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THE EFFECT OF IDH2 GENE VARIATION AND TERT GENE VARIATION IN PROSTATE CANCER

DOCTOR OF PHILOSOPHY THESIS

FERDA OZKAN, M.D.

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APPROVAL

This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated ..... and numbered .....

Prof. Dr. Bayram YILMAZ

Director of Institute of Health Sciences

DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for an award of any other degree except where due acknowledgment has been made in the text.

06/06/2023

Ferda Ozkan

DEDICATION

I dedicate my thesis to my lovely Family

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Ferda Ozkan

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

aKG	Alpha Ketoglutarate
ATRX	X-linked helicase II
CST	Charge Switch Technology
D2HG	D-2-Hydroxyglutarate
DNA	Deoxyribonucleic Acid
EGFR	Epidermal Growth Factor Receptor
IDH	Isocitrate Dehydrogenase
MGMT	O6-Methylguanine-DNA Methyltransferase
miRNA	MicroRNA
NADP+	Oxidised nicotinamide adenine dinucleotide phosphate
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
NGS	Next-Generation Sequencing
OD	Optical Density
PCR	Polymerase Chain Reaction
PSA	Prostate-Specific Antigen
PTEN	Phosphatase and tensin homolog
RB	Retinoblastoma
TERT	Telomerase Reverse Transcriptase

ABSTRACT

Ozkan, F. (2023). The Effect of Isocitrate Dehydrogenase 2 (IDH2) Gene Variation and TERT Gene Variation in Prostate Cancer. Yeditepe University, Institute of Health Sciences, Department of Molecular Medicine, PhD Thesis, Istanbul.

Worldwide, prostate cancer is the leading cause of death from cancer in males. It is still a chemotherapy-resistant disease with a highly metastatic potential, despite its pathological classification and the availability of numerous treatments because its molecular foundation has not yet been completely understood. The primary objective of this research was to use next-generation sequencing technology to identify variations in the IDH2 and TERT target genes in prostate cancer. For the study, blood samples from 27 patients at Yeditepe University Hospital who had been identified with prostate cancer were used. The mean age of the study population was 68 years of age; 88.89% of them were 60 years and older, and 11.11% were individuals under 60 years of age. The mean prostate volume and PSA level for the patient with prostate cancer were 43.59 cc and 31.28 ng/mL, respectively, according to clinical data. It was determined that there were fourteen patients with a Gleason score of less than seven and thirteen patients with a Gleason score of seven or higher.

The IDH2 and TERT genes were examined using next-generation sequencing and five mutations were identified. One of these mutations was discovered in the IDH2 gene and four others within the TERT gene. One variant detected in IDH2 was seen in 2 heterozygous patients. Four variants located in the exonic region of the TERT gene have been identified. Of these, c.3039C>T mutation was heterozygous in 6 patients, c.1392 C>T heterozygous in 1 patient, c.915G>A in 18 patients, 4 of which were homozygous and 14 heterozygous, and c.261G> T was heterozygous in one prostate cancer patient. Among these five variants, TERT c.261G>T was identified as potentially harmful. It was confirmed by Sanger sequencing that the patient carrying the potentially harmful variant was heterozygous mutant. The five variations we found in our study hold a potential to serve as new prostate cancer biomarkers, and great majority of the mutations have not been associated with this cancer type. It will help to rearrange pathological categorization by explaining the molecularly inexplicable characteristics of prostate cancer in accordance with further validation of our findings with studies to be conducted with a larger sample size.

Key Words: Next Generation Sequencing, Prostate Cancer, IDH2, TERT

ÖZET

Özkan, F. (2023). IDH2 ve TERT Gen Varyasyonlarının Etkisinin Prostat Kanserinde Araştırılması. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, Moleküler Tıp ABD., Doktora Tezi. İstanbul.

Prostat kanseri dünya genelinde en yaygın görülen kansere bağlı ölümlerin başında gelmektedir. Patolojik sınıflandırmanın ve çok sayıda tedavi seçeneğinin varlığına rağmen, moleküler alt yapısı tam olarak aydınlatılamamış olması nedeniyle, hala yüksek metastatik yapıya sahip, kemoterapi direnç bir hastalıktır. Prostat kanserinde yeni nesil dizileme yöntemini ile IDH2 ve TERT hedef genlerinde varyasyonların varlığını saptamak bu çalışmanın ana hedefidir. Yeditepe Üniversitesi Hastanesi'nde prostat kanseri tanısı alan yirmi yedi hastanın kan örnekleri analizler için kullanılmıştır. Çalışma populasyonun yaş ortalaması 68 olup, %88.89'u 60 yaş ve üzeri iken %11.11'i 60 yaşın altı bireylerdir. Hasta grubunun klinik verileri incelendiğinde, ortalama prostat hacmi 43.59 cc, PSA seviyesi 31.28 ng / mL, Gleason skoru yedi ve üzerinde olan on üç hasta ve yediden küçük on dört hasta olduğu belirlenmiştir.

Yeni nesil dizileme ile IDH2 ve TERT genleri incelenmiş ve beş varyasyon tespit edilmiştir. Bu varyasyonların 1'i IDH2 geninde, 4'ü ise TERT geninde saptanmıştır. IDH2'de tespit edilen 1 varyant heterozigot 2 hastada görülmüştür. TERT geninde ise ekzonik bölgede yer alan dört varyant tespit edilmiştir. Bunlardan c.3039C>T varyantı heterozigot 6 hastada, c.1392C>T varyantı heterozigot 1 hastada, c.915G>A varyantı 4'ü homozigot ve 14'ü heterozigot olmak üzere toplam 18 hastada, c.261G>T varyantı ise heterozigot bir hastada belirlenmiştir. Bu beş varyant arasında klinik önemi belirsiz olarak belirlenen varyant muhtemel zararlı olarak tanımlanmıştır. Muhtemel zararlı olduğu belirlenen bu varyantı taşıyan hastanın TERT c.261G>T heterozigot mutant olduğu Sanger sekanslaması ile teyit edilmiştir. Çalışmamızda tespit edittiğimiz 5 varyant prostat kanseri için yeni biyobelirteç niteliği taşımakta olup, mutasyonların büyük çoğunluğu bu kanser türünde gösterilmemiştir. Verilerimizin daha geniş örneklem ile gerçekleştirilecek araştırmalar ile doğrulanması sonucunda, prostat kanserinin moleküler düzeyde aydınlatılamamış noktalarının açıklığa kavuşmasını sağlayarak patolojik sınıflandırmanın yeniden düzenlenmesine katkı sunacaktır.

Anahtar Kelimeler: Yeni Nesil Dizileme, Prostate Kanseri, IDH2, TERT

1. STATEMENT AND PURPOSE

One of the leading causes of cancer-related mortality among men globally is prostate cancer (1). Despite its well-defined pathological classification, biochemical parameters, and the existence of multiple therapies, it is still a chemotherapy-resistant malignancy with a high risk of metastasis since the current understanding of its molecular basis is still limited.

Prostate cancer, which primarily affects older males, is the second-ranking cancer in the globe (2). Age, race, and family history are among the factors associated with the development of prostate cancer; for instance, this is a disease that primarily affects older males. The age-adjusted incidence rate of prostate cancer in Turkey is 35 cases per 100,000 people (3-5). Prostate cancer is one of the most curable cancers in the world when detected in its early stages, but when it progresses to a deadly state, tumors become androgen resistant. Although research on prostate cancer and its evolution has been going on for a while, the processes behind prostate cancer are still a significant medical conundrum (6).

In recent years, with the developing technology such as next generation sequencing, molecular evaluation of prostate cancer has become increasingly common in the staging of the disease and in the planning of treatment strategies. Epidemiological and genome-wide research results have identified and linked mutations in different genes with prostate cancer (7, 8).

The three isoforms of the enzymes called isocitrate dehydrogenase (IDH) are essential to accurately pursue many crucial metabolic activities, including the Krebs cycle (9-11). One of these isoforms, IDH2, resides in the mitochondrial matrix and, like IDH1, contains a catalytic site with an affinity for isocitrate (12, 13). As a result of the catalytic activity of wild-type IDH2, its substrate, isocitrate is converted to alpha-ketoglutarate (13). Mutated IDH2 exhibits neomorphic activity, leading to the conversion of alpha-ketoglutarate (aKG) into D-2-Hydroxyglutarate (D2HG). The accumulation of D2HG in cells at the supraphysiological level is a kind of result of this activity (14-18). Although IDH mutations are among the common mutations in human cancers, they are rare to detect in a few types, including prostate cancer (18-24).

One of the most well-known characteristics of malignancies is the immortality of the cells, and one of the essential mechanisms in carcinogenesis is telomerase reactivation, which results in telomerase maintenance (25). Although telomerase reactivation has been linked to the development of human malignancies, the underlying mechanisms are still not fully understood. The catalytic part of the telomerase complex, which exhibits RNA-dependent DNA polymerase activity, oversees telomere elongation and is called telomerase reverse transcriptase (TERT) (26).

Changes in the coding region of the TERT gene have been identified as rare events in cancers. On the other hand, mutations leading to novel binding motifs within the TERT gene's core promoter are of great interest in cancer research, due to the identification of multiple malignancies and their association with higher in vitro transcriptional activity (27, 28). In addition, it has been reported that prostate cancer patients with TERT expression have a worse prognosis and a higher risk of recurrence. For this reason, it is one of the genes that should not be ignored (29, 30).

Next-Generation Sequencing is a method often used to investigate pre-existing mutations and uncover new findings in malignancies, including prostate cancer (31). This doctoral thesis put forth an effort to highlight IDH2 and TERT variations and their existance in prostate cancer by this method. Given the roles of the IDH2 and TERT genes we analyzed in prostate cancer, we anticipate that this study will add to the scientific literature by offering a new viewpoint.

2. LITERATURE REVIEW

2.1. Molecular Pathology of Prostate Cancer

The second most common malignancy among males is prostate cancer. It is a diverse illness that can range from being asymptomatic to having a systemic cancer that is swiftly deadly (32).

Unfortunately, despite the rapidly advancing flow of information regarding better treatment for prostate cancer, the development of new treatment modalities is not in line with the speed of information flow. Depending on the clinical situation, different therapeutic approaches are needed. Decisions regarding two different options for patients with newly diagnosed prostate cancer that is localized are largely influenced by tumor-related factors, such as the size of the tumor, the histological subtype, the Gleason grade, and the level of serum prostate-specific antigen (PSA) (33).

Since its introduction as a marker, there has been an increase in the number of men diagnosed with very early-stage prostate cancer with the widespread use of prostate-specific antigen (PSA) testing. Both healthy and malignant prostate cells generate the PSA protein, and a high PSA level may indicate malignancy (34). Finding characteristics that may precisely predict how prostate cancer will behave in a particular patient is an uphill battle. Treatment plans for prostate cancer are determined by the clinical stage and histological diagnosis of biopsies, including the Gleason grade determined by pathologists (35). The best prognostic indicator for prostate cancer to date is Gleason grading obtained from a histopathological examination (34).

Prostate cancer has a complicated molecular pathophysiology, with numerous genes and other environmental variables contributing to its development. The development of new research techniques such as gene sequencing technologies in the study of human malignancies reveal the responsible genes and illuminate the disease at the molecular level. Research into the genetic alterations underlying the onset, growth, and advancement of prostate cancer is moving forward quickly (34).

Hereditary and sporadic types of prostate cancer can be distinguished from one another epidemiologically, however these two categories are impossible to differentiate at the molecular level. There are currently no known highly penetrant hereditary genes that cause prostate cancer (36). Despite the discovery of putative inherited prostate cancer susceptibility genes such as the cell cycle checkpoint genes, and infection genes in some families, the percentage of cases of hereditary prostate cancer caused by germline

mutations in these genes remains low (34). Prostate cancer is sporadic for the most part (34). In terms of the molecular pathology of sporadic prostate cancer, an increased risk of developing the disease has been connected, to date, to factors that contribute to prostate cancer progression, such as polymorphisms and somatic genetic alterations (34).

It is known that changes in these genes can provide information about the course of prostate cancer, apart from determining whether prostate cancer is hereditary or sporadic (33).

Prostate carcinogenesis involves a significant number of genetic regions, and the mechanisms are intricate and poorly understood (37, 38).

TLR4, CDKN1B, and AR gene polymorphisms have been linked to an increased risk of prostate cancer, while chromosomal loss and gain have been shown to occur often in the epidermal growth factor receptor (EGFR), phosphatase and tensin homologue (PTEN), c-myc, and retinoblastoma (Rb) genes (34).

Among the commonly altered genes in prostate cancer, the tumor suppressor gene PTEN is one of the most common. The inactivation of this gene is twice more common in metastatic prostate cancers than organ-confined ones (39, 40). PTEN loss is a suitable prognostic biomarker since it has a strong correlation with both clinical findings and deleterious pathogenic factors (40). Not just in prostate cancer but in many other human malignancies, c-myc and bcl-2 are well-known and significant oncogenes. Numerous studies have shown that myc expression is elevated in prostate cancer and that there is a strong link between myc overexpression and Gleason grade (34).

Morover, the length of the telomere in prostate cancer, has been observed to be noticeably shorter than in a healthy prostate. Telomeres stabilize and safeguard the ends of chromosomes, although they get shorter due to cell division as well as oxidative stress. Having chromosomal instability due to chromosome fusions, subsequent breaking, and rearrangement can raise the risk of developing cancer. Critically short telomeres have been found to do this in the presence of weakened DNA damage checkpoints (34, 41). By incorporating repeating telomeric sequences onto chromosomal ends, telomerase keeps telomere length constant. Contrary to the normal prostate epithelium, most prostate tumors have telomerase activity (42, 43).

MicroRNAs (miRNAs) can be identified as targets or therapeutic agents in prostate cancer illness, so their functional role goes beyond that of serving as diagnostic or prognostic indicators. The therapeutic potential of these drugs has also been the subject of numerous investigations. Since miRNAs control a wide range of target genes, their abnormal expression offers a possible treatment benefit for restoring normal gene expression (44).

There are several miRNAs that have been linked to prostate cancer (45). Prostate cancer was discovered to have elevated levels of the miRNA -130b/ miRNA -301b complex (46). The level of miRNA-301b-3p expression in prostate tumor tissue was found to be significantly higher than in surrounding healthy tissue (47). Because of this, targeting miR-301b will be a wise course to follow in the treatment of prostate cancer. Up-regulating miRNA-340 and miRNA-361-5p might be beneficial, as they function as tumor suppressors in prostate cancer, and are a potential strategy for future therapeutic development (45).

In a research study using more than two hundred miRNAs from prostate cancer patients, Wang et al. discovered a correlation between Gleason score and miRNA transcripts. By means of the study, hot spots were identified. Morover, prostate cancer patients with high Gleason had decreased miRNA -331-3p and miRNA -145 expression (48). There are also miRNAs specific to liquid samples such as plasma or serum. These are associated with high and low Gleason scores and mediate their differentiation.

MicroRNA content molecular analysis gives excellent opportunities for understanding the processes of cancer development. Additionally, these analytes have significant promise for the earlier identification of prostate cancer, for more precise prostate cancer staging, and the choice of strategies for treatment (49).

2.2. IDH2 and TERT Genes in Prostate Cancer

2.2.1. Isocitrate dehydrogenases 2 (IDH2)

Isocitrate dehydrogenase 1 (IDH1), IDH2, and IDH3 are the three isoenzymes of IDH found in mammalian cells (50). The position of IDH2 is on chromosome 15q26.1 (51). Isocitrate is reversibly converted to KG in the mitochondria by the homodimeric enzyme IDH2, which also simultaneously reduces NADP+ to NADPH (Figure 1) (52). NADPH and KG both have detoxifying abilitiy and play important roles in cellular detoxification processes (53). IDH2 is able to convert KG to isocitrate in hypoxic

conditions (54). Almost 60 known KG-dependent enzymes may be competitively inhibited by the accumulating D-2-Hydroxyglutarate (D2HG), leading to cellular changes in epigenetics (55).

IDH1 and IDH2 mutations, among other causal molecular abnormalities, have been found in different types of malignancies during the past decade using genome-wide sequencing of tumors in humans (56).

Many cellular processes are inhibited by IDH2 mutations, however many of these might not be significant for oncogenesis (57). On the otherhand, a reduction in NADPH generation due to the absence of one wild-type IDH2 means that the capacity of the cells to resist oxidative stress is decreased (58). IDH2 mutations have been clearly characterized in gliomas and have been found at various frequency in other cancers, including prostate cancer (59). When IDH mutations are evaluated specifically for prostate cancer, it is seen that they cannot be detected frequently in this disease. IDH2 mutations that are related with cancer nearly always occur at specific arginine residues in the active regions of the enzyme. IDH2R140Q or IDH2R172K substitutions are the most common missense mutations in the IDH2 codon for Arg140 or Arg172.

IDH2 mutations that have been found have particular missenses in conserved codons. Mutations are heterozygous for the wild-type allele and do not cause premature termination or frameshifts (60).

The wild-type IDH activity of the enzymes is impaired by cancer-associated IDH1/2 mutations, but they also enable new enzymatic capabilities to convert a-ketoglutarate to 2-hydroxyglutarate, which fosters DNA hyper-methylation and increased angiogenesis (61).

The importance of identifying mutant IDH1 and IDH2 enzymes as targets for unique and innovative cancer therapeutics was underlined by Yen et al. (62). The localized prostate cancer, which exhibits high variability in genomic stability, malignant cells metabolism, and clinical prognosis, has been found to include IDH1/2 mutations (63). IDH1 and IDH2 mutation-related cancers are increasingly showing excessive epigenetic changes, which may affect the differentiation of stem cells and ultimately lead to carcinogenesis. IDH1 and IDH2 mutations are useful indicators and possible pharmacological targets in therapeutics because of their distinctive characteristics (64).

It is also known that certain gene mutations are accompanied by IDH mutations. Mutations in IDH1/2 and TERT (65), IDH2, and miRNA-142 are examples that may work to affect the development of cancer (66).

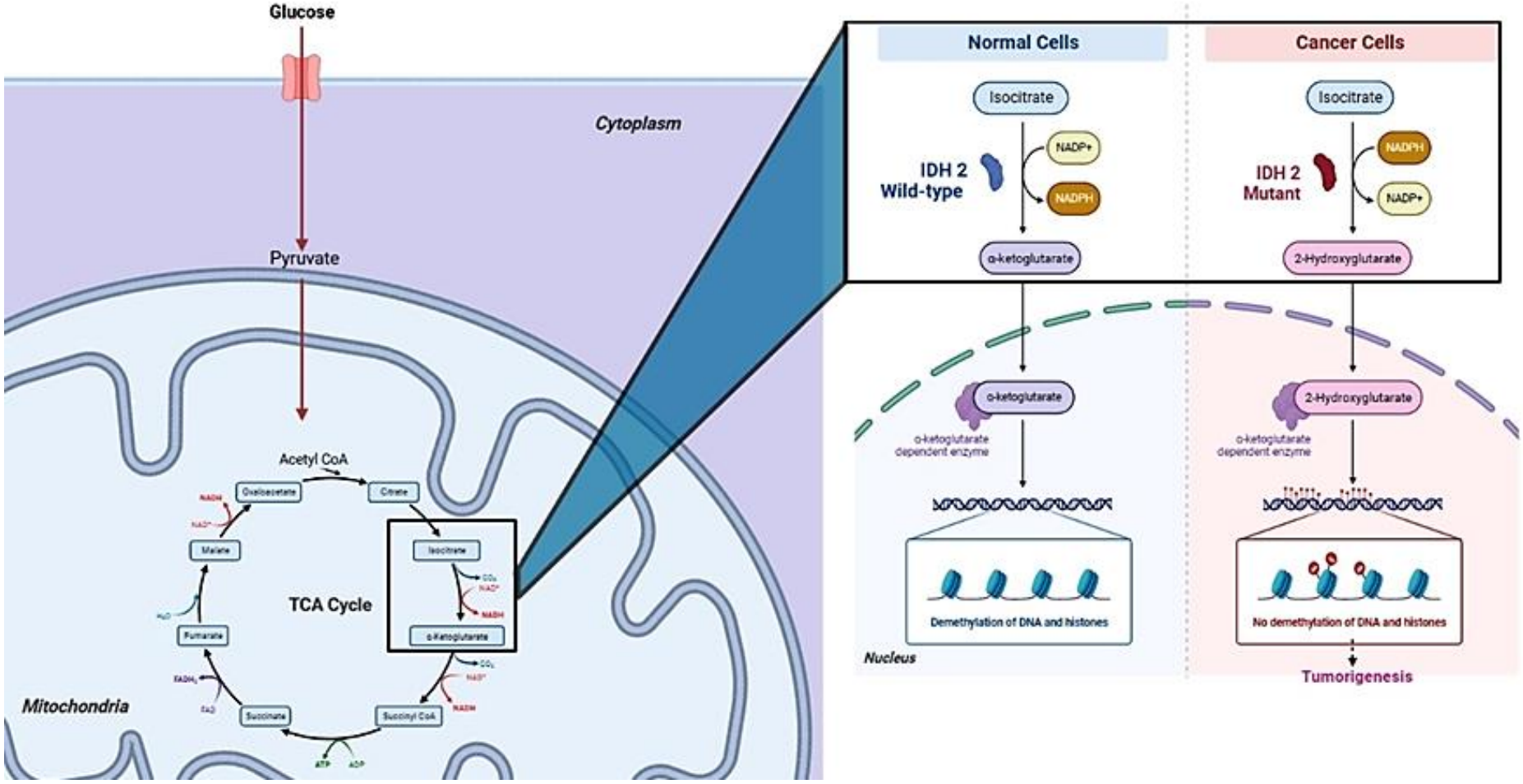

Figure 1. Role of wild-type and mutant forms of the IDH2 and its possible effects in normal and cancer cells.
IDH, isocitrate dehydrogenase; NADP+, oxidised nicotinamide adenine dinucleotide phosphate; NADPH, reduced nicotinamide adenine dinucleotide phosphate; TCA, tricarboxylic acid (Created with BioRender).

2.2.2. Telomerase Reverse Transcriptase (TERT)

A ribonucleoprotein enzyme called telomerase encloses DNA with the 5'-TTAGGG-3' DNA repetitive pattern at the end of human chromosomes (67). The major function of this enzyme is to protect chromosome ends intact (67). Telomerase reverse transcriptase is one of the key catalytic components of this enzyme. In addition, there is an RNA component (TERC) that acts as a template (68). The expression of the TERT is influenced by factors like time and quantity (69). Because of this characteristic, TERT is also an element that manages the telomerase enzyme's rate. The ability of cancer cells not to cease proliferating at the hayflick-limit is one of their distinct behavioral peculiarities when compared with normal cells. Cancer cells are able to avoid senescence through to continued telomerase activity and preservation of telomere length in this manner (70). There are differences in TERT expression of differentiated human cells, germ line cells and cancer cells. TERT activity contributes to preserving the length of telomeres in cancer cells and germ cells, even though its expression is almost undetectable in differentiated human cells (70). This process highlights that normal cells undergo senescence when their telomere length falls below a specific threshold, whereas cancer cells and germ cells continue to divide. As it can be understood from this information, TERT expression plays a role in gaining unlimited replication features of cancer cells. Studies have shown that TERT is among the determining factors in the evolution of a normal cell into a malignant form (71). Therefore, studies continue to elucidate the mechanisms governing TERT expression. There are 16 exons in the 40 kb TERT gene, which is found on chromosome 5p15.3340 (72). This gene's expression has been regulated by a variety of variables (73). Abnormalities arising from the TERT gene, which is found in duplicate in the cells of healthy individuals under normal conditions, may occur due to the loss or gain of this gene (74).

The TERT gene has been shown to be present in three copies in most human malignancies (75, 76). In addition, it is known that the polymorphism and mutations existing in the regulatory regions of the TERT gene affect its expression.

A relation between poor prostate cancer prognosis, disease recurrence and TERT expression has been reported (77). TERT promoter mutations frequently occur in a wide range of cancer types. It has been reported that TERT promoter mutations are detected more frequently in prostate cancer. The most frequent, cancer-specific, promoter mutations in various cancer types have been identified to be -124C > T and -146C > T hotspot mutations (78).

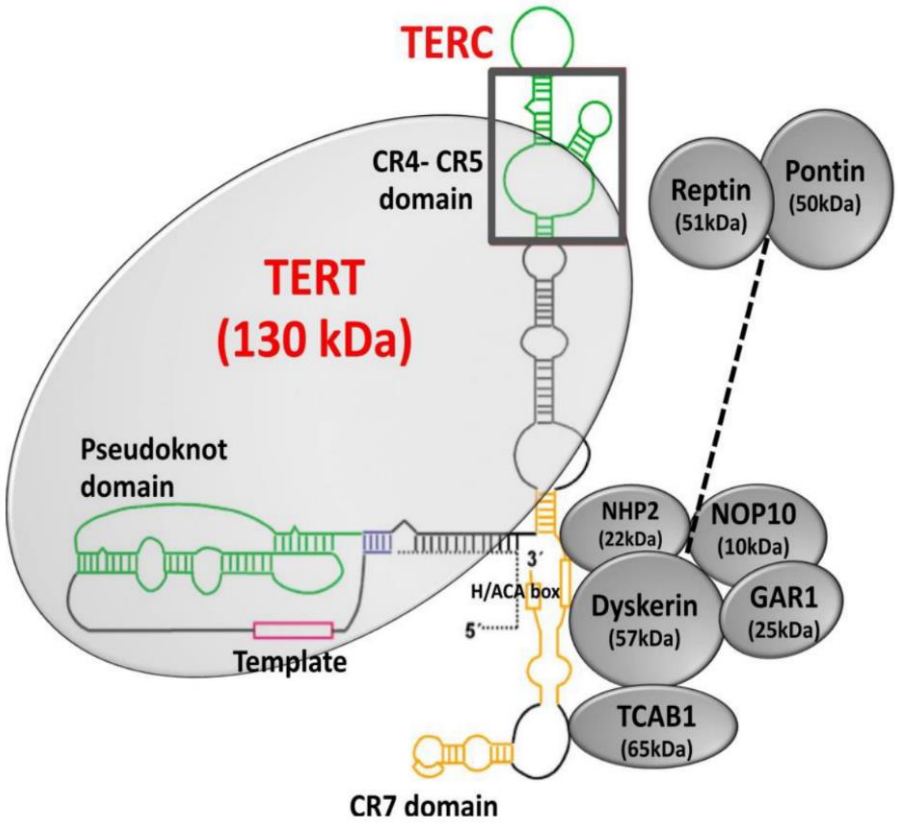

Figure 2. Complex of the telomerase system. It includes a rate-limiting, catalytic subunit called TERT, an RNA template called TERC, as well as other elements. The telomerase complex's two main components, TERT and TERC, are all that is required for telomerase activity (87).

Tumorigenesis is hypothesized to be influenced by promoter TERT mutations in two different ways. The shortest telomeres are repaired by promoter TERT mutations at the start of the stage, lengthening the lifespan of cells that carry them, but they are unable to stop the overall shortening of telomeres. This results in phase two, which requires more telomerase expression since the severely short telomeres cause genomic instability and additional cell growth (79).

The potential interaction of the promoter mutation in TERT with other alterations, such as those in IDH genes, is another intriguing feature of this mutation (80). Diplas et al. claim that a great proportion of diffuse gliomas may be classified using the combined information of promoter mutation in TERT and IDH mutation status (81). According to a report, in glioma cases, having both of these mutations and other alterations together is linked to a better prognosis as well as a better response to treatment (82).

As a result, the TERTp mutation status may be utilized to identify and discriminate between different types of cancers, as well as to predict prognosis and outcome, whether alone or in cooperation with mutations in other genes. Although the presence of TERTp mutations seems to have a substantial influence on the development of cancer, other tumors, such as prostate, exhibit telomerase activity despite having few TERTp mutations (83).

The majority of current research focuses on the transcriptional control of TERT (84). MiRNAs work as gene regulatory agents in cancer, suppressing tumor growth or promoting it (85, 86).

TERT is regulated by a number of miRNAs. TERT is regulated by a number of miRNAs. It has the ability to inhibit TERT excision and, accordingly, telomerase activity. Numerous miRNAs (ex. miR-541-3p, miR-422a, miR-1266) have been associated with changes in TERT expression in different cancer types.

3. MATERIALS AND METHODS

3.1. Study Population and Purpose

This doctoral study involved testing the blood samples of 27 males who had been identified as having prostate cancer after thorough examinations by the physicians of the Urology Department at Yeditepe University Faculty of Medicine. After the patients' signatures were obtained on the informed consent form, clinical data was accurately recorded. The specialists collected peripheral blood samples from the patients who were willing to participate in the research. The Helsinki Declaration of 1975 was followed during the entirety of the study, and the appropriate ethics committee permission was acquired from the relevant institution's ethics committee with the number 1733 in 2023. Tumors were assessed via the D'Amico risk classification. In assessing the test results, variables like the age at which the disease was discovered, family history of cancer, the presence of another cancer in addition to prostate cancer, the volume of the prostate, the PSA level, the pathological stage, the Gleason score, the presence of metastasis, and the type of treatment used were taken into account. By using NGS technology, this study's primary objective was to determine variations in the IDH2 gene and TERT gene related to prostate cancer.

3.2. Explanation of Genomic DNA Isolation from Blood Protocol

Prior to beginning the experimental process, the patients' blood samples were kept in 5cc EDTA tubes at +4°C. The iPrep DNA extraction instrument (Invitrogen), which can extract DNA from 13 samples at once, and the iPrep genomic DNA isolation kit, both of which are manufactured by Thermo Fisher Scientific Corporation, were utilized during the DNA isolation. This instrument performs the isolation of genomic DNA from a 350 ml blood sample by using a magnetic-bead-based technology that removes unwanted molecules depending on the pH change at the end of the isolation process.

Two main steps of this process can summarize the basis of this technology: The first is the separation of unattached molecules in low pH conditions. The second is the use of a buffer with a pH of 8.5 to purify nucleic acids. Low salt elution buffer mediates the increase in pH and neutralizes the bead surface, which is the fundamental part of the technology to clean nucleic acids. Liquid wash buffer mediates the unwanted molecules' removal. Consequently, DNA samples were acquired purely and preserved at +4°C for use in subsequent stages of the study.

3.3. Measurement of the DNA Purity and Concentration

The NanoDrop200 device was used to confirm that the DNA samples were sufficiently purified to be used for this research.

To accomplish the study, it was required that double-stranded DNA concentrations of 50 g/ml should have an optical density (OD) of 1 at a wavelength of 260 nm. The OD260/OD280 ratio had to be between 1.7 and 1.9 for a sample to be eligible for inclusion in the experiment. All these metrics were measured with the instrument.

3.4. Design of the Gene Panel, Library Preparation and Next Generation Sequencing

The Qiaseq-targeted DNA panel goes through the phases of fragmentation, UMI assignment, target enrichment, sample indexing, and amplification to generate a genomic library. Primers required in the aforementioned procedures when utilizing Illumina NGS technology, should be specially designed. The panel was made for the target genes and areas of interest that could be enhanced by 10–40 ng Fresh DNA.

As previously noted, this system contains essential stages. Details on these stages are given below:

Following end-repair and the addition of an A-tail, the genomic DNA sample is fragmented using a controlled multi-enzyme method. In order to use them at the 5’ ends, certain adapters are used to attach eligible DNA fragments to the sequencing device (including UMI and sample index).

Each DNA molecule is assigned a unique sequence or index known as the UMI before the target enrichment and library creation. This assignment is achieved by combining a 12-base random sequence adaptor with fragmented DNA. According to statistics, this process produces 412 different markers per adaptor since each DNA molecule in the sample adopts a different UMI sequence. Moreover, the first sample index is likewise present in this connected adapter.

After enough UMI-containing DNA molecules have been adequately obtained, the target enrichment stage is started. Targeted PCR is used to amplify UMI-linked DNA molecules for enrichment. This technique uses an adaptor complementary, one site-specific primer, and one universal primer. Using Universal PCR, extra sample indices and platform-specific adaptor sequences are added before the library is created.

For NGS, the Ion Personal Genome Machine® (PGM®), Ion Proton®, and Ion S5® systems from Thermo Fisher Scientific and the Illumina NGS systems (MiniSeq®, MiSeq® Personal Sequencer, NextSeq® 500, HiSeq® 1000, HiSeq® 1500, HiSeq 2000, and HiSeq 2500) were used which are all compatible with Qiaseq Targeted DNA panels (Figure 3).

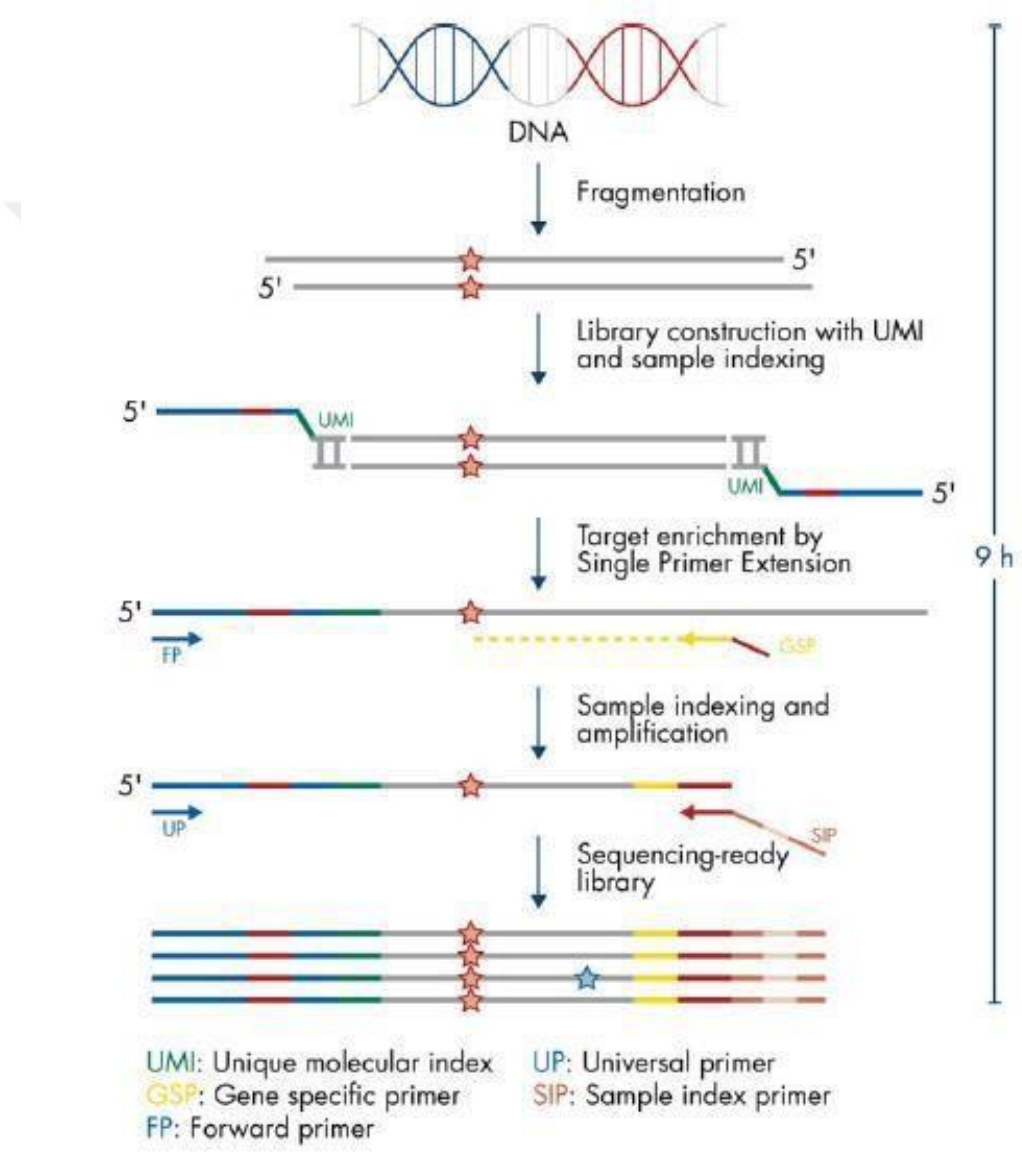

Figure 3. Synopsis of the Qiaseq Targeted DNA Panel Process Diagram

3.4.1. Fragmentation, end-repair, and addition

This entire process, of which details are given in Table 1, was carried out on ice. Following the table below, the fragmentation, End-repair, and A-addition mixture were prepared, and after ensuring that the Thermocycler device had reached a temperature of 4 degrees Celcius (°C), it was pipetted a few times. The process included a quick centrifugation.

Table 1. Fragmentation, End-Repair, and A-addition Reaction Mix

Contents	**Volume (μL)**	
	Standard, FFPE or pure cfDNA	**cfDNA contaminated with cellular DNA**
DNA	Variable	Variable
Fragmantation Buffer, 10x	2.5 μL	2.5 μL
Nuclease-free Water	Variable	Variable
FERA solution	0.75 μL	0.75 μL
FG solution	-	1.25 μL
Total	**20 μL**	**20 μL**

The Fragmentation Enzyme Mix was pipetted through numerous times before adding the 2.5 μl that was used in each reaction. Ice had to be used for the process. The PCR program was then started, and samples were inserted into the machine when it reached 4°C. The samples were taken on the ice after the program ended. The process of adapter ligation started as soon as feasible.

3.4.2. Adapter ligation of Samples

The reaction mixture was prepared in line with Table 2. Pipetting, a maximum of ten times, was used to gently blend all the ingredients. The ligation solution was excluded from the following combination because of its high viscosity, but it was included in each reaction that came after. The residual ligation solution on the pipette tip was scraped off before adding the ligation solution. The thermocycler was used for 15 minutes of incubation at 20°C. After the reaction was completed and the mixture was placed on ice, the QIAseq Beads cleaning process began.

Table 2. Adapter Ligation of Samples

Content	Volume		
	Standard DNA	**FFPE DNA**	**cfDNA**
DNA	25 µL	25 µL	25 µL
Ligation Buffer, 5x	10 µL	10 µL	10 µL
IL-N7 Adapter	2.8 µL	2.8 µL	0.5 µL
DNA Ligase	5 µL	5 µL	5 µL
Ligation Solution	7.2 µL	7.2 µL	7.2 µL
Nuclease-free Water	-	-	2.3 µL
Total	**50 µL**	**50 µL**	**50 µL**

3.4.3. Clearance of adapter-bound DNA with QIAseq Beads

The 50 µl of DNA that was extracted during the ligation procedure was put in a 1.5 ml LoBind tube. It was maximized to a volume of 100 µl by adding 50 µl of nuclease-free water. 100 µl of QIAseq Beads were added to the experiment. To help in mixing, the mixture was pipetted many times. The incubation time lasted five minutes at room temperature. After being put on a magnetic rack, the beads were left for ten minutes to separate from the supernatant. Before carefully removing and discarding the supernatant, the solution was allowed to become clear. We handled the target DNA-containing beads with the utmost care. 200 µl of the newly prepared 80% ethanol was added to the magnetic rack. The tube was spun while the beads were washed. The supernatant was finely discarded. 200 µl and 10 µl pipettes were used to remove EtOH and the leftover liquid. Once the ethanol was taken out of the magnetic rack, the beads were left to dry for 10 minutes. The DNA from the beads outside the magnetic rack was eluted using 52 µl of nuclease-free water. A sufficient amount of time was spent pipetting before the solution was placed on the magnetic rack to become purified. For the transfer of 50 µl of the supernatant, a neat 1.5 ml LoBind tube or PCR plate well was used. For standard-FFPE samples, 50 µl of QIAseq Beads were added to 50 µl of DNA solution; for cfDNA samples, 70 µl of QIAseq Beads were added to the DNA solution. Following that, the proper pipetting was performed. At room temperature, the incubation period was five minutes. It took five minutes to separate the beads from the supernatant after they had been put on a magnetic rack.

The supernatant was carefully taken out and discarded when the solution had turned clear. 200 µl of the newly prepared 80% ethanol was added to the magnetic rack. The tube was spun while the beads were washed. The supernatant was discarded very carefully. The ethanol washing procedure was repeated one more time. Ethanol was removed using 200 µl and then 10 µl pipettes. As the beads must be dry before elution for the target enrichment stage to be successful, the beads were dried for 15 minutes while remaining on the magnetic rack. 12 µl of nuclease-free water was used to elute the DNA target beads after they were taken off the magnetic rack. It was completely mixed using a pipette. It was maintained on the rack while the solution became clear. 9.4 µl of supernatant were transferred to a clean PCR tube or PCR plate well to commence the target enrichment procedure.

3.4.4. Target enrichment PCR

The reaction mixture was prepared according to the Table 3. It was briefly centrifuged, pipetted roughly ten times, and centrifuged once more. Following this process, the thermocycler instrument was set. After the procedure was finished and held on ice, the QIAseq Beads cleaning procedure began.

Table 3. Target Enrichment Reaction Mix

Contents	**Volume (µL)**
DNA library	9.4 µL
TEPCR Buffer, 5x	4 µL
QIAseq Targeted DNA panel	5 µL
IL-Forward primer	0.8 µL
HotStar Taq DNA Polymerase	0.8 µL
Total	**20 µL**

3.4.5. Clearance of the target enrichment PCR with QIAseq Beads

The PCR reaction was transferred to a 1.5 ml LoBind tube. It was then diluted with nuclease-free water by 80 µl until it reached a totol volume of 100 µl. By adding 70 µl of nuclease-free water to 20 µl of the sample, the cfDNA sample was completed to 90 µl. 100 µl of diluted PCR solution was mixed with 100 µl of QIAseq Beads (1.0 x volume). It was incubated for 5 minutes at room temperature after pipetting. After putting the sample on the magnetic rack, the beads were allowed to separate from the supernatant for around 5 minutes. The supernatant was carefully collected and discarded when the mixture had become transparent. 200 µl of the newly prepared 80% ethanol was added to the magnetic rack. The tube was spun while the beads were washed. The supernatant was completely discarded. The ethanol washing process was repeated once more. Ethanol was thrown using 200 µl and then 10 µl pipettes. The beads were allowed to dry for 10 minutes. 16 µl of nuclease-free water was required to elute the DNA beads. It was thoroughly mixed via pipetting. The mixture was kept on the rack until it became clear. Following the transfer of 13.4 µl of supernatant to a sterile PCR tube, the Universal PCR amplification process was started.

3.4.6. Universal PCR amplification

The centrifugation method was kept quick, and pipetting was performed several times while in the beginning of the Universal PCR, as mentioned below. Following this, the thermocycler instrument was calibrated. The QIAseq Beads cleaning phase started when the reaction was completed, and the solution was placed on ice.

Table 4. Universal PCR Amplification

Component	Volume
Enriched DNA	13.4 µl
UPCR Buffer, 5x	4 µl
HotStarTaq DNA Polymerase	1 µl
Nuclease-free Water	1.6 µl
Total	**20 µL**

3.4.7. Clearance of the Universal PCR with QIAseq Beads

20 µl of the PCR reaction was transferred into a 1.5 ml LoBind tube. 80 µl of nuclease-free water was added to get the volume up to 100 µl. 100 µl of 1.0x volume QIAseq Beads and 100 µl of diluted PCR solution were combined. The mixture might have been blended perfectly by pipetting it several times. At room temperature, it was incubated for five minutes, the tube was placed on the magnetic rack for five minutes, and the supernatant was thrown away. 200 µl of fresh 80% ethanol was added while it was still placed on the magnetic rack. Supernatant was discarded very carefully. One more, the ethanol washing process was performed. When the ethanol was removed, the beads were dried for 15 minutes on the magnetic rack using 200 µl and then 10 µl pipettes. 30 µl of nuclease-free water was used to elute the DNA library beads. The solution was carefully pipetted, then placed back on the magnetic rack and allowed to clear entirely. We transferred 28 µl of supernatant into a fresh 1.5 ml LoBind tube or PCR tube.

3.4.8. Qiaseq Quant Assay Protocol

The ILMN DNA standard was defrosted using an ice bath. The details of five sequential dilutions of the ILMN DNA standard are displayed in the table below. For the ILMN standard dilution, five tubes were prepared. 45 µl of dilution buffer were added to each tube. Pipetting was used to mix 5 µl of ILMN standard stock into the first tube. 5 µl of the solution was then taken from the first tube and added to the second. The following tubes had the same procedure.

Table 5. Qiaseq Quant Assay Protocol

Standard	Ion Torrent or Illuina DNAStandard	Dilution Buffer
Std1	5 µl undiluted	45 µl
Std2	5 µl Std1	45 µl
Std3	5 µl Std2	45 µl
Std4	5 µl Std3	45 µl
Std5	5 µl Std4	3 µl

The dilution ratios were 1:50, 1:5.000, and 1:50.000, respectively. The PCR mixture was prepared, and a cold block was used. Centrifuging of SYBR Green ROX FAST Mastermix was performed for 15 seconds. Three copies were prepared for five standards and one NTC (non-template control).

The real-time PCR instrument (RotorGene) was set up. Data for fluorescence light was recorded after 30 cycles. After 30 cycles, flouresent light was detected and collected data were entered into the "Illumina Data Analysis Program".

3.4.9. QiaSeq Upload Protocol

By means of the Illumina Experiment Manager, the Sample Sheet was prepared before the dilution began. MSxxx prefix was coded to the Reagent Cartridge Barcode section. A section was labeled as "custom primer for read 1" on the right. 800 µl of nuclease-free water was used to dilute 1N stock NaOH to 0.2N. Samples were determined as four molars based on the results of the Quant assay protocol. A mega pool was produced by filling 0.2 µl PCR tubes to the appropriate amounts based on the number of primers from each library. 5 µl were transferred from the tube containing the giant pool to another sterile tube. It was dilıuted with 5 µl of NaOH to 0.2N. At 20–22°C, it was gently vortexed and spun down for 5 minutes to allow it to denaturate. During this time, 990 µl of the Hyb buffer solution with yellow-capped from the cartridge was transferred into the 1.5 ml tube marked "20 pM pool". 10 µl of the NaOH solution and our sample were added into the 1.5 ml tube marked "20 pM pool" when the five minutes were over. The samples' dilution was kept consistent with the cartridge and library. "Advance primer" was written on one additional 1.5 ml tube. It was filled with 597 µl of HT1. From the index box, 3 µl of orange-capped primers were labeled as "Qiaseq A Read1 Custom Primer1". Before they were loaded, tubes were kept on the cold block. The instrument was turned off before the sequencing began via the MiSeq Control Software. The 600 µl of the samples, which had been concentrated to the required level, were loaded into well 17. The 600 µl of the tube marked "Forward primer" was added into well number 18. Once reagents and the flow cell had been loaded, the run configurations were reviewed, and a pre-run check was performed. Concentrations and quality values for the run have been monitored in the Sequencing panel as it progresses. Outcomes of the Sequencing monitor were evaluated immediately.

3.5. Validation of Mutations by Sanger Sequencing

Detected variations were validated through Sanger sequencing. For this sequencing technique, primes were designed to target specific.

3.5.1. Designing Primers for Sanger Sequencing

While targeting specific primers designed for Sanger sequencing, previously known single nucleotide variants were not included. Repeating sequences were excluded, and care was taken to ensure that the binding temperatures of the primers were not close to each other. Additionally, distribution of purines and pyrimidines were kept equal. Table 6 shows information obtained from NCBI Blast regarding primers.

Table 6. Designed Primer Sequences

Gene	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
TERT c.261G>T	CTTTCCGCGCGCTGGTG	CCCTGACGCTATGGTTCCAG

3.5.2. Polymerase Chain Reaction (PCR)

Target-specific primer sequences were used in PCR to amplify the TERT gene variants. Reaction mixture details can be found in Table 7.

Table 7. The Reaction Mix Prepared for Each Sample

Component	Reaction
1X Dream Taq Master Mix*	12.5 µl (2X)
200 nM Forward Primer (10 pmol/µl)	0.5 µl
200 nM Reverse Primer (10 pmol/µl)	0.5 µl
dH_2O	-
DNA (100-150 ng)	-
Total	**25 µL**

*DreamTaq PCR Master Mix (2X) Thermo Scientific™ Catalog number: K1072

PCR-based amplification was conducted using a thermal cycler (Applied Biosystems, Veriti 96-Well). PCR program details are shown in Table 8.

Table 8. Thermal Cycle Program

Step	Temperature	Time	Cycle
Denaturation	95°C	2 minutes	
	95°C	30 seconds	35 cycle
Annealing	60°C	30 seconds	
	72°C	60 seconds	
Final Extension	72°C	5 minutes	
	4°C	10 minutes	
	10°C	∞	

3.5.3. Agarose gel electrophoresis, PCR purification and Sequence PCR

After the PCR step, agarose gel electrophoresis was performed. A 2% agarose gel was used and the electrophoresis was run at 90 volts for 60 minutes with 1X TBE (Tris-borate-EDTA) buffer. After the procedure, a gel image was recorded.

After the agarose gel electrophoresis, PCR purification (Exosap) was performed. The construction of the Exosap step is as Table 9 and Table 10.

Table 9. PCR Purification (Exosap) Components

PCR Component	1x Reaction
Exonuclease I (20U/µl)	0.5 µl
Shrimp Alkaline Phosphatase (1U/µl)	1 µl
Total	**1.5 µl**

Table 10. Thermal Profile

Temperature	Time
37°C	30 minutes
80°C	15 minutes
10°C	∞

Following the Exosap stage sequence PCR step was performed. The preparation of the sequence PCR mix and its thermal profile can be seen in the tables below.

Table 11. Sequence PCR

PCR Component	1x Reaction
BigDye v.3.1	2 µl
5XReaksiyon Buffer	2 µl
Forward or Reverse (2pmol/µl) primer	2 µl
All of the exosap purified product	2 µl
dH2O	2 µl
Total	**10 µl**

Table 12. Sequence PCR Thermal Profile

Temperature	Time	Cycle
96°C	1 minutes	
96°C	10 seconds	25 Cycles
50°C	5 seconds	
60°C	4 minutes	
4°C	∞	

After sequence PCR, the study was completed by Ethanol Purification and loading the samples into the ABI 3500 Genetic Analyzer device. Sequence analysis results of the loaded samples were obtained.

3.5.4. Sequence Analysis

The "Bigdye Terminator v3.1 Cycle Sequencing kit" (Applied Biosystems, Foster City, CA, USA) containing the labeled dideoxynucleotides and the "Cycle Sequence" PCR using site-specific primers were used to obtain the PCR amplification products. After electrophoresis of the reaction products on the "ABI PRISM 3130XL Genetic Analyzer" (Applied Biosystems, Foster City, CA, USA) automated DNA sequence analysis instrument, chromatograms representing DNA sequence analysis data were obtained. Chromatography analyzes the color/base relation for each reaction's waveform, enabling the secure recording and storing of sequence analysis data. After the sequence analysis, the chromatogram wave peak lengths were compared. Corrections were made where necessary. CLUSTALX and GENEDOC multiple sequence analysis tools were used to analyze the chromatographic results.

In a CLUSTAL X alignment, the two chains were placed facing each other (version 1.83). The ends of the closely matched sequences were trimmed using the GENDOC (version 2.6.002) software, and the final consensus sequence was recorded.

3.6. Patient Demographics

The age, gender, tumor location, pathology findings, PSA values, and other clinical information of each patient involved in the study were recorded. The relationship between IDH2 and TERT genes and prostate cancer was investigated using the sequencing findings.

3.7. Statistical Analysis Method

Software known as QIAGEN Clinical Insight (QCI) was used to interpret variants. This bioinformatics tool uses the AMP/ASCO/CAP classification system to find emerging mutations.

The results of the study were statistically analyzed using the Microsoft Excel, GraphPad Prism version 9 (GraphPad Software, La Jolla, CA, USA) and MedCalc software (NY, USA). Descriptive statistical methods (number, percentage, median, etc.) were used to analyze the study's data. To evaluate the distribution and the difference of all these mutations on different risk grades of the disease, we used Chi-square and Fisher's exact tests. The 95% confidence level and $p>0.05$ threshold of significance were used to analyze the results.

4. RESULTS

4.1. Demographic Charcteristics of the Study Population

The clinical and pathological features of 27 males with prostate cancer who were diagnosed and followed up by the Department of Urology at Yeditepe University Faculty of Medicine are stated below:

Table 13. Patient Demographic Biochemical and Genetic Test Characteristics

Parameters	All (n=27)
Age at diagnosis (yr)	68 ± 7.65
Body Mass Index (kg/m2)	27.85±3.65
PSA level (ng/mL)	31.28±35.66
D'Amico Risk	
High	19 (70.37%)
Intermediate	8 (29.63%)
Family history of cancer, n (%)	
Yes	13
No	14
Second Cancer	
Yes	2
No	25
Prostate Volume	43.59±22.91
Comorbidities	
Yes	17
No	10
Clinical T-stage n (%)	
cT1c	9
cT2	15
cT3	3
Pathological T-stage, n (%)	
PT2a	2
PT2b	5
PT2c	12
PT3a	5
PT3b	3
Gleason Score	
≤7	14
>7	13

Table 14. Assessment of clinical information according to the D'Amico Classification

Parameters	All (n=27)	D'Amico Risk		P value
		High (n=19) 70.37%	Intermediate (n=8) 29.63%	
PSA level (ng/mL)	31.28±35.66	40.85±39.91	10.95±5.00	**0.048**
Prostate Volume	43.59±22.91	51.47±21.37	24.87±14.39	**0.003**
Clinical T-stage n (%)				
cT1c	9	3	6	**0.000**
cT2	15	13	2	0.087
cT3	3	3	0	0.532
Gleason Score				**0.001**
≤7	14	6	8	
>7	13	13	0	
Tm percentage	50.85±26.56	61.32±21.91	26±19.57	**0.0006**

In prostate cancer patients, the age of the disease diagnosis was 68 years. While 88.89% of our study population was equal and over sixty, just 11.11% of them were under 60 years of age. Body mass index was 27.85 kg/m^2. The presence of cancer in the family history and existence or lack thereof second cancer type in the patients were among the parameters evaluated. While 13 out of 27 prostate cancer cases had a family history of cancer, second cancer existences were present in only 2 patients. According to the D'Amico et al. classification introduced in 1998, 19 out of 27 prostate cancer patients were classified as high-risk, and 8 were classified as intermediate-risk.

The median PSA concentration was 31.28 ng/mL. The prostate volume in all patients is 43.59 cc. Additionally, clinical and pathological T-stages were analyzed. Three clinical T stages, cT1c, cT2, and cT3, were identified in patients. A total of 9 (33.3%), 15 (55.6%), and 3 (11.1%) men had Stage cT1c, cT2 and cT3 disease, respectively. Also, five pathological T-stage PT2a, PT2b, PT2c, PT3a, and PT3b subgroups were defined under this heading. A total of 2 (7.43%), 5 (18.51%), 12 (44.44%), 5 (18.51%), and 3 (11.11%) men had stage PT2a, PT2b, PT2c, PT3a and PT3b, respectively. Gleason scores of 7 or less were obtained by the majority of males (51.8%). 17 prostate cancer patients had comorbities like hypertension, diabetes mellitus or both.

Additionally, PSA level ($p = 0.048$), prostate volume ($p = 0.003$), cT1c stage ($p = 0.000$), Gleason score ($p = 0.001$) and Tm percentage ($p = 0.0006$) were statistically significant for D'Amico risk classification.

4.2. Next Generation Sequencing (NGS) Analysis

After the targeted gene sequencing was performed on the prostate cancer patients, 5 mutations were detected. One of these mutations was found in the IDH2 gene. Twenty out of 27 prostate cancer patients had this mutation, but it was not found in 7 other individuals.

The variant detected in the IDH2 gene was located in the exonic region. As a result of the investigation, c.996C>T (p.S332S, rs61737003) variant in 2 heterozygous patients was detected in the IDH2 gene. In addition, when the distribution of mutations was evaluated using the D'Amico classification, the distribution of the IDH2 gene mutation found in these two groups did not significantly differ from one another (p=0.512).

To enhance the efficacy of the investigation, the TERT gene was also analyzed in addition to the IDH2 gene. Out of the 5 mutations, four were located in the TERT gene. Among the five variants examined in prostate cancer patients, given in the Table 15, the highest rate of variation was found in TERT c.915G>A with 67%. As a result of the analysis of the TERT gene; c.3039C>T in 6 heterozygous patients, c.1392C>T in 1 heterozygous patient, c.915G>A in 18 patients (4 homozygous, 14 heterozygous), and c.261G>T in 1 patient were observed in the TERT gene. In addition, when the distribution of variations were evaluated using the D'Amico classification, the distribution of the TERT gene (c.3039C>T, p=0.319; c.1392C>T, p=0.296; c.915G>A, p=0.675; c.261G>T, p=0.508) variations found in these two groups did not significantly differ from one another (Table 15).

Table 15. IDH2 and TERT gene variant assessment according to the D'Amico Risk Groups

Variables	Category	n	D'Amico Risk		p-value
			High 70.37% (n=19)	Intermediate 29.63% (n=8)	
TERT					
C3039CT	*positive vs negative*	6 vs 21	11.11% vs 59.26%	11.11% vs 18.52%	0.319
C1392CT	*positive vs negative*	1 vs 26	0% vs 70.37%	3.7% vs 25.93%	0.296
C915GA	*positive vs negative*	18 vs 9	44.44% vs 25.93%	22.22% vs 7.41%	0.675
C261GT	*positive vs negative*	1 vs 26	3.7% vs 66.67%	0% vs 29.63	0.508
IDH2					
C996CT	*positive vs negative*	2 vs 25	3.7% vs 66.67%	3.7% vs 25.93%	0.512

Two prostate cancer patients carried variants of both the IDH2 and TERT genes. One of the two patients was at high risk, and the other was at intermediate risk. The IDH2 gene variant rs61737003 was identified along with the TERT gene variants rs33954691 and rs2736098 (Figure 4).

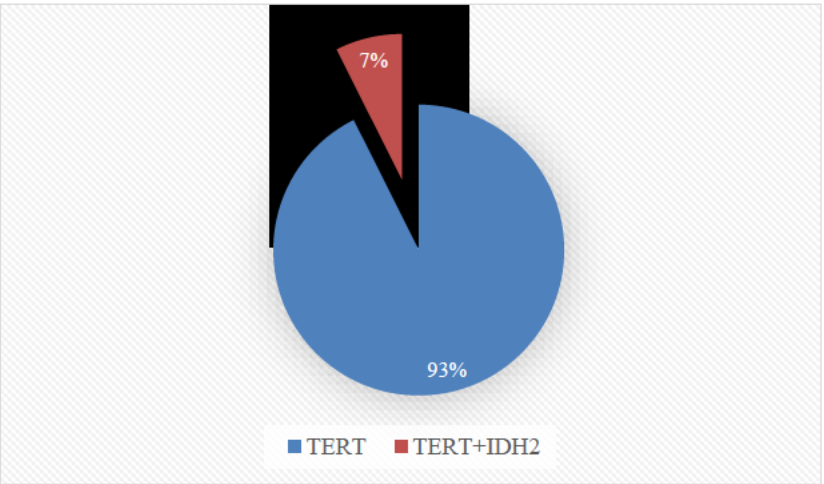

Figure 4. Distribution of TERT and IDH2 Variations

Table 16. Summary of 5 variations detected with NGS

Gene Symbol	Chromosome	Transcript Variant	Protein Variant	Normal	Population Frequency	Chromosome	Gene Region	Translation Impact	Classification	Phenotype	Transcripts	Patient ID
IDH2	15	c.996C>T c.840C>T c.606C>T	p.S280S p.S332S p.S202S	Normal	20.87%	90, 628, 591	Exonic	synonymous	Benign	Cancers and Tumors	NM_001289910.1	11, 17
TERT	5	c.2850C>T c.3039C>T	p.H950H p.H1013H	Normal	24.36%	1, 255, 520	Exonic; ncRNA	synonymous	Benign	Dyskeratosis congenita	NM_001193376.2	3, 11, 12, 17, 20, 24
TERT	5	c.1392C>T n.1471C>T	p.F464F	Normal	0.81%	1, 293, 609	Exonic; ncRNA	synonymous	Likely Benign	Dyskeratosis congenita	NM_001193376.2	27
TERT	5	c.915G>A n.994G>A	p.A305A	Normal	47.88%	1, 294, 086	Exonic; ncRNA	synonymous	Benign	Dyskeratosis congenita	NM_001193376.2	1, 3, 5, 6, 9, 10, 11, 13, 15, 16, 17, 18, 19, 20, 23, 24, 26, 27
TERT	5	c.261G>T n.340G>T	p.R87S	Normal	0%	1, 294, 740	Exonic; ncRNA	missense	Uncertain Significance	Dyskeratosis congenita	NM_001193376.2	7

Table 16. Summary of 5 variations detected with NGS (Continuing)

Gene Symbol	Chromosome	Transcript Variant	Protein Variant	SIFT Function Prediction	PolyPhen-2 Function Prediction	CADD Score	dbSNP ID	1000 Genomes Frequency	NHLBI ESP Frequency	ExAC Frequency	gnomAD Frequency	Patient ID
IDH2	15	c.996C>T c.840C>T c.606C>T	p.S280S p.S332S p.S202S	-	-	< 10	61737003	7.867	7.608	4.916	3.523	11, 17
TERT	5	c.2850C>T c.3039C>T	p.H950H p.H1013H	-	-	< 10	33954691	11.781	7.381	12.388	12.529	3, 11, 12, 17, 20, 24
TERT	5	c.1392C>T n.1471C>T	p.F464F		-	< 10	186596886	0.100	-	0.256	0.102	27
TERT	5	c.915G>A n.994G>A	p.A305A	-	-	< 10	2736098	26.558	20.753	40.447	27.537	1, 3, 5, 6, 9, 10, 11, 13, 15, 16, 17, 18, 19, 20, 23, 24, 26, 27
TERT	5	c.261G>T n 340G>T	p.R87S	Damaging	Benign	14.140	1033845124	-	-	-	-	7

4.3. Validation of Variations by Sanger Sequencing

DNA sequence change detected by NGS was confirmed by Sanger sequencing using primer sequences. The sequencing of the TERT variation in chromosome 5 was performed using the ABI 3130XL Genetic Analyzer (Life Technologies) by designing a primer suitable for the region. As a result of the studies, c.261G>T (p.R87S; rs1033845124) heterozygous missense variant was detected in the TERT gene in one prostate cancer patient. The variant we detected was Sanger sequencing and it was confirmed that the case was heterozygous. The results of the TERT gene variant agarose gel electrophoresis and the forward image of the detected variant confirmed by Sanger sequencing are shown in Figures 5 and 6.

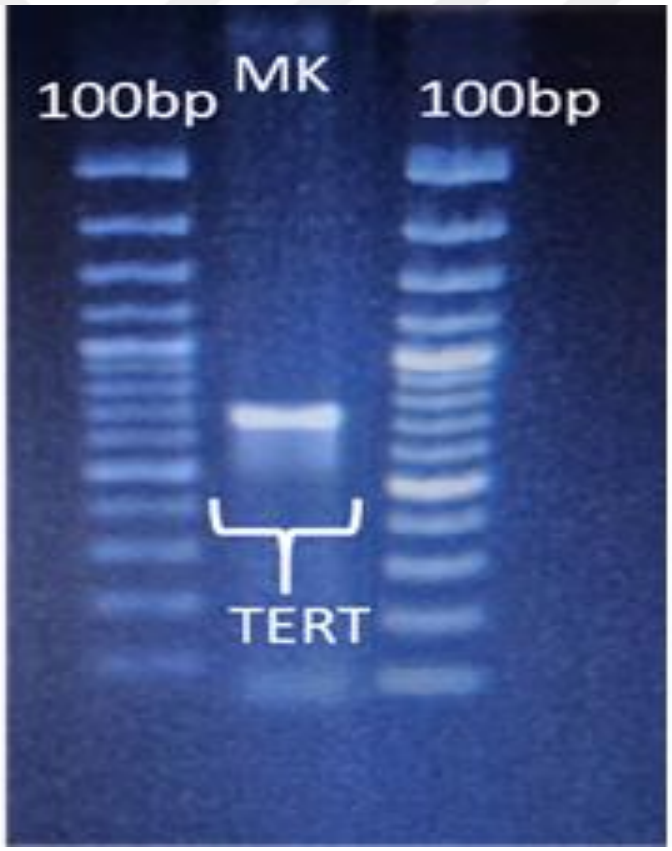

Figure 5. TERT gene variant agarose gel electrophoresis result

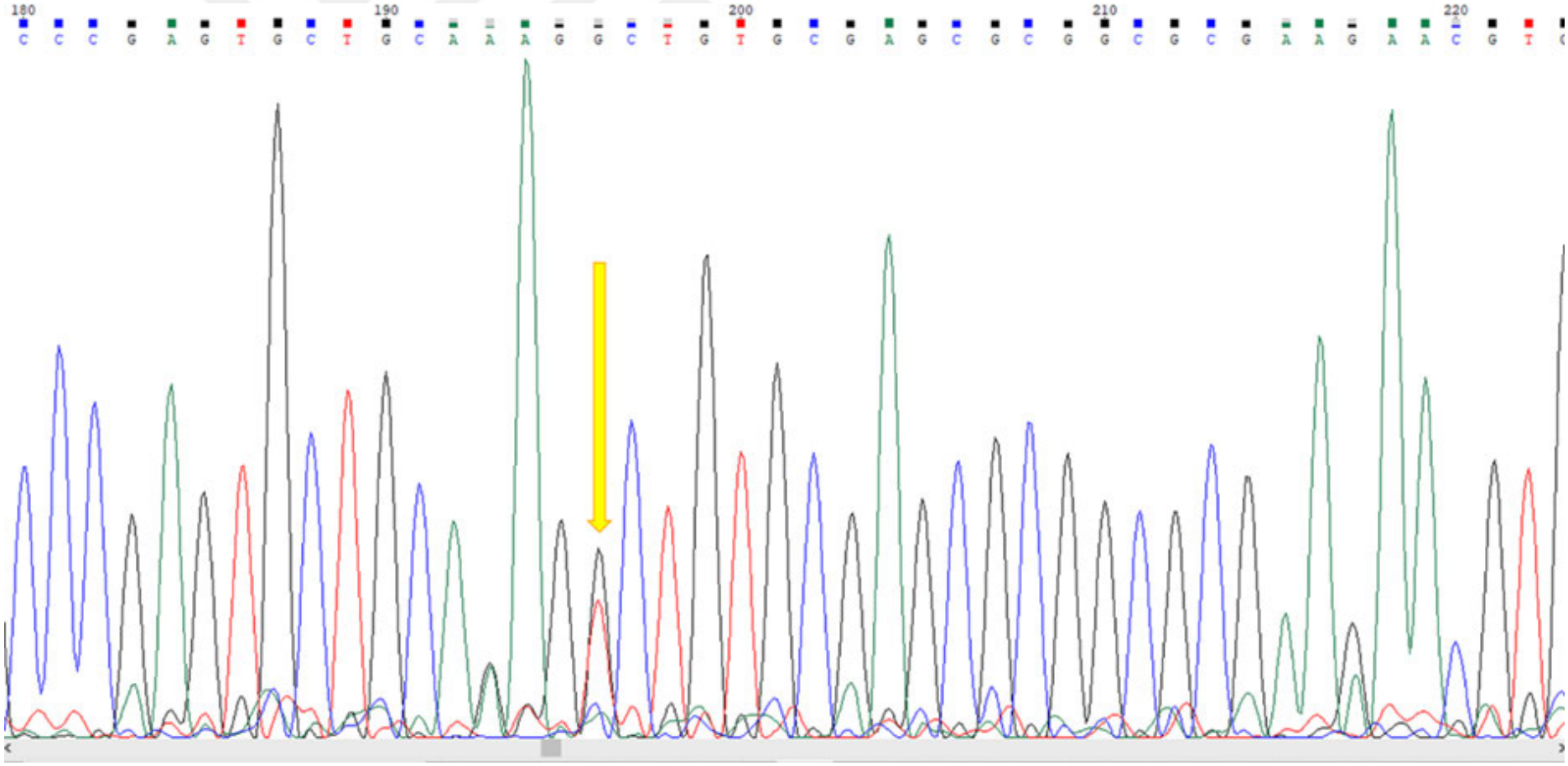

Figure 6. TERT gene c.261G>T forward image and confirmation by Sanger Sequencing
Our Sanger sequencing study displays A in green fluorescence, T in red, G in black, and C in blue, as is standard.

5. DISCUSSION AND CONCLUSION

The fact that the data that can support the evaluation by clinicians is limited to classifications based on pathological and biochemical parameters is among the reasons why this cancer continues to be one of the deadliest types of cancer diagnosed worldwide.

Molecular markers, in addition to being prognostic indicators, may also be targets for novel treatment approaches and predictors of response in a variety of malignancies. The prostate cell and its environment play a key role in the formation and progression of prostate cancer through a number of genetic and epigenetic changes. The last ten years have seen the emergence of several innovative therapeutic approaches for the treatment of prostate cancer, all of which require the discovery of prognostic biomarkers. Personalized medicine, the principle of tailoring treatment to each patient's specific tumor profile, is important in a complex disease such as prostate cancer. Identification of biomarker candidates, such as cancer variations, is key to the effective clinical application of this principle.

The use of molecular markers for drawing the line of tumors originating from the central nervous system elaborates the classification and facilitates the implementation of specific treatment applications by clearly determining the subclasses of the disease. Although prostate cancer is heterogeneous, as in the cancer types mentioned above, very few reported mutations have been confirmed to be associated with prostate cancer, and the molecular processes underlying prostate cancer have not yet been fully elucidated.

A deeper understanding of the underlying molecular pathways that sustain tumor growth and progression has recently been made possible in big part by technological innovations and the creation of new approaches. Currently, there are techniques that can unravel the puzzle, confirming genes linked to prostate cancer and identifying new ones. The invention of technologies like NGS has given scientists the ability to clarify most of the alterations associated with prostate cancer to date. In addition to mutations, it should be noted that this technology can also identify gene fusions and copy number alterations.

There are studies stating that the scope of the current categorization, which is determined by various features, especially the Gleason score and PSA level, should be revised to include parameters at the molecular level according to the reports.

Our research was designed in pursuit of a deeper understanding of the molecular biology behind prostate cancer. Two target genes, IDH2 and TERT, were examined for variations for this purpose using blood samples from individuals with prostate cancer. Next-generation sequencing technology was used for this analysis, and Sanger sequencing was used to validate the existence of the detected variants.

In total, 5 variants were detected in our study population with prostate cancer in two genes. Among those detected, a variant was identified as potentially harmful, and it was observed for the first time in the literature, not only in prostate cancer.

IDH2 mutations can be found extremely seldom in cases of prostate cancer, despite being a very unique marker in gliomas.

One of the synonymous variants we detected as a result of our analyses was in the IDH2 gene. This was rs61737003 (c.996C>T, p.S332S) benign variation detected in two heterozygous patients. NGS analysis of DNA samples from Polish patients diagnosed with myeloid leukemia detected 49 germline variants in six genes. One of these variants was the benign IDH2 variant, which was also identified in prostate cancer patients (88).

TERT plays a significant role in many oncogenic signaling networks, and its amplification frequently promotes tumorigenesis. Several factors, including TERT gene duplication, TERT gene polymorphism, TERT promoter mutation and methylation, and miRNA interference, can cause the excess of the TERT gene, which is frequently found in tumors.

As part of the purview of our thesis study, four of the 5 mutations were detected in the TERT gene. In this gene, two benign, one likely benign and one possibly harmful variation were determined: c.3039C>T (rs33954691), c.1392C>T (rs186596886), c.915G>A (rs2736098) and c.261G>T (rs1033845124).

c.3039C>T (rs33954691) and c.915G>A (rs2736098) were identified as benign variants which were found in the sixth and eighteenth patients.

As with the variant detected in IDH2, the TERT c.3039C>T (p.His1013, rs33954691) variants were also found by Bak et al. to has been shown to be present in patients with acute myeloid leukemia (88). Studies show the relationship of the rs33954691 variant, which we detected in prostate cancer patients, with leukocyte telomere length. Although the results of two different study groups, which published their results in 2010 and 2012, did not confirm each other, Atzmon et al. reported that this variant was associated with longevity, due to increased telomere length by regulating the TERT expression (89). Soerensen et al. linked rs33954691 to a lower risk of mortality depending on age

(<85 years), despite not being able to corroborate the association discovered by Altmon et al. (90). Subsequently, Shen et al. showed an association between papillary thyroid carcinoma cases in the radioiodine-refractory group and rs33954691, which also poses a risk (91). rs2736098 (c.915G>A) variation was observed in eighteen patients (four homozygous and fourteen heterozygous) in our study population. Many publications are reporting their results on this variant. Variants thought to be involved in the proven relationship between telomerase activity and carcinogenesis have been demonstrated in various cancer types. Zhang et al. (2022) performed a meta-analysis to elucidate TERT variants associated with cancer. They revealed the rs2736098 variant is associated with cancer in all populations, besides its dual effect according to the cancer type. This bidirectional effect is associated with an increase or decrease in risk, and the types of cancer observed include lung (increased risk), bladder (increased risk), colorectal (decreased risk) and breast (decreased risk) (92). Another meta-analysis was previously performed by Wang et al. This study identified the genes and variations linked to lung cancer by examining more than 200 variants and more than 100 genes, offering compelling cumulative epidemiological data. Among them, rs2736098 was the only variant in the TERT gene to have been associated with lung cancer susceptibility (93). The relationship between risk of lung cancer and rs2736098 was intensified by Li et al. (94). The rs2736098 variant has also been shown to be present in acute myeloid cases (88). Additionally, it has been noted by Pandith et al (2020) that the rs2736098 variant is one of those variants linked to an increased risk of glioma development (95). Diseases in which the presence of this variant has been demonstrated include Non-Small Cell Lung Cancer and associated with the disease risk (96). Moreover, increased TERT expression and this variant of the gene AA genotype in renal cell carcinoma was associated with poor survival (97).

More than the other variations of our study, we found that studies have questioned the linkage between the rs2736098 variation and prostate cancer. Rather than investigating whether or not this variant poses a risk for prostate cancer, its relationship with PSA level in important parameters of prostate cancer has been clarified (98, 99, 100).

Described as likely benign, c.1392C>T (rs186596886) variant was observed in one case, and c.261G>T (rs1033845124) was defined as a possible deleterious variant, which was identified in only one patient with prostate cancer. They were not reported prior to our investigation.

Out of a total of 5 variations in IDH2 and TERT genes that were detected using NGS, one was also validated by Sanger sequencing. In the TERT gene, we discovered a potentially harmful missense variant, c.261G>T (p.R87S, rs1033845124), which we disclosed in one patient. This variant has in no way been identified and associated with any disease. Since it is important to confirm the presence of this variant, analysis was performed with Sanger sequencing, which is the gold standard.

During *in vitro* DNA replication, DNA polymerase specifically incorporates chain-terminating dideoxynucleotides (ddNTPs), which are essential components of the Sanger sequencing technique developed by Frederick Sanger and other researchers in the 1970s. In general, current Sanger sequencing methods create a sequence chromatogram with associated fluorescent peaks by binding four separate fluorescent dyes to ddATP, ddCTP, ddGTP, and ddTTP (101). Sanger sequencing has proven to be a valuable tool for detecting single nucleotide mutations, insertions, and deletions in a variety of genes, including oncogenes and tumor suppressor genes (101).

It is well-known that recent developments in massive parallel sequencing (NGS) have accelerated and improved molecular pathology's ability to analyze mutations. We attempted to demonstrate the presence of the TERT gene mutation by utilizing these two methods.

The individual carrying this variant was confirmed to be a TERT c.261G>T (rs1033845124) heterozygous mutant.

Additionally, we know from the literature that carrying an IDH mutation in addition to mutations in the promoter region of the TERT gene is a mediator to a better prognosis in glioma. We sought to assess the patients who had variants of these two genes at the same time. Two patients carried variants of both genes together. One of the two patients was at high risk and the other was at an intermediate risk. The IDH2 gene rs61737003 was identified accompanying TERT rs33954691 and rs2736098 variants. As detailed above, these variants are synonymous and, with the exception of rs2736098, we have no information regarding their clinical significance in prostate cancer.

Our sample size was too small to comment on prostate cancer prognosis. Additionally, the fact that patients carrying the variations together were in different risk groups prevents us from drawing a clear conclusion regarding prostate cancer prognosis. However, as a result of our study, we identified prostate cancer patients carrying different variants of both genes.

The present dissertation examined the IDH2, and TERT gene sequences in prostate cancer patients, and as a result five variations were detected. All of these variations except TERT rs2736098 were not known to exist in prostate cancer.

For the first time, c.261G>T (rs1033845124) missense variation in the TERT gene (CADD score > 10, no ExAC frequency) was identified as possibly pathogenic for prostate cancer.

The observation of previously unidentified variants in prostate cancer demonstrates how much we still do not know about the molecular basis of this disease. At the same time, it has been revealed that molecular parameters are of vital importance in the diagnosis and treatment of prostate cancer. With the cooperation of molecular biologists, pathologists, and urologic oncologists, it is possible to bring these markers to the clinic and determine the optimal treatment strategy for each patient.

Due to the absence of a control group and the small number of patients in this thesis study, further studies are needed to clarify the clinical significance of the variations detected. Nevertheless, valuable results have been reported by our study to help elucidate the molecular biology of prostate cancer.

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7. APPENDICES

7.1. Ethical Approval

T.C. YEDİTEPE ÜNİVERSİTESİ

Sayı: 37068608-6100-15- 2518 16.Mart.2023
Konu: Klinik Araştırmalar
Etik Kurul Başvurusu hk.

Sorumlu Araştırmacı Prof. Dr.Ferda Özkan
(Yeditepe Üniversitesi, Tıp Fakültesi Patoloji AD., ISTANBUL)

Klinik Araştırma başvurunuz ve ek belgeniz Yeditepe Üniversitesi Klinik Araştırmaları Etik Kurulu (KAEK) tarafından değerlendirildi. Etik Kurulumuz, başvuru ve ek bilgileri değerlendirdikten sonra **15 Mart 2023** tarihli toplantısında **"IDH2 ve TERT Gen Varyasyonlarının Etkisinin Prostat Kanserinde Araştırılması"** başlıklı klinik araştırma protokolünüzü ve başvurunuzu onaylanmıştır.

Onay bilgileri:

Referans No : 2023/1733
Onay Süresi : Mart 2023 - Mart 2024
Yetkili Personel : Prof. Dr. Turgay İsbir

Yeditepe Üniversitesi Klinik Araştırmalarda Etik Kurulu Sağlık Bakanlığı, TİTCK Başkanlığın onayı ile oluşturulmuş ve bu projenin onaylanması, "İlaç ve Biyolojik Ürünlerin Klinik Araştırmaları Hakkında Yönetmeliği, Tıbbi Cihaz Yönetmeliği ve uluslararası etik ilkeler çerçevesinde değerlendirilmiştir. Bu araştırmaya ilişkin raporların, onay tarihinden itibaren her 12 ayda bir sunulması gerekmektedir. Raporların sunulmaması, projenin devam etmediğinin onayı olarak değerlendirilecektir.

Saygılarımla

Prof. Dr. Turgay Çelik

Klinik Araştırmaları Etik Kurul Başkanı

7.2. Patient Data

	GRUP	tanıyaş	boy	kilo	bmii	sigarapaketyıl	sisthastalık	ailedeprostat	ailedekanser	ikincikanser
hasta 1	hasta	62	178	73	23	30	yok		var	yok
hasta 2	hasta	53	178	77	24	60	HT		var	yok
hasta 3	hasta	68	165	81	30	30	HT		yok	mes
hasta 4	hasta	66	163	78	29	35	yok		var	yok
hasta 5	hasta	63	161	74	29	20	HT, DM		var	yok
hasta 6	hasta	63	170	83	29		yok		var	yok
hasta 7	hasta	70	175	85	28	20	HT, DM		var	yok
hasta 8	hasta	64	173	96	32	100	yok		yok	yok
hasta 9	hasta	77	178	105	33	20	KAH		yok	yok
hasta 10	hasta	79	170	94	33	20	DM		yok	yok
hasta 11	hasta	70	165	67	25		HT, DM		var	yok
hasta 12	hasta	83	170	86	30	30	KAH		yok	yok
hasta 13	hasta	55	175	80	26		DM		yok	yok
hasta 14	hasta	56	170	60	21		yok		yok	yok
hasta 15	hasta	69	165	90	33	35	yok		var	yok
hasta 16	hasta	61	170	85	29	20	HT		yok	yok
hasta 17	hasta	64	165	66	24		yok		var	yok
hasta 18	hasta	69	168	85	30	20	yok		yok	yok
hasta 19	hasta	77	183	82	25	20	HT, KAH		yok	yok
hasta 20	hasta	66	178	80	25	5	HT		yok	yok
hasta 21	hasta	78	165	70	26	30	yok		yok	yok
hasta 22	hasta	74	165	63	23	50	HT, KAH		yok	yok
hasta 23	hasta	67	170	90	31		HT		var	yok
hasta 24	hasta	63	167	90	32	52	HT		var	kem
hasta 25	hasta	75	155	79	33	40	HT, DM, KAH		var	yok
hasta 26	hasta	77	170	70	24	15	yok		var	yok
hasta 27	hasta	67	167	69	25	55	DM, KAH		yok	yok

prostathacim	psaa	evre	cT1c	cT2	cT3	dAmicorisk	patolojikevre	pT2a	pT2b	pT2c	pT3a	pT3b	evre23
43	20	cT1c	VAR	YOK	YOK	yüksek	pT3a	YOK	YOK	YOK	VAR	YOK	evre3
20	11	cT1c	VAR	YOK	YOK	orta	pT2c	YOK	YOK	VAR	YOK	YOK	evre2
15	7	cT1c	VAR	YOK	YOK	orta	pT2c	YOK	YOK	VAR	YOK	YOK	evre2
72	647	cT3	YOK	YOK	VAR	yüksek	pT2c	YOK	YOK	VAR	YOK	YOK	evre2
60	6,1	cT1c	VAR	YOK	YOK	yüksek	pT3a	YOK	YOK	YOK	VAR	YOK	evre3
90	11	cT2	YOK	VAR	YOK	yüksek	pT2c	YOK	YOK	VAR	YOK	YOK	evre2
78	9	cT2	YOK	VAR	YOK	yüksek	pT2a	VAR	YOK	YOK	YOK	YOK	evre2
25	26	cT2	YOK	VAR	YOK	yüksek	pT3b	YOK	YOK	YOK	YOK	VAR	evre3
90	137	cT2	YOK	VAR	YOK	yüksek	pT2c	YOK	YOK	VAR	YOK	YOK	evre2
75	7,4	cT1c	VAR	YOK	YOK	yüksek	pT2c	YOK	YOK	VAR	YOK	YOK	evre2
30	21	cT2	YOK	VAR	YOK	yüksek	pT2c	YOK	YOK	VAR	YOK	YOK	evre2
25	20	cT2	YOK	VAR	YOK	yüksek	pT2b	YOK	VAR	YOK	YOK	YOK	evre2
55	515	cT3	YOK	YOK	VAR	yüksek	pT2c	YOK	YOK	VAR	YOK	YOK	evre2
36	38	cT2	YOK	VAR	YOK	yüksek	pT2c	YOK	YOK	VAR	YOK	YOK	evre2
40	24	cT2	YOK	VAR	YOK	yüksek	pT3a	YOK	YOK	YOK	VAR	YOK	evre3
45	59	cT2	YOK	VAR	YOK	yüksek	pT3b	YOK	YOK	YOK	YOK	VAR	evre3
25	11	cT1c	VAR	YOK	YOK	orta	pT3a	YOK	YOK	YOK	VAR	YOK	evre3
35	127	cT2	YOK	VAR	YOK	yüksek	pT3b	YOK	YOK	YOK	YOK	VAR	evre3
50	59	cT2	YOK	VAR	YOK	yüksek	pT2c	YOK	YOK	VAR	YOK	YOK	evre2
45	22	cT2	YOK	VAR	YOK	yüksek	pT2c	YOK	YOK	VAR	YOK	YOK	evre2
26	22	cT2	YOK	VAR	YOK	orta	pT2b	YOK	VAR	YOK	YOK	YOK	evre2
24	81	cT3	YOK	YOK	VAR	yüksek	pT2c	YOK	YOK	VAR	YOK	YOK	evre2
58	9,4	cT1c	VAR	YOK	YOK	orta	pT2a	VAR	YOK	YOK	YOK	YOK	evre2
26	10	cT2	YOK	VAR	YOK	orta	pT3a	YOK	YOK	YOK	VAR	YOK	evre3
60	27	cT2	YOK	VAR	YOK	yüksek	pT2b	YOK	VAR	YOK	YOK	YOK	evre2
12	12	cT1c	VAR	YOK	YOK	orta	pT2b	YOK	VAR	YOK	YOK	YOK	evre2
17	5,2	cT1c	VAR	YOK	YOK	orta	pT2b	YOK	VAR	YOK	YOK	YOK	evre2

primergleason	sekondergleason	gleasonskoru	gleason7	tmyuzdesi	tanızamanımetastaz	tedavi	ektedavi	takiptemetastaz	takipsuresiay
4	5	9	>7	30	yok	radikal pro	RT,	yok	39
3	4	7	<esit7	20	yok	radikal pro	yok	yok	24
3	4	7	<esit7	40	yok	ADT	yok	yok	76
4	3	7	<esit7	90	kem	ADT	yok	var	15
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4	5	9	>7	90	yok	küratif rad	ADT	yok	204
4	4	8	>7	25	yok	radikal pro	yok	yok	4
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4	4	8	>7	70	kem	ADT	yok	yok	27
3	5	8	>7	80	yok	ADT	yok	yok	37
4	3	7	<esit7	70	yok	küratif rad	ADT	yok	17
4	4	8	>7	40	kem	ADT	yok	yok	14
4	4	8	>7	80	kem	ADT	doc	var	120
3	4	7	<esit7	80	yok	küratif rad	ADT	kem	204
4	5	9	>7	40	yok	radikal pro	yok	yok	2
4	5	9	>7	60	yok	radikal pro	RT,	yok	16
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5	4	9	>7	90	yok	radikal pro	ADT	yok	3
3	4	7	<esit7	50	kem	ADT	doc	var	17
4	3	7	<esit7	60	yok	radikal pro	yok	yok	51
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3	4	7	<esit7	75	kem	ADT	yok	yok	60
3	4	7	<esit7	2	yok	radikal pro	ADT	yok	76
4	3	7	<esit7	55	yok	radikal pro	RT,	yok	51
4	4	8	>7	50	kem	ADT	yok	yok	17
3	4	7	<esit7	10	yok	küratif rad	ADT	yok	46
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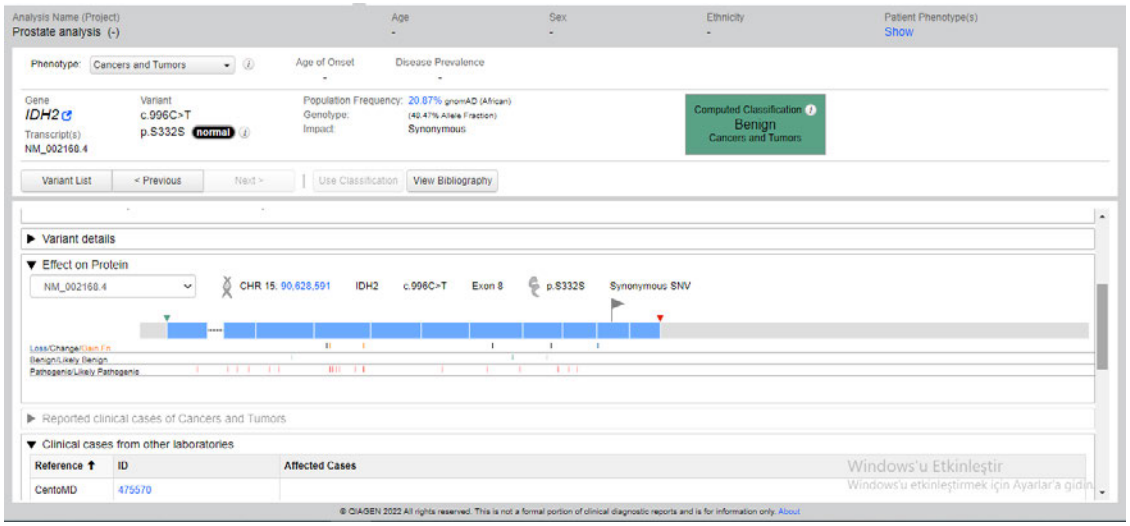
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Sex
Ethnicity
Patient Phenotype(s)
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Age of Onset
Disease Prevalence
Gene
IDH2
Variant
c.996C>T
p.S332S normal
Transcript(s)
NM_002168.4
Population Frequency: 20.87% gnomAD (African)
Genotype: (49.47% Allele Fraction)
Impact: Synonymous
Computed Classification
Benign
Cancers and Tumors
Variant List
< Previous
Next >
Use Classification
View Bibliography
Variant details
Effect on Protein
NM_002168.4
CHR 15: 90,628,591
IDH2
c.996C>T
Exon 8
p.S332S
Synonymous SNV
Loss/Change/Gain Fn
Benign/Likely Benign
Pathogenic/Likely Pathogenic
Reported clinical cases of Cancers and Tumors
Clinical cases from other laboratories
Reference
ID
Affected Cases
CentoMD
475570
Windows'u Etkinleştir
Windows'u etkinleştirmek için Ayarlar'a gidin.
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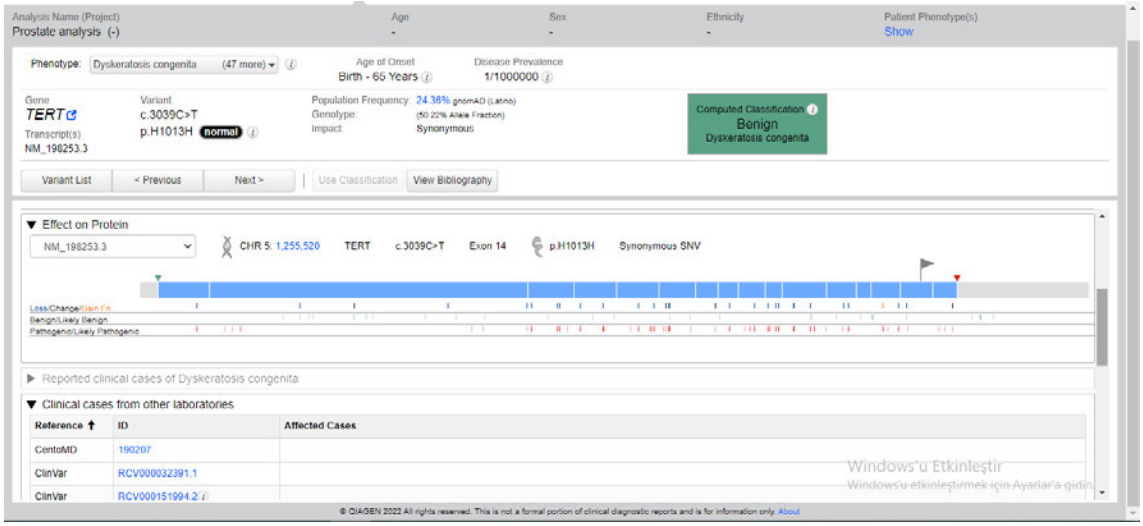
Analysis Name (Project)
Prostate analysis (-)
Age
Sex
Ethnicity
Patient Phenotype(s)
Show
Phenotype: Dyskeratosis congenita (47 more)
Age of Onset
Birth - 65 Years
Disease Prevalence
1/1000000
Gene
TERT
Variant
c.3039C>T
p.H1013H normal
Transcript(s)
NM_198253.3
Population Frequency: 24.36% gnomAD (Latino)
Genotype: (50.22% Allele Fraction)
Impact: Synonymous
Computed Classification
Benign
Dyskeratosis congenita
Variant List
< Previous
Next >
Use Classification
View Bibliography
Effect on Protein
NM_198253.3
CHR 5: 1,255,520
TERT
c.3039C>T
Exon 14
p.H1013H
Synonymous SNV
Loss/Change/Gain Fn
Benign/Likely Benign
Pathogenic/Likely Pathogenic
Reported clinical cases of Dyskeratosis congenita
Clinical cases from other laboratories
Reference
ID
Affected Cases
CentoMD
190207
ClinVar
RCV000032391.1
ClinVar
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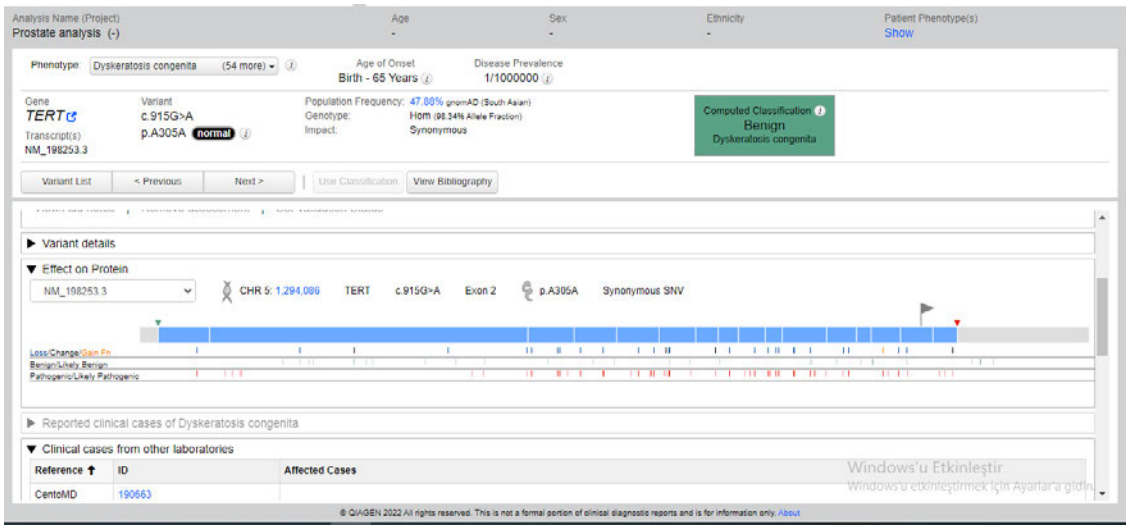
Analysis Name (Project)
Prostate analysis (-)
Age
Sex
Ethnicity
Patient Phenotype(s)
Show
Phenotype: Dyskeratosis congenita (54 more)
Age of Onset
Birth - 65 Years
Disease Prevalence
1/1000000
Gene
TERT
Variant
c.915G>A
p.A305A normal
Transcript(s)
NM_198253.3
Population Frequency: 47.66% gnomAD (South Asian)
Genotype: Hom (98.34% Allele Fraction)
Impact: Synonymous
Computed Classification
Benign
Dyskeratosis congenita
Variant List
< Previous
Next >
Use Classification
View Bibliography
Variant details
Effect on Protein
NM_198253.3
CHR 5: 1,294,086
TERT
c.915G>A
Exon 2
p.A305A
Synonymous SNV
Loss/Change/Gain Fn
Benign/Likely Benign
Pathogenic/Likely Pathogenic
Reported clinical cases of Dyskeratosis congenita
Clinical cases from other laboratories
Reference
ID
Affected Cases
CentoMD
190663
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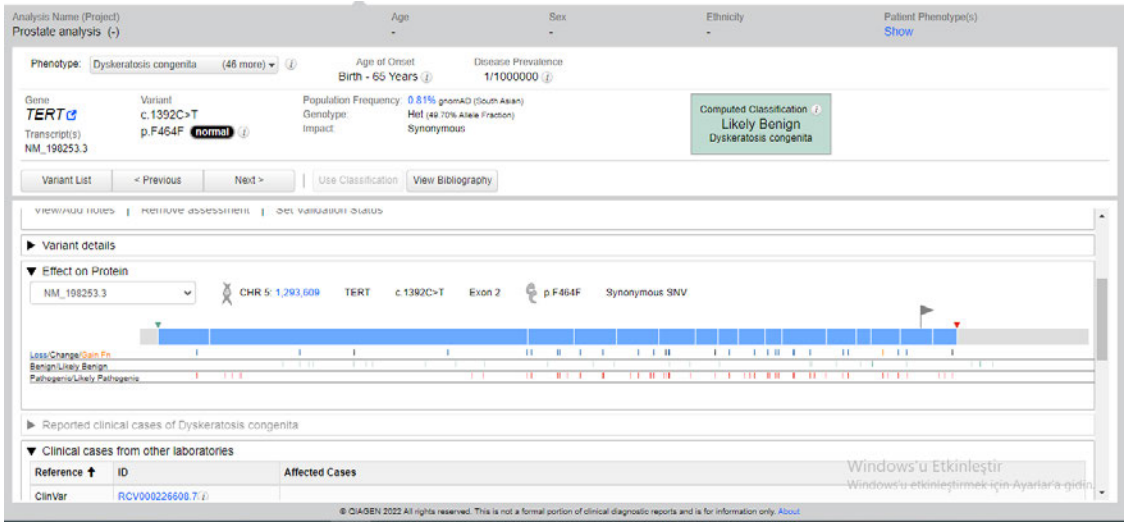
Analysis Name (Project)
Prostate analysis (-)
Age
-
Sex
-
Ethnicity
-
Patient Phenotype(s)
Show
Phenotype: Dyskeratosis congenita (46 more)
Age of Onset
Birth - 65 Years
Disease Prevalence
1/1000000
Gene
TERT
Transcript(s)
NM_198253.3
Variant
c.1392C>T
p.F464F normal
Population Frequency: 0.81% gnomAD (South Asian)
Genotype: Het (49.70% Allele Fraction)
Impact: Synonymous
Computed Classification
Likely Benign
Dyskeratosis congenita
Variant List
< Previous
Next >
Use Classification
View Bibliography
Variant details
Effect on Protein
NM_198253.3
CHR 5: 1,293,609
TERT
c.1392C>T
Exon 2
p.F464F
Synonymous SNV
Loss/Change/Gain Fn
Benign/Likely Benign
Pathogenic/Likely Pathogenic
Reported clinical cases of Dyskeratosis congenita
Clinical cases from other laboratories
Reference
ID
Affected Cases
ClinVar
RCV000226600.7
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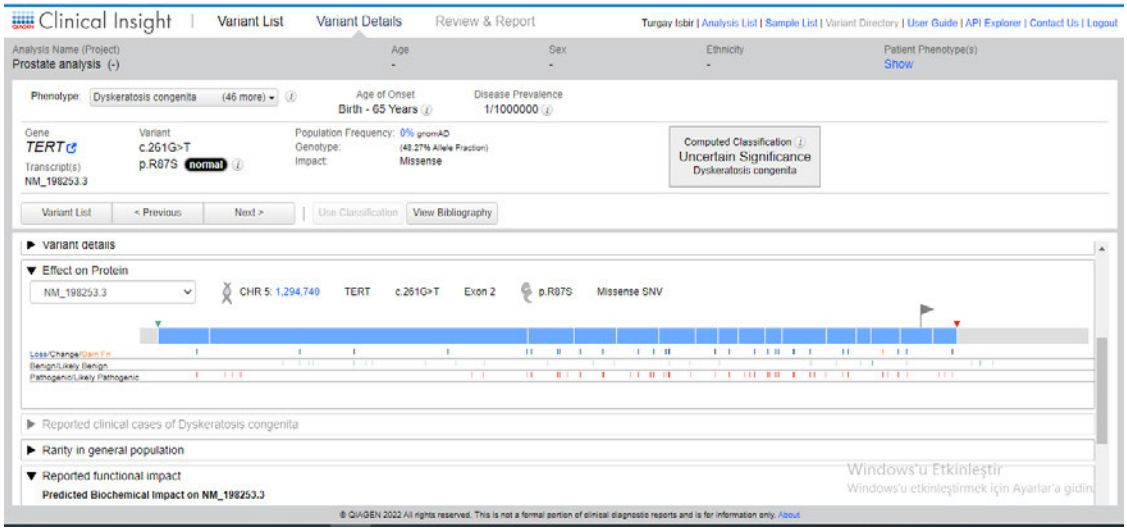
Clinical Insight
Variant List
Variant Details
Review & Report
Turgay Isbir | Analysis List | Sample List | Variant Directory | User Guide | API Explorer | Contact Us | Logout
Analysis Name (Project)
Prostate analysis (-)
Age
-
Sex
-
Ethnicity
-
Patient Phenotype(s)
Show
Phenotype: Dyskeratosis congenita (46 more)
Age of Onset
Birth - 65 Years
Disease Prevalence
1/1000000
Gene
TERT
Transcript(s)
NM_198253.3
Variant
c.261G>T
p.R87S normal
Population Frequency: 0% gnomAD
Genotype: (48.27% Allele Fraction)
Impact: Missense
Computed Classification
Uncertain Significance
Dyskeratosis congenita
Variant List
< Previous
Next >
Use Classification
View Bibliography
Variant details
Effect on Protein
NM_198253.3
CHR 5: 1,294,740
TERT
c.261G>T
Exon 2
p.R87S
Missense SNV
Loss/Change/Gain Fn
Benign/Likely Benign
Pathogenic/Likely Pathogenic
Reported clinical cases of Dyskeratosis congenita
Rarity in general population
Reported functional impact
Predicted Biochemical Impact on NM_198253.3
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7.3. Cirruculum Vitae

Personal Informations

Name	Ferda	**Surname**	OZKAN

Educational Informations

Title	Name of Institution	Year
Doctorate	Yeditepe University Institute of Health Sciences, Department of Molecular Medicine	2017- 2023
Medical Expertise	Istanbul University Cerrahpaşa School of Medicine, İstanbul	1989
Medical School	Istanbul University School of Medicine, İstanbul	1983- 1989

Work Experience

Job Title	Name of Institution	Year
Intern Doctor		
Assoc Prof	Yeditepe University School of Medicine, Department of Pathology	
Head of Department	Yeditepe University School of Medicine, Department of Pathology	- Present
Professor	Yeditepe University School of Medicine, Department of Pathology	- Present

Computer skills

Program	Ability to use
Microsoft Office	Very Good