

T.C.
YEDİTEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF MOLECULAR MEDICINE

**MUTATION ANALYSIS of IDH 1 GENE in
PROSTATE CANCER PATIENTS BY NEXT
GENERATION SEQUENCING METHOD**

DOCTOR OF PHILOSOPHY THESIS

FARUK YENCİLEK, M.D.

İstanbul-2023

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FARUK YENCİLEK, M.D.

SUPERVISOR
Prof. Dr. Turgay İSBİR

İstanbul-2023

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This study have approved as a Doctorate Thesis in regard to content and quality by the Jury.

	Title, Name-Surname (Institution)
Chair of the Jury	Prof. Dr. Turgay İSBİR Yeditepe University
Supervisor:	Prof. Dr. Turgay İSBİR Yeditepe University
Member/Examiner:	Prof. Dr. S. Ayşe ÖZER Yeditepe University
Member/Examiner:	Prof. Dr. Haydar Kamil ÇAM Marmara University
Member/Examiner:	Prof. Dr. Arzu ERGEN İstanbul University
Member/Examiner:	Prof. Dr. Bedia ÇAKMAKOĞLU İstanbul University

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

Faruk Yencilek



DEDICATION

I dedicate my thesis to my lovely Family

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TABLE OF CONTENTS

APPROVAL	ii
DECLARATION	iii
DEDICATION	iv
ACKNOWLEDGEMENTS	v
TABLE OF CONTENTS	vi
LIST OF TABLES	viii
LIST OF FIGURES	ix
LIST OF SYMBOLS AND ABBREVIATIONS	xi
ABSTRACT	xii
ÖZET	xiii
1. STATEMENT AND PURPOSE.....	1
2. LITERATURE REVIEW	3
2.1. Overview of Prostate Cancer	3
2.2. Molecular Bases of Prostate Cancer	7
2.2.1. Isocitrate dehydrogenase 1 (IDH1).....	9
2.2.2. O6-Methylguanine-DNA methyltransferase (MGMT)	11
2.2.3. Alpha-thalassemia/Mental retardation, X linked (ATRX)	12
3. MATERIALS and METHODS.....	13
3.1. The Study Population and Aim.....	13
3.2. Explanation of Genomic DNA Isolation from Blood Protocol	13
3.3. Measurement of DNA Purity Measurement and Concentration.....	14
3.4. Design of the Gene Panel, Library Preparation and Next Generation Sequencing .	14
3.4.1. Fragmentation, end-repair, and addition.....	15
3.4.2. Adapter ligation of samples	16

3.4.3. Clearance of adapter-bound DNA with QIAseq Beads	16
3.4.4. Target enrichment PCR	18
3.4.5. Clearance of the target enrichment PCR with QIAseq Beads	18
3.4.6. Universal PCR amplification.....	19
3.4.7. Clearance of the Universal PCR with QIAseq beads	19
3.4.8. QIAseq Quant Assay Protocol	20
3.4.9. QIAseq Upload Protocol	21
3.5. Validation of Mutations by Sanger Sequencing	22
3.5.1. Designing Primers for Sanger Sequencing.....	22
3.5.2. Polymerase Chain Reaction (PCR).....	23
3.5.3. Agarose gel electrophoresis, PCR purification and Sequence PCR	23
3.5.4. Sequence Analysis	25
3.6. Statistical Analysis Method	25
4. RESULTS	26
4.1. Demographic Characteristics of the Study Population	26
4.2. Next Generation Sequencing (NGS) Analysis	28
4.3. Validation of Mutations by Sanger Sequencing	31
5. DISCUSSION and CONCLUSION	40
6. REFERENCES	46
7. APPENDICES	57
7.1. Ethical Approval	57
7.2. Patient Data	58
7.3. Curriculum Vitae	64

LIST OF TABLES

Table 1.	Grading and Staging System for prostate cancer	6
Table 2.	Fragmentation, End-Repair, and Addition.....	15
Table 3.	Adapter Ligation of Samples	16
Table 4.	Target Enrichment	18
Table 5.	Universal PCR Amplification	19
Table 6.	Qiaseq Quant Assay Protocol	20
Table 7.	Designed Primer Sequences.....	22
Table 8.	The Reaction Mix Prepared for Each Sample	23
Table 9.	Thermal Cycle Program.....	23
Table 10.	PCR Purification (Exosap) Components	24
Table 11.	Thermal Profile	24
Table 12.	Sequence PCR	24
Table 13.	Thermal Profile of Sequence PCR	24
Table 14.	Patient Demographic and Clinical Characteristics	27
Table 15.	IDH1, MGMT and ATRX gene mutations and their clinical value for the D'Amico Risk Groups	28
Table 16.	Summary of 11 mutations detected with NGS	29

LIST OF FIGURES

Figure 1.	Most commonly diagnosed cancer among men worldwide, 2020.....	3
Figure 2.	An illustration of the progression of prostate cancer	4
Figure 3.	Wild-type IDH1 and Mutant IDH1 and cellular consequences	11
Figure 4.	Synopsis of the Qiaseq Agarose gel electrophoresis image of IDH1 and MGMT gene mutations in prostate cancer patients	31
Figure 5.	Heterozygous mutation in IDH1 c.548A>G (p.Y183C) (rs34599179) (Patient: HG)	32
Figure 6.	Wild-type (normal) MGMT c.343C>T (rs12917) (Patient: AH) forward image and confirmation by Sanger Sequencing.....	32
Figure 7.	Wild-type (normal) MGMT c.343C>T (rs12917) (Patient: FY) forward image and confirmation by Sanger Sequencing.....	33
Figure 8.	Wild-type (normal) MGMT c.343C>T (rs12917) (Patient: SŞ) forward image and confirmation by Sanger Sequencing.....	33
Figure 9.	Wild-type (normal) MGMT c.343C>T (rs12917) (Patient: YM) forward image and confirmation by Sanger Sequencing.....	34
Figure 10.	Wild-type (normal) MGMT c.343C>T (rs12917) (Patient: ZC) forward image and confirmation by Sanger Sequencing	34
Figure 11.	Heterozygous mutation in the MGMT gene c.159C>T (rs1803965) reverse image and confirmation by Sanger Sequencing (Patient: AH).....	35
Figure 12.	Heterozygous mutation in MGMT c.250C>T (rs12917) and normal MGMT gene c.252C>T (rs1803965) variant (Patient: AH) reverse image and confirmation by Sanger Sequencing.....	35
Figure 13.	Heterozygous mutation in MGMT c.159C>T (rs1803965) (Patient: FY) reverse image and confirmation by Sanger Sequencing	36
Figure 14.	Heterozygous mutation in MGMT c.250C>T (rs12917) and normal MGMT gene c.252C>T (rs1803965) variant (Patient: FY) reverse image and confirmation by Sanger	36
Figure 15.	Heterozygous mutation in MGMT c.159C>T (rs1803965) (Patient: SŞ) reverse image and confirmation by Sanger Sequencing	37
Figure 16.	Heterozygous mutation in MGMT c.250C>T (rs12917) and normal MGMT gene c.252C>T (rs1803965) variant (Patient: SŞ) reverse image and confirmation by Sanger Sequencing.....	37

Figure 17. Heterozygous mutation in MGMT c.159C>T (rs1803965) (Patient: YM) reverse image and confirmation by Sanger Sequencing	38
Figure 18. Heterozygous mutation in MGMT c.250C>T (rs12917) and normal MGMT gene c.252C>T (rs1803965) variant (Patient: YM) reverse image and confirmation by Sanger Sequencing.....	38
Figure 19. Heterozygous mutation in MGMT c.159C>T (rs1803965) (Patient: ZC) reverse image and confirmation by Sanger Sequencing	39
Figure 20. Heterozygous mutation in MGMT c.250C>T (rs12917) and normal MGMT gene c.252C>T (rs1803965) variant (Patient: ZC) reverse image and confirmation by Sanger Sequencing.....	39



LIST OF SYMBOLS AND ABBREVIATIONS

ATRX	Alpha-thalassemia/mental retardation, X-linked
BRCA1	Breast cancer 1
BRCA2	Breast cancer 2
CST	Charge Switch Technology
D2HG	D-2-Hydroxyglutarate
DNA	Deoxyribonucleic Acid
IDH	Isocitrate Dehydrogenase
MGMT	O6-Methylguanine-DNA Methyltransferase
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
SNP	Single nucleotide polymorphism
TET	Ten-eleven translocation methylcytosine dioxygenase

ABSTRACT

Yencilek, F. (2023). Mutation Analysis of IDH1 Gene in Prostate Cancer Patients by Next Generation Sequencing Method. Yeditepe University, Institute of Health Sciences, Department of Molecular Medicine, PhD thesis, Istanbul.

The second most common type of cancer among men is prostate cancer. The aim of our study was to evaluate MGMT and ATRX genes, especially IDH1, with next-generation sequencing, to detect potential mutations and their relationship with risk classes of the disease. Analyses were performed using peripheral blood samples from 27 patients diagnosed with prostate cancer. IDH1, MGMT and ATRX genes were evaluated by next generation sequencing. In addition, analysis was performed to evaluate the effects of detected mutations in D'Amico intermediate-risk and high-risk groups. The mean age of the study group was 68 years (53-83); 88.89% of the patients were aged 60 years or older, and 11.11% were under 60 years of age. The prostate volume, PSA level, cT1c, Gleason score, and Tm % of the patient group's clinical data were significantly different between the intermediate-risk and high-risk patient groups ($p=0.003$, $p=0.048$, $p=0.000$, and $p=0.001$, respectively). The target genes were sequenced, and as a result, 11 mutations were found in total: 3 in IDH1, 6 in MGMT, and 2 in ATRX genes. The majority of the detected mutations in prostate cancer were identified for the first time. According to D'Amico risk groups, it was found that the c.159C>T (rs1803965) and c.250C>T (rs12917) MGMT gene mutations varied significantly from one another ($p=0.017$). The heterozygous mutant genotypes of the patient with the IDH1 c.548 A>G (rs34599179) missense mutation, the five patients with the MGMT gene c.159C>T synonymous mutation, and the five prostate cancer patients with the MGMT gene c.250C>T missense mutation were confirmed by Sanger sequencing. These identified mutations should undoubtedly be included in the classification of prostate cancer. Whether or not they can be assessed in clinical applications is debatable. However, integrating molecular parameters with standard evaluation criteria will contribute to the application of personalized treatment methods by evaluating the clinical data of prostate cancer patients with a higher accuracy rate.

Key Words: Next Generation Sequencing, Prostate Cancer, IDH1, MGMT, ATRX

ÖZET

Yencilek, F. (2023). Prostat Kanseri Hastalarında IDH 1 Gen Mutasyonlarının Yeni Nesil Dizileme Metodu ile Belirlenmesi. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, Moleküler Tıp ABD., Doktora Tezi. İstanbul.

Erkekler arasında en sık görülen ikinci kanser türü prostat kanseridir. Çalışmamızın amacı, potansiyel mutasyonları ve onların hastalığın risk sınıfları ile ilişkisini saptamak için IDH1 başta olmak üzere MGMT ve ATRX genlerini yeni nesil dizileme ile değerlendirmektir. Analizler prostat kanseri tanısı almış 27 hastanın periferik kan örnekleri kullanılarak gerçekleştirilmiştir. Yeni nesil dizileme ile IDH1, MGMT ve ATRX genleri değerlendirildi. Ayrıca, tespit edilen mutasyonların D'Amico orta-risk ve yüksek-risk grupları için belirteç adaylığını değerlendirmek için analiz yapılmıştır. Çalışma grubunun ortalama yaşı 68 (53-83) olmakla birlikte, hastaların %88.89'u 60 yaş ve üzeri iken, %11.11'i 60 yaşın altındaydı. Hasta grubunun prostat hacmi ($p=0.003$), PSA seviyesi ($p=0.048$), cT1c ($p=0.000$), Gleason skoru ($p=0.001$), Tm yüzdesi (0.0006) gibi klinik veriler orta-riskli ve yüksek-riskli hasta grupları arasında anlamlı olarak farklılık göstermiştir. Araştırmaya konu olan hedef genlerin dizilimi sonucunda, 3'ü IDH 1'de, 6'sı MGMT'de, 2'si ATRX'te olmak üzere toplamda 11 mutasyon tespit edilmiştir. Tespit edilen mutasyonların büyük çoğunluğu prostat kanserinde ilk defa tanımlanmıştır. D'Amico risk gruplarına göre MGMT geninde tespit edilen c.159C>T (rs1803965) ve c.250C>T (rs12917) mutasyonlarının anlamlı olarak farklılık gösterdiği belirlenmiştir ($p=0.017$). IDH1 c.548A>G missense mutasyonunu taşıyan hastanın heterozigot mutant, MGMT geni c.159C>T sinonim varyantını taşıyan beş hastanın heterozigot mutant ve MGMT geni c.250C>T missens mutasyonunu taşıyan beş prostat kanseri hastasının heterozigot mutant karakterde olduğu Sanger sekanslaması ile doğrulanmıştır. Tanımlanan mutasyonların klinik uygulamalarda değerlendirmeye alınabilmesi tartışmaya açık olmasına rağmen, moleküler belirteçlerin prostat kanserinin sınıflandırmasında değerlendirmeye alınması gerektiği açıktır. Bununla birlikte, moleküler parametrelerin standart değerlendirme kriterlerine dahil edilmesinin prostat kanseri hastalarının klinik verilerinin daha yüksek doğruluk oranıyla değerlendirilerek, kişiye özel tedavi yöntemlerinin daha etkin bir şekilde uygulanabilmesine katkı sunacaktır.

Anahtar Kelimeler: Yeni Nesil Dizileme, Prostate Kanseri, IDH1, MGMT, ATRX

1. STATEMENT AND PURPOSE

According to statistics on cancer worldwide, prostate cancer is the leading type of cancer in men (1). Statistics in Türkiye also show compatibility with the world, and it is revealed by the data that prostate cancer comes second to lung cancer among men (1, 2). Many factors can become risk factors for prostate cancer, including being 50 years of age or older, race, family history, and diet (3-5). The fact that prostate cancer is a disease that has various risk factors and remains unresolved worldwide increases the importance of finding cross-point diagnosis and treatment methods. Today, despite some parameters such as prostate-specific antigen (PSA), Gleason score and pathological stages in the detection of prostate cancer, and advances in surgical techniques, none alone is sufficient (6). The presence of some molecular parameters that are stated to provide diagnostic sensitivity and clinical benefit in prostate cancer are rapidly reported with genome-wide analyses every day. For this reason, there is an increasing interest in studies that try to clarify the questions about the relationship between the target genes brought into the literature and the disease. Thanks to numerous methods, including next-generation sequencing, fusions, mutations, deletions, and amplifications have been shown in detail in various genes, thus enabling molecular classification (7, 8).

Although the frequency of mutation of the isocitrate dehydrogenase 1 (IDH 1) gene, which encodes an enzyme to pursue citrate metabolism neatly, is below 3% in prostate cancer, it has been reported that it is among the genes that lie behind the epigenome alterations reported in cancer types (9, 10). IDH1 from the IDH enzyme family, which has three isoforms, one of which is not fully elucidated, mediates the production of cytoplasmic alpha-ketoglutarate (aKG), which is necessary for epigenetic regulation (10). Mutations in IDH1 are known to mediate the formation of D-2-hydroxyglutarate (2-DHG) instead of aKG. This ultimately leads to genome hypermethylation. The reason is that the mechanisms that progress in the presence of aKG are affected by the change in enzymatic activity (10-12). The most common type of cancer with IDH1 mutations is gliomas. As mentioned earlier, these mutations, which are not frequently seen in prostate cancer, are thought to be one of the oncogenic drivers that can be identified in prostate cancer.

O6-Methylguanine-DNA-methyltransferase, MGMT, as a part of deoxyribonucleic acid (DNA) repair mechanism, can inactivate itself as well as perform the repair process stoichiometrically. Loss of expression has been reported in prostate cancer. It has been reported that variations in the gene that produces this enzyme both pose a risk and have no effect on prostate cancer (13).

Alpha thalassemia/mental retardation syndrome X-linked (ATRX), which plays a role in the regulation of DNA damage response and is one of the most mutated genes in cancer, provides chromosome stability (14). Although reported mutations of the ATRX gene have been detected, the relationship between this gene, which has a high mutation frequency, and prostate cancer, has not been demonstrated. There is no information to clearly explain the relationship between mutations in the genes briefly summarized above and prostate cancer. For this reason, it is of great importance to detect mutations in prostate cancer in terms of discovering new diagnostic markers and laying the groundwork for more advanced techniques in addition to existing methods.

This thesis aims to determine the importance of MGMT and ATRX genes, which have been investigated in addition to the IDH 1 gene, whose mutations have been reported to play a role in various cancer types including prostate cancer, and to bring the mutations in this gene to the medical literature in detail with the next-generation sequencing method.

2. LITERATURE REVIEW

2.1. Overview of Prostate Cancer

While prostate cancer is the second most common type of cancer in men, it ranks third among cancer types regardless of gender (1). Prostate cancer, which is diagnosed in 112 of 185 countries around the world, ranks second in Türkiye as well (1, 2) (Figure 1). While the countries with the highest incidence are in Northern and Western Europe, the incidence for those countries is between seventy and one hundred per hundred thousand people. This rate is less than half in our country (35 per 100,000 men) (1, 15). It is so rare to detect prostate cancer cases at the age of 40 and below, and it has been reported that cases intensify between the ages of 65 and 74 (15, 16).

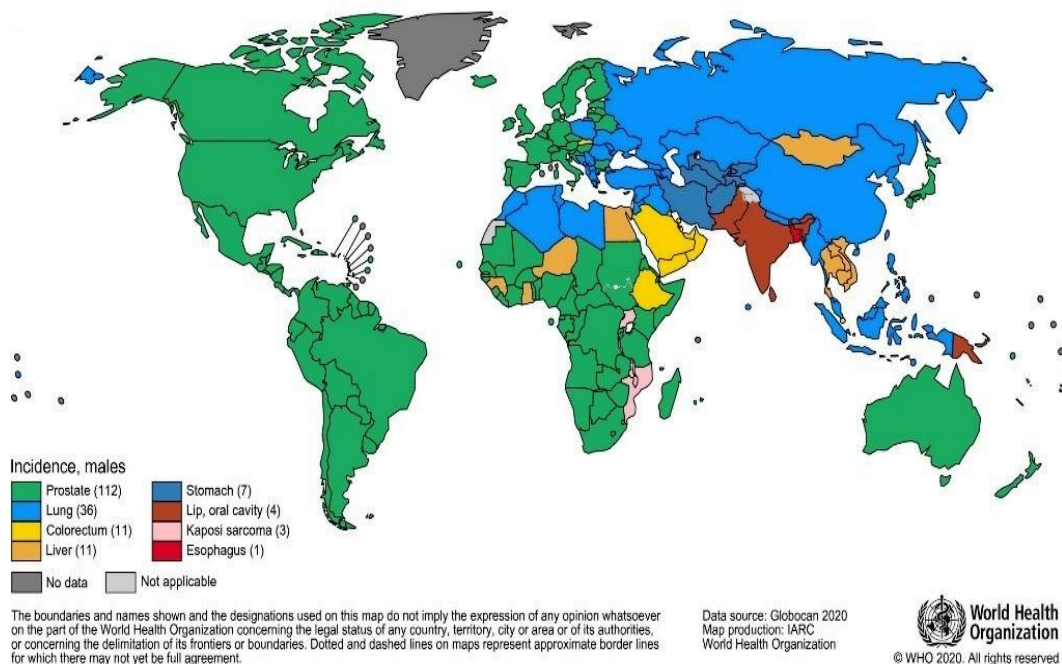


Figure 1: Most commonly diagnosed cancer among men worldwide, 2020 (1)

The peripheral, transition, and central zones form the anatomical contours of the human prostate (17). Sixty to seventy-five percent of human prostate cancer originates in the peripheral zone (18, 19). This is followed by the transition zone, where benign prostate enlargement originates, and the central zone with approximately twenty-five percent and ten percent, respectively (20-22).

If we look at the prostate gland at the cellular level, three epithelial cells are seen: luminal, basal, and neuroendocrine (23, 24). Although the origin of prostate cancer is not clearly defined, some studies hold luminal or basal cells responsible for the origination of cancer (25). One study reported that prostate tumors originating from the luminal cell have an aggressive character and a worse prognosis (26). Another one has suggested that being of basal stem cell origin causes it to draw a more aggressive prostate cancer image (27). Although there is no clarity regarding the cell of origin, changes are observed in both luminal and basal cells during the formation process of prostate cancer. The general observation is an increase in luminal cells accompanying the basal loss (25). Three stages in which changes in these cells illuminate the formation of prostate tumors are detailed. Among these, basal cell loss and hyperplasia in luminal cells are seen in the precancerous period, also known as intraepithelial neoplasia. Androgen-dependent adenocarcinoma is the stage in which the luminal cell phenotype is completely lost with the dominance of the basal cells. The next stage is the androgen-independent or castration-resistant process in which adenocarcinoma pursues to transform (25). Since it is reported that they can provide clues about both the origin of prostate cancer and the process of the disease, there are many cells in the prostate epithelium, from fibroblasts to immune cells, which have the potential to play a role in the course of prostate cancer, even though they are the two most prominent cells in prostate cancer (28).

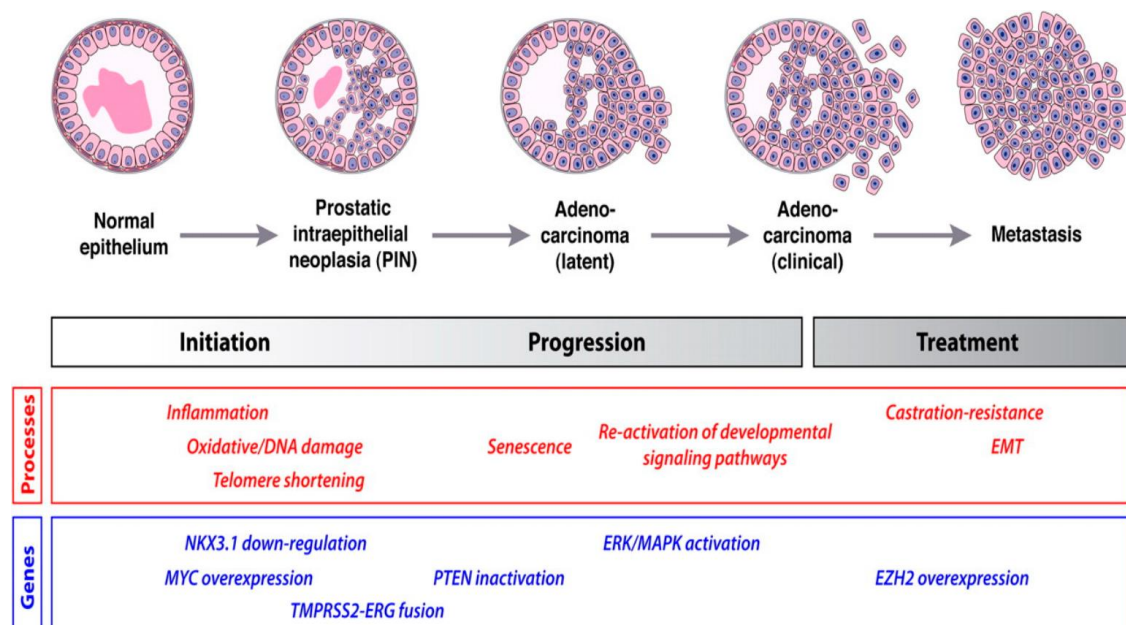


Figure 2. An illustration of the progression of prostate cancer (29).

As with other cancer types, there is a great need for prognostic data in prostate cancer. The absence of any specific prostate cancer is due to the presence of non-widespread symptoms that indicate benign prostatic disorders. There are very few biochemical and pathological criteria of clinical value for the detection and diagnosis of this cancer. Serum prostate-specific antigen (PSA) level, rectal examination, and prostate biopsy are some of them (30). The prevalence of research at the molecular level and the methods used increase immensely. On the other hand, there are very few biochemical and pathological criteria of clinical value for the detection and diagnosis of this cancer.

Although the proliferation of studies at the molecular level and the increased sensitivity of the methods used gradually bring the discovery of biomarkers closer, a biomarker that can be specific for prostate cancer has not yet been brought to the clinic. Examination of tumor architecture and interpretation by pathologists is possible by performing histological grading. The Gleason score was introduced to the medical world by Gleason and Mellinger in 1974 (31). This criterion, which enables the grading of prostate cancer to be completed in a standardized way, has been a determinant that provides an understanding of the behavior of cancer (32). The Gleason score provides ratings over five categories. A value of 1 represents the least aggressive, normal prostate tissue, while a value of 5 indicates how high the aggressiveness is and represents abnormal architecture (33). Although this rating independently is not adequate, it supplies valuable clinical data for shaping the course of treatment and prognosis of the disease. TNM staging is a classification system that determines the extent of cancer by determining tumor size (T), spread to nearby lymph nodes (N), and metastasis (M). Categorizing tumors from T1 to T4, this staging is also used to determine the clinical stage of the disease (34).

Table 1. Grading and Staging System for prostate cancer (35, 36)

System	Category	Description
Gleason Grading ^a	Gleason 1	Well-differentiated, closely packed, uniform, medium-sized glands
	Gleason 2	Glands somewhat loosely arranged, there may be minimal infiltration
	Gleason 3	Smaller, discrete glands, marked variation in size and shape of the glands with some infiltration
	Gleason 4	Fused glands with an irregular border
	Gleason 5	Poorly-differentiated, no glandular differentiation
TNM Staging ^b	Tumour (T)	T1 Clinically inapparent tumour (neither palpable nor visible by imaging)
		T1a Tumour in ≤5% of resected tissue
		T1b Tumour in >5% of resected tissue
		T1c Tumour identified by needle biopsy
		T2 Tumour confined within prostate
		T2a Unilateral, ≤one-half of one lobe
		T2b Unilateral, >one-half of one lobe
		T2c Bilateral
		T3 Tumour extends through the prostate capsule
		T3a Extracapsular extension
		T3b Invasion into seminal vesicle(s)
		T4 Tumour invades adjacent structures other than seminal vesicles
	Node (N)	N0 No regional lymph node metastases
		N1 Regional lymph node metastases
	Metastasis (M)	M0 No distant metastases
		M1 Distant metastases
		M1a Nonregional lymph node(s)
		M1b Bone(s)
		M1c Other site(s)

Since prostate cancer is organ-confined or has the potential to metastasize, it is important to limit the determining exact criteria. The PSA level of 0.15 ng/mL, the absence of a Gleason score of 4 or 5, and a tumor volume of less than 0.5 cm³ are the criteria taken into consideration for the detection of the clinically indolent state of prostate cancer. This cancer can also have an aggressive profile that is resistant to treatment and can have fatal consequences. Among the criteria for the presence of this type are the detection of N1 and M1 while the tumor is T3 or worse according to TNM staging, and the calculation of a Gleason score greater than 7 (37-39).

Because prostate cancer has a heterogeneous structure and its aggressiveness varies, there is a need for biomarkers that will enable the diagnosis of this cancer and the proper treatment to be applied with great precision by making the distinction between

its subtypes. No more adequate biomarker candidate has been found to date than the criteria that continue to be used, such as the Gleason score. Therefore, studies aimed at elucidating the genetics and biology of prostate cancer at the molecular level are of great value.

Planning the treatment of prostate cancer can be accomplished by determining the stage of the disease, as well as evaluating factors such as the age of the patient and whether there is any other concomitant disease (40, 41). Compliance with the temperament of prostate cancer is also a determining factor in the appropriate treatment selection. Prostatectomy, radiotherapy, chemotherapy, and hormone therapy are the main applications that take care of increasing the quality of life of the patient, as well as controlling the disease. On the other hand, preventive measures are generally preferred for low-risk prostate cancer (42).

2.2. Molecular Bases of Prostate Cancer

The reason why prostate cancer is a complicated disease is the involvement of many molecules and molecular pathways both in its development and in its progression. At the helm of the development and progression of cancer, which is called carcinogenesis, there are changes in the genetic material. These changes can be inherited or occur through various events, from oxidative stress to carcinogen exposure. As one of the results, mutations occurring in protooncogenes, tumor suppressor genes, and DNA repair genes contribute to the course of the carcinogenesis process (43). Data from a wide range of studies, from twin studies to epidemiological studies, are increasing daily to elucidate the molecular mechanisms of prostate cancer (25). While the family history of prostate cancer as one of the factors that increase the risk of prostate cancer has been reported by epidemiological studies (44), it has been revealed by studies conducted with twins that it is one of the hereditary cancers (45). In addition, the fact that studies conducted on ethnic groups report that African men have the highest data when both the incidence of the disease and the mortality rate are evaluated is one of the biggest signs that prostate cancer is based on genetics (46, 47). Prostate cancer-specific studies have demonstrated the presence of single nucleotide polymorphisms (SNPs) in genes associated with prostate cancer (48). One of the indicated SNPs, rs339331, causes an increase in the expression of Regulatory Factor X6 (RFX6) as a gene that triggers cancerogenesis because of its association with a genes associated with

susceptibility to prostate cancer, such as Homeobox B13 (HOXB13) (49). In addition, many loci have been brought to the scientific world through the recent genome-wide association (GWAS) studies that question the susceptibility to prostate cancer (50, 51).

Studies are showing the presence of familial mutations in both prostate cancer susceptibility genes and DNA repair genes (52, 53). Detection of alterations that occur in the formation and progress of prostate cancer is valuable in terms of enabling the subtypes of the disease to be separated from each other more precisely, thus enabling clearer treatment strategies to be devised. Genome-wide large-scale studies have determined that changes in DNA include mutations, gene fusions, and copy number changes (25).

Translocation observed in androgen-regulated promoters, and Erythroblast Transformation Specific (ETS) family members such as ETS-Related Gene (ERG), ETV genes rank first among the genomic alterations that can be detected in prostate cancer (54). Fusion between Transmembrane Serine Protease 2 (TMPRSS2) and members of the ERG family is seen in more than half of non-metastasized prostate cancers (55). In addition, deletion of NK3 Homeobox 1 (NKX3.1) gene, which is considered among the tumor suppressor genes by playing a role in the response to DNA damage, is associated with prostate cancer. As with the 8p of the NKX3.1 gene, the deletion in the 10q of the Phosphatase and tensin homolog (PTEN) gene is also responsible for the formation of prostate cancer. As with the 8p of the NKX3.1 gene, the deletion in the 10q arm of the PTEN gene is also involved in prostate carcinogenesis. The main reason why these changes detected in PTEN remain in the carcinogenesis process is that this gene is a tumor suppressor that plays a role in the cell survival pathway as a regulator (56-58). In 40% of prostate cancer, there is a loss of this gene due to mutations in the gene as well as PTEN deletion (25, 59). Among the mutations detected in prostate cancer, the relationship between mutations in genes localized in DNA repair pathways and susceptibility to the disease is seen in breast cancer 1 (BRCA1) and breast cancer 2 (BRCA2) gene mutations, which are also commonly detected in ovarian and breast cancer (60). Germline mutations in these genes can result in the development of prostate cancer as well as a more aggressive phenotype (25). Genomic investigations have found abnormalities in genes involved in DNA repair, such as RAD51, ATM, and ATR, in close to 35% of metastatic prostate malignancies (61).

Epigenetic changes in some gene regions, including the IDH1 gene, for which mutation analyses were performed in this thesis, have been described in prostate cancer (62-64). The role of DNA methylation in gene regulation has led to questioning its relationship with carcinogenesis. Studies have reported that hypermethylation occurs especially in CpG islands, which are regions rich in cytosine and guanine, and this shows its effect on carcinogenesis through the silencing of genes. IDH1 is among the 15 genes found to be involved in prostate carcinogenesis (64, 65).

2.2.1. Isocitrate dehydrogenase 1 (IDH1)

IDH 1 gene codes for one of the metabolic enzymes, isocitrate dehydrogenase 1, which performs a variety of activities within the cell, the most significant of which is its participation in the Krebs cycle, which is involved in energy generation. This critical mechanism allows living cells to obtain energy from food. During the Krebs cycle, alpha-ketoglutarate (aKG) is produced, which is a determinative factor of the rate of this cycle, by isocitrate oxidative decarboxylation catalyzed by IDH1. In this activity of IDH1, NADP⁺, producer of oxidative damage defender NADPH, is employed as a cofactor. This enzyme is found in peroxisomes and the cytoplasm; it is a homodimeric enzyme with two active sites per dimer. In general, subunits of IDH enzymes form three domains, which are identified as: large, small and clasp domains, whereas IDH2 has an extra mitochondrial targeting sequence. In IDH1, known to be involved in activities such as glucose metabolism, redox control, lipogenesis and glutamine production, its active site has NADPH and isocitrate binding sites, as in other isoforms (66).

Hyperlipidemia and obesity were associated with IDH1 overexpression in the liver and adipose tissues by a study performed in transgenic mice (67). Another animal experiment showed that astrocyte development is among the conditions under the control of IDH1 (68). In addition to these, IDH1 is also associated with the oxidative stress mechanism. As mentioned earlier, NADPH is a defender against oxidative stress and achieves this by reducing oxidized glutathione and thioredoxin (69). The producer of this molecule, IDH1, prevents lipid peroxidation and oxidative DNA damage in this way (70). Therefore, wild-type IDH1 is required for the control of oxidative stress in response to various cellular stressors.

The presence of IDH1 gene mutations has been detected in various types of cancer (71). As a result of the mutation of IDH1, the activity of the enzyme is changed, and αKG is transformed into another intermediate molecule, D-2-hydroxyglutarate (D-2-HG). The accumulation of D-2-HG interferes with the actions of enzymes involved in cellular processes, alters cellular metabolism and epigenetic regulation, and promotes tumor formation by blocking the DNA repair process. It also disturbs the cell division cycle by blocking the action of enzymes that remove methyl groups from histone and DNA. Additionally, it promotes genes that help cells survive in low-oxygen situations, which leads to tumor growth.

Tumors with IDH1 mutations frequently exhibit aberrant histone and DNA methylation patterns, which may result in stem cell changes and carcinogenesis (71). According to the findings of various clinical research, CpG island hypermethylation and IDH mutation are strongly related. The best example that can be given on this subject is the CpG island methylator phenotype (CIMP). This epigenetic marker is counted as a sign of IDH mutant in solid glioma tumors (72, 73). Neomorphic activity as a feature of IDH1 mutation has been reported to mediate histone methylation and DNA hypermethylation. It is also known that different tumors have differences in hypermethylation patterns (74). Methyltransferase and demethylases are in charge of regulating DNA methylation. An enzyme named Ten-eleven translocation methylcytosine dioxygenase (TET) participates in the demethylation of cytosine. Under the responsibility of this enzyme, 5-methylcytosine is converted with co-substrates and cofactors (α-ketoglutarate and iron, respectively) (75). Due to D-2-HG's structural resemblance to αKG, a component required for TET demethylase activity, the activity of TET is inhibited by its presence (Figure 3) (76, 77). Studies by different research groups have reported that induction of the hypermethylation phenotype is associated with mutant IDH associated with carcinogenesis (78, 79).

The presence of high levels of D-2-HG affects not only DNA but also histone methylation. In the context of histone methylation, as seen in Figure 3, lysine-specific demethylase (KDM) is also a histone demethylase that is inhibited via D-2-HG. In IDH mutant cancers, this inhibition has consequences leading to oncogenic cellular differentiation (80).

As a result of genetic studies, it has been determined that altered frequency of IDH1 mutations are observed in 1.2% of prostate cancer (25).

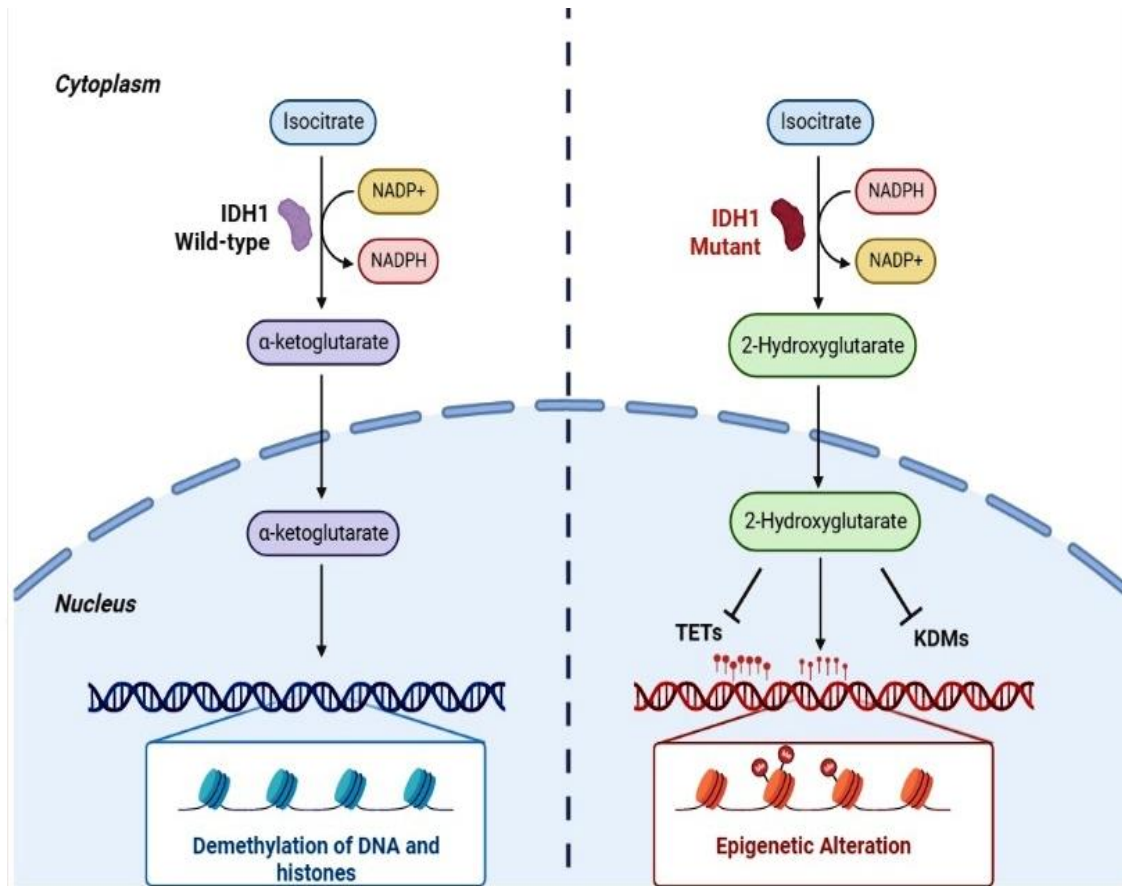


Figure 3: Wild-type IDH1 and Mutant IDH1 and cellular consequences (Created with BioRender).

One of the reason of alterations to the epigenome are caused by IDH1 mutations. D-2-HG produced by IDH mutations acts as a competitive inhibitor of KDM or TET because of structural similarities. Demethylation of nucleotides and histones, respectively, is therefore blocked. Abbreviations: IDH, isocitrate dehydrogenase; KDM, histone lysine demethylase; NADP⁺, oxidised nicotinamide adenine dinucleotide phosphate; NADPH, reduced nicotinamide adenine dinucleotide phosphate; TET, ten-eleven translocation oxygenase

2.2.2. O6-Methylguanine-DNA methyltransferase (MGMT)

An enzyme called MGMT, which is localized on chromosome 10q26, also known as O6-Methylguanine-DNA Methyltransferase, removes pro-mutagenic alkyl groups from the O6 position of guanine. Hence, it hinders cells from reacting with alkylating substances. As it combines with the methyl group, the MGMT complex is broken down by ubiquitin (81). Since O6 methylguanine connects to thymine during replication when a cell lacks the MGMT molecule, the base pair pairing is faulty. These base pairs are targeted by the DNA mismatch repair pathway proteins.

At the end of this process, either the DNA is repaired, or the cell goes into apoptosis. Loss of MGMT expression may occur as a result of methylated promoter CpG islands (82-84).

DNA hypermethylation was the main topic of the majority of earlier publications on prostate cancer epigenetics. So far, it has been shown that almost thirty genes may play a part in prostate cancer. By altering the activity of these genes, a condition known as promoter hypermethylation, prostate cancer can develop and spread. (85). A number of malignancies have been associated with aberrant promoter methylation, which is a critical tumor suppressor gene for the MGMT gene. However, there are conflicting reports of both low and high MGMT methylation in tissues from prostate tumors. Findings point to MGMT promoter methylation as a possible biomarker for prostate cancer monitoring and early diagnosis (85).

2.2.3. Alpha-thalassemia/Mental retardation, X-linked (ATRX)

The alpha thalassemia mental retardation X-linked disease is caused by inherited mutations in the SNF2 family chromatin remodeler ATRX, after which this protein is called. Moreover, numerous cancer types typically possess mutations in the tumor suppressor gene ATRX. It functions as a histone chaperone to deposit the histone variation H3.3 in a replication-independent manner in heterochromatic regions of the genome in collaboration with DAXX (Death domain-associated protein 6). ATRX also reduces genomic instability at telomeres in addition to being engaged in various processes that maintain genome stability in cells. Loss of ATRX therefore causes more significant genomic instability (86).

The ATRX gene enables the synthesis of a protein that is essential for developing and growing properly. While studies suggest that ATRX protein affects the expression of other genes through chromatin remodeling mechanisms, researchers do not put forward any specific roles for ATRX protein. For the preservation of heterochromatin at repeated sequences, such as telomeres, ATRX/ DAXX's chromatin remodeling role is crucial (86).

In a prostate cancer instance that it was previously uncovered, chromosomal inversions affecting ATRX were discovered in several distant metastases but not in the primary cancer. These results are interpreted to indicate that the ATRX loss-of-function mutation benefits lethal metastasis (87).

3. MATERIALS AND METHODS

3.1. Study Population and Aim

The is a study conducted by examining the blood samples of twenty-seven individuals diagnosed with prostate cancer, all of whom were determined as a result of detailed examinations by the clinicians of Yeditepe University Faculty of Medicine, Department of Urology. All demographic and clinical data were properly recorded after the patients signed the informed consent form. The peripheral blood samples of the patients who declared their participation in the study were taken by the experts. All stages of the investigation, from the supply of biological samples required for the research to the realization of the experiments, were carried out in a way that did not conflict with the 1975 Declaration of the Ethical Guidelines of Helsinki and the necessary ethics committee approval was obtained from the ethics committee of the relevant institution with the number 1732 in 2023.

Tumors were also assessed through the D'Amico risk classification. Parameters such as age at which the disease was detected, family history of cancer, presence of another cancer accompanying prostate cancer, prostate volume, PSA level, pathological stage, Gleason score, presence of metastasis, and treatment applied were the parameters considered in the evaluation of the test results.

The primary aim of this study is to reveal the candidacy of mutations in the IDH1 gene to prostate cancer by the NGS method. In addition to evaluating this gene, the MGMT and ATRX genes were also analyzed.

3.2. Explanation of Genomic DNA Isolation from Blood Protocol

The blood samples of the patients were stored in 5cc EDTA tubes at +4°C until the stage of performing the experiments was started. In the stage of DNA isolation, the iPrep DNA extraction device (Invitrogen) which can isolate DNA from 13 samples at once, and the iPrep genomic DNA isolation kit were used, both belonging to Thermo Fisher Scientific Company. This instrument, which performs the isolation of genomic DNA from a 350 ml blood sample, uses a magnetic-bead-based technology based on the unwanted molecules removal depending on the pH change. The basis of this technology consists of roughly two stages: (1) binding of (+ charged) nucleic acids to (- charged) CST in the presence of low pH and purification from unbound molecules (2) purification of nucleic acids with the help of a buffer at pH 8.5.

In these processes where buffers with different properties are used, liquid wash buffer mediates the process of cleaning from unwanted molecules, and low salt elution buffer mediates the increase in pH and neutralizes the bead surface, which is the main element of the technology, to clean nucleic acids. As with the storage of blood samples, DNA samples obtained in pure form were stored at +4°C for use in other steps of the investigation.

3.3. Measurement of the DNA Purity and Concentration

Verification of whether the DNA samples were pure enough to be used in experiments was completed via the NanoDrop200 instrument.

The baseline information when performing these measurements is that double-stranded DNA concentrations of 50 µg/ml should have an optical density (OD) of 1 at a wavelength of 260 nm. The selection criterion for the samples that can be included in the experiments was determined as having an OD₂₆₀/OD₂₈₀ ratio between 1.7 and 1.9. The instrument can perform all of these measurements.

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

Fragmentation, UMI assignment, target enrichment, sample indexing, and amplification are steps for the Qiaseq-targeted DNA panel to create a library. When using Illumina NGS systems, specially designed primers are needed for the aforementioned processes. 10 to 40 ng of fresh DNA was required for the targeted gene and region of interest.

This system has four fundamental phases as mentioned above. In order to clarify these:

Fragmentation: After performing end-repair and adding an A-tail, the genomic DNA sample is fragmented using a controlled multi-enzyme procedure. Eligible DNA fragments are attached to the sequencing platform using particular adapters for use at the 5' ends (including UMI and sample index).

UMI assignment: Prior to the steps of target enrichment and library formation, each DNA molecule is given a distinctive sequence or index known as the UMI. By fusing a 12-base random sequence adaptor with fragmented DNA, this assignment is made possible. Each DNA molecule in the sample adopts a different UMI sequence, and statistically, this procedure results in 412 distinct markers per adaptor. Moreover, this linked adapter also has the first sample index.

Target enrichment and creation of the final library: The target enrichment stage is the point at which UMI-containing DNA molecules are sufficiently enriched. Targeted PCR using an Adaptor complement, one site-specific primer, and one universal primer is used to amplify UMI-linked DNA molecules for enrichment. In addition, platform-specific adaptor sequences and additional sample indices are added using Universal PCR, which is then utilized to generate the library.

Next Generation Sequencing: Qiaseq Targeted DNA panels are compatible with Thermo Fisher Scientific Ion Personal Genome Machine® (PGMTM), Ion ProtonTM, and Ion S5TM systems as well as Illumina NGS systems (MiniSeq®, MiSeq® Personal Sequencer, NextSeq® 500, HiSeq® 1000, HiSeq® 1500, HiSeq 2000, and HiSeq 2500).

3.4.1. Fragmentation, end-repair, and addition

1) This entire procedure, the contents of which are shown in Table 2, was performed on ice. The fragmentation, End-repair, and A-addition mix was prepared following the table below and pipetted a few times after confirming that the Thermocycler device had attained a temperature of 4°C. Quick centrifugation was completed.

Table 2. Fragmentation, End-Repair, and Addition

Contents	Standard DNA, FFPE DNA, cfDNA Volume (µL)	CfDNA Contaminated by Cellular DNA Volume (µL)
DNA	Various	Various
Fragmentation Buffer, 10x	2.5 µL	2.5 µL
Nuclease- Free Water	Various	Various
FERA solution	0.75	0.75 µL
FG solution	-	1.25 µL
TOTAL	20 µL	20 µL

2) Each reaction contained 2.5 µl of the Fragmentation Enzyme Mix, which was subsequently pipetted through multiple times to mix it. It was ensured that the procedure was performed on ice.

3) From this point on, actions were arranged using the PCR program shown below.

4) After starting, the PCR procedure was halted at 4°C and samples were put into the device.

5) Following the conclusion of the program and when the block had reached 4°C, the samples were taken on the ice.

6) As soon as possible, the adapter ligation process began.

3.4.2. Adapter ligation of Samples

1) The reaction mixture's specifics were accomplished in accordance with Table 3. Gently, pipetting maximum of 10 times was the way to combine all of the components. Due to its high viscosity, the ligation solution was omitted from the following mixture, but was added to each subsequent reaction. Upon adding the ligation solution, the remaining ligation solution from the pipette tip was scraped off.

2) 15 minutes of incubation were spent with the thermocycler at 20°C.

3) The QIAseq Beads cleaning procedure started when the reaction was ended and the mixture was put on ice.

Table 3. Adapter Ligation of Samples

Content	Standard DNA Volume (μL)	FFPE DNA Volume (μL)	cfDNA Volume (μL)
DNA	25	25	25
Ligation Buffer x5	10	10	10
IL-N7 Adapter	2.8	2.8	0.5
DNA Ligase	5	5	5
Ligation Solution	7.2	7.2	7.2
Nuclease Free Water	-	-	2.3
TOTAL	50 μL	50 μL	50 μL

3.4.3. Clearance of adapter-bound DNA with QIAseq Beads

1) A 1.5 ml LoBind tube was used to hold the 50μl of DNA that was taken from the ligation process. By adding 50 μl of nuclease-free water, it was brought to a volume of 100 μl.

2) Experiment was supplemented with 100μl of QIAseq Beads. The mixture was properly blended by pipetting it many times.

3) At room temperature, the incubation period ran for five minutes.

4) The beads were allowed to separate from the supernatant for 10 minutes after being placed on a magnetic rack. The solution was left to become transparent before the

supernatant was carefully removed and discarded. The target DNA-containing beads were handled with extreme caution.

5) 200 μ l of the newly prepared 80% ethanol was added to the magnetic rack. The tube was spun while the beads were washed. The supernatant was extracted and finely discarded.

6) EtOH was eliminated at this stage when the ethyl alcohol washing procedure was repeated. To extract the residual liquid, 200 μ l, and 10 μ l pipettes were employed.

7) The beads were let to completely dry for 10 minutes after the ethanol was removed from the magnetic rack.

8) 52 μ l of nuclease-free water was used to elute the DNA from beads outside the magnetic rack. Before putting the solution to the magnetic rack until pure, enough time it was pipetted.

9) A clean 1.5 ml LoBind tube or PCR plate well was used to transfer 50 μ l of the supernatant.

10) 50 μ l of QIAseq Beads were added to 50 μ l of DNA solution for standard-FFPE samples, and 70 μ l of QIAseq Beads were added to the DNA solution for cfDNA samples. After addition, appropriate pipetting was performed.

11) Five minutes were spent incubating at room temperature.

12) The beads were removed from the supernatant after being placed on a magnetic rack, and the process took five minutes. Once the solution had become clear, the supernatant was carefully removed and discarded.

13) 200 μ l of the newly prepared 80% ethanol was added to the magnetic rack. The tube was spun while the beads were washed. The supernatant was extracted and finely discarded.

14) One further round of the ethanol washing process was conducted.

15) Using 200 μ l and then 10 μ l pipettes, ethanol was eliminated. The beads were dried for 15 minutes while staying on the magnetic rack since it is crucial for the effectiveness of the target enrichment stage that the beads be completely dry before elution.

16) The DNA target beads were taken off the magnetic rack and eluted with 12 μ l of nuclease-free water. By pipetting, it was fully blended. As the solution became clear, it was kept on the rack.

17) 9.4 μ l of supernatant were transferred to a clean PCR tube or PCR plate well to begin the target enrichment process.

3.4.4. Target enrichment PCR

- 1) Pipetting was performed while the reaction mixture was being prepared, which was conducted as outlined below (Table 4), in between two quick centrifugation processes.
- 2) The thermocycler instrument was then adjusted after this procedure.
- 3) The QIAseq Beads cleaning process started once the reaction was completed and kept on ice.

Table 4. Target Enrichment

Contents	Volume (μL)
DNA library (in item 28)	9.4
TEPCR Buffer, 5x	4
QIAseq Targeted DNA panel	5
IL-Forward primer	0.8
HotStar Taq DNA Polymerase	0.8
Total	20 μL

3.4.5. Clearance of the target enrichment PCR with QIAseq Beads

- 1) A 1.5 μl LoBind tube received 20 μl of the PCR reaction. It was then diluted by 80 μl of nuclease-free water until it reached 100 μl. The cfDNA sample was completed to 90 μl by adding 70 μl of nuclease-free water to 20 μl of sample.
- 2) To 100 μl of diluted PCR solution, 100 μl of QIAseq Beads (1.0x volume) were added. It was blended gently by pipetting it many times. At room temperature, it was incubated for 5 minutes.
- 3) After placing the sample on the magnetic rack, the beads were given about 5 minutes to separate from the supernatant. After the mixture was clear, the supernatant was carefully removed and discarded.
- 4) 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 extracted and finely discarded.
- 5) One further round of the ethanol washing process was conducted.
- 6) Using first 200 μl then 10 μl pipettes, ethanol was removed. The beads were dried for 10 minutes.

7) To elute the DNA beads, 16 μL of nuclease-free water was needed. By pipetting, it was fully blended. Until it turned clear, the solution was left to stand on the rack. The Universal PCR amplification process was initiated following the transfer of 13.4 μL of supernatant were transferred to a sterile PCR tube.

3.4.6. Universal PCR amplification

- 1) The centrifugation procedure was kept brief, and pipetting was done repeatedly while preparing the appropriate research design for the Universal PCR stage as stated below.
- 2) Following this process, the thermocycler instrument was set.
- 3) When the reaction was finished and the mixture was held on ice, the QIAseq Beads cleaning procedure began.

Table 5. Universal PCR Amplification

Gradient	Volume (μL)
Enriched DNA	13.4
UPCR Buffer, 5x	4
Hot Start Taq DNA polimerase	1
Nuclease Free Water	1.6
Total	20 μL

3.4.7. Clearance of the Universal PCR with QIAseq Beads

- 1) A 1.5 ml LoBind tube received 20 μL of the PCR reaction. To get it up to 100 μL , 80 μL of nuclease-free water was added.
- 2) 100 μL of QIAseq Beads (1.0x volume) and 100 μL of diluted PCR solution were mixed. Pipetting the mixture many times allowed for perfect blending. It was incubated for five minutes at room temperature.
- 3) Five minutes were given for the tube to sit on the magnetic rack so that the beads could separate from the supernatant. The solution was allowed to become clear just before the supernatant was carefully thrown away.
- 4) While still standing on the magnetic rack, 200 μL of the freshly made 80% ethanol was added. In the meantime, the beads were being cleaned in the tube, extracting and discarding the supernatant very carefully.

- 5) One more procedure for the ethanol washing process was performed.
- 6) The beads were dried for 15 minutes on the magnetic rack after the ethanol was removed using 200 μ l and then 10 μ l pipettes.
- 7) 30 μ l of nuclease-free water was used to elute the DNA library beads away from the magnetic rack. After careful pipetting, the solution was repositioned on the magnetic rack until it became completely clear. A new 1.5 ml LoBind tube or PCR tube was used to transfer 28 μ l of supernatant.

3.4.8. Qiaseq Quant Assay Protocol

- 1) An ice bath was used to defrost the ILMN DNA standard.
- 2) The table below shows the results of 5 serial dilutions of the ILMN DNA standard. There were five tubes ready for the ILMN standard dilution. Each tube received 45 μ l of dilution buffer. 5 μ l of ILMN standard stock were blended into the first tube using pipetting. Afterwards, 5 μ l of the solution were taken from the first tube and put in the second one. The same process was applied to the following tubes.

Table 6. Qiaseq Quant Assay Protocol

Standard	Ion Torrent or Illumina DNA Standard	Dilution Buffer
Std1	5 μ l undiluted	45 μ l
Std2	5 μ l Std1	45 μ l
Std3	5 μ l Std2	45 μ l
Std4	5 μ l Std3	45 μ l
Std5	5 μ l Std4	3 μ l

- 3) The dilution ratios of 1:50, 1:5,000, and 1:50,000 were applied to the samples for the first, second, and third times, respectively. The preparation of the first dilution provided preparation for what follows.
- 4) The PCR mixture was prepared as described below, and a cold block was used to hold it cold.
 - a) Centrifuging was performed for 10 to 15 seconds using SYBR Green ROX FAST Mastermix.
 - b) Three copies were created for five standards and one NTC (non-template control). The standards and NTC would thus be found in the first 18 Rotor Gene tubes. Each sample's dilutions (1:5,000 and 1:50,000) were placed in a single Rotor Gene tube.

- 5) In the cycle after, the real-time PCR instrument (RotorGene) was put up. After 30 cycles, data for fluorescence irradiation were recorded.
- 6) After the PCR cycle, the pertinent sample values were acquired and inputted into the "Illumina Data Analysis Software".

3.4.9. QiaSeq Upload Protocol

- 1) The SampleSheet was prepared using the Illumina Experiment Manager prior to starting the dilution.
- 2) The Reagent Cartridge Barcode section contained a code with the prefix MSxxx.
- 3) On the right, there was an area labeled "custom primer for read 1."
- 4) Using nuclease-free water (800 μ l water + 200 μ l NaOH), 1N stock NaOH was diluted to 0.2N.
- 5) All of the samples were calibrated to be 4 molar based on the outcomes of the Quant assay protocol.
- 6) 0.2 μ l PCR tubes were filled to the proper levels based on the number of primers from each library in order to create a mega pool.
- 7) From the tube containing the giant pool, 5 μ l were removed and transferred to another tube. We diluted 5 μ l of NaOH to 0.2N before adding it. It was allowed to denature for 5 minutes at 20–22°C by gently vortexing and spinning down.
- 8) During this time, 990 μ l of the Hyb buffer solution with yellow-capped from the cartridge was transferred into the 1.5 ml tube marked "20 pM pool".
- 9) In the 1.5 ml tube with 990 μ l of Hyb, which we labeled as the "20 pM pool," 10 μ l of the solution from the tube containing NaOH and our sample was added when the five minutes were over.
- 10) The samples' dilution was kept consistent with the cartridge and library that were being utilized.
- 11) One other 1.5 ml tube was given the name "Advance primer". To this tube, 597 μ l of HT1 were added. From the index box, 3 μ l orange-capped primers with the label "QiaSeq A Read1 Custom Primer1" were added.
- 12) Before they were loaded, both tubes were placed on the cold block.
- 13) Through the MiSeq Control Software, the device was turned off before the sequencing began. It was switched back on after a time using the button on the rear, and the gadget was anticipated to turn on.

14) As seen in the image below, 600 µl of our samples, which had been concentrated to the required level, were loaded into well 17. The tube marked "Forward primer" held 600 µl, which was poured into well number 18.

15) After loading the flow cell and reagents, review the run configurations and perform a pre-run check. Concentrations and quality values for the run were shown in the Sequencing panel as it progressed. We immediately evaluated the outcomes from the Sequencing monitor. Once the run was ended, we continued to a post-run wash. Run metrics were displayed on the Sequencing screen at various points during a run.

3.5. Validation of Mutations by Sanger Sequencing

In order to validate the variant found in patients, target-specific primers were designed and synthesized for the Sanger sequencing.

3.5.1. Designing Primers for Sanger Sequencing

It is crucial to take into account the absence of previously reported single-nucleotide variants in the chosen primers when designing primers for Sanger sequencing.

In addition, there shouldn't be any repeating sequences in these areas. The primers' binding temperatures in the areas for which they are intended shouldn't be too nearby to one another.

While the primers were designed, care was taken to ensure that purines and pyrimidines were evenly distributed. The sequencing primers and data from NCBI Blast are shown in Table 7.

Table 7. Designed Primer Sequences

Gene	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
IDH1	TAGGTCATTTGGTTGTGGTGGGT	GGTGCTCAGATACAAAGGCCAAC
MGMT	CTTTTCCGATGTGTGGAGGC	GTCCACGCACAAGTCTCGTT
MGMT	CTAGAAGAGTGGCACACGCA	CCCCATCACAGATATCGCCC

3.5.2. Polymerase Chain Reaction (PCR)

While amplifying the candidate novel variants in the IDH1 and MGMT genes, site-specific primer sequences were used in PCR. Details of PCR with a total volume of 25 μ l are given in Table 8.

Table 8. The Reaction Mix Prepared for Each Sample

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

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

25 μ l of the reaction mixture was added for each sample. The thermal cycler (Applied Biosystems, Veriti 96 Well) was used to perform PCR-based amplification. The PCR program in Table 9 was applied.

Table 9. Thermal Cycle Program

Step	Temperature	Time	Cycle
Denaturation	95°C	2 minutes	35 cycle
	95°C	30 seconds	
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, the Agarose gel electrophoresis step was started. 2% agarose gel was used in this step. It was operated at 90 Volts for 60 minutes with 1X TBE (Tris-borate-EDTA). After the procedure, gel image was recorded.

After Agarose Gel Electrophoresis, PCR Purification (Exosap) was performed. The construction of the exosap step is as follows.

Table 10. PCR Purification (Exosap) Components

PCR Component	1x Reaction
Exonuclease I (20U/ μ l)	0.5 μ l
Shrimp Alkaline Phosphatase (1U/ μ l)	1 μ l
Total	1.5 μL

Table 11. 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 can be seen in the table below.

Table 12. Sequence PCR

PCR Component	1x Reaction
BigDye v.3.1	2 μ l
5X Reaksiyon Buffer	2 μ l
Forward or Reverse (2pmol/ μ l) primer	2 μ l
All of the exosap purified product	2 μ l
dH ₂ O	2 μ l
Total	1.5 μL

Table 13. Sequence PCR Thermal Profile

Temperature	Time	Cycle
96°C	1 minutes	25 Cycles
96°C	10 seconds	
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 GENEDOC (version 2.6.002) software, and the final consensus sequence was recorded.

3.6. Statistical Analysis Method

Microsoft Excel, GraphPad Prism version 9 (GraphPad Software, La Jolla, CA, USA) and MedCalc software (NY, USA) programs were utilized for statistical analysis for analyzing the study's findings. While analyzing the study's data, descriptive statistical techniques (number, percentage, median, etc.) were employed. 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 Characteristics 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 listed in Table 14 below. The median age of the patients was 68 years. While 88.89% of our study population was over sixty, just 11.11% of them were under 60 years of age. The average height of all patients was 169.59 cm, and the average weight was 79.92 kg. Body mass index was 27.85 kg/m². They consumed an average of 33.66 packs of cigarettes per year. The presence of cancer in the family history and second cancer existence in the patients were among the parameters evaluated. While 48.14% of the patients had a family history of cancer, a second cancer accompanying prostate cancer in only 7.40%. Based on the classification introduced by D'Amico et al. in 1998, prostate cancer patients included in our study were divided into two main groups, as intermediate and high risk. According to this classification, 29.63% of prostate cancer patients were in the intermediate-risk group, and 70.37% were in the high-risk group. The median PSA concentration was 72 ng/mL. The PSA level of the D'Amico intermediate-risk group was 10.95 ng/mL, and the D'Amico high-risk group had a PSA level of 40.85 ng/mL. The mean prostate volume in all patients was 43.59 cc. Patients at high-risk had a significantly higher prostate volume (51.47 cc) when compared to intermediate-risk (24.87 cc) patients ($p=0.003$). 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%). According to the data on the percentage of TM, this rate was 50.85% in all patients. This parameter differed significantly between the high-risk (61.32%) and intermediate-risk (26%) groups ($p=0.0006$). When evaluating the presence of metastases before and after the follow-up, metastasis was detected in 25.92% of the patients during the diagnosis and in 14.81% of the patients during the follow-up period, at a median follow-up of 4.14 years. Of the 27 patients with prostate cancer, the high-risk group had 4.3 years of follow-up and the intermediate risk group had 3.7 years.

Table 14. Patient Demographic and Clinical Characteristics

Parameters	All (n=27)	D'Amico Risk		P value
		High (n=19) 70.37%	Intermediate (n=8) 29.63%	
Age at diagnosis (yr)	68 ± 7.65	68.37 ± 72	67.125±7.95	0.708
Height (cm)	169.59±6.22	170.11±6.89	168.38 ± 4.41	0.520
Weight (kg)	79.92±10.69	81.32±11.10	76.63±9.53	0.307
Body Mass Index (kg/m2)	27.85±3.65	28.21±3.78	27±3.42	0.443
Smoking (pack year), mean±SD	33.66±20.88	31±21.97	40.33±17.85	0.368
PSA level (ng/mL)	31.28±35.66	40.85±39.91	10.95±5.00	0.048
Family history of cancer, n (%)				
Yes	13	8	5	0.419
No	14	11	3	
Second Cancer				
Yes	2	0	2	0.079
No	25	19	6	
Prostate Volume	43.59±22.91	51.47±21.37	24.87±14.39	0.003
Comorbidities				
Yes	17	12	5	0.974
No	10	7	3	
Clinical T-stage n (%)				
cT1c	9	3	6	0.000
cT2	15	13	2	0.087
cT3	3	3	0	0.532
Pathological T-stage, n (%)				
PT2a	2	1	1	0.512
PT2b	5	2	3	0.135
PT2c	12	10	2	0.235
PT3a	5	3	2	0.615
PT3b	3	3	0	0.532
Gleason Score				
≤7	14	6	8	0.001
>7	13	13	0	
Tm percentage	50.85±26.56	61.32±21.91	26±19.57	0.0006
Metastasis				
Yes	8	8	0	0.060
No	19	11	8	
Treatment				
Radical prostatectomy	12	8	4	-
ADT	10	8	2	-
Curative radiotherapy	5	3	2	-
Follow-up	49.74±53.40	51.94±62.24	44.5±24.22	0.747

The bolded p-values are significant (< 0.05)

4.2. Next Generation Sequencing (NGS) Analysis

After the targeted gene sequencing was performed on the prostate cancer patients, 11 mutations were detected. Three of these mutations were found in IDH1. In 3 of 27 prostate cancer patients, these mutations were found. In addition, when the distribution of mutations was evaluated using the D'Amico classification, 1 of the 3 variations found in the IDH1 gene were found in the high-risk group and 2 in the intermediate-risk group. All of the variants detected in the IDH1 gene were in exonic regions. As a result of our study, uncertain significant c.548A>G (rs34599179) variant in 1 heterozygous patient, benign c.532G>A (rs34218846) variant in 2 heterozygous patients, and benign c.315C>T (rs11554137) variant in 2 heterozygous patients were observed in the IDH1 gene.

To enhance the efficacy of the investigation, the MGMT and ATRX genes were also analyzed in addition to the IDH1 gene. Out of the 11 mutations, six were in MGMT and two in ATRX. Among the 11 chromosomes examined in prostate cancer patients, given in the Table 16, the highest rate of anomaly was found in benign MGMT c.427A>G variant with 25.93% and benign MGMT c.533A>G variant with 25.93% (Table 15).

Table 15. IDH1, MGMT, ATRX gene mutations and their clinical value for D'Amico Risk Groups

Variables	Category	n	D'Amico Risk		p-value
			High	Intermediate	
Age group	<60 vs ≥60	3 vs 24	7.41% vs 62.96%	3.70% vs 25.93%	0.881
IDH1					
C548AG	positive vs negative	1 vs 26	0% vs 70.37%	3.70% vs 25.93%	0.296
C532GA	positive vs negative	2 vs 25	3.70% vs 66.67%	3.70% vs 25.93%	0.512
C315CT	positive vs negative	2 vs 25	3.70% vs 66.67%	3.70% vs 25.93%	0.512
MGMT					
C-55CT	positive vs negative	1 vs 26	3.70% vs 66.67%	0% vs 29.63%	1.0
C-28CT	positive vs negative	4 vs 23	3.70% vs 66.67%	11.12% vs 18.51%	0.064
C159CT	positive vs negative	5 vs 22	3.70% vs 66.67%	14.815% vs 14.815%	0.017*
C250CT	positive vs negative	5 vs 22	3.70% vs 66.67%	14.81% vs 14.81%	0.017*
C427AG	positive vs negative	7 vs 20	14.81 vs 55.56%	11.12% vs 18.51%	0.633
C533AG	positive vs negative	7 vs 20	14.81 vs 55.56%	11.12% vs 18.51%	0.633
ATRX					
C2671CG	positive vs negative	11 vs 16	29.63% vs 40.74%	11.12% vs 18.51%	1.0
C1353CT	positive vs negative	1 vs 26	3.70% vs 66.67%	0% vs 29.63%	1.0
Number of chromosome anomalies	≤2 vs >2	20 vs 7	59.26% vs 11.11%	11.12% vs 18.51	0.026*

As a result of the analysis of the MGMT gene; likely benign c.-55C>T (5 Prime UTR Variant, rs750932227) variant in 1 heterozygous patient, benign c.-28C>T (5 Prime UTR Variant, rs16906252) variant in 4 heterozygous patients, benign c.159C>T (rs1803965) variant in 5 heterozygous patients, benign c.250C>T (rs12917) variant in 5 heterozygous patients, benign c.427A>G (rs2308321) variant in 7 patients (2 homozygous, 1 heterozygous and 1 hemizygous), and benign c.533A>G (rs2308327) variant in 7 patients (6 heterozygous, 1 homozygous) was detected.

Benign c.2671C>G (rs3088074) variant in 11 homozygous patients and c.1353C>T (rs199929884) in 15 homozygous patient were found as outcomes of the sequencing of the ATRX gene.

A significant difference was identified between the groups regarding the two variations of the MGMT gene when the mutations were evaluated in the high-risk patient group and the intermediate-risk patient group according to the D'Amico risk categorization.

- For patients who were MGMT c.159C>T (rs1803965) and c.250C>T (rs12917) mutation carrier, there was a significant difference between those patients who were classified as having D'Amico high- or intermediate- risk groups ($p=0.017$) (Table15).

Table 16. Summary of 11 mutations detected with NGS

Gene Symbol	Chromosome	Transcript Variant	Protein Variant	Normal	Population Frequency	Chromosome	Gene Region	Translation Impact	Classification	Phenotype	Transcripts	Patient ID
IDH1	2	c.548A>G	p.Y183C	Normal	1.69%	209,108,301	Exonic	missense	Uncertain Significance	Cancers and Tumors	NM_005896.4	3
IDH1	2	c.532G>A	p.V178I	Normal	8.67%	209,108,317	Exonic	missense	Benign	Cancers and Tumors	NM_005896.4	13, 17
IDH1	2	c.315C>T	p.G105G	Normal	10.53%	209,113,192	Exonic	synonymous	Benign	Cancers and Tumors	NM_005896.4	13, 17
MGMT	10	c.39C>T c.-55C>T	p.S13S	Normal	0.004%	131,265,518	Exonic; 5'UTR	synonymous	Likely Benign	Familial Pancreatic Cancer	NM_002412.5	6
MGMT	10	c.66C>T c.-28C>T	p.R22R	Normal	7.82%	131,265,545	Exonic; 5'UTR	synonymous	Benign	Familial Pancreatic Cancer	NM_002412.5	14, 23, 24, 26
MGMT	10	c.159C>T c.252C>T	p.L84L p.L53L	Normal	22.80%	131,506,192	Exonic	synonymous	Benign	Familial Pancreatic Cancer	NM_002412.5	2, 12, 21, 23, 24
MGMT	10	c.250C>T c.343C>T	p.L115F p.L84F	Normal	23.17%	131,506,283	Exonic	missense	Benign	Familial Pancreatic Cancer	NM_002412.5	2, 12, 21, 23, 24
MGMT	10	c.427A>G c.520A>G	p.I143V p.I174V	Normal	12.67%	131,565,064	Exonic	missense	Benign	Familial Pancreatic Cancer	NM_002412.5	2, 5, 9, 10, 15, 26, 27
MGMT	10	c.626A>G c.533A>G	p.K178R p.K209R	Normal	12.81%	131,565,170	Exonic	missense	Benign	Familial Pancreatic Cancer	NM_002412.5	2, 5, 9, 10, 15, 26, 27
ATRX	X-Linked	c.2785C>G c.2671C>G	p.Q891E; p.Q929E; p.E929E; p.E891E	Normal	87.73%	76,937,963	Exonic	synonymous	Benign	X-Linked Alpha Thalassemia-mental	NM_138270.4	1, 3, 4, 7, 10, 11, 15, 20, 21, 25, 27
ATRX	X-Linked	c.1353C>T c.1467C>T	p.T489T p.T451T	Normal	0.29%	76,939,281	Exonic	synonymous	Likely Benign	X-Linked Alpha Thalassemia-mental	NM_138270.4	15

Table 16. Summary of 11 mutations 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
IDH1	2	c.548A>G	p.Y183C	Damaging	Probably Damaging	26.700	34599179	0.359	0.846	1.055	1.004	3
IDH1	2	c.532G>A	p.V178I	Tolerated	Probably Damaging	25.000	34218846	4.932	6.251	5.003	4.951	13, 17
IDH1	2	c.315C>T	p.G105G	-	-	13.260	11554137	5.691	7.104	5.240	5.211	13, 17
MGMT	10	c.39C>T c.-55C>T	p.S13S	-	-	< 10	750932227	-	-	0.000	0.001	6
MGMT	10	c.66C>T c.-28C>T	p.R22R	-	-	15.700	16906252	2.456	4.369	6.857	5.605	14, 23, 24, 26
MGMT	10	c.159C>T c.252C>T	p.L84L p.L53L	-	-	< 10	1803965	14.716	13.471	13.149	13.203	2, 12, 21, 23, 24
MGMT	10	c.250C>T c.343C>T	p.L115F p.L84F	-	-	12.340	12917	14.836	13.471	14.204	14.308	2, 12, 21, 23, 24
MGMT	10	c.427A>G c.520A>G	p.I143V p.I174V	-	-	12.520	2308321	5.132	9.251	9.880	9.171	2, 5, 9, 10, 15, 26,27
MGMT	10	c.626A>G c.533A>G	p.K178R p.K209R	-	-	12.650	2308327	5.132	9.250	9.473	9.264	2, 5, 9, 10, 15, 26, 27
ATRX	X-Linked	c.2785C>G c.2671C>G	p.Q891E; p.Q929E; p.E929E; p.E891E	-	-	< 10	3088074	55.232	50.833	39.236	38.534	1, 3, 4, 7, 10, 11, 15,20,21,25,27
ATRX	X-Linked	c.1353C>T c.1467C>T	p.T489T p.T451T	-	-	< 10	199929884	0.026		0.024	0.029	15

4.3. Validation of Mutations by Sanger Sequencing

According to the NGS results, 11 variants were identified, and five of them were missense mutations. Confirmation of a missense IDH1 mutation with uncertain significance and a synonymous mutation, and a missense mutation determined in the MGMT gene, which was found to be significant in the D'Amico classification, were performed by Sanger sequencing.

According to Sanger sequence results, it was clarified that 1 patient had heterozygous mutant for IDH1 c.548A>G (rs34599179).

In addition, a synonymous mutation, and a missense mutation in the MGMT gene, which were significantly associated with D'Amico risk classification, were detected in five patients. All five patients were confirmed to be heterozygous carriers of the MGMT c.250C>T (rs12917) missense mutation. It was confirmed that all five patients with the synonymous variant in the MGMT gene were heterozygous carriers of the c.159C>T (rs1803965) mutation.

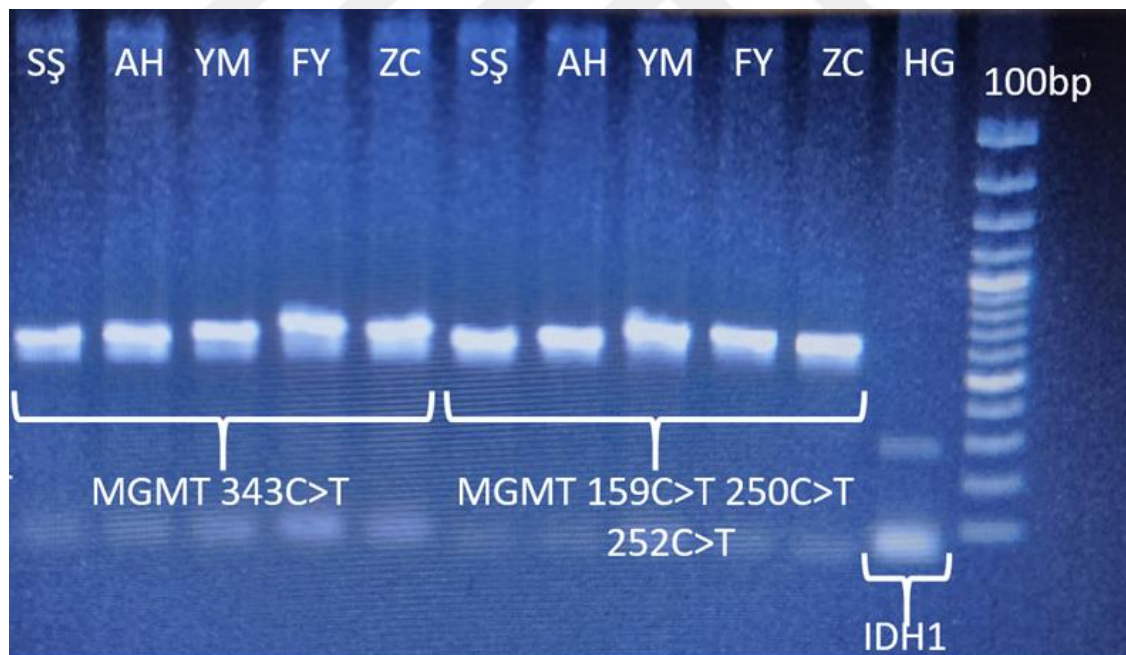


Figure 4: Agarose gel electrophoresis image of IDH1 and MGMT gene mutations in prostate cancer patients.

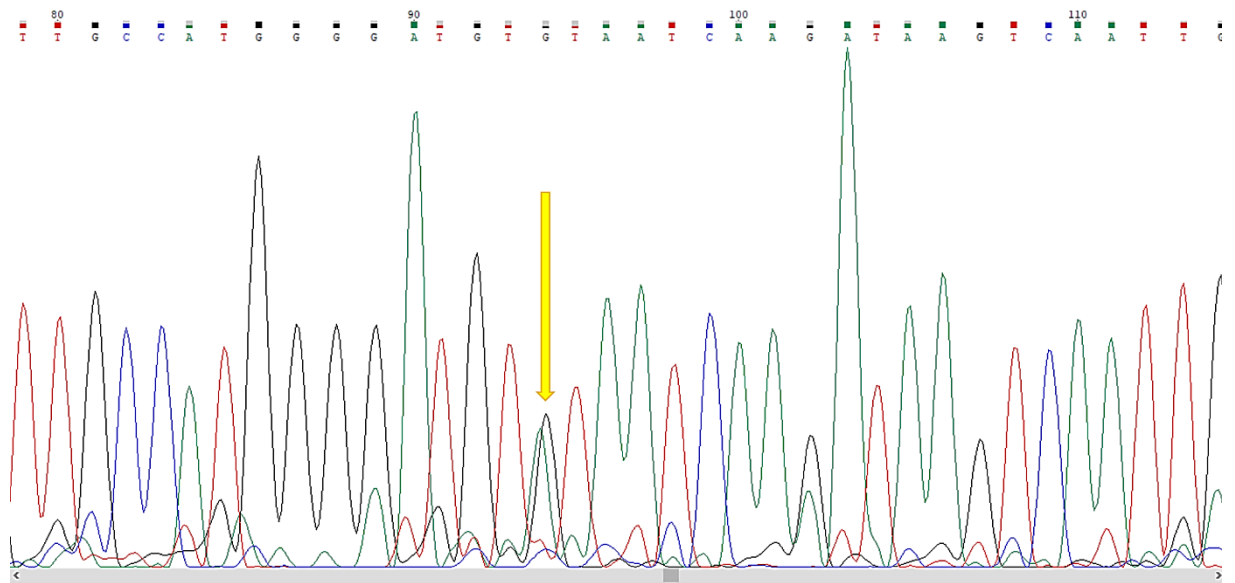


Figure 5: Heterozygous mutation in IDH1 c.548A>G (rs34599179) (Patient: HG) forward image and confirmation by Sanger Sequencing.

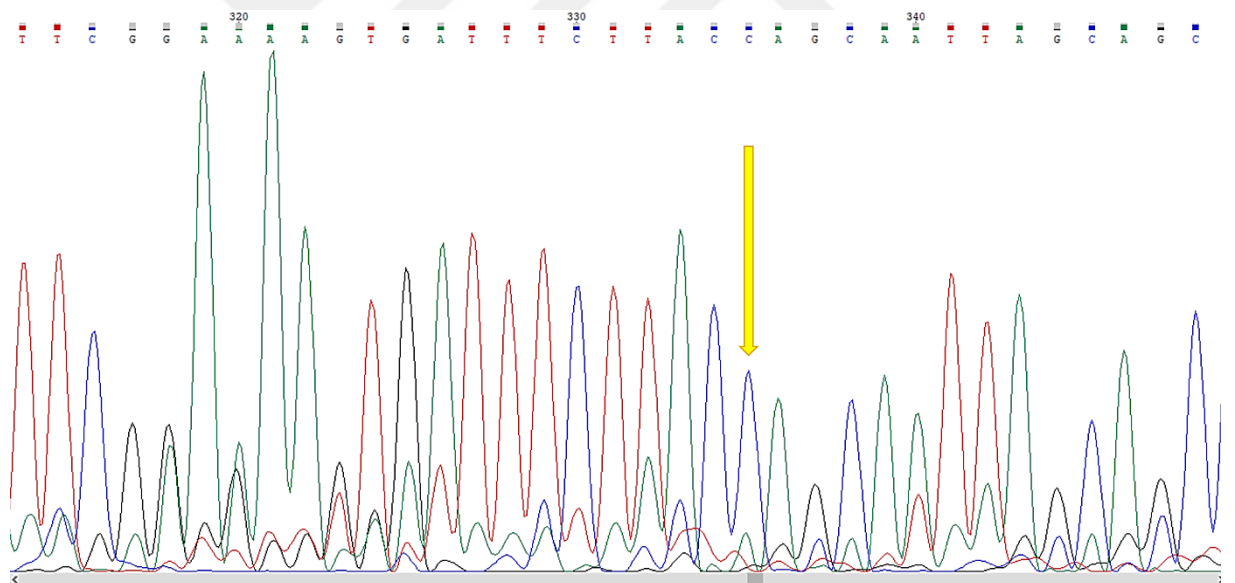


Figure 6: Wild-type (normal) MGMT c.343C>T (rs12917) (Patient: AH) forward image and confirmation by Sanger Sequencing.

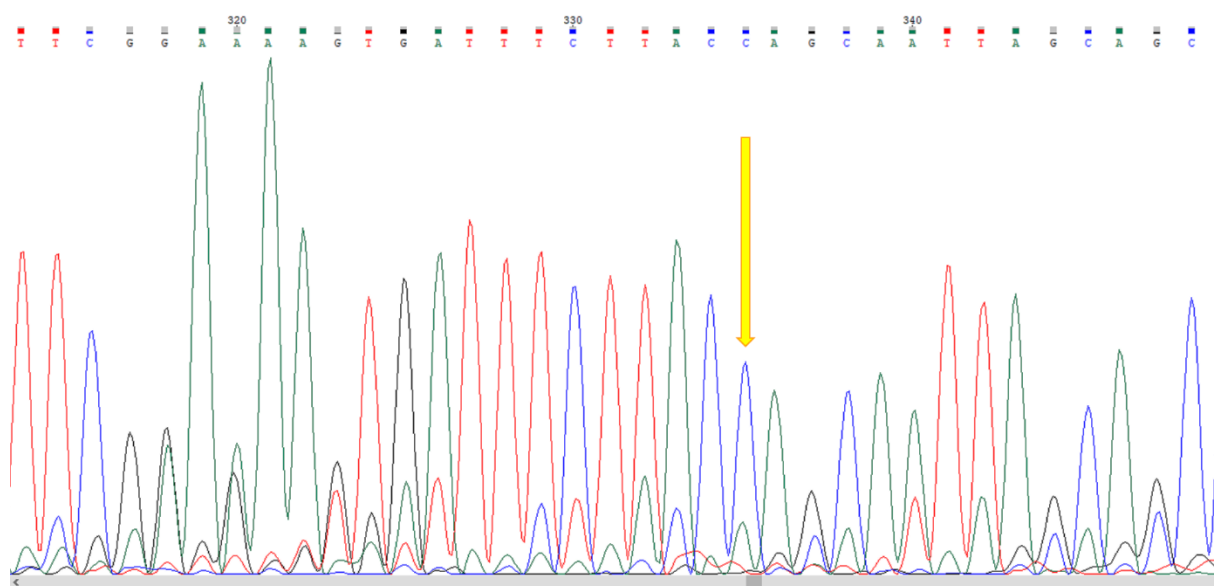


Figure 7: Wild-type (normal) MGMT c.343C>T (rs12917) (Patient: FY) forward image and confirmation by Sanger Sequencing.

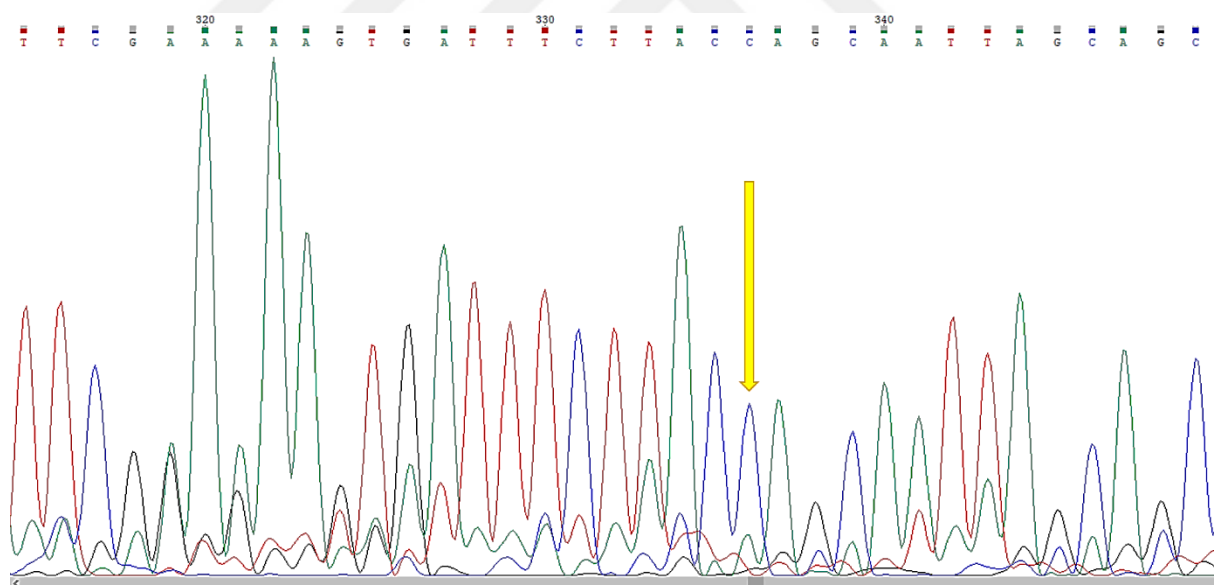


Figure 8: Wild-type (normal) MGMT c.343C>T (rs12917) (Patient: SŞ) forward image and confirmation by Sanger Sequencing.

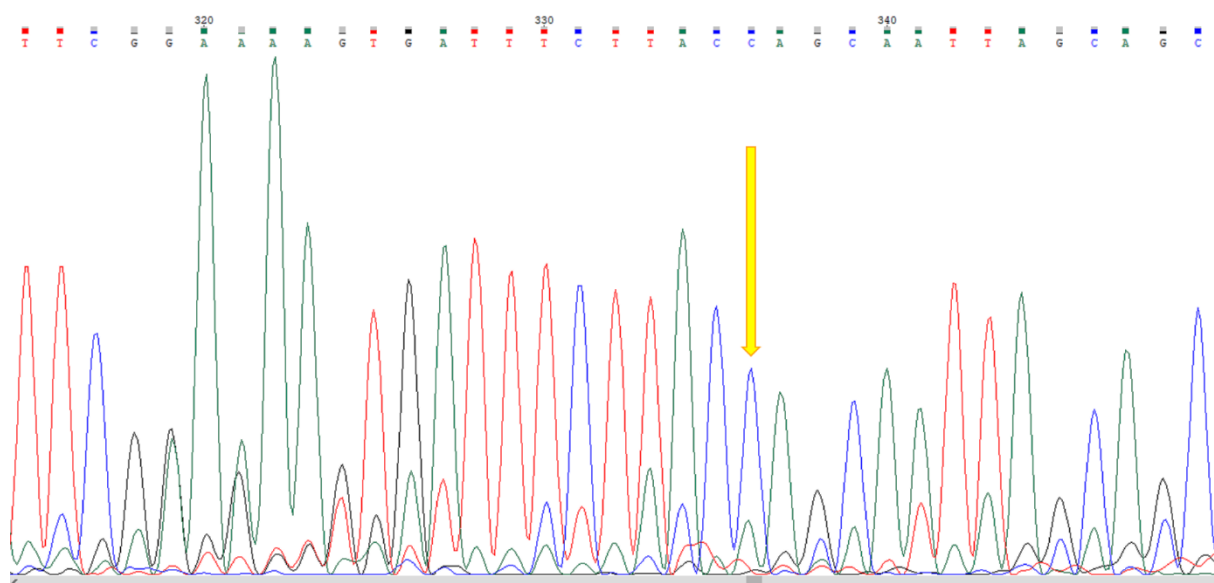


Figure 9: Wild-type (normal) MGMT c.343C>T (rs12917) (Patient: YM) forward image and confirmation by Sanger Sequencing.

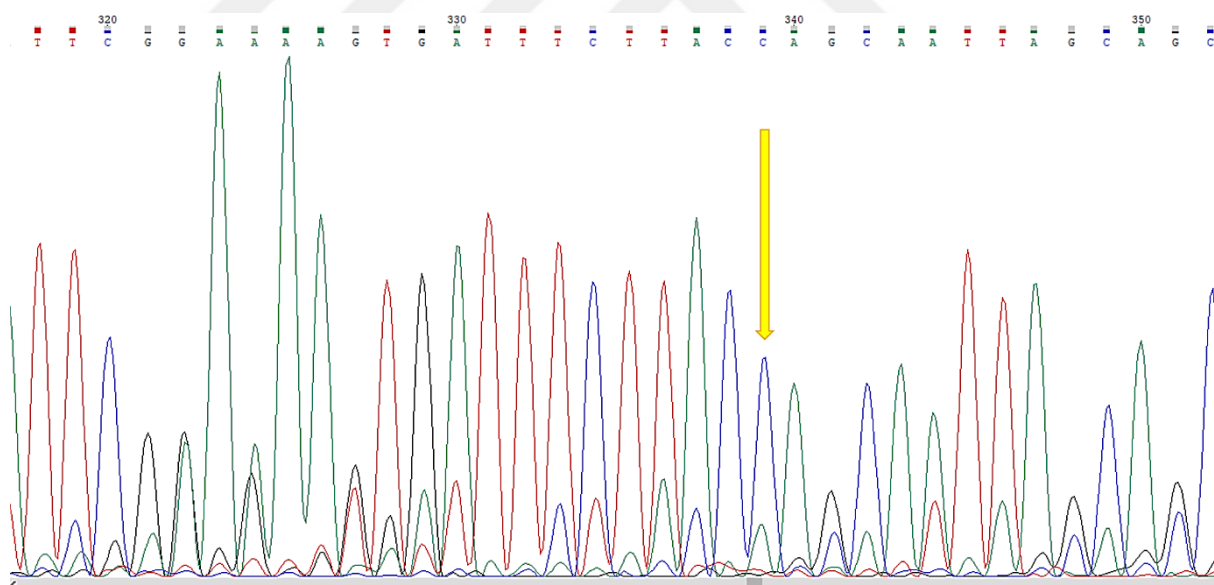


Figure 10: Wild-type (normal) MGMT c.343C>T (rs12917) (Patient: ZC) forward image and confirmation by Sanger Sequencing.

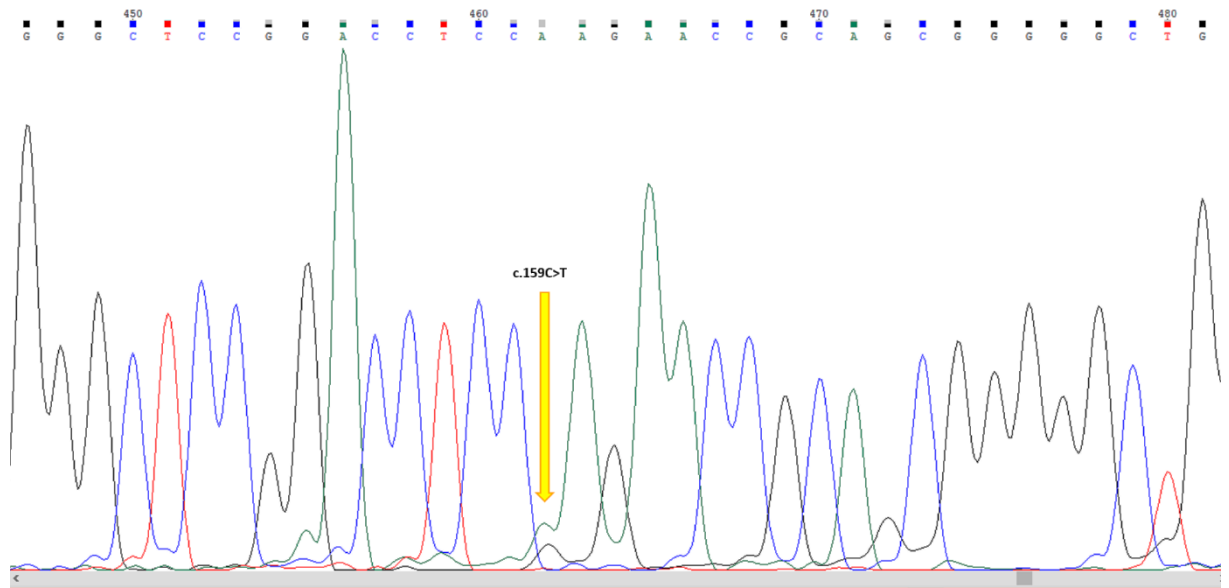


Figure 11: Heterozygous mutation in the MGMT gene c.159C>T (rs1803965) reverse image and confirmation by Sanger Sequencing (Patient: AH).

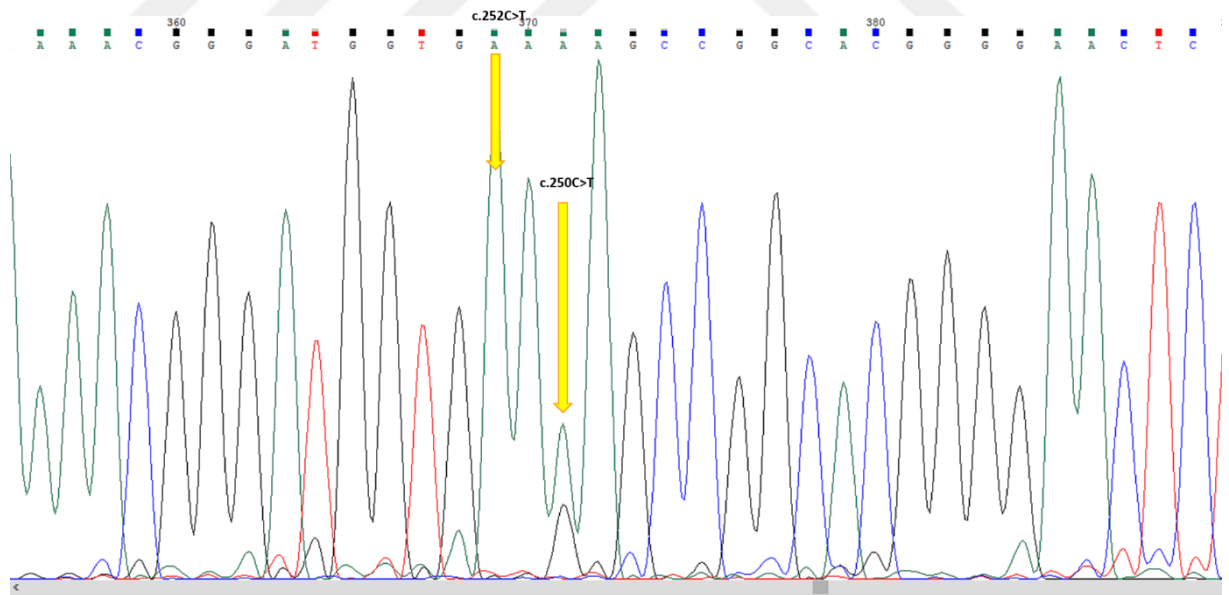


Figure 12: Heterozygous mutation in MGMT c.250C>T (rs12917) and normal MGMT gene c.252C>T (rs1803965) variant (Patient: AH) reverse image and confirmation by Sanger Sequencing.

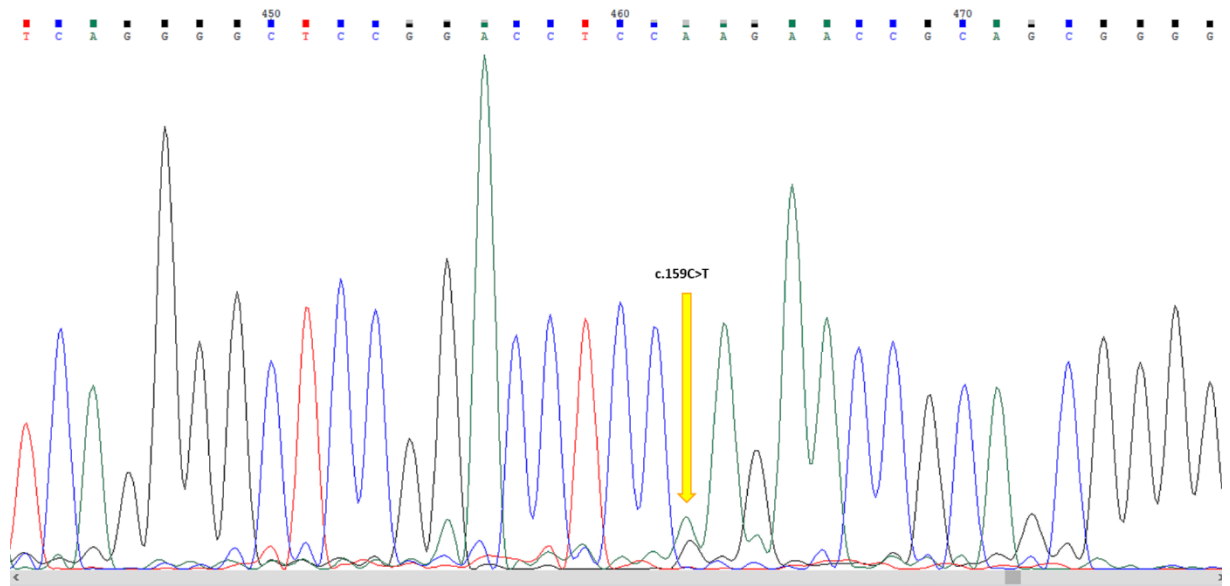


Figure 13: Heterozygous mutation in MGMT c.159C>T (rs1803965) (Patient: FY) reverse image and confirmation by Sanger Sequencing.

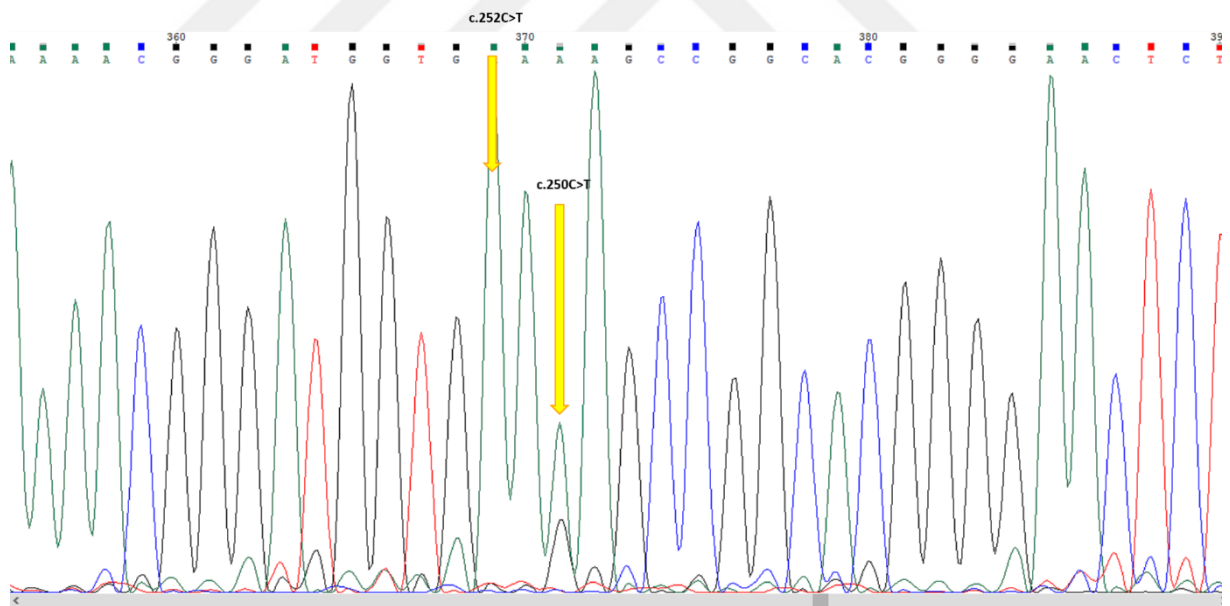


Figure 14: Heterozygous mutation in MGMT c.250C>T (rs12917) and normal MGMT gene c.252C>T (rs1803965) variant (Patient: FY) reverse image and confirmation by Sanger Sequencing.

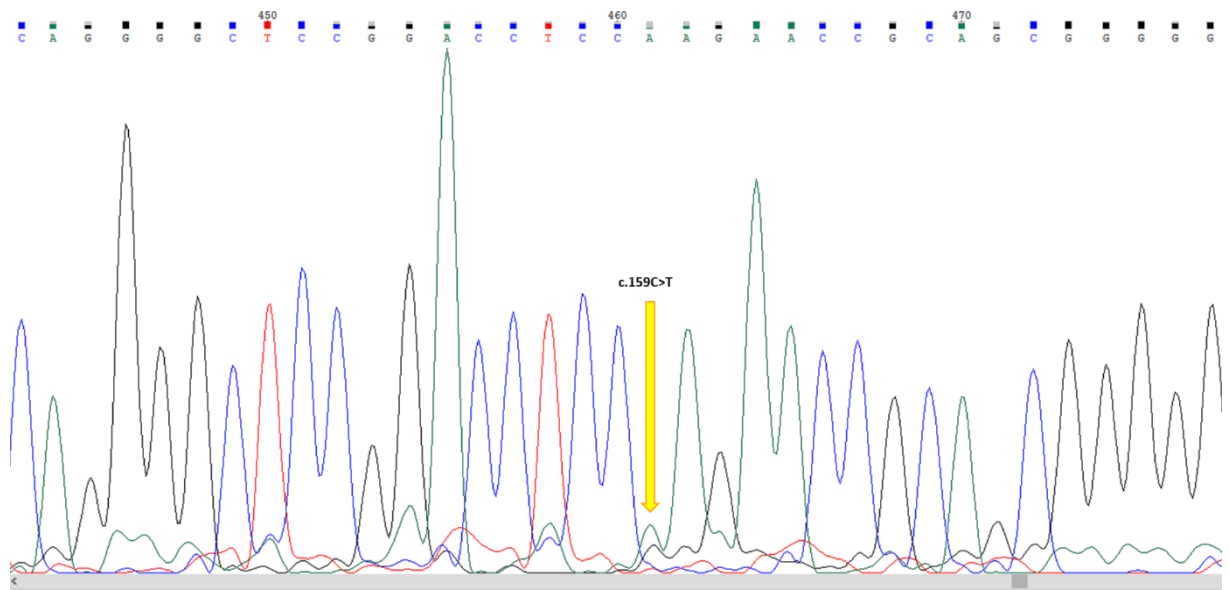


Figure 15: Heterozygous mutation in MGMT c.159C>T (rs1803965) (Patient: SŞ) reverse image and confirmation by Sanger Sequencing

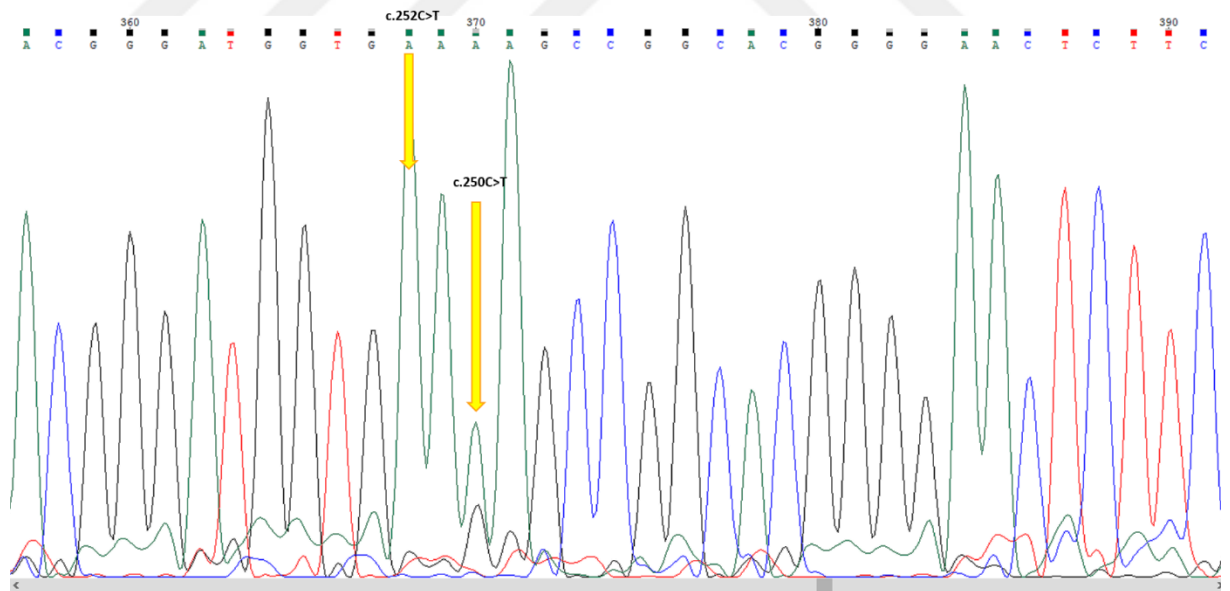


Figure 16: Heterozygous mutation in MGMT c.250C>T (rs12917) and normal MGMT gene c.252C>T (rs1803965) variant (Patient: SŞ) reverse image and confirmation by Sanger Sequencing

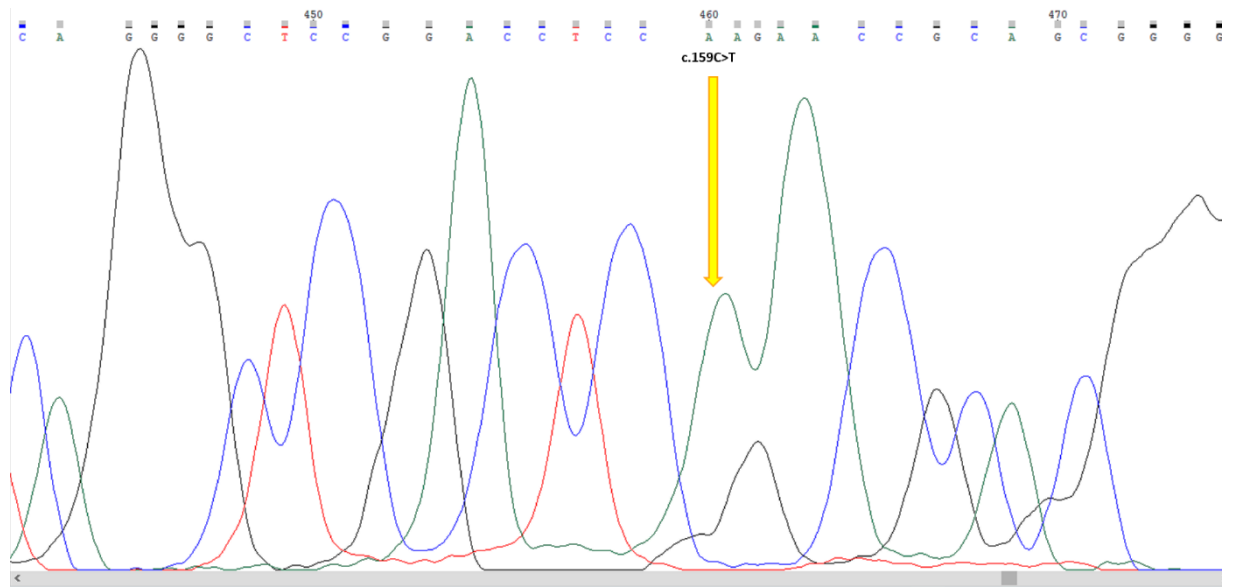


Figure 17: Heterozygous mutation in MGMT c.159C>T (rs1803965) (Patient: YM) reverse image and confirmation by Sanger Sequencing

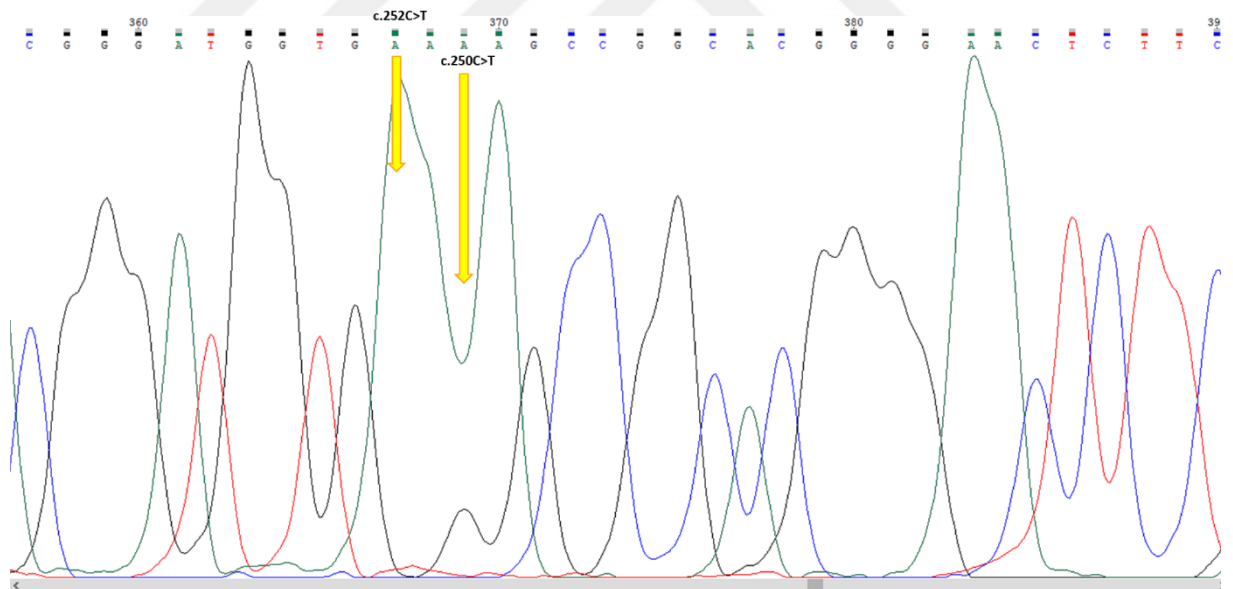


Figure 18: Heterozygous mutation in MGMT c.250C>T (rs12917) and normal MGMT gene c.252C>T (rs1803965) variant (Patient: YM) reverse image and confirmation by Sanger Sequencing

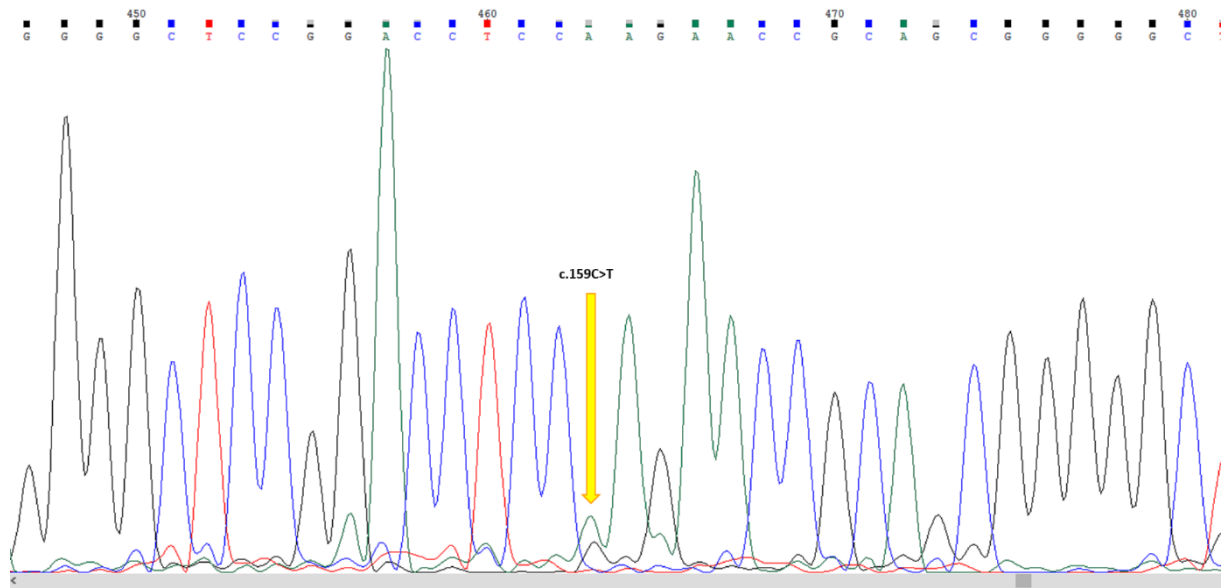


Figure 19: Heterozygous mutation in MGMT c.159C>T (rs1803965) (Patient: ZC) reverse image and confirmation by Sanger Sequencing

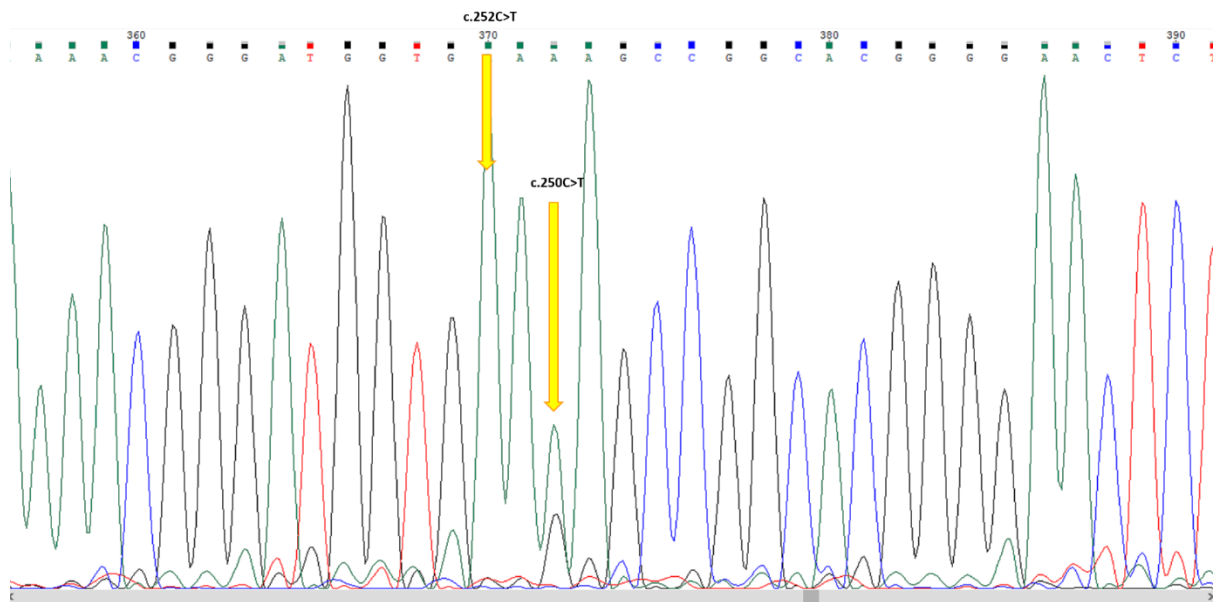


Figure 20: Heterozygous mutation in MGMT c.250C>T (rs12917) and normal MGMT gene c.252C>T (rs1803965) variant (Patient: ZC) reverse image and confirmation by Sanger Sequencing

5. DISCUSSION AND CONCLUSION

Despite new treatment approaches developed by immense molecular biology knowledge integrated with medicine and visualization techniques, unlighted genetic background and environmental factors continue to contribute to the development and progression of human cancers.

Prostate cancer, a highly deadly disease, is ranked second among all male cancers (1). For this reason, many scientific groups from all over the world have put efforts into finding a solution to cure this male cancer by working on the disease's unclarified characteristics in the scientific world; however there are still a lot of things that need to be explained. Among these unknowns, issues related to the molecular infrastructure of prostate cancer occupy a large place.

The presence of mutations in genes with many different functions have been detected in prostate cancer, as in all types of cancer, and the relationship between the disease and the alterations has been investigated by applying several methods and it continues to be studied (88). Although a new mutations are added to the reported mutations, one that has the potential to be specific and susceptible to prostate cancer has not been reported. At this point, there are methods that can untie the knot, verifying genes associated with prostate cancer and revealing new ones.

The majority of the alterations that have been clarified to date with prostate cancer have been made possible by the discovery of technologies such as NGS (89). It should be underlined that besides mutations, gene fusions and copy number changes can also be determined by this method. Today, it is known that there are molecular parameters that are recommended to be included in the D'Amico risk classification, which is shaped by certain parameters, especially the Gleason score and PSA level (89, 90). It is reported that the current classification may be insufficient and the necessity of including molecular markers in the classification has arisen (91).

Alterations defining the genetic profile of prostate cancer include TMPRSS2-ERG fusion, GSTP1 promoter hypermethylation, loss of p27, NKX3.1 and PTEN, mutation and amplification in AR, C-myc alteration, and telomerase activation (92, 93). Although the above-mentioned are the main ones, it is known that there are many unexplained changes associated with the presence and progression of prostate cancer, including epigenetic mechanisms, disorders in the apoptosis mechanism and chromosomal changes (92).

Our study was designed to contribute to the clarification of the molecular biology of prostate cancer. For this purpose, mutation analysis was performed by using blood samples from patients with prostate cancer in 3 target genes, including MGMT and ATRX genes, the main target being the IDH1 gene. This examination was accomplished via the next-generation sequencing method, and the existence of the detected mutations was confirmed by Sanger sequencing.

In total, 11 mutations were detected in patients with prostate cancer in three different genes. Although no pathological variant identification has been made, almost all mutations have been associated with this cancer for the first time.

Three of the 11 mutations were detected in the exonic regions of the IDH1 gene. While IDH1 mutations are distinctive, especially in gliomas, they can be detected very rarely in cases of prostate cancer.

Within the scope of our thesis research, within the IDH1 gene, one clinical significance unknown missense variation, and two benign variations were determined: c.548A>G (p.Y183C; rs34599179), c.532G>A (p.V178I; rs34218846), c.315C>T (p.G105G; rs11554137). c.548A>G (p.Y183C; rs34599179) was only detected in one heterozygous prostate cancer patient. c.532G>A (p.V178I; rs34218846) and c.315C>T (p.G105G; rs11554137) were benign variations which were both detected in heterozygous patients with number of 13 and 17. The CADD scores of detected rs34599179, rs34218846 and rs11554137 mutations were 26.70, 25.00 and 13.26, respectively. According to this score and PolyPhen-2 results, rs34599179 and rs34218846 mutations can be interpreted as harmful for prostate cancer.

The IDH1 SNP rs11554137 has been identified as a possible novel prognostic marker in acute myeloid leukemia (94). According to the results of another study conducted with Colombian prostate patients, the synonym SNP rs11554137 IDH1105GGT was detected in tumor tissue (95).

No studies are reporting the results of studies on rs34218846 missense variant in IDH1 in prostate cancer. On the other hand, including previous genome studies to elucidate the molecular biology underlying obesity in Hispanic Americans, the target genes identified by Gao et al. include the IDH1 gene. The IDH1 rs34218846 variant, which we detected in our prostate cancer patients, was significantly associated with various parameters associated with obesity (96).

A missense variant whose clinical significance has not yet been elucidated identified in IDH1 c.548A>G (p.Y183C, rs34599179). According to our results, this variant may be a threatening variant for prostate cancer. This variation was observed in only two samples by Snezhkina AV et al., who investigated carotid body tumors (97).

In our study, as a result of our MGMT gene analysis, six variations were identified as benign and likely benign in bioinformatics databases. Additionally, according to the D'Amico risk classification, which was created to better understand the marker characteristics of variants, the variants that we found to be significantly different between our high-risk and intermediate-risk patient groups were detected in the MGMT gene.

Three synonymous (c.-55C>T, rs750932227; c.-28C>T, rs16906252; c.159C>T, rs1803965) and three missense variants (c.250C>T, rs12917; c.427A>G, rs2308321; c.533A>G, rs2308327) were found in MGMT. Among these variants, a synonymous (c.159C>T, rs1803965; p=0.017) and a missense variant (c.250C>T, rs12917; p=0.017) differed significantly between high-risk and intermediate-risk groups, which were detected in five patients.

Sylvester et al. (2011), as in our study group, rs1803965 and missense rs12917 variation were detected in two patients diagnosed with glioma who received TMZ and radiation (98). Additionally, rs12917 alterations, in head and neck cancer and prostate cancer, are associated with disease susceptibility (99, 100). On the other hand, rs1803965 has not been identified in prostate cancer.

According to the 2016 study by Kuroiwa-Trzmielina et al., rs16906252C>T is linked to an elevated risk for MGMT-methylated colorectal cancer (101). Its mutant form has been identified as a risk factor for adenocarcinoma (102). In addition, its relationship with colon cancer was revealed by the study of Pardini et al. (103). Moreover, a significant relationship was found between MGMT methylation and those carrying the T allele of rs16906252 in glioblastoma patients and B-cell lymphoma, and it was concluded that it predisposed patients for glioblastoma (104, 105).

No data could be found in any medical database for the 5 Prime UTR variant; rs750932227 was observed in one heterozygous prostate cancer patient. This variant has an ExAC frequency of 0.000 and a gnomAD of 0.001. Therefore, it can be said that it is a very rare variant. However, the CADD score is less than 10. This allows us to speculate that this variant may be benign. This variant is a very rare benign variant and

was first described in prostate cancer.

MGMT missense variant (rs2308321) was identified in seven cases of our study population. Another missense variation in MGMT (rs2308327) gene was observed in seven prostate cancer patients who were the same cases detected in rs2308321. There are few studies that question their relationship with prostate cancer. On the other hand, Zhang et al. did not find an association between these two variations and prostate cancer (106).

ATRX variants were detected in twelve prostate cancer patients. Eleven of these twelve patients had rs3088074 (c.2671C>G, p.E891E, rs3088074).

There was no information reported for prostate cancer and this variation relation previously. We identified eleven homozygous prostate cancer cases for the first time. However, it is not a variant that has not been shown in other diseases. Some subtypes of brain cancers, which were classified according to the molecular parameters, are linked to ATRX mutations (107). Glioma cases are one of best examples for understanding the variant (108, 109). Moreover, besides mutations, also loss of ATRX has been shown in neurofibromatosis (110, 111). ATRX rs3088074 has also been reported in ovarian cancer patients (112) and in one male with lung carcinoma (113). Song et al. analyzed pleura's solitary fibrous tumor by NGS technology; they have found ATRX rs3088074 missense variation in their study individuals (114).

Unlike the ATRX rs3088074 variant, there is no reported association or reported function in the literature for the rs199929884 (c.1353C>T, p.T451T) variant.

This variant was detected in one of 37 of our prostate cancer patients. Since this synonymous variant of germline origin has a CADD score of less than 10, it can be interpreted as benign or possibly benign, which is confirmed by the ClinVar database.

In addition, Sanger sequencing was performed for an IDH1 mutation, which was identified as uncertain significance, and for two mutations of the MGMT gene, which was determined to be a significant potential marker according to the D'Amico risk classification.

As mentioned previously, the c.548A>G missense mutation in the IDH1 gene has been reported in a few studies, neither demonstrating clinical significance in prostate cancer patients.

The heterozygous genotype of the prostate cancer patient carrying the IDH1 c.548A>G missense mutation was confirmed by Sanger sequencing. c.159C>T, c.252C>T variations were determined as a result of next generation sequencing for rs1803965, one of the MGMT gene mutations that were found to be significantly different between D'Amico risk groups. According to the Sanger sequencing result, it was clarified that this mutation, which was detected in 5 patients, was a c.159C>T (rs1803965) variant. c.250C>T and c.343C>T (rs12917) variants were determined as a result of NGS analysis under the umbrella of rs12917 in MGMT gene. It was determined by Sanger sequencing that all five patients were heterozygous and had c.250C>T (rs12917) variant.

In this case, the c.548A>G (p.Y183C, rs34599179) variant, which is possibly pathogenic for the IDH1 gene, was shown for the first time in a prostate cancer case and was brought to the literature. In addition, it has been reported for the first time that the MGMT gene c.159C>T (p.L53L, rs1803965) and c.250C>T (p.L845F, rs12917) variations have the potential to be a determinant in D'Amico risk classification.

As a result of this thesis study, which we designed to examine IDH1, MGMT, and ATRX gene sequences in prostate cancer patients, 11 variants were identified. None of these variants detected, except for IDH1 rs11554137 and MGMT rs12917, have been reported in prostate cancer.

The class of the rs34599179 variant in the IDH1 gene is unknown, but the missense variant was detected in a prostate cancer patient with a CADD score of 26.700.

The rs1803965 synonymous MGMT gene variant, whose clinical significance has not been shown in any disease before, and the rs12917 missense MGMT variant associated with prostate cancer risk were detected in our prostate cancer patients. In addition, it was determined that according to the D'Amico risk classification, these MGMT gene variants were significantly higher in those with intermediate risk group than those with high-risk group. We have revealed molecular marker candidate variants that can be added to the D'Amico risk classification criteria have been revealed by us.

The fact that the analysis of variation was performed on a small sample and the absence of a control group are two factors limiting the study.

Our study brought meaningful molecular information to the medical literature by identifying the great majority of IDH1, MGMT, and ATRX gene variants, not only for the first time in prostate cancer but also in any human disease. Therefore, it is important to carry out studies that complement the deficiencies we have pointed out in our study in terms of prostate cancer and cancer molecular biology.

Our results show that there are many unidentified variants in the molecular infrastructure of prostate cancer. These variants have potentially been shown to be of clinical importance in the diagnosis and treatment of prostate cancer.



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7. APPENDICES

7.1. Ethical Approval

 T.C. YEDİTEPE ÜNİVERSİTESİ	
Sayı: 37068608-6100-15- 2519 Konu: Klinik Araştırmalar Etik Kurul Başvurusu hk.	16.Mart.2023
Sorumlu Araştırmacı Prof. Dr.Faruk Yencilek (Yeditepe Üniversitesi, Tıp Fakültesi Üroloji AD., İSTANBUL)	
Klinik Araştırma başvurunuz ve ek belgeniz Yeditepe Üniversitesi Klinik Araştırmaları Etik Kurulu (KA EK) tarafından değerlendirildi. Etik Kurulumuz, başvuru ve ek bilgileri değerlendirdikten sonra 15 Mart 2023 tarihli toplantısında “Prostat Kanseri Hastalarında IDH1 Geninin Yeni Nesil Dizileme Yöntemiyle Mutasyon Analizi” başlıklı klinik araştırma protokolünüzü ve başvurunuzu onaylanmıştır. Onay bilgileri: Referans No : 2023/1732 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 ÇELİK	

7.2. Patient Data

	GRUP	taniyaş	boy	kilo	bmii	sigarapaketyl	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	patolojikere	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	tanzamanımetas taz	tedavi	ektedavi	takiptemetastaz	takipsuresiy
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
4	4	8	>7	25	yok	radikal pro	RT,	yok	37
4	5	9	>7	90	yok	küratif rad	ADT	yok	204
4	4	8	>7	25	yok	radikal pro	yok	yok	4
4	4	8	>7	60	yok	radikal pro	RT,	yok	103
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
4	3	7	<esit7	45	yok	radikal pro	yok	yok	11
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
4	3	7	<esit7	30	yok	ADT	yok	yok	50
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
4	3	7	<esit7	6	yok	küratif rad	yok	yok	22

Analysis Name (Project) **Prostate analysis (-)** Age Sex Ethnicity

Phenotype: **Cancers and Tumors** Age of Onset Disease Prevalence

Gene **IDH1** Variant **c.315C>T** Population Frequency: **10.53%** gnomAD (African) **normal** Computed Classification: **Benign** Cancers and Tumors

Transcript(s) **NM_005896.4** Genotype: **p.G105G** Impact: **Synonymous**

Variant List < Previous Next > Use Classification View Bibliography

Variant details

Effect on Protein: **NM_005896.4** **CHR 2: 209,113,192** **IDH1** **c.315C>T** **Exon 4** **p.G105G** **Synonymous SNV**

Loss/Change/Gain Fx Benign/Likely Benign Pathogenic/Likely Pathogenic

Reported clinical cases of Cancers and Tumors

Clinical cases from other laboratories

Reference	ID	Affected Cases
ClinVar	RCV000454437.1	

Analysis Name (Project) **Prostate analysis (-)** Age Sex Ethnicity

Phenotype: **Cancers and Tumors** Age of Onset Disease Prevalence

Gene **IDH1** Variant **c.532G>A** Population Frequency: **8.67%** gnomAD (Ashkenazi Jewish) **normal** Computed Classification: **Benign** Cancers and Tumors

Transcript(s) **NM_005896.4** Genotype: **p.V178I** Impact: **Missense**

Variant List < Previous Next > Use Classification View Bibliography

Variant details

Effect on Protein: **NM_005896.4** **CHR 2: 209,108,317** **IDH1** **c.532G>A** **Exon 6** **p.V178I** **Missense SNV**

Loss/Change/Gain Fx Benign/Likely Benign Pathogenic/Likely Pathogenic

Reported clinical cases of Cancers and Tumors

Clinical cases from other laboratories

Reference	ID	Affected Cases
ClinVar	RCV000121207.1	
COSMIC	97131	

Analysis Name (Project) **Prostate analysis (-)** Age Sex Ethnicity

Phenotype: **Cancers and Tumors** Age of Onset Disease Prevalence

Gene **IDH1** Variant **c.548A>G** Population Frequency: **1.69%** gnomAD (European) **normal** Computed Classification: **Benign** Cancers and Tumors

Transcript(s) **NM_005896.4** Genotype: **p.Y183C** Impact: **Missense**

Variant List < Previous Next > Use Classification View Bibliography

Variant details

Effect on Protein: **NM_005896.4** **CHR 2: 209,108,301** **IDH1** **c.548A>G** **Exon 6** **p.Y183C** **Missense SNV**

Loss/Change/Gain Fx Benign/Likely Benign Pathogenic/Likely Pathogenic

Reported clinical cases of Cancers and Tumors

Clinical cases from other laboratories

Reference	ID	Affected Cases
ClinVar	RCV000121204.1	

Analysis Name (Project) Prostate analysis (-) Age Sex Ethnicity Patient Phenotype(s) Show

Phenotype: X-linked alpha-thalassemi... (142 more) Age of Onset Birth - 1 Year Disease Prevalence 200 Individuals Mode of Inheritance X-Linked

Gene **ATRX** Variant c.2671C>G * Transcription p.E891E normal Computed Classification Benign X-linked alpha-thalassemi-mental ...

Population Frequency: 87.73% gnomAD (African) Genotype: Hom (98.19% Allele Fraction) Impact: Synonymous

Variant List < Previous Next > Use Classification View Bibliography

Variant details

Effect on Protein NM_138270.5 CHR X: 76,937,963 ATRX c.2671C>G Exon 8 p.E891E Synonymous SNV

Loss/Change/Gain Fx Benign/Likely Benign Pathogenic/Likely Pathogenic

Reported clinical cases of X-linked alpha-thalassemi-mental retardation syndrome

Clinical cases from other laboratories

Reference	ID	Affected Cases
ClinVar	RCV00078959.1	

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Analysis Name (Project) Prostate analysis (-) Age Sex Ethnicity Patient Phenotype(s) Show

Phenotype: X-linked alpha-thalassemi... (142 more) Age of Onset Birth - 1 Year Disease Prevalence 200 Individuals Mode of Inheritance X-Linked

Gene **ATRX** Variant c.1353C>T Transcription p.T451T normal Computed Classification Likely Benign X-linked alpha-thalassemi-mental ...

Population Frequency: 0.29% gnomAD (Ashkenazi Jewish) Genotype: (95.65% Allele Fraction) Impact: Synonymous

Variant List < Previous Next > Use Classification View Bibliography

Variant details

Effect on Protein NM_138270.5 CHR X: 76,939,281 ATRX c.1353C>T Exon 8 p.T451T Synonymous SNV

Loss/Change/Gain Fx Benign/Likely Benign Pathogenic/Likely Pathogenic

Reported clinical cases of X-linked alpha-thalassemi-mental retardation syndrome

Clinical cases from other laboratories

Reference	ID	Affected Cases
ClinVar	RCV000502718.1	

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Analysis Name (Project) Prostate analysis (-) Age Sex Ethnicity Patient Phenotype(s) Show

Phenotype: Familial pancreatic cancer (3 more) Age of Onset >19 Years Disease Prevalence 1/111111

Gene **MGMT** Variant c.-28C>T Transcription normal Computed Classification Benign Familial pancreatic cancer

Population Frequency: 7.82% gnomAD (European) Genotype: (83.79% Allele Fraction) Impact: -

Variant List < Previous Next > Use Classification View Bibliography

Variant details

Effect on Protein NM_002412.5 CHR 10: 131,265,545 MGMT c.-28C>T 5'UTR SNV

Loss/Change/Gain Fx Benign/Likely Benign Pathogenic/Likely Pathogenic

Reported clinical cases of Familial pancreatic cancer

Clinical cases from other laboratories

Reference	ID	Affected Cases
ClinVar	RCV000431439.1	
HGMD	CR075258 (FP)	

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Analysis Name (Project)
Prostate analysis (-)

Age
-

Sex
-

Ethnicity
-

Patient Phenotype(s)
[Show](#)

Phenotype: Familial pancreatic cancer (3 more) [i](#) Age of Onset >19 Years [i](#) Disease Prevalence 1/111111 [i](#)

Gene
MGMT [i](#)

Variant
c.159C>T
p.L53L **normal** [i](#)

Population Frequency: 22.80% gnomAD (Latino)
Genotype: (44.67% Allele Fraction)
Impact: Synonymous

Computed Classification [i](#)
Benign
Familial pancreatic cancer

Variant List < Previous Next > Use Classification View Bibliography

Variant details

Effect on Protein
NM_002412.5
CHR 10: 131,506,192 MGMT c.159C>T Exon 3 p.L53L Synonymous SNV

Loss/Change/Gain Fx
Benign/Likely Benign
Pathogenic/Likely Pathogenic

Reported clinical cases of Familial pancreatic cancer

Rarity in general population

Reported functional impact
Predicted Biochemical Impact on NM_002412.5

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Analysis Name (Project)
Prostate analysis (-)

Age
-

Sex
-

Ethnicity
-

Patient Phenotype(s)
[Show](#)

Phenotype: Familial pancreatic cancer (3 more) [i](#) Age of Onset >19 Years [i](#) Disease Prevalence 1/111111 [i](#)

Gene
MGMT [i](#)

Variant
c.250C>T
p.L84F **normal** [i](#)

Population Frequency: 23.17% gnomAD (Latino)
Genotype: (48.28% Allele Fraction)
Impact: Missense

Computed Classification [i](#)
Benign
Familial pancreatic cancer

Variant List < Previous Next > Use Classification View Bibliography

View/Add notes | Remove assessment | Set Validation Status

Variant details

Effect on Protein
NM_002412.5
CHR 10: 131,506,283 MGMT c.250C>T Exon 3 p.L84F Missense SNV

Loss/Change/Gain Fx
Benign/Likely Benign
Pathogenic/Likely Pathogenic

Reported clinical cases of Familial pancreatic cancer

Clinical cases from other laboratories

Reference	ID	Affected Cases

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Analysis Name (Project)
Prostate analysis (-)

Age
-

Sex
-

Ethnicity
-

Patient Phenotype(s)
[Show](#)

Phenotype: Familial pancreatic cancer (3 more) [i](#) Age of Onset >19 Years [i](#) Disease Prevalence 1/111111 [i](#)

Gene
MGMT [i](#)

Variant
c.427A>G
p.I143V **normal** [i](#)

Population Frequency: 12.67% gnomAD (European)
Genotype: Het (55.63% Allele Fraction)
Impact: Missense

Computed Classification [i](#)
Benign
Familial pancreatic cancer

Variant List < Previous Next > Use Classification View Bibliography

Variant details

Effect on Protein
NM_002412.5
CHR 10: 131,565,064 MGMT c.427A>G Exon 5 p.I143V Missense SNV

Loss/Change/Gain Fx
Benign/Likely Benign
Pathogenic/Likely Pathogenic

Reported clinical cases of Familial pancreatic cancer

Clinical cases from other laboratories

Reference	ID	Affected Cases
HGMD	CM004820 (DFP)	

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Analysis Name (Project)
Prostate analysis (-)

Age
-

Sex
-

Ethnicity
-

Patient Phenotype(s)
[Show](#)

Phenotype: Familial pancreatic cancer (3 more) [i](#) Age of Onset >19 Years [i](#) Disease Prevalence 1/111111 [i](#)

Gene
MGMT [c](#)

Variant
c.533A>G

Transcript(s)
NM_002412.5

Population Frequency: 12.81% gnomAD (European) [i](#)

Genotype: Het (47.22% Allele Fraction)

Impact: Missense

Computed Classification [i](#)
Benign
Familial pancreatic cancer

Variant List < Previous Next > Use Classification View Bibliography

Variant details

Effect on Protein
NM_002412.5

CHR 10: 131,565,170 MGMT c.533A>G Exon 5 p.K178R Missense SNV

Loss/Change/Gain/Fn
Benign/Likely Benign
Pathogenic/Likely Pathogenic

Reported clinical cases of Familial pancreatic cancer

Clinical cases from other laboratories

Reference	ID	Affected Cases
ClinVar	RCV001616833.1 i	

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Analysis Name (Project)
Prostate analysis (-)

Age
-

Sex
-

Ethnicity
-

Patient Phenotype(s)
[Show](#)

Phenotype: Familial pancreatic cancer (3 more) [i](#) Age of Onset >19 Years [i](#) Disease Prevalence 1/111111 [i](#)

Gene
MGMT [c](#)

Variant
c.-55C>T

Transcript(s)
NM_002412.5

Population Frequency: 0.004% gnomAD (Latino) [i](#)

Genotype: -

Impact: -

Computed Classification [i](#)
Uncertain Significance
Familial pancreatic cancer

Variant List < Previous Next > Use Classification View Bibliography

Effect on Protein
NM_002412.5

CHR 10: 131,265,518 MGMT c.-55C>T 5'UTR - SNV

Loss/Change/Gain/Fn
Benign/Likely Benign
Pathogenic/Likely Pathogenic

Reported clinical cases of Familial pancreatic cancer

Rarity in general population

Reported functional impact
Predicted Biochemical Impact on NM_002412.5

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7.3. Curriculum Vitae

Personal Informations

Name	Faruk	Surname	YENCILEK
Place of Birth	Adana		
Nationality	T.C.		
Email			

Educational Informations

Title	Name of Institution	Year
Doctorate	Yeditepe University Institute of Health Sciences, Department of Molecular Medicine	2016- 2023
Medical Expertise	Istanbul University Cerrahpaşa School of Medicine, İstanbul	1996-2001
Medical School	Marmara University School of Medicine (English), İstanbul	1988- 1996

Work Experience

Job Title	Name of Institution	Year
Intern Doctor	İstanbul University Cerrahpaşa School of Medicine, Department of Urology	1996-2001
Urologist (6 months)	SSK Aksaray Hospital	2002-2002
Urologist	Yeditepe University School of Medicine, Department of Urology	2002-2003
Asst Prof	Yeditepe University School of Medicine, Department of Urology	2003- 2011
Post Doc Researcher	Heidelberg University, SLK Heilbronn Klinikum, Urologie, Heilbronn, Almanya	2008-2009
Assoc Prof	Yeditepe University School of Medicine, Department of Urology	2011-2016
Head of Department	Yeditepe University School of Medicine, Department of Urology	2012- Present

Member of the Board of Directors and Executive Board	Yeditepe University / Health Services Educational Research And Application Center	2013- Present
Head Physician	Yeditepe University Hospitals	2013- Present
Professor	Yeditepe University School of Medicine, Department of Urology	2016- Present

Computer skills

Program	Ability to use
Microsoft Office	Very Good

Publications / Notices Certificates /

A1. International Articles (SCI, SCI-Expanded) :

A1.1 Alıcı, B., M.K. Gümüştas, H. Özkara, E. Akkuş, G. Demirel, **F. Yencilek**, H. Hattat, “Apoptosis in the erectile tissues of diabetic and healthy rats” BJU Int., 85, 326-329, (2000).

A1.2 **Yencilek, F.**, Baykal C, “46 XX Male: A case report” Clin Exp Obstet Gynecol., 32, 263-264, (2005).

A1.3 Gürses, B., N. Kabakçı, H. Z. Akşit, **F. Yencilek**, A. Kovanlıkaya, I. Kovanlıkaya, “Cystic splenosis mimicking a renal mass: a case report and review of the literature” Australas Radiol., 51, 52-55, (2007).

A1.4 **Yencilek, F.**, C. Aktas, C. Goktas, C. Yilmaz, U. Yilmaz, K. Sarica, “Role of papaverine hydrochloride administration in patients with intractable renal colic: randomized prospective trial” Urology, 72, 987-990, (2008).

A1.5 Yildirim, G., C. Ficicioglu, O. Akcin, R. Attar, N. Tecellioglu, **F. Yencilek**, “Can pentoxifylline improve the sperm motion and ICSI success in the primary ciliary dyskinesia?” Arch Gynecol Obstet., 279, 213-215, (2009).

A1.6 Sarica, K., B. Eryildirim, **F. Yencilek**, U. Kuyumcuoglu, “Role of overweight status on stone-forming risk factors in children: a prospective study” *Urology*, 73, 1003-1007, (2009).

A1.7 Mutlu, N., L. N. Türkeri, **F. Yencilek**, A. Demir, K. Emerk, “Complexed prostate specific antigen: better test in the diagnosis of prostate cancer for the clinically relevant 2.5-4 ng/ml total PSA range” *Can J Urol.*, 16, 4558-4567, (2009).

A1.8 Erol, B., H. Tokgoz, V. Hanci, S. Bektas, B. Akduman, **F. Yencilek**, G. Mungan, A. Mungan, “Vardenafil reduces testicular damage following ischemia/reperfusion injury in rats” *Kaohsiung J Med Sci.*, 25, 374-380, (2009).

A1.9 Teber, D., A. S. Gözen, J. Cresswell, A. E. Canda, **F. Yencilek**, J. Rassweiler, “Prevention and management of ureteral injuries occurring during laparoscopic radical prostatectomy: the Heilbronn experience and a review of the literature” *World J Urol.*, 27, 613-618, (2009).

A1.10 Teber, D., S. Guven, T. Simpfendorfer, M. Baumhauer, E. O. Güven, **F. Yencilek**, S. Gözen, J. Rassweiler, “Augmented reality: a new tool to improve surgical accuracy during laparoscopic partial nephrectomy? Preliminary in vitro and in vivo results” *Eur Urol.*, 56, 332-338, (2009).

A1.11 **Yencilek, F.**, B. Onal, A. Erozcı, Z. Talat, S. Ataus, “Intermediate-risk group in patients with transitional cell carcinoma of the bladder: prediction of high-risk patients in this heterogeneous group” *Urol Int.*, 83, 295-299, (2009).

A1.12 **Yencilek, F.**, K. Sarica, B. Eryildirim, S. Erturhan, M. Karakok, U. Kuyumcuoglu, “Hyperoxaluria-induced tubular ischemia: the effect of verapamil on the limitation of tissue HIF-1 alpha levels in renal parenchyma” *Int Urol Nephrol.*, 42, 361-367 (2010).

A1.13 Kilickesmez, O., E. Inci, S. Atilla, N. Tasdelen, B. Yetimoglu, **F. Yencilek**, N. Gurmen, “Diffusion-Weighted Imaging of the Renal and Adrenal Lesions”, *J Comput Assist Tomogr.*, 33, 828-833, (2009).

A1.14 **Yencilek, F.**, K. Sarica, S. Erturhan, F. Yagci, A. Erbagci, “Treatment of ureteral calculi with semirigid ureteroscopy: Where should we stop?” Urol Int., 84, 260-264, (2010).

A1.15 Aydın, H., **F. Yencilek**, N. Mutlu, N. Çomunoğlu, H. H. Koyuncu, K. Sarica, “Ethylene Glycol-Induced Hyperoxaluria Increases Plasma and Renal Tissue Asymmetrical Dimethylarginine Levels in Rats: A New Pathogenetic Link in Hyperoxaluria-Induced Disorders” J Urol., 183, 759-764, (2010).

A1.16 Canguven, O., G. Aykose, S. Albayrak, C. Goktas, R. Horuz, **F. Yencilek**, “Efficacy of Testosterone gel in the Treatment of Erectile Dysfunction in Hypogonadal Hemodialysis Patients. A pilot study” Int J Impot Res., 22, 140-145, (2010).

A1.17 **Yencilek F**, Attar R, Erol B, Narin R, Aydın H, Karateke A, Sarica K. “Factors affecting sexual function in premenopausal age women with type 2 diabetes: a comprehensive study”. Fertil Steril., 94, 1840-1843 (2010).

A1.18 Koyuncu HH, **Yencilek F**, Eryildirim B, Sarica K. “Family history in stone disease: how important is it for the onset of the disease and the incidence of recurrence?” Urol Res., 38, 105-109 (2010).

A1.19 **Yencilek F**, Erturhan S, Canguven O, Koyuncu H, Erol B, Sarica K. “Does tamsulosin change the management of proximally located ureteral stones?” Urol Res., 38, 195- 199. (2010).

A1.20 Gürses B, Tasdelen N, **Yencilek F**, Kılıckesmez NO, Alp T, Fırat Z, Albayrak MS, Uluğ AM, Gürmen AN. “Diagnostic utility of DTI in prostate cancer”. Eur J Radiol., 79, 172-176. (2011).

A1.21 Tanriverdi O, **Yencilek F**, Koyuncu H, Yencilek E, Sarica K. “Emergent stenting after uncomplicated ureteroscopy: evaluation of 23 patients”. Urology., 77, 305-308. (2011).

A1.22 Koyuncu H, Ergenoglu M, **Yencilek F**, Gulcan N, Tasdelen N, Yencilek E, Sarica K. “The evaluation of saphenofemoral insufficiency in primary adult varicocele”. J Androl., 32, 151-154. (2011).

A1.23 Koyuncu H, **Yencilek F**, Erturhan S, Eryildirim B, Sarica K. “Clinical course of pediatric urolithiasis: follow-up data in a long-term basis”. Int Urol Nephrol., 43, 7- 13. (2011).

A1.24 Aydin H, **Yencilek F**, Erihan IB, Okan B, Sarica K. “Increased 10-year cardiovascular disease and mortality risk scores in asymptomatic patients with calcium oxalate urolithiasis”. Urol Res., 39, 451-458. (2011).

A1.25 Sarica K, Aydin H, **Yencilek F**, Telci D, Yilmaz B. “Human umbilical vein endothelial cells accelerate oxalate-induced apoptosis of human renal proximal tubule epithelial cells in co-culture system which is prevented by pyrrolidine dithiocarbamate”. Urol Res., 40, 461-466. (2012).

A1.26 Menda F, Temur S, Bilgen S, **Yencilek F**, Koyuncu H, Sancar N, Koner O. “Effect of morphine on lower urinary tract discomfort after transurethral resection of prostate under general anesthesia: a randomised clinical study”. J Anesth., 27, (2013).

A1.27 Atis G, Koyuncu H, Gurbuz C, **Yencilek F**, Arikan O, Caskurlu T. “Bilateral single- session retrograde intrarenal surgery for the treatment of bilateral renal stones”. Int Braz J Urol., 39, 387-392. (2013).

A1.28 Koyuncu H, **Yencilek F**, Kalkan M, Bastug Y, Yencilek E, Ozdemir AT. “Intrarenal Surgery vs Percutaneous Nephrolithotomy in the Management of Lower Pole Stones Greater than 2 cm”. Int Braz J Urol., 41, (2015).

A1.29 **Yencilek F**, Yildirim A, Yilmaz SG, Altinkilic EM, Dalan AB, Bastug Y, Isbir T. “Investigation of Interleukin-1 β Polymorphisms in Prostate Cancer”. Anticancer Res., 35, 6057-6061. (2015).

A1.30 **Yencilek F.**, Yilmaz SG., Yıldırım A., Gormus U., Altinkilic EM., Dalan AB, Bastug Y., Turkmen S., Turkan S., Isbir T. Apolipoprotein E Genotypes in Patients with Prostate Cancer. *Anticancer Res.* 36 (2), (2016). (kabul tarihi 19.11.2015)

A2. International Articles:

A2.1 Sarica, K., **F. Yencilek**, “Prevention of shockwave induced functional and morphological alterations: an overview” *Arch Ital Urol Androl.*, 80, 27-33, (2008).

A2.2 Canguven, O., C. Goktas, **F. Yencilek**, C. Cetinel, S. Albayrak, “A new technique for simple renal cyst: cystoretroperitoneal shunt” *Adv Urol.*, 2009 906013, doi:10.1155/2009/906013, 2009.

A2.3. Koyuncu H, Serefoglu EC, Karacay S, Ozdemir AT, Kalkan M, **Yencilek F.** “Is there a correlation between intravaginal ejaculatory latency time and enuresis? An exploratory study”. *Cent European J Urol.*, 67, 74-78. (2014).

A2.4 Kalkan M, Uzun H, Turkan S, Sahin C, Fidan F, **Yencilek F**, Koyuncu H. “Change in Smoking Habits and Contribution of Physicians to Smoking Cessation in Patients with Non-muscle-Invasive Bladder Cancer”. *Eur J Gen Med.*, 12, 101-103. (2015).

B. International Oral Presentations and Proceedings:

B.1 Kalkan, M., O. Demirkesen, B. Önal, H. Uzun, **F. Yencilek**, A. Öner, “Extracorporeal shockwave lithotripsy in childhood ureteral stones,” 2nd Eurolithiasis Society (EULIS) Meeting (10th European Symposium on Urolithiasis), June 11-14, 2003, Istanbul- Turkey.

B.2 **Yencilek, F.**, M. Kalkan, H. Uzun, E. Akkuş, A. Öner, V. Solok, “The Relationship between crystalluria and sialic acid in hyperoxaluric rats,” 2nd Eurolithiasis Society (EULIS) Meeting (10th European Symposium on Urolithiasis), June 11-14, 2003, Istanbul- Turkey.

B.3 Öbek, C., T. Kocagöz, S. Aktaş, **F. Yencilek**, B. Oyan Uluc, Y. Koc, “Hemorrhagic cystitis due to viral infection following hematopoietic stem cell transplantation,” 25th World Congress of Endouroloji & SWL, 30 October – 3 November, 2007, Cancun-Mexico.

B.4 Gözen A. S., D. Teber, A. E. Canda, M. Ateş, **F. Yencilek**, J. Rassweiler, “Does Body Mass Index affect operative measurements, functional results, oncological outcomes and quality of life of patients who underwent laparoscopic radical prostatectomy? The Heilbronn experience,” 26th World Congress of Endourology & SWL, November 30- December 3, 2008, P. Shangai-P. R. China.

B.5 Gözen A. S., D. Teber, J. Cresswell, A. E. Canda, **F. Yencilek**, J. Rassweiler, “Management of ureteral injuries occurring during laparoscopic radical prostatectomy,” 26th World Congress of Endourology & SWL, November 30- December 3, 2008, Shangai-P. R. China.

B.6 Teber, D., Ö. Yaycioglu, A. Cardi, A. Gözen, **F. Yencilek**, J. Rassweiler, “Single knot running suture anastomosis in laparoscopic dismembered pyeloplasty: Training model and clinical results,” 26th World Congress of Endourology & SWL, November 30- December 3, 2008, Shangai, P. R. China.

B.7 **Yencilek, F.**, K. Sarica, S. Erturhan, M. Karakök, U. Kuyumcuoglu, “Hyperoxaluria induced tubular ischemia: Evaluation of tissue HIF-1 alpha levels in renal parenchyma in rabbit model,” 24th Annual EAU Congress, 17-21 March, 2009, Stockholm-Sweden.

B.8 **Yencilek, F.**, H. Aydin, N. Mutlu, N. Comunoglu, H. Koyuncu, K. Sarica, “Ethylene glycol-induced hyperoxaluria increases plasma and renal tissue asymmetrical dimethylarginine levels in rats: A new pathogenic link in hyperoxaluria-induced disorders,” 27th World Congress of Endourology & SWL, October 6- 10th, 2009, Munich- Germany.

B.9 Gözen, A., T.Kalogeropoulos, L. Tunc, **F. Yencilek**, J. Rassweiler, D. Teber, “Laparoscopic upper pole heminephroureterectomy in children: The Heilbronn experience,” 27th World Congress of Endourology & SWL, October 6- 10th, Munich, 2009.

B.10 **Yencilek, F.**, S. Erturhan, H. Koyuncu, O. Canguven, C. Goktas, K. Sarica, “Does Tamsulosin change the management of proximally located ureteral stones? A Comprehensive study,” 27th World Congress of Endourology & SWL, October 6- 10th , 2009, Munich-Germany.

B.11 Sarica, K., **F. Yencilek**, S. Erturhan, B. Eryildirim, H. Koyuncu, “Clinical course of pediatric urolithiasis: Follow-up data in a long-term basis,” 6th Eurolithiasis Society Symposium (eULIS) Meeting (14th European Symposium on Urolithiasis), 15-17 October, 2009, Como-Italy.

B.12 Koyuncu, H., **F. Yencilek**, B. Eryildirim, S. Erturhan, U. Kuyumcuoglu, K. Sarica, “The impact of positive family history on the age at onset and natural course of stone disease,” 6th Eurolithiasis Society Symposium (eULIS) Meeting (14th European Symposium on Urolithiasis), 15-17 October, 2009, Como-Italy.

B.13 Sarica, K., B. Okan, **F. Yencilek**, H. Koyuncu, “Impact of dietary habit in recurrent stone formation,” 6th Eurolithiasis Society Symposium (eULIS) Meeting (14th European Symposium on Urolithiasis), 15-17 October, 2009, Como-Italy.

B.14 **Yencilek, F.**, H. Koyuncu, S. Erturhan, K. Sarica, “How did medical expulsion treatment change our strategies in ureteral stone management?,” 6th Eurolithiasis Society Symposium (eULIS) Meeting (14th European Symposium on Urolithiasis), 15-17 October, 2009, Como-Italy.

B.15 Erol B, Amasyalı A.S, Ozkanlı S, Sari U, Efiloglu O, Danacioglu Y.O, **Yencilek F**, Atis G, Yildirim A, Caskurlu T. The preventive effects of antioxidant drugs in experimental testis ischemia/reperfusion injury: Which one is superior, combined antioxidants or thymoquinone? EAU 15th SEEM (South Eastern European Meeting, 6-8 November 2015, Antalya, Turkey.

C2. National/International Book Chapters:

C2.1 Öbek, C., **F. Yencilek**, “Transrektal prostat ultrasonografisi, transrektal ultrasonografi eşliğinde prostat biyopsisi,” Üroonkoloji Kitabı, Editörler: Haluk Özen, Levent Türkeri, Ertem Basım Yayın, Birinci basım, 633-648, (2007).

C2.2 Teber, D., **F. Yencilek**, Ö. Şanlı, “Açık ve laparoskopik radikal prostatektomi: Güncel bir değerlendirme,” Ürolojide Yeni Ufuklar, Editör: Sümer Baltacı, Güneş Tıp Kitabevi, Ankara, 81-100, (2009).

C2.3 Koyuncu H, **F Yencilek**. Benign Prostat Hiperplazisi’nin Cerrahi Tedavisinde Transüretral Rezeksiyon Hala Altın Standart mıdır? Türk Üroloji Seminerleri. 3: 10- 13. (2012).

C2.4 **F. Yencilek**, H. Koyuncu. Ürogenital Sisteme ait Semptomlar ve Fizik Muayene. Semiyoloji. Editör: Yaşar Küçükardalı. Nobel Tıp Kitabevi. ss345-354. (2012)

C2.5 S. Albayrak, **F. Yencilek**. Mesane Augmentasyon: Güncel ve Gelecek Teknikler. Pediatrik Üroloji.(Kelalis-King-Belma). Çeviri Editörü Mete Kilciler, Murat Dayanç. Habitat Yayıncılık. ss 821-855. (2013).

C2.6 **F. Yencilek**, H. Koyuncu, A.T. Özdemir. Testis Germ Hücre Tümörlerinde Serum Tümör Belirteçleri ve Klinik Kullanımı. Ss 749-755. Üroonkoloji. Editör: Çetin Dinçel. Habitat yayıncılık. (2013)

C2.7 H. Koyuncu, A.E. İşgören, **F. Yencilek**. Fleksibl Üreterorenoskopide Kullanılan Cihazlar ve Lazer Tedavileri. Üriner Sistem Taş Hastalığının Tedavisi. Editör: Turhan Çaşkurlu. Ss 229-236. (2013).

D. National Articles:

D1. Alıcı, B., K. Gümüştas., E. Akkuş., **F. Yencilek**, İ. Karaman, H. Hattat, “Sperm kreatinin kinaz aktivitesi: Sperm fertilizasyon potansiyelinin göstergesi mi?,” Türk Üroloji Dergisi, 25, 136-140, (1999).

- D2. Kalfazade, N., B. Alıcı, T. İrez, H. Özkara, İ. Karaman, **F. Yencilek**, Ergenekon E, “İnfertil erkeklerde indirgenmiş glutatyon ve pentoksifilin’in sperm fonksiyon testleri ve motilitesi üzerine etkilerinin kıyaslanması,” Türk Üroloji Dergisi, 25, 146-150, (1999).
- D3. Mihmanlı, İ., F. Kantarcı, S. Kuruoğlu, G. Ögüt, **F. Yencilek**, “Akut anaerob bakteriyel prostatit: Olgu sunumu,” Bilgisayarlı Tomografi Bülteni, 5, 221-222, (1999).
- D4. Önder, A. U, Ö. Yayıoğlu, **F. Yencilek**, Ö. Çetiner, V. Yalçın, V. Solok, “Transrektal ultrasonografi ile saptanan prostat ve adenomun volumünün piyes volümü ile korelasyonu: Eliptik ve sferikal volümün karşılaştırılması,” Türk Üroloji Dergisi, 26, 94-99, (2000).
- D5. Ataus, S., **F. Yencilek**, C. Demirkese, E. Akkuş, A. Akaydın, “Glans penis izole kaposi sarkomu,” Türk Üroloji Dergisi, 26, 253-255, (2000).
- D6. İrez, T., B. Alıcı, H. Özkara, E. Akkuş, **F. Yencilek**, P. Öcal, A. U. Özalp, H. Hattat, “Ureaplazma urealyticum ve chlamydia trachomatis enfeksiyonlarının semen parametrelerine etkisi,” Cerrahpaşa Tıp Dergisi, 31, 23-27, (2000).
- D7. **Yencilek, F.**, M. Kalkan, H. Uzun, E. Akkuş, A. Öner, V. Solok, “Hiperkalsiürik ve hiperoksalürik sıçanlarda kristalüri ile sialik asit ilişkisi,” Türk Üroloji Dergisi, 29, 126-132, (2003).
- D8. Öbek, C., **F. Yencilek**, “Laparoskopik retroperitoneal lenf nodu diseksiyonu,” Türkiye Klinikleri Cerrahi Tıp Bilimleri Dergisi, 3, 43-48, (2007).
- D9. Öbek, C., **F. Yencilek**, “Ürolojik laparoskopikide komplikasyonlar ve önlenmesi,” Türkiye Klinikleri Cerrahi Tıp Bilimleri Dergisi, 3, 109-116, (2007).
- D10. Öbek, C., **F. Yencilek**, “Böbrek tümörlerinde organ koruyucu yaklaşım,” Yeditepe Tıp Dergisi, 1, 81-88, (2007).

D11. Gürses, B., N. Kabakçı, N. Soydan, **F.Yencilek**, H. Z. Akşit, M. M. Cihangiroğlu, “Appendiks epididim torsiyonu: Olgu sunumu,” Yeditepe Tıp Dergisi, 3, 11-13, (2007).

D12. Cangüven, Ö., **F. Yencilek**, “İlaçların reproduktif sisteme olan yan etkileri,” Androloji Bülteni, 32, 29-31, (2008).

D13. **F. Yencilek**, K. Sarıca, T. Gürpınar, C. Göktaş, Ö. Cangüven, S. Albayrak, “ A comparision of shock wave lithotripsy, semirigid and flexible ureteroscopy in the management of proximal ureteral calculi,” Türk Üroloji Dergisi, 35, 101-107, (2009).

D14. **F. Yencilek**, H. Koyuncu. Altmış cc'den küçük prostatlarda fotoselektif vaporizasyon ile holmium lazer ablasyonunun karşılaştırılması: Randomize bir çalışmanın uzun dönem sonuçları. (Makale-Yorum). Üroonkoloji Bülteni. 80-83. (2010).

D15. **F. Yencilek**, H. Koyuncu. Prostat kanseri için idrar belirteçlerinde son durum analizi. Üroonkoloji Bülteni. 2, 11-16. (2011).

D16. **F. Yencilek**, S. Erturhan, Ö. Cangüven, B. Erol, H. Koyuncu, C. Göktaş, K. Sarıca. The effect of indomethacin on hyperoxaluria-induced renal tubular epithelial injury Turkish Journal of Urology. 35, 298-303, (2009)

E. International Oral Presentations and Proceedings:

E1. Alıcı, B, H. Özkara, E. Akkuş, B. Önal, **F. Yencilek**, H. Hattat, “Total motil sperm sayısının intrauterin inseminasyon başarısına etkisi,” 15. Ulusal Üroloji Kongresi, 25-29 Ekim, Antalya, 1998.

E2. İrez, T., B. Alıcı, H. Özkara, E. Akkuş, **F. Yencilek**, H. Hattat, “Ureaplazma Urealyticum ve Chlamydia Trachomatis Enfeksiyonlarının Semen Parametrelerine Etkisi,” 15. Ulusal Üroloji Kongresi, 25-29 Ekim, Antalya, 1998.

E3. Tansu, N., B. Önal, C. Öbek, M. Kalkan, O. Demirkese, K.K. Kantay, **F. Yencilek**, V. Yalçın, V. Solok, “Ekstrakorporeal şok dalga litotripsinin izole alt kaliks taşlarındaki etkinliği,” 4. Ulusal Endoüroloji Kongresi, 1-2 Haziran, İstanbul, 2000.

E4. Ataus, S., **F. Yencilek**, C. Öbek, M. Kalkan, A. Öner, A. Erözenci, V. Solok, “Yüzeyel mesane tümörlü hastalarda ilk kontrol sistoskopisinin rekürrens açısından prognostik önemi,” 16. Ulusal Üroloji Kongresi, 8-12 Ekim, İzmir, 2000.

E5. Yalçın, V., A. U. Önder, B. Önal, M. Kalkan, **F. Yencilek**, A. R. Kural, V. Solok, “Üreter taşlarında üreterorenoskopi: 929 uygulama” 16. Ulusal Üroloji Kongresi, 8-12 Ekim, İzmir, 2000

E6. Kalkan, M., S. Ataus, H. Uzun, **F. Yencilek**, B. Önal, Z. Talat, “Yüzeyel mesane tümörlerinde rekürrensi öngörmede kullanılan prognostik etmenlerin analizi,” 17. Ulusal Üroloji Kongresi, 5-10 Ekim, Antalya, 2002.

E7. Gürpınar, T., **F. Yencilek**, V. İsmayilov, Ü. Gül, A. U. Özalp, T. İsayev, F. Memmedov, “Perkütan nefrolitotomi: Azerbaycan deneyimimiz,” 18. Ulusal Üroloji Kongresi, 2-7 Ekim, Antalya, 2004.

E8. **Yencilek, F.**, V. İsmayilov, A. U. Özalp, F. Memmedov, T. Gürpınar, “Azerbaycanda transüretal üreterolitotripsi deneyimleri,” 18. Ulusal Üroloji Kongresi, 2-7 Ekim, Antalya, 2004.

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F. Projects:

F1. TUBİTAK projesi: ABT-737 ve RAD-001(Everolimus) İlaç Kombinasyonunun Böbrek Kanseri Üzerinde Kemoteropatik Özelliğinin RenCA Hücre Modeli ile Balb-C Fareleri Üzerinde İncelenmesi

F2. TUBİTAK projesi: Ulusal imkanlarla “Nefrostomi Balon Dilatasyon Sistemi Geliştirilmesi” (Sanayi AR-GE projesi)