ORIGINAL ARTICLE

Detection of BRCA2 Genes Polymorphism among Prostate Cancer Patients in Sudan 2020: A Case-Control Study

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Abstract

Background: Prostate cancer (PCa) has been associated with BRCA2 mutations; however, reported risk estimates differ greatly, mainly because of variations in retrospective study designs. Determining a consistent level of risk associated with BRCA2 mutations in PCa is difficult due to variabilities in population selection, study size, and genetic risk assessment techniques.

Objective: To evaluate the contribution of BRCA2 gene variability to prostatic cancer susceptibility in patients recruited to the National Cancer Institute in Sudan.

Methods: Prostate cancer patients were the subject of this case-control study conducted between 2020 and 2023 at the National Cancer Institute (NCI), University of Gezira, Sudan. The study included 81 confirmed cases of prostate cancer (whose diagnoses were confirmed by histopathological examination) as the case group and 81 healthy individuals as the control group. Samples of blood were taken. DNA was extracted using the Intron, G-spin total DNA extraction kit. Allele-specific primers for PCR were used (ASP). SPSS (version 21) was used for data analysis.

Results: Among patients with prostate cancer from Sudan, BRCA2 rs4942486 was frequently found (62%), whereas BRCA2 rs144848 was less common (38%). In dominant genetic models, the BRCA2 rs144848 polymorphism is significantly correlated with both family history (OR=0.2, 95% CI: 0.04-0.96; p-value=0.022) and cancer metastases (OR=0.57, 95% CI: 0.23-1.40; p-value=0.022). Additionally, patients with a Gleason score of greater than 7 showed a higher frequency of mutations than patients with a score of less than 7. This implies that mutations might be linked to prostate cancer in its more advanced stages.

Conclusion: In summary, there is insufficient evidence to establish a correlation between Sudanese prostate cancer patients and the BRCA2 rs144848 and rs4942486 polymorphisms. Nonetheless, rs144848, and rs4942486, two SNPs in BRCA2, displayed some correlations with metastases and Gleason score.

Keywords: *BRCA2, Gene polymorphisms, rs144848, rs4942486, Sudan, Prostate cancer.*

INTRODUCTION

Worldwide prostate cancer is a major public health concern. It is the fifth most common cause of cancer-related deaths among men globally and the second most common type of cancer diagnosed globally. Prostate cancer was the cause of 359,000 deaths and 1.3 million new cases in 2018 [1]. Prostate cancer mortality rates have decreased in many developed regions, including North America, Northern and Western Europe, Oceania, and advanced areas of Asia, due to advancements in screening, early detection, and improved treatment. On the other hand, mortality rates have been rising in several regions, such as Central and South America, Central and Eastern Europe, and several Asian nations [2]. A more Westernized lifestyle, shifting risk factors, and restricted access to efficient treatment options could be contributing factors to this rise [3]. The most common cancer among Sudanese men is prostate cancer, which affects all of the nation's tribes equally [4]. Even with early intervention, metastases occur in up to 40% of patients with prostate cancer; these most often lead to Metastatic Castration-Resistant Prostate Cancer (mCRPC), an extremely difficult and intricate disease. Reversible mutations in DNA damage repair genes, including those implicated directly or indirectly in homologous recombination repair (HRR), have been discovered in about 25% of patients with metastatic colorectal cancer [5]. Germline mutations of DNA homologous recombination repair (HRR) genes, particularly BRCA2, are typically present in advanced prostate cancers (PCs) and are indicative of a poor prognosis for conventional therapy [6]. One of the biggest risk factors for prostate cancer is a family

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history of the condition. At an 8.6-fold increased risk in males under 65 years of age, germline mutations in the BRCA2 gene are currently known to be the genetic factor linked to the highest risk of developing prostate cancer [7]. 1995 saw the discovery that the 13q12-linked gene BRCA2 encodes a 3418 amino acid protein through the use of positional cloning. Its C-terminal region is conserved, and the protein's middle contains repeats referred to as BRC repeats [8]. BRCA genes function as tumour suppressors in prostate cancer and are crucial for the cellular damage response. They regulate transcription, the course of the cell cycle, the division of cells, and the induction of apoptosis. Their mutation leads HR to fix DNA incorrectly, which sets up the ideal environment for cancer development. BRCA mutations raise the risk of several cancers, including breast, ovarian, prostate, and other visceral tumours [9]. Double-strand DNA breaks must be repaired, and transcription regulation and repair depend on the BRCA2 gene. In healthy cells, it is essential for preserving DNA stability and regulating cell division [10]. The BRCA2 gene's exon 10 contains the rs144848 non-synonymous polymorphism, which is frequently found [8]. So this study aimed to evaluate the contribution of BRCA2 gene variability to prostatic cancer susceptibility in patients recruited to the National Cancer Institute in Sudan.

METHODS

Study Population

Between November 2020 and August 2023, a case-control population-based study was carried out at the National Cancer Institute (NCI), University of Gezira, Gezira State, Sudan. There were 162 participants in this study: 81 patients who had an incident and a histologically confirmed PC diagnosis, who made up the case group, and 81 healthy individuals who made up the control group according to sample calculation. Men aged 45 and older were eligible to participate, as were case subjects who had just received a diagnosis and were not receiving any treatment at the time of sample collection. Patients with prostatitis, STDs, chronic renal failure, or those receiving finasteride or dutasteride therapy for prostatic disease were not included. Form cases were given a unique questionnaire that included personal demographics and clinical data from the patient's hospital record, such as the histological stage grade at diagnosis and correlation with the Gleason score [5]. Males between the ages of 45 and 75 who had no prior history of prostatic disorders were chosen as the study's control groups.

Sample Size

To calculate the sample size for our case-control study on prostate cancer in Sudan, we calculated according to the following parameters [11] for the power analysis:

- **Study type:** Case-control study
- **Statistical test:** Chi-square test for association or t-test.
- **Effect size:** Medium (Cohen's d = 0.5) based on expected differences.
- **Significance level (α):** 0.05
- **Power (1 - β):** 0.80 (80%)

Based on the power analysis, for a medium effect size (Cohen's d = 0.5), an 80% power, and a significance level of 0.05, the study would require approximately 64 participants per group (cases and controls), which results in a total sample size of 128.

Sample Collection

Five millilitres of venous blood were drawn and placed in two separate containers: an EDTA container containing the whole blood for DNA extraction and a plain container for serum after centrifugation [12].

Prostate Specific Antigen (PSA)

Cobas e411 was used to measure the PSA level in the serum [13].

DNA Extraction

DNA was extracted using the Intron G-spin Total DNA Extraction Kit following the manufacturer's instructions.

Detection, SNPs Primers Sequence, and Product Size

Through the use of allele-specific polymorphism (PCR), the BRCA2 gene's rs144848 mutation (delete) was screened for among study participants. Every analysis was done in blindness with regard to the attributes of the various groups, and every subject underwent two independent reactions.

To genotype the BRCA2 (rs144848 A>C) SNP, separate PCR reactions were conducted using forward primers specific to either the A or C alleles in conjunction with a single reverse primer as follows:

- **BC2Asp-A:** 5`- ACTCTCAAAGGGCTTCTGGTT -3`
- **BC2Asp-C:** 5`- ACTCTCAAAGGGCTTCTGGTG -3`
- **BC-CP:** 5`- CAATGCCAAATGTCCTAGAAG -3`

Internal control primers were used in the same reaction tubes as the following sequence:

- Internal Control F 5`- GCCTTCCCAACCATTCCCTTA-3`
- Internal Control R 5`- TCACGGATTTCTGTTGTGTTTC-3`

A co-amplified 426 bp human growth hormone sequence was used to verify that the PCR amplification was successful. 5 µl of Master mix, 2 µl DW, 2 µl DNA sample, 0.5 µl primer (BC2Asp-A, BC2Asp-C, and BC-CP), and 0.2 µl primer (internal control) were added to the PCR mixture.

The amplification process involved a first denaturation step at 95°C for 2 min; subsequent denaturation steps at 95°C for 20 s, 55°C for 20 s, and 72°C for 40 s for 35 cycles; the last cycle included a final extension of 2 min at 72°C. On a 2% agarose gel, every reaction was confirmed. The product size for the A or C alleles was 278 bp.

Study participants' BRCA2 (rs4942486 T > C) polymorphism was screened, and allele-specific PCR was used to genotype them. BRCA2 (rs4942486) SNP, T-, or C allele-specific forward primers were utilized in separate PCR reactions along with a single reverse primer for BRCA2 genotyping as follows:

- **BC2-AST:**5'-GTA CTT GTC CTG TTT AAA GCC TTC T-3'
- **BC2-ASC:**5'-GTA CTT GTC CTG TTT AAA GCC TTC C-3'
- **BC2-CP:** 5`- ACTGGATCTGAGCTTGTTTCT -3`

Also, internal control primers were used in the same reaction tubes as the following sequence:

- Internal Control F 5`- GCCTTCCCAACCATTCCCTTA-3`
- Internal Control R 5`- TCACGGATTTCTGTTGTGTTTC-3`

A co-amplified 426 bp human growth hormone sequence was used to verify that the PCR amplification was successful. The PCR mixture contained the following components: 0.2 µl internal control primer, 0.8 µl primer (BC2-AST, BC2-ASC, or BC2-CP), 2.5 µl DW, and 2 µl DNA sample.

The PCR amplification protocol involved denaturation at 95˚C for 30s, annealing at 57˚C for 20s, and polymerization at 72˚C for 45s for 35 cycles. The size of the product for the T or C alleles was 172 bp.

Commercial PCR premix (AccuPower PCR PreMix; Bioneer, Daejeon, Korea) that was prepared in accordance with the manufacturer's instructions was used for the PCR process. The master mix concentration was 2X, and the PCR mix's total volume ranged from 10 µL to 15 µL.

By electrophoresising 2% agarose concentration in 1.x TBE (Tris-borate-EDTA buffer) at 120 Volts for approximately 30 minutes and visualizing the results under a gel documentation system, the amplification's efficiency was evaluated (GDS) [14].

The Ministry of Health in Gezira State's Research and Ethics Committee granted ethical clearance. The National Cancer Institute (NCI) and, the University of Gezira's Research and Ethics Committee granted ethical permission Before the collection of samples, each participant provided written and informed consent.

Statistical Analysis

Data analysis was done with SPSS (version 21) software. The patients' clinico-demographic details were shown as means with standard deviations and value ranges, or as counts and percentages. By calculating odds ratios, associations between genotypes and alleles among various groups were evaluated. Binary logistic regression was applied to calculate the odds ratio. A statistically significant result was defined as a p-value of less than 0.05. SNP Stats were used to analyze the significance of various genotype frequency combinations in cases compared to controls using codominant, dominant, over-dominant, and recessive models. Each genotype was classified based on the presence of particular alleles.

RESULTS

Among the 230 case and control subjects (rs144848) SNP, the A allele frequency is the most common (71%). Additionally, it makes up 122 (75%) of the cases group compared to 108 (67%) in the control group. Under the Dominant, SNP rs144848 was found to be inversely related to PCa (OR=1.43, 95% CI: 0.76-2.66; p=0.27 for A/C-C/C). Additionally, Codominant with p-values of 0.28, 013, and 0.14, respectively, the recessive and overdominant models did not demonstrate a significant correlation between SNP and prostate cancer (**Table 1**).

The most common SNP T allele frequency in the BRCA2 (rs4942486) was 197 (61%), with 94 (58%), in the case group and 103 (64%) in the control group. Under the codominant (OR= 1.18, 95% CI: 0.60-2.33; and OR= 0.54, 95% CI: 0.22-1.35; p-value = 0.22) and C/T and C/C genotypes, respectively, dominant (OR=

Table 1: Association between BRCA2 (rs144848) and study subjects.

Model	Genotype	Cases n(%)	Controls n(%)	p-value	OR (95%)
Allele effect	A	122(75)	108(67)	-	-
	C	40(25)	54(33)		
Codominant	A/A	50 (61.7)	43 (53.1)	0.28	Reference category
	A/C	22 (27.2)	22 (27.2)		1.16 (0.57-2.38)
	C/C	9 (11.1)	16 (19.8)		2.07 (0.83-5.15)
Dominant	A/A	50 (61.7)	43 (53.1)	0.27	Reference category
	A/C-C/C	31 (38.3)	38 (46.9)		1.43 (0.76-2.66)
Recessive	A/A-A/C	72 (88.9)	65 (80.2)	0.13	Reference category
	C/C	9 (11.1)	16 (19.8)		1.97 (0.81-4.76)
Overdominant	A/A-C/C	59 (72.8)	59 (72.8)	0.14	1.00
	A/C	22 (27.2)	22 (27.2)		1.00 (0.50-2.00)
Log-additive	-	-	-	-	1.37 (0.90-2.09)

Table 2: Association between BRCA2 (rs4942486) and study subjects.

Model	Genotype	Cases n(%)	Controls n(%)	p-value	OR (95%)
Allele effect	T	94(58)	103(64)	-	-
	C	68(42)	59(36)		
Codominant	T/T	31 (38.3%)	32 (39.5)	0.22	Reference category
	C/T	32 (39.5)	39 (48.1)		1.18 (0.60-2.33)
	C/C	18 (22.2)	10 (12.3)		0.54 (0.22-1.35)
Dominant	T/T	31 (38.3)	32 (39.5)	0.87	0.95 (0.50-1.79)
	C/T-C/C	50 (61.7)	49 (60.5)		
Recessive	T/T-C/T	63 (77.8)	71 (87.7)	0.09	0.49 (0.21-1.15)
	C/C	18 (22.2)	10 (12.3)		
Over dominant	T/T-C/C	49 (60.5)	42 (51.9)	0.27	1.42 (0.76-2.65)
	C/T	32 (39.5)	39 (48.1)		
Log-additive	-	-	-	0.32	0.81 (0.52-1.24)

Table 3: Association between BRCA2 (rs144848) and disease metastases.

Model	Genotype	Met=No n(%)	Met=Yes n(%)	OR (95% CI)	p-value
Codominant	A/A	22 (55)	28 (68.3)	Reference category	0.38
	A/C	12 (30)	10 (24.4)	0.66 (0.24-1.80)	
	C/C	6 (15)	3 (7.3)	0.39 (0.09-1.75)	
Dominant	A/A	22 (55)	28 (68.3)	0.57 (0.23-1.40)	0.022
	A/C-C/C	18 (45)	13 (31.7)	Reference category	
Recessive	A/A-A/C	34 (85)	38 (92.7)	0.45 (0.10-1.93)	0.27
	C/C	6 (15)	3 (7.3)	Reference category	
Overdominant	A/A-C/C	28 (70)	31 (75.6)	0.75 (0.28-2.01)	0.57
	A/C	12 (30)	10 (24.4)	Reference category	
Log-additive	-	-	-	0.64 (0.33-1.22)	0.17

1.05, 95% CI: 0.50-1.79; P-value = 0.87) and recessive (OR= 2.02, 95% CI: 0.21-1.15; P-value = 0.13) for C/C genotype, and log additive models (OR=1 .42, 95% CI: 0.76-2.65; P-value = 0.27) and C/T-C/C genotypes, respectively, and recessive (OR= 2.02, 95% CI: 0.21-1.15; P-value = 0.13) and C/C genotypes, were found to be insignificantly associated with PCa (**Table 2**).

The rs144848 SNP was found to be significantly associated with metastases under the following genotypes: dominant (OR=0.57, 95% CI: 0.23-1.40; P-value = 0.022) for A/C -C/C genotype, recessive (OR=0.45, 95% CI: 0.10-1.93; P-value = 0.27) for C/C genotype, over-dominant (OR=0.57, 95% CI: 0.28-2.01; P-value = 0.57) for A/C, and the log additive models (OR=0.64, 95% CI: 0.33-1.22; P-value = 0.17) for A/C and C/C genotypes, respectively (**Table 3**).

DISCUSSION

Complex molecular mechanisms underlie the development of prostate cancer (PCa), which is caused by complex interactions between multiple genes and environmental factors. These changes in DNA and epigenetics can appear at various phases of the cancer's development. The aggressive progression of high-risk prostate cancer has been highlighted by the recognition of mutations in BRCA1 and BRCA2 as significant contributors to the disease's advancement [11]. Prostate cancer has a highly aberrant genetic pathway with several abnormalities. These consist of changes in the number of chromosomes, point mutations, somatic copy number variations, and structural rearrangements. These genetic changes impair regular cellular functions and add to the intricacy and development of the illness [15]. In the current study, we found that among patients with prostate cancer who carried the rs144848 mutation, wild-type BRCA2 (62%) was more common than mutant-type (38%). This outcome was lower than what Agalliu *et al.* [16] discovered. This variation across the studies could be due to the demographic variability between them. In the presence of BRCA2 (rs144848), A allele was common among cases, controls, and all patients. This is consistent with recent studies conducted by Martínez-Nava *et al.* [17]. In contrast to the control group, the BRCA2 rs144848 polymorphism did not demonstrate any discernible correlation with prostate cancer [17, 18].

Considering the correlation between the rs144848 single nucleotide polymorphism (SNP) and disease metastases, metastases under the codominant for the A/C and C/C Genotypes, respectively, were highly associated. These results corroborate the claims of multiple previous, updated studies that over 10% of males with metastatic PCa have genetic variations in DNA repair genes [19, 20]. According to our findings, the frequency of BRCA2 rs4942486 alteration among patients with prostate cancer was higher in the mutant type (62%) than in the wild type (38%), indicating a high prevalence of the mutation in the Sudanese population. Castro *et al.* [21] also reported on this finding. One of our longstanding conclusions is that the BRCA2 (rs4942486) SNP did not show any associations with the risks of prostate cancer when tested in additive, dominant, or recessive genetic models. This finding aligns with the research conducted by Legge, which also revealed no association between it and the likelihood of developing prostate cancer [22]. Additionally, there were three genotypes: two were mutant and one was a wide type (T/T). When comparing prostate cancer patients to controls in the (C/T-C/C) genotype, the CC variant was found more frequently, suggesting that it may offer some protection against the disease. Furthermore, there is a strong theory that the relationship between BRCA2 mutations and the risk of prostate cancer may vary depending on the age of diagnosis. According to earlier studies, men who are diagnosed with prostate cancer before the age of 65 and have BRCA2 mutations have a higher incidence of the disease [23]. This result is in line with our finding that people over 65 are more likely to have the rs4942486 mutation. Furthermore, some research has shown that men who receive a prostate cancer diagnosis later in life are more likely to have BRCA2 mutations linked to the disease. The complex relationship between BRCA2 mutations and the age at which the disease manifests is highlighted by Agalliu *et al.,* which indicates that men with BRCA2 mutations are more likely to develop prostate cancer at a younger age [24]. Half of the six prostate cancer patients with BRCA2 mutations were diagnosed before the age of 50, according to Edwards *et al.* analysis of the average estimated prevalence of disease-associated BRCA2 mutations in the general UK population (0.12% and 0.07%) [25]. Agalliu *et al.* discovered that aggressive prostate tumours frequently manifest early, especially in those under the age of 55 [26]. The discrepancies observed amongst studies regarding the association between age at prostate cancer and BRCA2 mutations can be ascribed to differences in study populations, sample sizes, study designs, diagnostic standards, procedures, and follow-up times.

STUDY LIMITATION

Despite many methodological biases the study did not consider other genetic or environmental factors that may interact with the BRCA2 rs144848 gene disease severity. These limitations suggest the need for future research with larger, more diverse populations to fully understand the role of BRCA2 rs144848 in Prostate cancer.

CONCLUSION

The BRCA2 rs144848 polymorphism was found to be significantly correlated with cancer metastases and family history in patients with prostate cancer. It is interesting to note that although the BRCA2 rs4942486 polymorphism did not significantly correlate with the risk of prostate cancer overall, patients with Gleason scores higher than 7 had a higher frequency of this mutation, which may indicate that the disease is in a more advanced stage. The finding of these mutations can help tailor treatment plans and offer important insights into how prostate cancer develops.

ETHICAL APPROVAL

The National Cancer Institute (NCI) and, the University of Gezira's Research and Ethics Committee granted ethical permission (REF letter No. 1/K/T/44 Dated; 06-06-2021). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/ or national research committee and the Helsinki Declaration.

CONSENT FOR PUBLICATION

Written informed consent was taken from the participants.

AVAILABILITY OF DATA

The data set may be acquired from the corresponding author upon a reasonable request.

FUNDING

None.

CONFLICT OF INTEREST

The authors have disclosed no financial conflict of interest related to this research.

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AUTHORS' CONTRIBUTION

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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