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Article

Analyzing the Mutational Landscape of Prostate Cancer Susceptibility Genes through Next-Generation Sequencing (NGS) Analysis

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Abstract

Prostate cancer, a significant public health concern, exhibits a complex genetic landscape influenced by a variety of susceptibility genes. This study deciphers the intricate genetic makeup of prostate cancer by examining mutations in 14 prostate cancer susceptibility genes. The Next-Generation Sequencing (NGS) approach was employed on a cohort of 45 prostate cancer patients to detect mutations. The gnomAD database was used to analyze mutational frequencies in the Asian population suffering from prostate cancer. The cBioPortal database was employed to check the presence of observed mutations in The Cancer Genome Atlas (TCGA) dataset. In addition, this study employed RT-qPCR and Immunohistochemistry (IHC) techniques were utilized to check the functional consequences of the observed pathogenic mutations. Finally, Metascape and DrugBank resources were used for gene enrichment and drug prediction analyses. Results of the NGS analysis revealed a total of 29 mutations (pathogenic and benign) within the examined prostate cancer cohort, distributed across four key genes (*BRCA1/2, TP53, and PMS2*) out of the total analyzed 14 genes. Pathogenic mutations in BRCA1/2 and TP53 genes are of particular interest due to their fundamental roles in DNA repair, cell cycle regulation, and tumor suppression. Functional consequence analyses (RT-qPCR and IHC) demonstrated the down-regulation of *BRCA1/2* and *TP53* genes in prostate cancer samples with pathogenic mutations, reinforcing the disruption of their tumor suppressor roles. Lastly, drug prediction analysis uncovered promising therapeutic options by identifying drugs capable of enhancing the mRNA expression of these genes. This opens new avenues for tailored treatment strategies aimed at restoring normal cellular functions of the *BRCA1/2* and *TP53* genes. In conclusion, this study provides a comprehensive view of genetic mutations in prostate cancer susceptibility genes, ranging from benign to pathogenic. It emphasizes the genetic complexity of prostate cancer and offers insights into potential mechanisms driving this malignancy. These findings lay the groundwork for further research, personalized treatment approaches, and enhanced clinical management of prostate cancer.

1. Introduction

Prostate cancer is a prevalent malignancy affecting men globally, characterized by the uncontrolled growth of prostate gland cells [1-3]. This disease begins when abnormal cells within the prostate start to multiply uncontrollably, forming tumors that may remain localized or spread to other parts of the body [4,5]. While some cases progress slowly and may not require aggressive treatment, others can be highly aggressive [6]. Early detection, risk assessment, and personalized treatment strategies are pivotal in managing this multifaceted cancer [7,8].

The frequency of prostate cancer varies across regions and populations, but it consistently ranks among the top cancers diagnosed [9-14]. The prevalence of prostate cancer is attributed to a combination of genetic, environmental, and lifestyle factors [15,16]. Among these factors, genetic predisposition has gained prominence, and *BRCA1* and *BRCA2* mutations have been implicated in prostate cancer susceptibility [17,18].

The *BRCA1* and *BRCA2* genes, well-established for their roles in breast and ovarian cancers among women, have garnered attention for their association with prostate cancer in men [19,20]. While germline mutations in established prostate cancer susceptibility genes have been uncovered, such mutations are infrequent in any single gene [21,22]. Consequently, the practice of testing one gene at a time is inefficient and costly. With the emergence of Next Generation Sequencing (NGS) [23], simultaneous assessment of numerous prostate cancer susceptibility genes is now possible via multiplex panels, offering a cost-effective testing solution. Nevertheless, concurrent testing of multiple genes may unveil genetic alterations with less clear clinical management, although further data will ultimately refine management and screening guidelines for prostate cancer patients.

In this mono-centric investigation, the TruSight Sequencing Cancer Panel was employed on an Illumina

MiSeq platform to comprehensively examine genetic mutations in 14 key genes (*BRCA1/2, ATM, CHEK2, PALB2, RAD51C, RAD51D, NBN, CDH1, TP53, MLH1, MSH2, MSH6,* and *PMS2*) associated with increased prostate cancer susceptibility. The primary objective of this study was to assess the prevalence of individuals carrying mutations in these genes within Pakistani study cohort. Furthermore, the study aimed to elucidate the functional implications of these observed mutations through a multi-faceted approach encompassing bioinformatics analysis and molecular experiments. By integrating high-throughput sequencing technology with in-depth bioinformatics and molecular methodologies, the objective was to uncover potential genetic drivers and their functional consequences in prostate cancer development and progression. This comprehensive investigation aimed to shed light on the underlying genetic landscape of prostate cancer susceptibility, paving the way for personalized therapeutic strategies and improved clinical management of this complex disease.

2. Methodology

2.1 Sample Collection and Ethical Approval

This study meticulously collected a total of 45 prostate cancer tissue samples from patients undergoing surgical resection at the Nishtar Hospital, Multan, Pakistan. This study was approved by the Institutional Review Board of Nishtar Medical University (Reference # 2038) and adhered to the Declaration of Helsinki. Written informed consent was obtained from all participants, ensuring voluntary involvement. Patient data and tissue samples were anonymized to protect privacy, with stringent data security measures in place.

2.2 Inclusion and Exclusion Criteria

The inclusion criteria for prostate cancer patients in this study encompassed individuals with a confirmed diagnosis of prostate cancer, as histologically verified by biopsy reports, who were willing to participate in the research. Exclusion criteria included patients with a history of other malignancies, those undergoing treatment for prostate cancer, and individuals unable or unwilling to provide informed consent. Patients with incomplete medical records or insufficient tissue samples for genetic analysis were also excluded from the study. Clinical information of the included prostate cancer patients is detailed in Table 1.

Table 1. An overview of prostate cancer patient's characteristics in the present study

Sr.no	Characteristics		Sample count (n)
1	Sex	Male	45
		Female	0
2	Age	>60	9
		<60	36
3	Treatment	Pre-treatment	45
		Post-treatment	0

2.3 Nucleic Acid Extraction

For DNA and RNA extraction, reliable kit-based methods were employed to ensure the purity and integrity of the genetic material. Specifically, the GeneJET Genomic DNA Purification Kit (cat # K0721, Thermo Fisher) was used for DNA extraction, and the GeneJET Genomic RNA Purification Kit (cat # K0732, Thermo Fisher) was used for RNA extraction. The DNA extraction process involved the following steps: cell lysis, which breaks down cell membranes to release DNA, followed by the removal of proteins and other contaminants using a protein precipitation solution. The DNA was then bound to a silica membrane within a spin column, washed with ethanol-based buffers to remove impurities, and finally eluted in a low-salt buffer. For RNA extraction, the procedure included the disruption of cells using a lysis buffer that contains guanidine thiocyanate to protect RNA from degradation. Following lysis, samples were homogenized and the RNA was selectively bound to a silica membrane. Contaminants were removed with a series of wash steps, and the purified RNA was eluted in nuclease-free water. After the extraction, the purity of the DNA and RNA was verified by assessing the A260/A280 absorbance ratio using a spectrophotometer. The absorbance was measured at 260 nm and 280 nm to determine the presence of protein or phenol contamination. Samples with an A260/A280 ratio within the range of 1.8 to 2.0 were considered pure and selected for subsequent analyses, as this ratio indicates minimal contamination by proteins or other organic compounds.

2.4 Next-Generation Sequencing (NGS)

For next-generation sequencing library preparation, the TruSight Cancer Sequencing Panel, a targeted resequencing kit, was utilized on the MiSeq platform by Illumina (San Diego, CA). All steps were performed in strict accordance with the manufacturer's recommended protocols. Library preparation was initiated with 50 ng of genomic DNA for each sample, using the TruSight Rapid Capture and TruSight Cancer kits. The resultant double-stranded DNA libraries were subsequently transformed into single-stranded DNA. To target specific regions, biotin-labeled probes were employed in the first rapid capture step. Streptavidin beads were introduced to enrich the pool of mixed samples for the desired target regions. Biotinylated DNA fragments bound to the streptavidin beads were then isolated from the solution using magnetic pull-down. After this, the enriched DNA fragments were eluted from the beads and subjected to a second rapid capture step. Finally, the prepared libraries were applied to the MiSeq Flowcell for sequencing. Subsequently, the paired sequences from each sample were aligned to the human genome reference GRCh37/hg19 using BWA-MEM version 0.7.7. Duplicate sequences were identified and marked with Picard's MarkDuplicates version 1.111 (available at https://github.com/broadinstitute/picard), and local InDel realignment was conducted using the Genome Analysis Tool Kit (GATK) version 3.1.1. The TruSight Cancer panel encompasses 94 genes associated with both common (e.g., breast, prostate) and rare cancers. Among

these genes, this study focused on 14 specific genes (*BRCA1/2, ATM, CHEK2, PALB2, RAD51C, RAD51D, NBN, CDH1, TP53, MLH1, MSH2, MSH6,* and *PMS2*) utilizing the SeqNext module within the Sequence Pilot software by JSI medical systems GmbH in Kippenheim, Germany. The sequencing achieved a medium sequence depth of 400x, with a minimum of 30x coverage for the coding regions and the first 10 base pairs of flanking intronic regions for each gene.

2.5 Interpretation of the Observed Mutations

The interpretation of genetic mutations adhered to the comprehensive guidelines established by the American College of Medical Genetics (ACMG) and the Association for Molecular Pathology (AMP), categorizing mutations as pathogenic, likely pathogenic, variants of uncertain significance (VUS), likely benign, or benign. This classification utilized a combination of in-silico prediction tools and clinical data. Specifically, this study employed SIFT (Sorting Intolerant From Tolerant) to evaluate amino acid substitutions based on sequence homology, PolyPhen-2 (Polymorphism Phenotyping v2) to assess the impact of amino acid changes on protein structure and function, and Mutation Taster to predict disease-causing potential using various computational methods. Additionally, the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) provided valuable clinical significance data, aggregating information about genomic variations and their health implications. Integrating these computational tools with ClinVar's clinical data ensured a robust and reliable classification of mutations, supporting accurate clinical decision-making and enhancing the understanding of genetic contributions to disease.

2.6 Frequencies of the Detected Mutations in gnomAD Database

The gnomAD database (https://gnomad.broadinstitute.org/) [24], a comprehensive resource of human genetic variations, played a pivotal role in this study. This invaluable database was harnessed to conduct an in-depth analysis of the frequencies of observed mutations within the Asian population. gnomAD provided a wealth of genetic data, enabling insights into the prevalence and distribution of these mutations in the Asian context. This extensive dataset allowed for a nuanced understanding of genetic variation across different populations, thereby enhancing the precision and relevance of the research findings. By leveraging gnomAD, the study was grounded in robust and representative genetic information, facilitating more accurate interpretations and conclusions.

2.7 Analysis of Detected Mutations in The Cancer Genome Atlas (TCGA)

In this study, cBioPortal database (https://www.cbioportal.org/) [25] was utilized to analyze detected mutations within TCGA cohorts. This invaluable platform offered us a robust and user-friendly interface to delve into genomic alterations across a variety of TCGA cohorts. By harnessing cBioPortal's extensive resources and analytical tools, this study gained deeper insights into the detected mutations across these cohorts, enhancing the precision and relevance of the research findings. The comprehensive data and sophisticated analysis capabilities provided by cBioPortal allowed us to explore the genetic landscape with greater detail and accuracy, thereby strengthening the overall impact of the study.

2.8 RT-qPCR Analysis

First-strand cDNA synthesis was carried out using the cDNA Synthesis SuperMix from TransGen Biotech Co., Ltd., following the manufacturer's recommended protocols. For the quantitative analysis of gene expression, RT-qPCR was conducted using the TB Green® Premix EX Taq™ II (Takara Bio, Inc.) on an Applied Biosystems 7900 Real-Time PCR System (Thermo Fisher Scientific, Inc.). The expression levels of RNA were quantified using the comparative $2^{-\Delta\Delta Cq}$ method, with *GAPDH* serving as the internal control to normalize the data. All reactions were repeated in triplicates. To identify statistically significant differences between the two groups, a Student's t-test was employed. This methodological approach ensured accurate and reliable quantification of RNA expression levels, facilitating the comparison of gene expression between experimental conditions.

2.9 ROC Analysis

ROC curves for the mutated gene's expression level were constructed using the SRPLOT web resource (https://www.bioinformatics.com.cn/en). This tool enabled us to assess the diagnostic performance of the gene expression levels by providing a clear graphical representation of sensitivity versus specificity, thus facilitating the evaluation of the gene's potential as a biomarker.

2.10 Immunohistochemistry (IHC) Analysis

A previously reported protocol for IHC analysis was adopted in this study [26]. In brief, tissue sections underwent deparaffinization, and antigen retrieval was achieved through heat treatment in an EDTA solution at pH 8.0. Protein expression levels of the mutated genes were assessed using 4-µm-thick sections obtained from formalin-fixed, paraffin-embedded (FFPE) specimens. Monoclonal antibodies targeting *BRCA1* (Cat # TA802618AM), *BRCA2* (Cat # TA802628), and *TP53* (TA502870) were utilized, and staining was carried out using the Ventana BenchMark XT staining system. A pathologist assessed tumor positivity based on the presence or absence of nuclear staining in tumor tissue, considering staining intensity.

2.11 Gene Enrichment Analysis

Metascape (https://metascape.org/gp/index.html), a comprehensive bioinformatics tool, played a crucial role in this study for gene enrichment analysis [27]. Designed to simplify the functional annotation and analysis of large-scale omics datasets, Metascape served as a

valuable resource for unraveling the biological significance of the findings. This user-friendly platform offers a comprehensive suite of tools for gene annotation, pathway enrichment, and protein interaction network analysis. By leveraging Metascape, researchers can efficiently interpret the functional implications of their data, uncovering key molecular pathways and biological processes underlying complex biological phenomena. The integration of Metascape into the study workflow enhanced the ability to extract meaningful insights from the genomic and transcriptomic data, ultimately contributing to a deeper understanding of the molecular mechanisms underpinning the research objectives.

2.12 Drug Prediction Analysis

The DrugBank database (https://go.drugbank.com/) stands as a comprehensive and accessible resource in the realm of pharmaceuticals [28]. It serves as a reservoir of meticulously curated information about drugs, their mechanisms, interactions, and associated targets. This resource proves invaluable for researchers, healthcare professionals, and the pharmaceutical industry, facilitating the exploration of drug properties, indications, side effects, and pathways. In this study, DrugBank database was used to explore drugs that regulate the expression of key genes such as *BRCA1, BRCA2, TP53*, and *PMS2*. By leveraging DrugBank's extensive database, drugs that may influence the expression levels of critical genes involved in cancer biology were identified and analyzed. This information enhances the understanding of the pharmacological landscape and aids in identifying potential therapeutic agents that could modulate gene expression in a clinical setting. The integration of DrugBank data provided a robust foundation for the research, enabling a detailed investigation into regulatory mechanisms and potential treatment strategies for gene-associated disorders.

3. Results

3.1 Mutations Across Prostate Cancer Patients

This study employed a NGS-based gene panel to concurrently identify mutations in 14 established prostate cancer susceptibility genes (*BRCA1/2, ATM, CHEK2, PALB2, RAD51C, RAD51D, NBN, CDH1, TP53, MLH1, MSH2, MSH6,* and *PMS2*) with varying levels of penetrance. This study involved the examination of 45 patients within a diagnostic context. The identified mutations were categorized into pathogenic, likely pathogenic, variants of uncertain significance (VUS), likely benign, or benign. A total of 29 mutations were identified within the examined prostate cancer cohort, spanning four genes: *BRCA1/2, TP53*, and *PMS2* (Table 2). Within the BRCA1 gene, ten mutations were found, comprising nine (90%) benign and one (10%) pathogenic mutation (Table 2). The *BRCA2* gene yielded eight mutations, with two being pathogenic (25%) and six (75%) benign (Table 2). *TP53* exhibited eight mutations, with one (12%) being pathogenic and seven (88%) being benign mutations (Table 2). In *PMS2*, three mutations were identified, all of which (100%) were of a benign nature (Table 2).

3.2 Pathogenic Mutations

Among the 45 prostate cancer patients, a subset of 9 samples displayed pathogenic mutations within *BRCA1/2* and *TP53*. Within the *BRCA1* gene, a single pathogenic mutation was identified, designated as p.Tyr1845Ter (Table 2). In the *BRCA2* gene, two distinct pathogenic mutations, p.Asp23Tyr and p.Tyr42Cys, were identified (Table 2). Additionally, a single pathogenic mutation, p.Glu339Ter, was observed in the TP53 gene (Table 2).

3.3 Frequencies of Pathogenic Mutations in Asian Prostate Cancer Patients

In the subsequent phase of this investigation, the frequencies of the identified pathogenic mutations within *BRCA1* (p.Tyr1845Ter), *BRCA2* (p.Asp23Tyr and p.Tyr42Cys), and *TP53* (p.Glu339Ter) genes were analyzed among Asian prostate cancer patients, leveraging the gnomAD database. The outcomes unveiled that the frequencies of these pathogenic mutations were conspicuously absent in the gnomAD database. These findings signify that these specific pathogenic mutations in within *BRCA1* (p.Tyr1845Ter), *BRCA2* (p.Asp23Tyr and p.Tyr42Cys), and *TP53* (p.Glu339Ter) had not been previously documented among prostate cancer patients of Asian descent.

3.4 Analysis of the Pathogenic Mutations in The Cancer Genome Atlas (TCGA)

Herein, this study meticulously examined the existence of the identified pathogenic mutations in *BRCA1* (p.Tyr1845Ter), *BRCA2* (p.Asp23Tyr and p.Tyr42Cys), and *TP53* (p.Glu339Ter), within the TCGA prostate cancer patient cohort, employing the cBioPortal database. The comprehensive analysis yielded conclusive results, affirming the absence of these specific pathogenic mutations in the TCGA prostate cancer patients, as these mutations were absent among the detected mutations in the TCGA dataset (Figure 1). This noteworthy discovery emphasizes the remarkable uniqueness and rarity of the pathogenic mutations observed in this study.

Table 2. Count and type of mutations observed in *BRCA1*, *BRCA2*, *TP53*, and *PMS2* genes across prostate cancer patients.

Sr. no	Gene	NM:c.DNA	Protein	Nature	No. patients
1	*BRCA1*	NM_007294.4:c.5535C>A	p.Tyr1845Ter	Pathogenic	9
2		NM_007294.4:c.5347A>T	p.Met1783Leu	Benign	17
3		NM_007294.4:c.5198A>G	p.Asp1733Gly	Benign	21
4		NM_007294.4:c.5158A>G	p.Thr1720Ala	Benign	34
5		NM_007294.4:c.5117G>C	p.Gly1706Ala	Benign	12
6		NM_007294.4:c.5456A>G	p.Asn1819Ser	Benign	25
7		NM_007294.4:c.4985T>C	p.Phe1662Ser	Benign	11
8		NM_007294.4:c.4955T>C	p.Met1652Thr	Benign	6
9		NM_007294.4:c.4913A>T	p.Glu1638Val	Benign	21
10		NM_007294.4:c.4910C>T	p.Pro1637Leu	Benign	4
11	*BRCA2*	NM_000059.4:c.67G>T	p.Asp23Tyr	Pathogenic	9
12		NM_000059.4:c.125A>G	p.Tyr42Cys	Pathogenic	9
13		NM_000059.4:c.440A>G	p.Gln147Arg	Benign	16
14		NM_000059.4:c.502C>A	p.Pro168Thr	Benign	15
15		NM_000059.4:c.978C>A	p.Ser326Arg	Benign	14
16		NM_000059.4:c.1040A>G	p.Gln347Arg	Benign	21
17		NM_000059.4:c.1151C>T	p.Ser384Phe	Benign	25
18		NM_000059.4:c.1181A>C	p.Glu394Ala	Benign	13
19	*TP53*	NM_000546.6:c.1014_1015insT	p.Glu339Ter	Pathogenic	9
20		NM_000546.6:c.970G>A	p.Asp324Asn	Benign	1
21		NM_000546.6:c.952C>T	p.Pro318Ser	Benign	6
22		NM_000546.6:c.935C>G	p.Thr312Ser	Benign	12
23		NM_000546.6:c.869G>A	p.Arg290His	Benign	16
24		NM_000546.6:c.805A>T	p.Ser269Cys	Benign	1
25		NM_000546.6:c.704A>G	p.Asn235Ser	Benign	22
26		NM_000546.6:c.386C>A	p.Ala129Asp	Benign	11
27	*PMS2*	NM_000535.7:c.1532C>T	p.Thr511Met	Benign	5
28		NM_000535.7:c.1531A>G	p.Thr511Ala	Benign	11
29		NM_000535.7:c.1408C>T	p.Pro470Ser	Benign	11

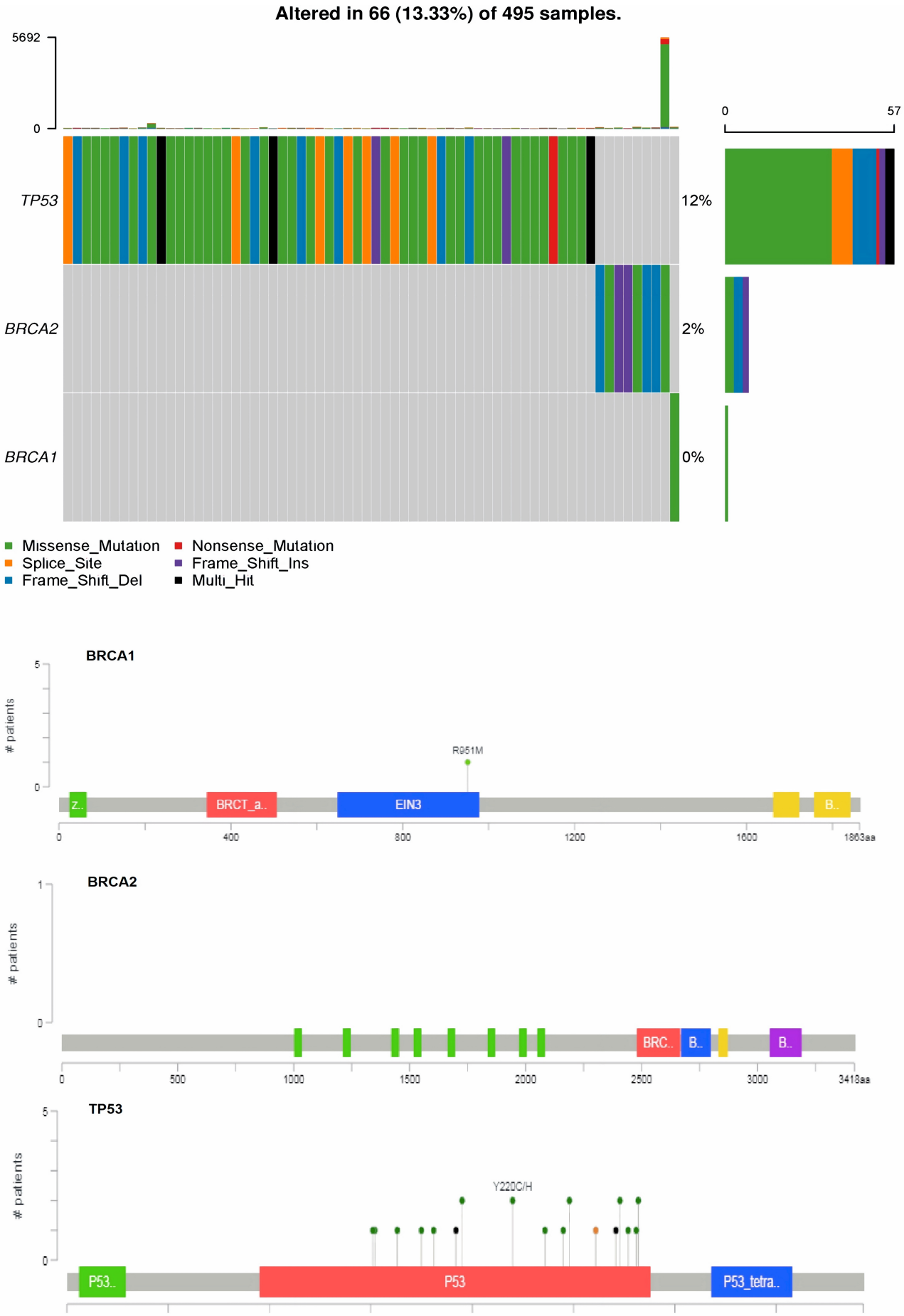

Figure 1. Assessment of mutations in *BRCA1/2* and *TP53* genes among prostate cancer patients using the cBioPortal resource within The Cancer Genome Atlas (TCGA) dataset.

3.5 Functional Consequence Analyses of the Observed Pathogenic Mutations

3.5.1 RT-qPCR Analysis

In this phase of the research, prostate cancer tissue samples were categorized into two distinct groups. The first group comprised nine prostate cancer samples with documented pathogenic mutations in *BRCA1/2* and *TP53* genes, while the second group encompassed 36 prostate cancer samples lacking such pathogenic mutations in these genes. Subsequently, an expression analysis of *BRCA1/2* and *TP53* genes was conducted in these two segregated cohorts, utilizing RT-qPCR. This comprehensive analysis revealed a compelling finding: the initial group of prostate cancer samples, having pathogenic mutations in *BRCA1/2* and *TP53* genes, exhibited a noteworthy down-regulation of these genes in comparison to the second group (Figure 2A).

Moreover, according to the ROC analysis of the *BRCA1/2* and *TP53* genes, it was observed that these genes have suitable diagnostic performance (AUC = 0.778, 0.773, and 0.771) (Figure 2B).

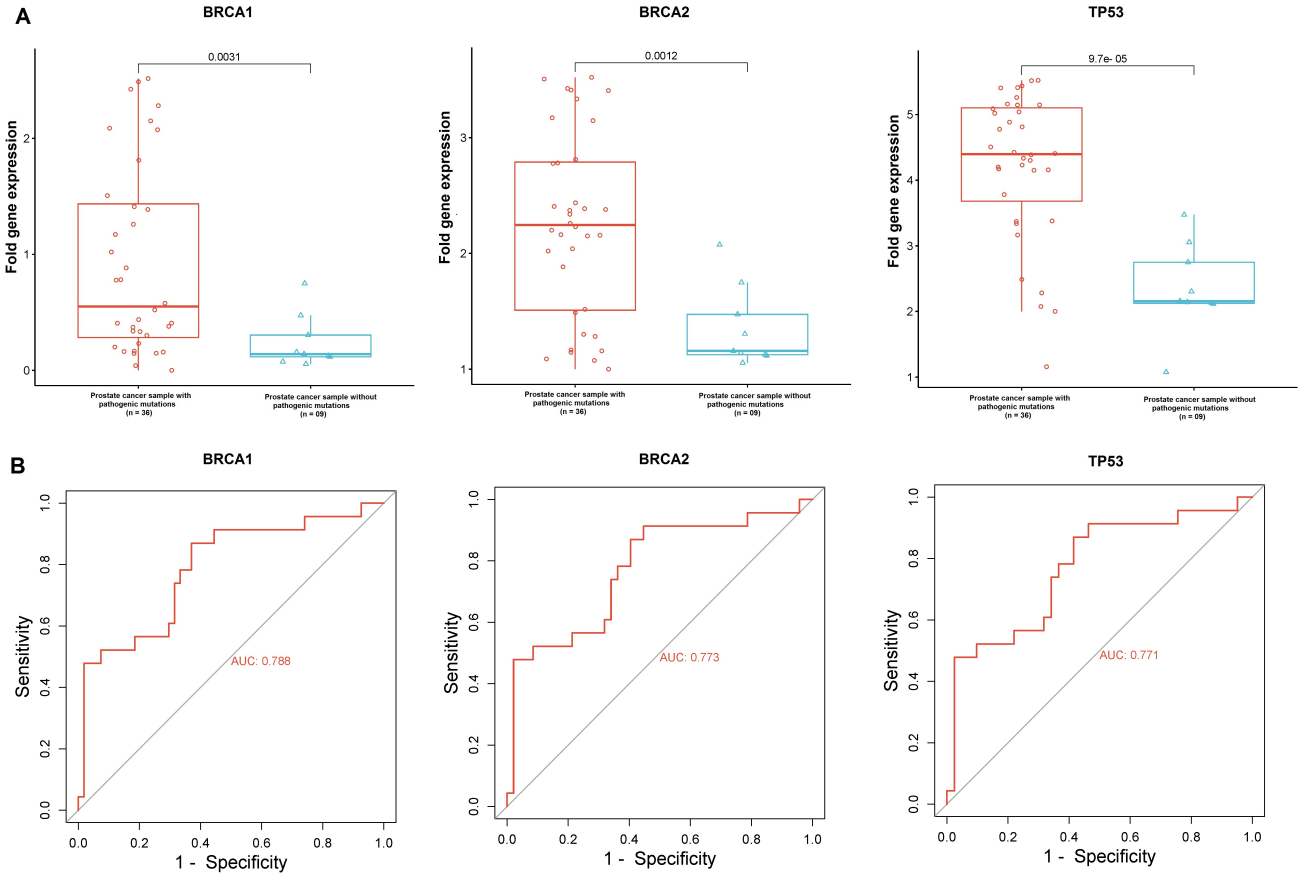

Figure 2. RT-qPCR and ROC analysis of the *BRCA1/2* and *TP53* genes among prostate cancer patients. (A) RT-qPCR-based expression of the *BRCA1/2* and *TP53* genes in two distinct groups of the prostate cancer patients. (B) RT-qPCR-based ROC analysis of the *BRCA1/2* and *TP53* genes. A p-value less than 0.05 was considered as significant.

3.5.2 Immunohistochemistry (IHC) Analysis

Next, the IHC analysis of *BRCA1, BRCA2*, and *TP53* proteins in two representative prostate cancer tissue samples was conducted. These samples were carefully chosen to represent two distinct genetic profiles: one with pathogenic mutations in the *BRCA1, BRCA2,* and *TP53* genes, and the other without mutations in these genes. IHC analysis revealed significant differences in the staining intensities of *BRCA1, BRCA2*, and *TP53* proteins between the two tissue samples. Notably, the tissue sample with pathogenic mutations in these three genes exhibited lower staining intensities compared to the counterpart sample, which lacked these mutations (Figure 3). These results collectively demonstrate a clear association between the presence of pathogenic mutations in *BRCA1, BRCA2, and TP53* genes and a reduction in the expression of their respective proteins in prostate cancer tissue.

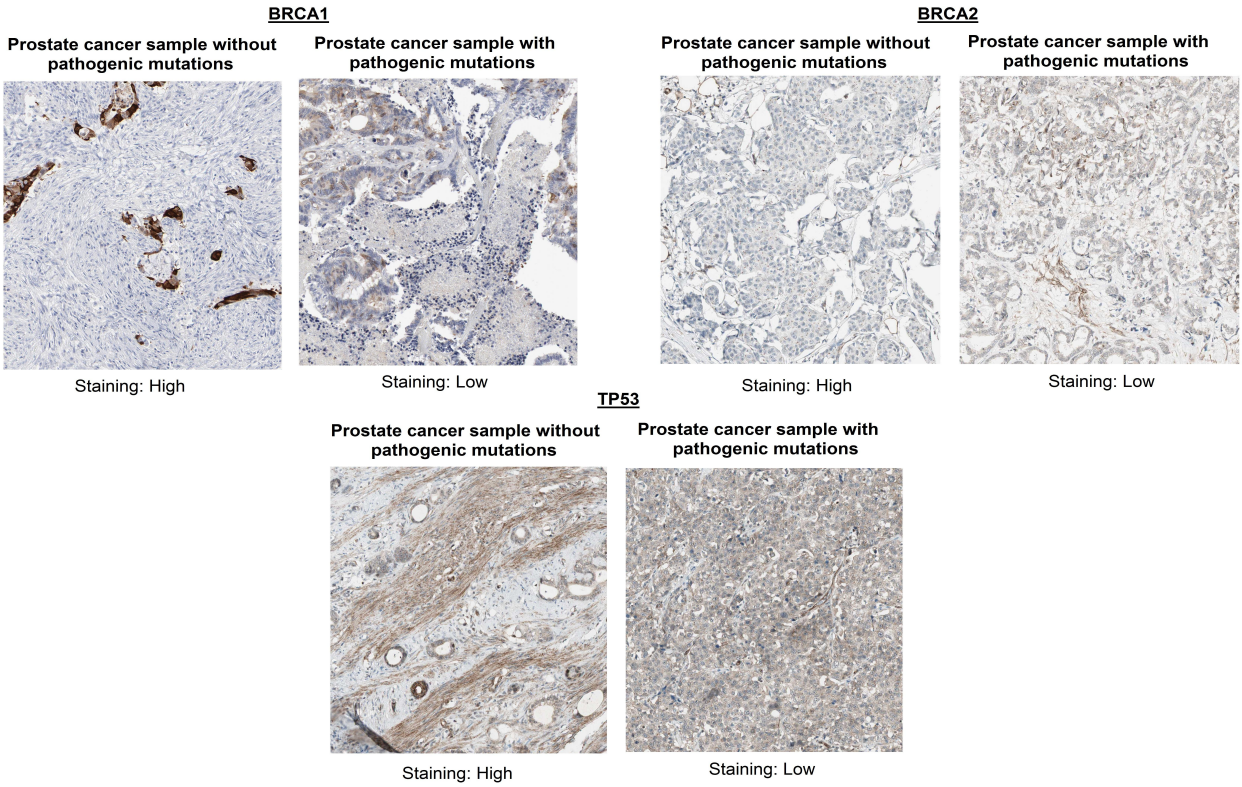

Figure 3. Immunohistochemical-based evaluation of *BRCA1/2* and *TP53* proteins expression in two different kinds of prostate cancer tissue samples. Expression differences were measured based on the staining intensities.

3.5.3 Gene Enrichment Analysis

Next, this study performed GO and KEGG enrichment analyses. Among GO, *BRCA1, BRCA2,* and *TP53* genes were enriched in "*BRCA1-BARD1* complex, lateral element, *BRCA1*-A complex, and beta-catenin destruction complex" etc., CC terms (Figure 4A), "Histone acetyltransferase regulator activity, H3 histone acetyltransferase activity, TFIID-class transcription factor complex binding, and *MDM2/MDM4* family protein binding" etc., MF terms (Figure 4B), "Response to UV-C, DNA damage response, signal transduction by p53 class mediator in tran, Response to X-ray, and Neg. reg. of DNA replication etc., BP terms (Figure 4C), and "Homologous recombination, fanconi anemia pathway, endometrial cancer, basal cell mcarcinoma, platinum drug resistance in cancer, and breast cancer" etc., KEGG terms (Figure 4D).

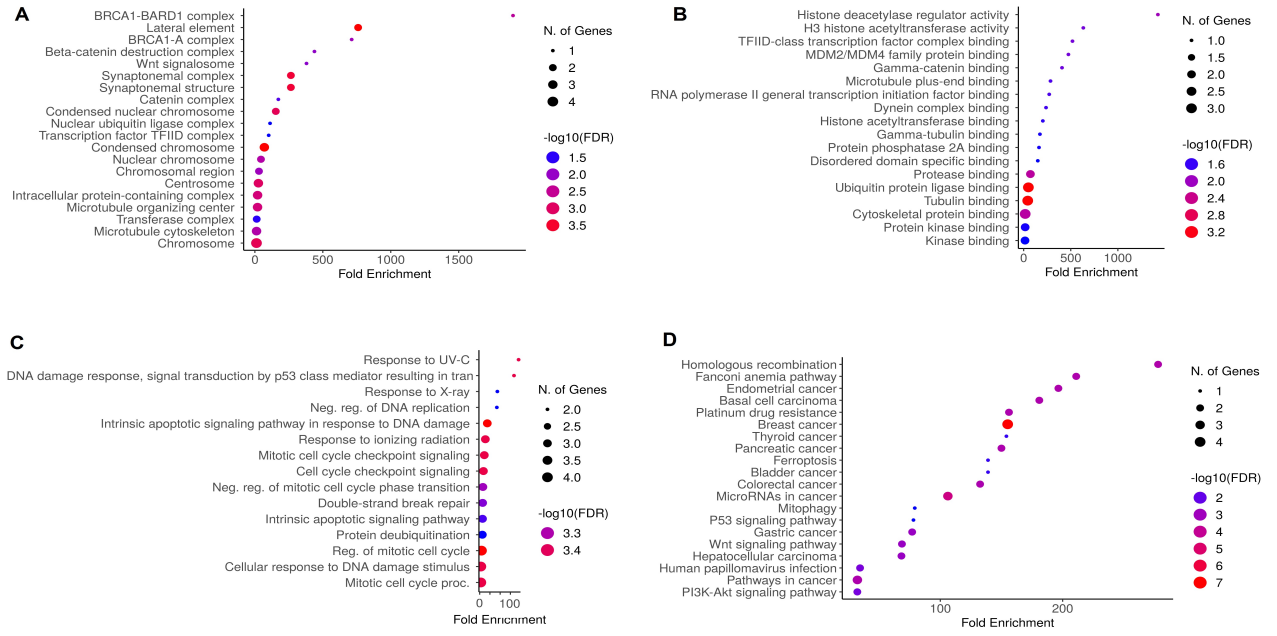

Figure 4. GO and KEGG analyses of *BRCA1/2* and *TP53* genes via Metascape. (A) *BRCA1/2* and *TP53* genes-related CC terms, (B) *BRCA1/2* and *TP53* genes-related MF terms, (C) *BRCA1/2* and *TP53* genes-related BP terms, and (D) *BRCA1/2* and *TP53* genes-related KEGG terms. A $P < 0.05$ was used as the cut-off criterion.

3.5.4 Drug Prediction Analysis

In the drug prediction analysis, the DrugBank database was explored to identify drugs with the potential to modulate the expression of *BRCA1, BRCA2,* and *TP53* genes at the mRNA level for the treatment of prostate cancer. Analysis results yielded a total of 14 drugs (Cisplatin, Estradiol, Tretinoin, Genistein. Acetaminophen, Quercetin, Resveratrol, Metribolone, Genistein, Teriflunomide, Berberine, Bleomycin, Celecoxib, and Cyclosporine) that exhibited the capability to enhance the expression of *BRCA1, BRCA2,* and *TP53* genes at the mRNA level, with the intention of potentially serving as treatment options for prostate cancer (Table 3). These findings are significant as they provide potential therapeutic options for prostate cancer patients with genetic alterations in *BRCA1, BRCA2,* and *TP53* genes. Enhancing the expression of these genes at the mRNA level may contribute to the restoration of normal cellular functions, DNA repair mechanisms, and cell cycle regulation. It is important to note that further research, including preclinical and clinical studies, is necessary to validate the efficacy and safety of these drugs in the context of prostate cancer treatment.

Table 3. DrugBank-based *BRCA1/2* associated drugs

Sr. No	Hub gene	Drug name	Effect	Reference	Group
1	*BRCA1*	Cisplatin	Increase expression of BRCA1 mRNA	A22234	Approved
		Estradiol		A21155	
		Tretinoin		A24464	
		Genistein		A22773	
2	*BRCA2*	Acetaminophen	Increase expression of BRCA2 mRNA	A20418	Approved
		Quercetin		A21498	
		Estradiol		A21155	
		Resveratrol		A23885	
		Metribolone		A23234	
		Genistein		A22773	
3	*TP53*	Teriflunomide	Increase expression of TP53 mRNA	A20413	Approved
		Berberine		A21082	
		Estradiol		A21155	
		Bleomycin		A21429	
		Celecoxib		A21562	
		Cyclosporine		A21092	

4. Discussion

Cancer is characterized by the accumulation of genetic mutations that confer a growth advantage to affected cells [29]. In the case of prostate cancer, several genetic alterations have been identified, but mutations in *BRCA1/2, ATM, CHEK2, PALB2, RAD51C, RAD51D, NBN, CDH1, TP53, MLH1, MSH2, MSH6, and PMS2* stand out due to their profound implications. These genes are well-established tumor suppressors, and their dysfunction can lead to unchecked cell proliferation, impaired DNA repair, and genomic instability, creating an environment conducive to cancer initiation and progression [30-32]. Several studies have emphasized the importance of these genes in various cancer types, making them prime candidates for investigation in prostate cancer [32-35].

This study utilized the TruSight Sequencing Cancer Panel with an Illumina MiSeq platform to scrutinize genetic mutations in *BRCA1/2, ATM, CHEK2, PALB2, RAD51C, RAD51D, NBN, CDH1, TP53, MLH1, MSH2, MSH6,* and *PMS2* associated with an increased risk of prostate cancer. In addition, the functional consequences of the observed pathogenic mutations were analyzed through additional molecular experiments. Results of the study revealed a total of 29 mutations within the examined prostate cancer cohort, distributed across four key genes: *BRCA1/2, TP53,* and *PMS2*. Within the *BRCA1* gene, ten mutations were found, comprising nine benign and one pathogenic mutation. The *BRCA2* gene yielded eight mutations, with two being pathogenic and six benign. *TP53* exhibited eight mutations, with one being pathogenic and seven being benign mutations. In *PMS2*, three mutations were identified, all of which were of a benign nature The presence of pathogenic mutations in *BRCA1/2* and *TP53* genes is of particular interest due to their well-established roles in DNA repair, cell cycle regulation, and tumor suppression. Pathogenic mutations in these genes can compromise their tumor-suppressive functions, potentially leading to the development and progression of prostate cancer, as explained by previous studies [36-39]. The high prevalence of benign mutations in *BRCA1/2* and *TP53* genes is also noteworthy. Although these mutations may not directly contribute to prostate cancer development, their presence highlights the genetic complexity of this disease.

RT-qPCR and IHC analyses further revealed that prostate cancer samples having pathogenic mutations in *BRCA1/2* and *TP53* genes, exhibited a noteworthy down-regulation of these genes at mRNA and protein levels in comparison to the other prostate cancer sample group lacking pathogenic mutations in these genes. The down-regulation of *BRCA1, BRCA2,* and *TP53* genes is known

to significantly contribute to the development of cancer by compromising fundamental cellular processes critical for genome integrity and tumor suppression [40,41]. As *BRCA1* and *BRCA2* are pivotal for DNA repair mechanisms, particularly homologous recombination [42-44], which is essential for maintaining genomic stability [43], the reduced expression or functional impairment of *BRCA1/2* can lead to the accumulation of DNA damage, creating an environment conducive to oncogenesis. *TP53*, on the other hand, is a master regulator of cell cycle progression and apoptosis [45-47]. The down-regulation can result in unchecked cell growth, impaired cell cycle control, and the evasion of apoptosis, allowing damaged cells to proliferate, a hallmark of cancer development. Overall, the down-regulation of these genes due to pathogenic mutations collectively can lead to a cascade of events contributing to the initiation and progression of prostate cancer.

Pathway analysis highlighted that *BRCA1/2* and *TP53* genes were involved in some important pathways, including Homologous recombination, fanconi anemia pathway, endometrial cancer, basal cell mcarcinoma, platinum drug resistance in cancer, and breast cancer" etc. The dysregulation of these pathways is already well-acknowledged in cancer development by previous studies [48-50].

Additionally, a drug prediction analysis was conducted to identify potential therapeutic agents capable of enhancing the mRNA expression of BRCA1, BRCA2, and TP53 genes. Utilizing the DrugBank database, which provides detailed information on drug mechanisms and interactions, the analysis revealed a total of 11 drugs with the potential to up-regulate the expression of these genes at the mRNA level. These findings are of significant clinical importance, as they provide potential therapeutic options for prostate cancer patients with genetic alterations in *BRCA1, BRCA2*, and *TP53* genes. Enhancing the expression of these genes at the mRNA level offers the possibility of restoring normal cellular functions, improving DNA repair mechanisms, and reestablishing proper cell cycle regulation, all of which are frequently disrupted in cancer [51,52]. However, all the drugs identified in this study warrant further investigation to determine their potential benefits in the management of prostate cancer with genetic mutations in these genes.

While this study showcased notable strengths, such as its comprehensive analysis of mutations in key prostate cancer susceptibility genes and the integration of diverse analytical approaches, it's imperative to acknowledge the inherent limitations that temper the interpretation and generalizability of the findings. First and foremost, the study's relatively small sample size of 45 patients warrants caution in extrapolating the results to broader populations. Larger cohorts would provide more robust data and strengthen the reliability of the conclusions. Furthermore, the study's mono-centric design introduces a potential bias inherent in single-center studies, limiting the diversity and representativeness of the patient population. Collaborative efforts across multiple centers could mitigate this limitation and enhance the generalizability of the findings.

Another important limitation lies in the focus on known susceptibility genes, which may have led to the oversight of mutations in other genes or pathways relevant to prostate cancer development. Expanding the scope of genetic analysis to include a broader spectrum of genes associated with cancer predisposition could uncover novel biomarkers and therapeutic targets. Additionally, while the study employed RT-qPCR and immunohistochemistry to assess the functional consequences of observed mutations, further functional validation through diverse experimental assays, such as cell-based assays or animal models, would provide deeper insights into the biological significance of these mutations.

Despite these limitations, the study's strengths lay in its multidimensional approach to dissecting the genetic landscape of prostate cancer. By leveraging next-generation sequencing technology, bioinformatics analysis, molecular experiments, and drug prediction analysis, the study provided a comprehensive understanding of genetic alterations and their potential implications in prostate cancer biology. Notably, the identification of pathogenic mutations in known susceptibility genes and the exploration of their frequencies in Asian prostate cancer patients offer clinically relevant insights that could inform personalized treatment strategies.

Moving forward, future research endeavors should focus on validating the observed mutations in larger and more diverse cohorts, exploring additional genetic alterations beyond known susceptibility genes, conducting further functional characterization using various experimental assays, and translating these discoveries into clinical practice through prospective studies and clinical trials. By addressing these limitations and pursuing these future directions, the understanding of prostate cancer genetics can be advanced, novel therapeutic targets can be identified, and patient outcomes can be ultimately improved in the fight against this disease.

5. Conclusion

In conclusion, this study provides valuable insights into the genetic landscape of prostate cancer and its clinical implications. Through the application of next-generation sequencing technology and comprehensive bioinformatics analysis, mutations in key prostate cancer susceptibility genes, including *BRCA1, BRCA2, TP53*, and *PMS2*, among others, were identified. While the study cohort was relatively small and mono-centric, the detection of pathogenic mutations in these genes underscores their potential role in prostate cancer development and progression. Importantly, findings of this study reveal a notable absence of certain pathogenic mutations in Asian prostate cancer patients, highlighting the need for further investigation into the genetic diversity of prostate cancer across different populations.

Functional analysis using molecular experiments and drug prediction analysis provided additional insights into the biological significance of observed mutations and potential therapeutic avenues. Notably, the down-regulation of *BRCA1, BRCA2*, and *TP53* genes in prostate cancer samples with pathogenic mutations underscores their relevance as diagnostic markers and therapeutic targets. The identification of drugs capable of modulating the expression of these genes offers promising avenues for personalized treatment approaches in prostate cancer patients with specific genetic alterations.

Despite the study's limitations, including its small sample size and mono-centric design, the findings of this contribute to a deeper understanding of the molecular mechanisms underlying prostate cancer.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Ethical Approval

This study was approved by the Institutional Review Board of Nishtar Medical University (Reference # 2038) and adhered to the Declaration of Helsinki. Written informed consent was obtained from all participants, ensuring voluntary involvement. Patient data and tissue samples were anonymized to protect privacy, with stringent data security measures in place.

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