# RECURRENT OF *TMPRSS2* GENETIC POLYMORPHISM AND ITS ROLE IN IRAQI PATIENTS WITH PROSTATE CANCER

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**ABSTRACT**

**The role of transmembrane protease serine 2(TMPRSS2) in prostate carcinogenesis relies on overexpression of ETS transcription factors. The aim of this article was to investigate the association of TMPRSS2 polymorphism (rs12329760 (C\T)) with prostate cancer (PCa) in sample of Iraqi patients. One hundred and two individuals were involved in this study for the period from February – 2019 to February – 2020. The sample type was formalin fixed paraffin embedded tissue samples (FFPE), which involved fifty-six samples of pre-diagnosed patients with prostate cancer, aged between 48 and 86 years, and forty-six samples were found to be controls (healthy group) dependent on Prostate Gland integrity, which is the same age as in a group of patients. Analysis of Hardy Weinberg equilibrium (HWE) exposed that the genotypes were compatible with equilibrium in both PCa and control samples with no significant differences among frequencies of (observed and expected) in which ($p > 0.05$). Surveying for the relation of (rs 12329760) with both allele and genotype frequencies appeared that there were no major differences between patients and controls frequencies, although there were decreasing and increasing in the percentage of C allele (67 vs 72.8 %) and T allele (33 vs 27.2 %) respectively.**

**Keywords: genotype, transmembrane protease serine 2, SNP.**



# تكرار تعدد الأشكال الوراثي لـ *TMPRSS2* ودوره في المرضى العراقيين المصابين بسرطان البروستات

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**المستخلص**

**يعتمد دور البروتياز الغشائي، سيرين 2 في تسرطن البروستات ، على الإفراط في التعبير في عوامل النسخ ETS . كان الغرض من هذه الدراسة هو التحقق من العلاقة بين تعدد اشكال النيوكلوتيدة المفردة (C \ T) TMPRSS2 rs12329760 وسرطان البروستات في عينة من المرضى العراقيين. شارك في هذه الدراسة مائة واثنتان من الأفراد للفترة من شباط – 2019 إلى شباط – 2020. وكان نوع العينة (النسيج المطمور في شمع البارافيين) وتضمنت الدراسه ستة وخمسون عينة من مرضى تم تشخيصهم مسبقًا بسرطان البروستات تتراوح أعمارهم مابين 48 و 86 عامًا. اضافة الى 46 عينة أخرى لتكون مجموعة سيطرة (مجموعة صحية مظهريا) ذات نفس المدى العمري لمجموعة المرضى. أظهر تحليل (هاردي واينيبيرغ) أن الأنماط الجينية كانت متوافقة مع التوازن في كل من عينات المرضى وا فراد السيطرة مع عدم وجود فروق ذات دلالة إحصائية بين الترددات المرصودة والمتوقعة ($p> 0.05$). وعند التحقق من وجود العلاقة مع كل من تكرار الأليلات و الانماط الجينية لتعدد اشكال النيوكلوتيدة المفردة في كل من المرضى والاصحاء, تبين أنه لا توجد فروق كبيرة ذات دلالات معنوية، على الرغم من وجود تناقص وزيادة في النسبة المئوية للأليل C (67 مقابل 72.8٪) وأليل T ( 33 مقابل 27.2٪) على التوالي.**

**كلمات مفتاحية:, الطراز الوراثي, البروتياز الغشائي سيرين 2, تعدد اشكال النيوكلوتيدة المفردة.**

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## INTRODUCTION

The prostate is an exocrine glandular shaped organ caudally in the bladder around the bladder's spine. It is part of the male system which relies on the stimulation of androgen, natural differentiation, development and physiological activities (16). Prostate cancer is one of the world's most common and leading causes of cancer-related cause of death for men, mainly in developing nations (13,19). Prostate cancer disease incidences have risen significantly in developing and Asian countries including Iraq. Rendering to the data published by World health organization (WHO) in 2017, PCa mortality in Iraq reached 0.25%. Moreover, the death rate is 7.02 per 100,000 of the population; which makes Iraq ranked no. 152 (18). The 2008 Iraqi Cancer Board recorded that prostate cancer is one of the top 10 cancers affecting men in Iraq, where new cases registered in 2008 were 246 by 3,73 and an average infected incidence of 1,56 per 100,000 population were registered either in the capital Baghdad, with 66 new cases in the same year (10). (PCa) emerges from a glandular component and is, with the case of not melanoma, the most diagnosed male malignant in the western world (6).

Environmental and hereditary considerations influence the occurrence and progression of prostate cancer. Sex, diet, family heritage and hormone status include risk factors for prostate cancer (20). Variants of certain genes have been linked with the increased occurrence of prostate cancer including (TMPRSS2, ATM, BRCA1-2, ATM, NBN) (2). TMPRSS2 (Transmembrane Serine Protease 2) is a protein encoding gene that has been described, mainly in fusion with ERG, which belongs to a family of transcription factor-specific ETS encodings for a protein named ERG (3). This merger happens in 90% of all prostate cancer ETS fusions (11). In 60% of instances, fusion arises since the sequence between the genes is lost (3 Mb) (15). The other cause of fusions is induced by more complicated types of translocation (5). In TMPRSS2, a single nucleotide polymorphism (SNP) was found to be positive for TMPRSS2 rs12329760 C>T (Met160Val) translocation fusion and several copies of gene fusion [6]. The level of this SNP is significantly different from race and ethnicity (17). The aim of the present study was to determine the association of TMPRSS2 SNP (C\T) rs12329760 with prostate cancer in Iraqi population.

## MATERIALS AND METHODS

### Sample size

One hundred and two individuals were involved in this study for the period from February – 2019 to February – 2020. The sample type was FFPE (formalin – fixed embedded tissue) from the government as well as private facilities and laboratories at Baghdad, Basra and Kufa/ Iraq. Fifty-six samples of pre-diagnosed patients with prostate cancer, aged between 48 and 86 years. The other 46 samples were found to be controls (healthy group) dependent on Prostate Gland integrity, which is at the same age as in the group of patients.

### DNA Extraction and Quantification

Extraction of DNA was done by cutting about (8) sections each about (5 mm) using QIAamp DNA FFPE Tissue Kit. For such samples, its DNA is mostly fragmented due to storing in the chemical materials that may disrupt the DNA integrity. According to that we measured the DNA concentration using Qubit fluorometer from Thermo Fisher (Applied Biosystem).

### Polymerase chain reaction

Conventional PCR was accomplished to get the suitable product for DNA sequencing to amplify the (205bp) segment of TMPRSS2 gene rs12329760. The primers as shown in Table 1, were dissolved in a Nuclease-free water to reach a concentration of 100 (pmol/µl) and remained as stock in (deep freeze). For work of PCR the primers then diluted to (10 pmol/µl) by dilution of (10 µl) of stock with (90 µl) of free nuclease water. The component of PCR reaction is shows in Table 2, and the optimization of primer annealing temperature was (52 °C) by using the free tool of (new England biolab) as shown in Table 3. Our result was improved using Gel-electrophoresis (2% agarose gel) by adding 4.0 g of agarose to 200 ml of 1x TBE (Tris/Borate/EDTA) buffer and using safe stain (Figure 1) (1).

**Table 1. Primer designed to amplify 205 bp**

| Gene name | SNP name | Sequence 5´ ⟶3´ | Tm (ºC) |
|---|---|---|---|
| *TMPRSS2* | Met160Val | F 5´ TCATGGATAATCCTCCCTCT 3´ | 52 |
| | rs 12329760 | R 5´ GTGTTTCTGCTGTCTGTTAC 3´ | |

**Table 2. PCR components**

| Component | Reaction size |
|---|---|
| DNA template | 5 µl |
| Primers | 1.5 µl for each |
| Master mix | 12.5 µl |
| Nuclease-free water | 4.5 µl |

**Table 3. PCR Amplification Program of Gene (repeated for 36 cycles)**

| Name of gene | Initial denaturation | Denaturation | Annealing | Extension | Final extension |
|---|---|---|---|---|---|
| TMPRSS2 Rs 12329760 | 95 C 5 min | 95 C 30 sec | 52 C 30 sec | 72 C 30 sec | 72 C 5min |

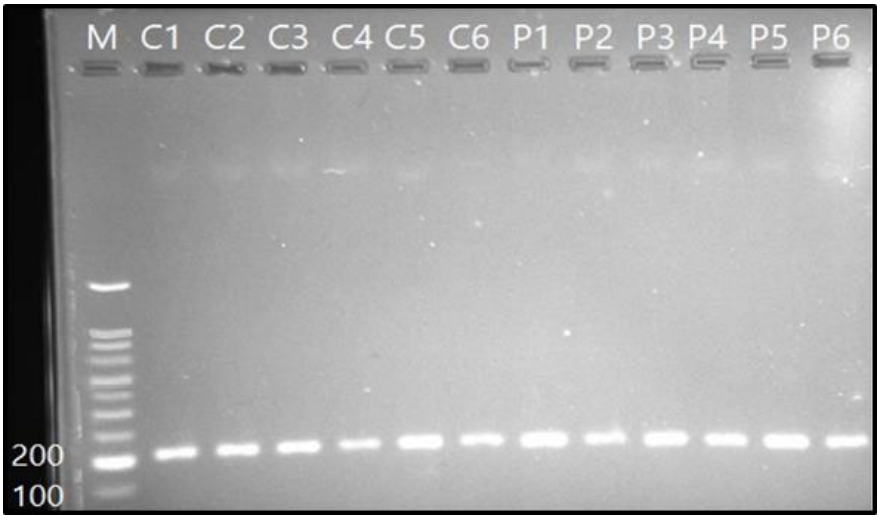


**Figure 1. PCR product of TMPRSS2 rs12329760 and the band was 205 bp while DNA ladder (Lane M) was 1000 bp (100 bp for each), Lane (C1 to C6) were samples of controls and Lane (P1 to P6) were samples of Prostate cancer patients**

**Genotyping**

Genotyping was carried out for TMPRSS2 rs12329760 by straight sequencing (Sanger method) using the product amplified according to the above primers. Sequencing was achieved unidirectionally by (ABI3730XL) (Macrogen-Corporation-Korea).

**Statistical analysis**

The frequencies of genotypes were checked for their approval with (HWE) Hardy-Weinberg equilibrium and a considerable difference between the frequencies of genotype in both (observed and expected) which was estimated by (Pearson's Chi-square test) from (https://wpcalc.com/en/equilibrium-hardy-weinberg/. SPSS software version 26 was used to show the association of TMPRSS2 (rs 12329760) and (PCa) in term of (odds ratio) and a significant difference was measured by Fisher exact test (9,14).

**RESULTS AND DISCUSSION**

The SNP of TMPRSS2 rs12329760 (C\T; chromosome 21; region 41480570) genotypes were (CC, CT and TT) with two alleles (C and T) (figure.2). Analysis of (HWE) exposed that the genotypes were compatible with equilibrium in both PCa and control samples with non-significant differences between both frequencies (Table 4).

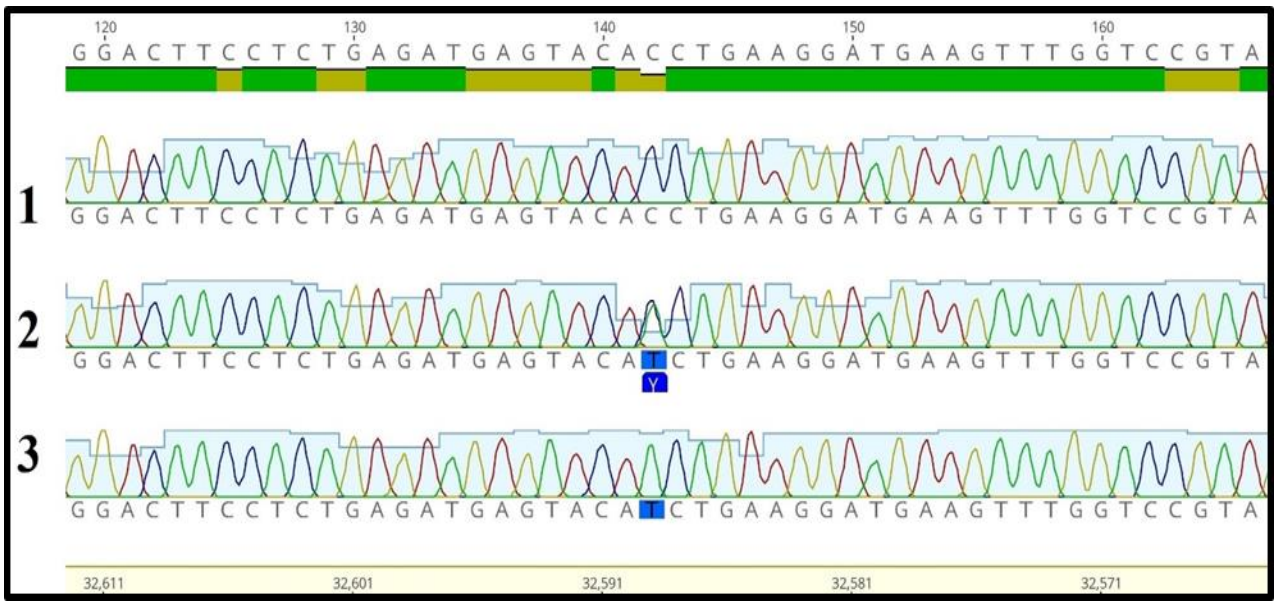


**Figure 2. DNA sequence chromatogram of TMPRSS2 SNP (C\T: rs12329760) showing three genotypes: CC (sample 1), CT (sample 2; Y) and TT (sample 3).**

**Table 4. Hardy-Weinberg equilibrium of (observed and expected) genotypes PCa patients and controls group**

| Genotype | Prostate cancer patients (No. = 56) | | | | Controls (No. = 46) | | | |
|---|---|---|---|---|---|---|---|---|
| | Observed | | Expected | | Observed | | Expected | |
| | No. | % | No. | % | No. | % | No. | % |
| CC | 28 | 50 | 25.1 | 44.8 | 26 | 56.5 | 24.4 | 53 |
| CT | 19 | 34 | 24.8 | 44.2 | 15 | 32.6 | 18.2 | 39.6 |
| TT | 9 | 16 | 6.1 | 11 | 5 | 10.9 | 3.4 | 7.4 |
| HWE analysis | $X^2 = 1.588$; D.F. = 1; $p > 0.05$ | | | | $X^2 = 0.7923$; D.F. = 1; $p > 0.05$ | | | |

Surveying for the relation of (rs 12329760) with both allele and genotype frequencies appeared that there were no major differences between patients and controls frequencies, although there were decreasing and increasing in the percentage of C allele (67 vs 72.8 %) and T allele (33 vs 27.2 %) respectively (Table 5).

**Table 5. Association of genotypes and alleles of TMPRSS2 (rs 12329760) with PCa patients and controls group**

| Genotype Or Allele | Patients No.= 56 | | Controls No. = 46 | | OD | CI | *P* |
|---|---|---|---|---|---|---|---|
| | NO. | % | NO. | % | | | |
| CC | 28 | 50 | 26 | 56.5 | 0.77 | 0.35 - 1.67 | 0.554 |
| CT | 19 | 34 | 15 | 32.6 | 1.06 | 0.47 - 2.41 | 1 |
| TT | 9 | 16 | 5 | 10.9 | 1.57 | 0.49 - 5.00 | 0.567 |
| C | 75 | 67 | 67 | 72.8 | 0.76 | 0.41 - 1.38 | 0.445 |
| T | 37 | 33 | 25 | 27.2 | 1.32 | 0.72 - 2.41 | 0.445 |

**OD: odds ratio, CI: 95% confidence interval, P: probability**

TMPRSS2 (rs12329760) (C\T) were explored by other studies in prostate cancer patients, but conflicted investigations were found. Bhanushali et al. reported the strong association between (T) allele and increased peril in an Indian population (4). While Fitzgerald et al. adduced no correlation between TMPRSS2 SNP and PCa patients in an American population (7). Moreover, García-Perdomo et al. did not find any remarkable relation of mentioned SNP with PCa patients and considered the most not frequent polymorphism in Southwestern-Colombian Population (8). Maekawa et al. showed that such polymorphism was significantly associated with the occurrence of sporadic PCa, but not with latent in Japanese men (12). The reason for the discrepancy between the results of these studies was due to the difference in ethnicity and race. In Conclusion Our results have been found no association between TMPRSS2 SNP rs12329760 (C\T) with increased risk of prostate cancer patients.

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