيضا (TT, CC, TC) لم تكن ترددات النمط الجيني هذهِ متوافقة مع معادلة هاردي–واينبيرغ في المرضى والاصحاء. لوحظ ان النمط الجيني AG كان اكثر ترددا في المرضى من مجموعة الاصحاء (68.7 % مقابل 36.4%) مع وجود فرق معنوي 0.01 > P، وكان معدل الارجحية التقديري لهذا الاختلاف 3.85 وكانت هذه القيمة موجبة الارتباط بمرضى سرطان البروستات والتي تعني ان هذا النمط الجيني AG كان عامل خطر لمرضى سرطان البروستات، وقد لوحظ أيضا ان allele G في مرضى سرطان البروستات مقارنة مع الكونترول 40.6 %مقابل 33.3% ولا تظهر فرقاً كبيراً 0.05 > P. تم الكشف عن ان OR=1.16 للاليل G مما يعني ان هذا الاليل له عامل خطورة ايجابي لمرضى سرطان البروستات. وفي الختام ارتبط النمط الجيني AG للطفرة rs13252298 بمرضى سرطان البروستات.

الكلمات المفتاحية: السرطان، سرطان البروستات ،دراسة الحالة , تفاعل البوليمر المتسلسل، عامل خطورة

## INTRODUCTION

Cancer is a molecularly diverse disease that is one of the leading causes of death around the world. It's difficult to comprehend this disease because there are so many different forms of tumors with different histopathology, genetic and epigenetic variations, and clinical implications. Chemotherapeutics' modes of action and the creation of new therapies (7).Prostate cancer is one of the most common type of cancer in male(15). Prostate cancer is a major extremely diagnosed malignancy and the second leading cause of cancer-related mortality in men in the United States (17) The PCa rate of death in black men its twice and in the latter stages of the PCa are much more likely to be diagnosed relative to white men (18). The PCa differs among populations, which show that genetic back-ground perform a significant role in susceptibility to PCa (10). The PCa risk has been found that related to single-nucleotide polymorphisms (SNPs) (2,8,11,15). From a human genome about one percentage is made up of protein-coding sequences, while approximately 4‑9% of a human genome's sequences are transcribed as noncoding RNAs (ncRNAs) (5). The ncRNAs have been discovered to control the number of biological processes, such as cell cycle, translation gene expression, splicing, and transcription (1,3,6,13,14), The PCa-associated noncoding RNA-1 (*PRNCR-1*) have been shown to be upregulated in PCa and to play a role in development of PCa by modulating androgen receptors (AR). So, the aim of this study was to find out if there were association between the SNP rs13252298 for *PRNCR1* gene polymorphisms and a risk of PCa patients in a sample of Iraqi population.

## MATERIALS AND METHODS:

Thirty two PCa patients were enrolled in the case control study during the period from 7-22 November 2019, these patients suffering from prostate cancer were taken from Iraqi populations with ages range from 51 to 95 years and BMI 19.7-36.8 were recruited at the Middle Euphrates cancer center in AL-Najaf both laboratory data and clinical of every patient were gathered from their clinical records which included ages , thirty three healthy Iraqi population samples were collected as a control group, whose ages ranged between 60 to 81 ,and with Body mass index 16.9-37.86.

**Sample Collection**: From each participating subject, by disposable syringe was collected blood from venous (5 ml). Dispensed the blood into an EDTA tube, and frozen at -20 C before DNA was extracted for *PRNCR-1* gene SNP genotyping.

**DNA extraction**: From blood sample the genomic DNA was isolated for thirty-two PCa patients and thirty-three healthy subjects as control group according to the protocol of kit (ReliaPrep™ Blood gDNA Miniprep System, Promega).

**Primers and optimization of PCR reaction**: Regions of *PRNCR1* gene were selected and amplified by PCR using primers (designed by the program PRIMER3) . PCR reaction system contained 12.5 µM of Master Mix, 1 µM each of oligonucleotide primers, 7.5µM Nuclease Free Water, 3µl DNA template. Thermocycling conditions of primers *PRNCR1* for SNP rs13252298-(F5`-ACT AAA CAT TGA TTG GCC ATC TCC ACT G-3`` and R5`- TGA ACT TTC CTA GCA CAC ACT TTT GCA C-3`, and primers for SNP were as follows: an initial denaturation at 95°C for 5min, then by 30cycles of denaturation at 95°C for 30second , annealing at 60°C for 30second, and extension at 72°C for 0.5min, final extension 72°C for 7min.

**Direct Sequencing analysis**: PCR products were directly sequenced by Macrogen Corporation of Korea, and the results were analyzed using a genious program. according to the protocol of manufacturer's. The samples were after that run on the ABI3730XL genetic analyzer (TF). The sequence is compared with the cDNA sequence of the human genome and gene.

**Statistical analysis**

The allele frequencies of the *PRNCR-1* gene was determined using the direct gene counting technique, and major deviation from HWE was measured for two alleles using the H-W calculator, which is free to use online at http://www.had2know.com/academics/hardy-weinberg-equilibrium-calculator-3-alleles.html. HWE is an expected genotype frequency if mating is nonassortative and no mutations from one allele to another. The respective population frequencies are p and q

when there are two alleles for a particular gene; A and B, then p², 2pq and q² is an expected frequencies of a genotypes AA, AB and BB respectively. Pearson's Chi-square test used to determine if there were significant differences between observed and expected frequencies.

The distributions of *PRNCR1* alleles and genotypes were presented as percentage frequencies, and significant differences between their distributions in PCa patients and controls were measured using two-tailed Fisher's exact chance (P). Furthermore, a preventive fraction (PF), etiological fraction (EF), and odd ratio (OR) were calculated to determine an association between *PRNCR-1* alleles and genotypes and disease. The range of OR value from less than 1 (association is negative) to more than 1 (association is positive). The EF was calculated if an association of OR was positive, while a PF was given if an association of OR was negative. The WINPEPI computer program for epidemiologists were used to calculated these estimates. The most recent WINPEPI package version (which includes a programs and their manuals) is available online free at http://www.brixtonhealth.com.

## RESULTS AND DISCUSSION
**Genotypes and allele frequencies of *PRNCR-1* polymorphisms.**
The PCR products for patients and controls group were show in Figure 1 and asequence of rs13252298 SNP of *PRNCR*1 gene shows in Figure 2.

Our study included 32 patients of PCa with the mean age±SD of 69.63±1.919years with BMI 28.08±0.799, and 33 as control group with the mean age±SD of 68.40±0.955years with BMI 26.17±0.17. Non-significant difference has founded between the two groups in terms of age and BMI 0.472, 0.094 respectively. The genotypes and allele frequencies of *PRNCR-1*

polymorphisms in PCa patients and controls are shown in table 1. As regards the rs13252298 A>G was presented with three genotypes (AA,AG,GG) that were corresponding to two alleles (*A* and *G*). It was observed that AG genotype was more frequent in patients than controls(68.7% *vs.* 36.4%), this genotype AG were not in agreement with HWE in PCa patients as there was a significant difference between observed and expected genotype frequency (p<0.01) , and also these genotypes AG has effect on PCa susceptibility since there was significant difference (p<0.01)in genotype between patients and control (Table 1).,and the estimated of such a difference was (OR =3.85 , PF =0.31 , 0.84-5.99 C.I 95%), this value was positive associated with PCa patients that mean this genotype was risk factor for PCa patients, it was also noticed that *G* allele in PCa patients compared with controls(40.6 *vs.*33.3%) show non-significant difference p>0.05 . It was revealed that *G* allele have (OR=1.44;PF=0.05; 0.71-2.95 C.I 95%) that mean this allele have positive risk factor for PCa patients, but it was non-significant difference( P=0.363) . while *A* allele showed a raise frequency (59.3% *vs.* 66.7%), in patients and control, but both differences failed to attend a significant level (*p*>0.05). The AA genotype frequency also observed to have a decreased frequency in PCa patients compared to controls (25.0 *vs.* 48.5%), but again the difference was non-significant. (Table 1) Was show that the AG genotype significantly a raise the risk of PCa patients (OR=5.16, 95% CI: 3.16‑ 8.41, *p*<0.0001) this was support our finding, receptor of Androgen, a member of the nuclear receptor family, is a ligand‑ activated transcription factor (9). It has been suggested lncRNA *PRNCR-1* promotes prostate carcinogenesis via activating AR (4).
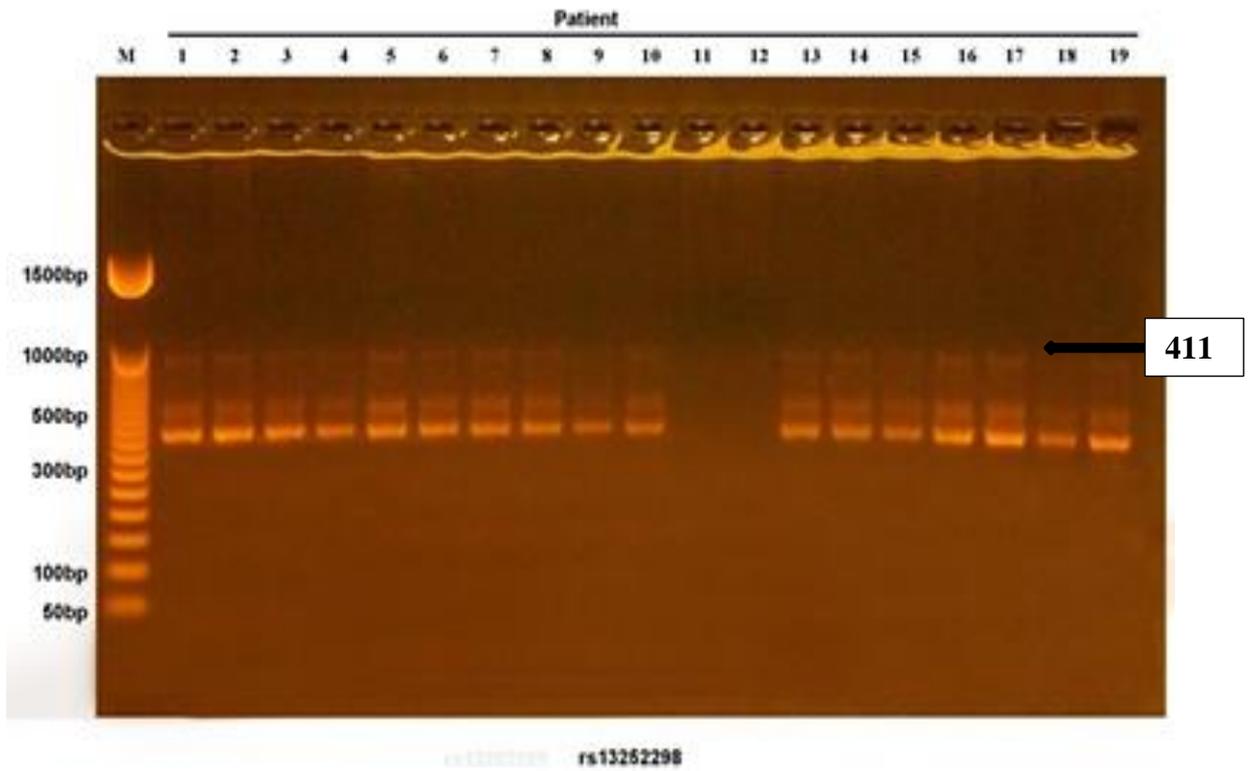
**(A)**
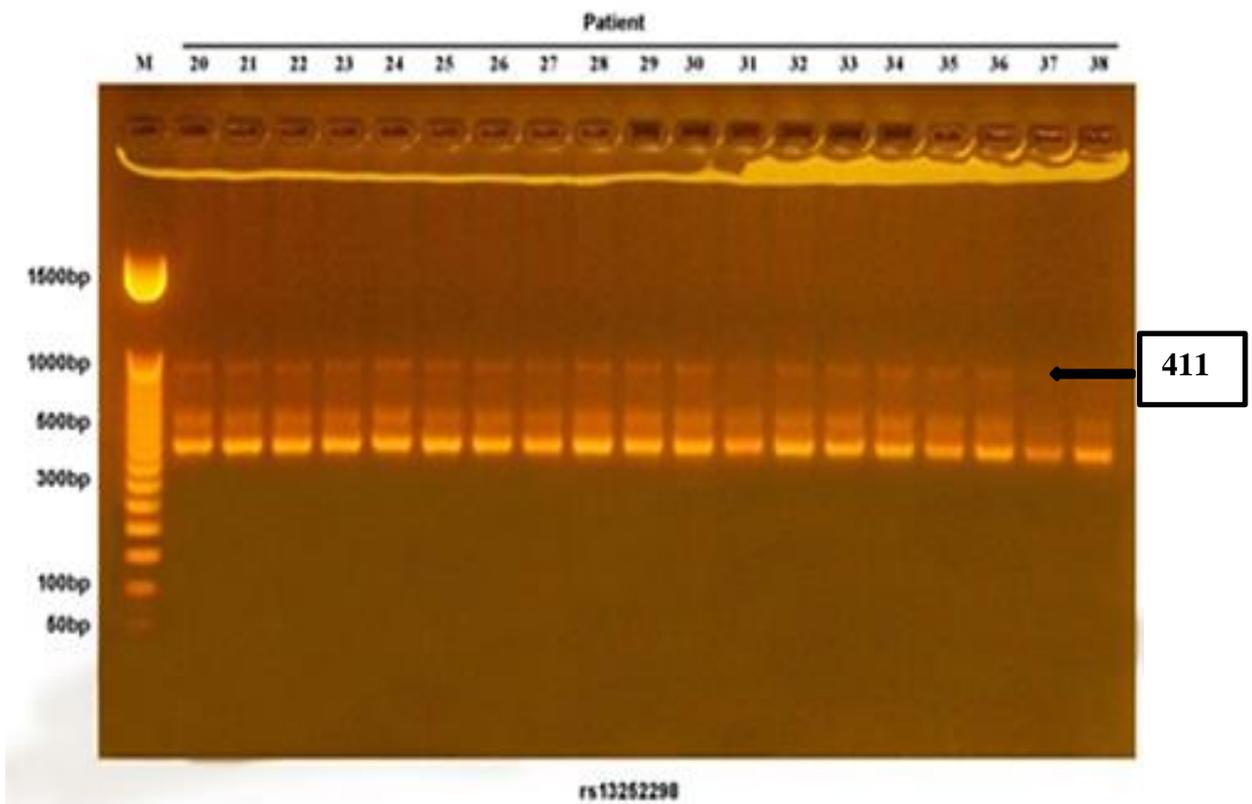


**(B)**
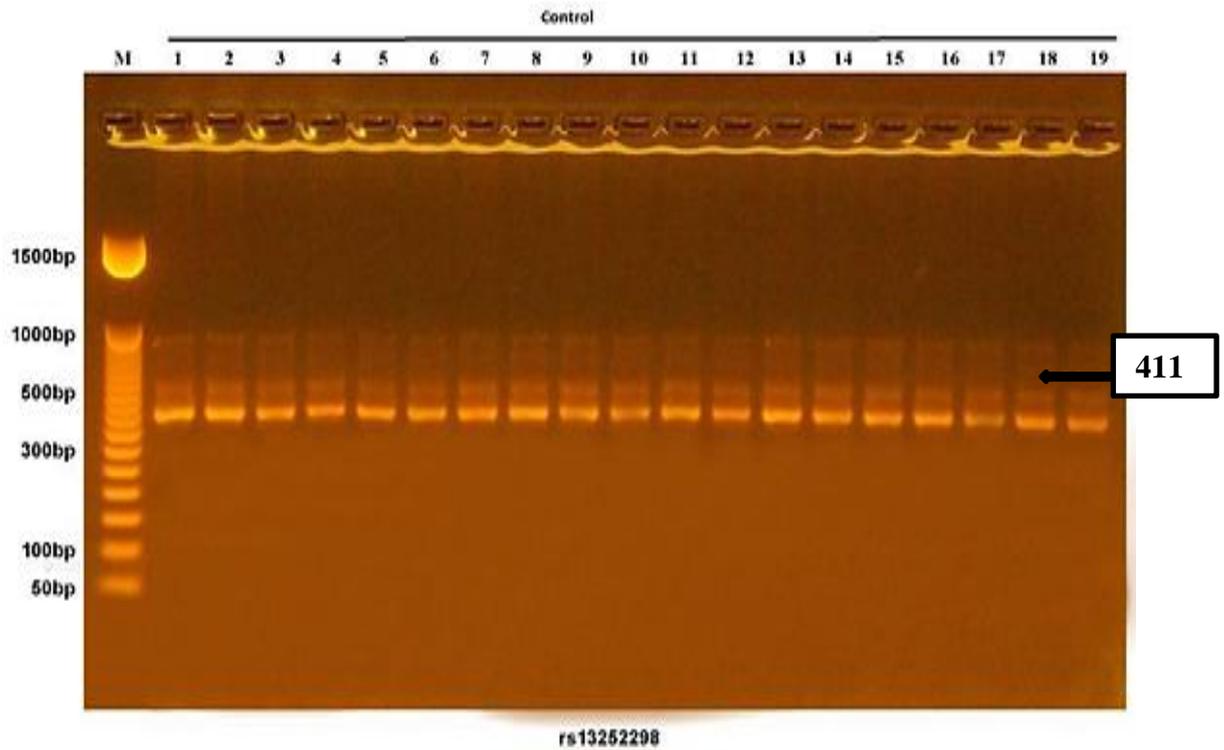
**(C)**
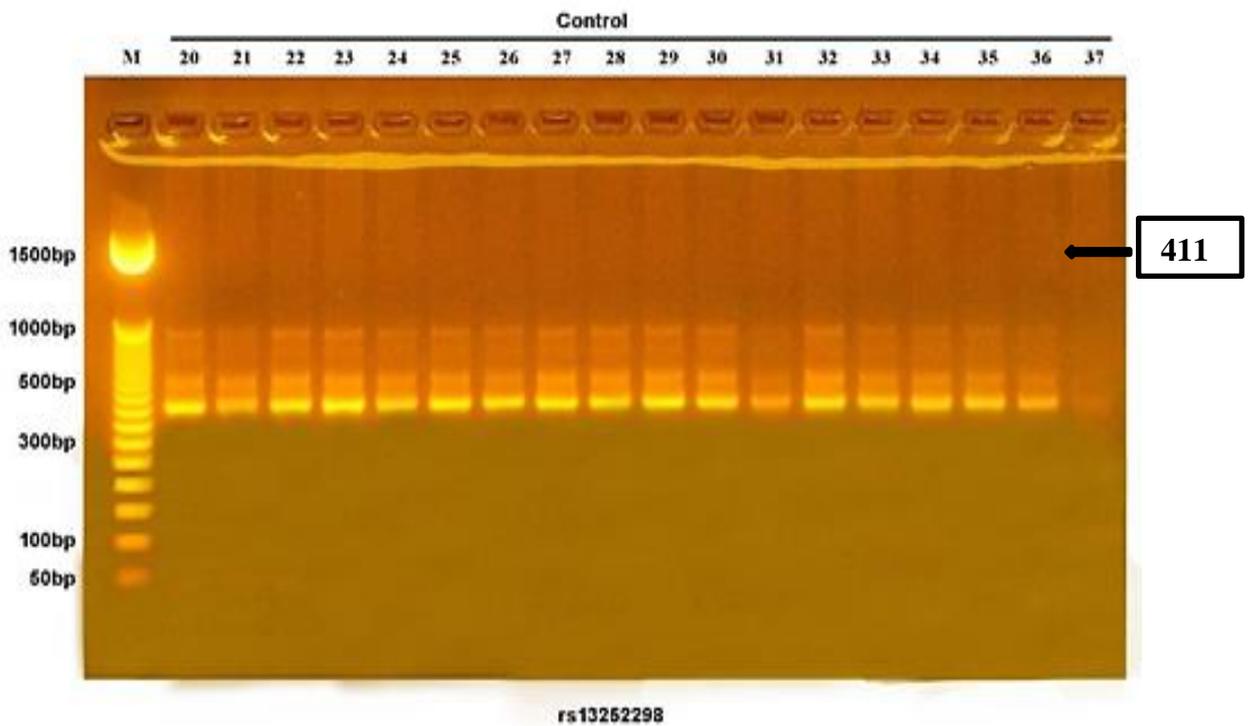


**(D)**

**Figure 1.(A,B,C,D) Results of the amplification of rs13252298 primer of Human samples were loaded on 2% agarose gel electrophoresis stained with Ethidium bromide. M: 100 bp ladder marker. Lanes 1-25 resemble 411bp PCR products for patients and controls group.**
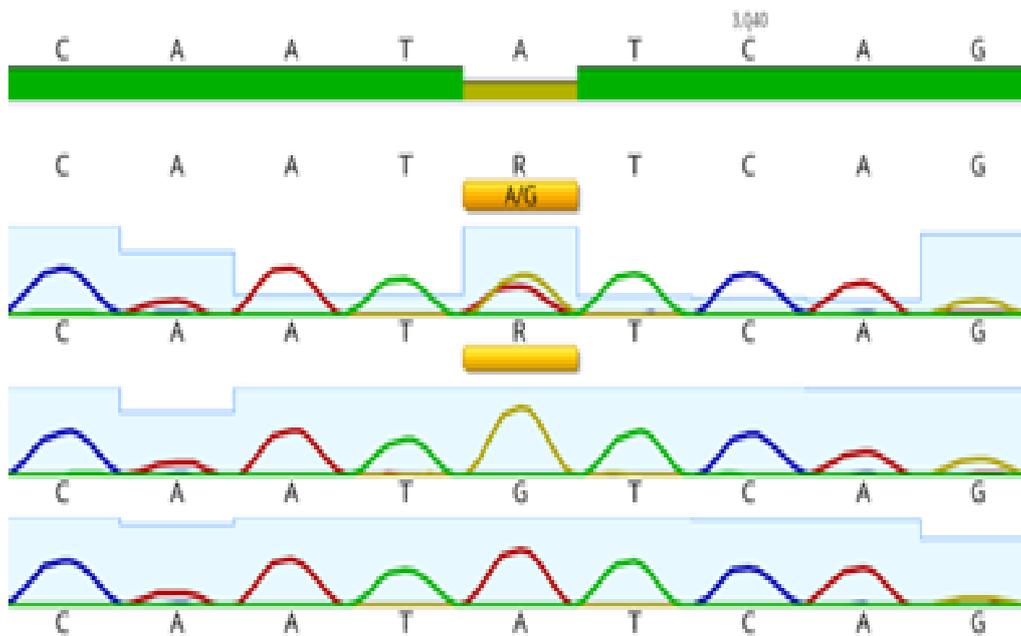
**Figure 2. Analysis of rs13252298 SNP of *PRNCR1* gene using Sanger sequencing. Single "A" peak indicative of the A homozygous allele. Single "G" peak indicative of the G homozygous allele**

**Table 1**. **Observed and expected genotype and allele frequencies for rs13252298 SNP in PCa patients and controls**

| Groups | | | | rs13252298 | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | | | | Genotypes | | HWE | Alleles | |
| | | | AA | AG | GG | $p \leq$ | A | G |
| Patients (No. = 32) | Observed | No. | 8 | 22 | 2 | 0.01 | 38 | 26 |
| | | % | 25 | 68.7 | 16.2 | | 59.3 | 40.6 |
| | Expected | No. | 11.2 | 15.4 | 5.2 | | Not estimated | |
| | | % | 35.4 | 48.4 | 16.3 | | | |
| Controls (No. = 33) | Observed | No. | 16 | 12 | 5 | N.S. | 44 | 22 |
| | | % | 48.5 | 36.4 | 15.1 | | 66.7 | 33.3 |
| | Expected | No. | 14.7 | 14.6 | 3.7 | | Not estimated | |
| | | % | 44.5 | 44.3 | 11.2 | | | |
| Odd Ratio (OR) | | | 0.35 | 3.85 | 0.37 | | 0.69 | 1.44 |
| Etiological Fraction(EF) | | | 0.25 | - | - | | 0.09 | - |
| Preventive Fraction(PF) | | | - | 0.31 | 0.10 | | - | 0.05 |
| Fisher's Exact Probability | | | 0.07 | 0.01. | 0.427 | | 0.363 | 0.363 |
| 95% Confidence interval(C.I.) | | | 0.18-1.31 | 0.84-5.99 | 0.20-3.22 | | 0.42-1.79 | 0.56-2.40 |

**HWE: Hardy-Weinberg Equilibrium; N.S.: Not significant**

**CONCLUSION.**
In conclusion, AG genotype for SNP rs13252298 was associated with prostate cancer patients positively association which that mean this genotype AG was risk factor for PCa patients in Iraqi population.

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