T.C.

YEDITEPE UNIVERSITY

INSTITUTE OF HEALTH SCIENCES

DEPARTMENT OF MOLECULAR MEDICINE

MUTATION ANALYSIS OF THE TERT GENE IN OVARIAN CANCER PATIENTS OF TURKISH POPULATION BY NEXT GENERATION SEQUENCING METHOD

DOCTOR OF PHILOSOPHY THESIS

BETÜL ÇAPAR GORALI

İSTANBUL, 2022

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İSTANBUL, 2022

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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.

BETÜL ÇAPAR GORALI
27/06/2022

DEDICATION

I dedicate my thesis to my father and mother

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BETÜL ÇAPAR GORALI

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LIST OF SYMBOLS AND ABBREVIATIONS

TERT: Telomerase Reverse Transcriptase
WHO: World Health Organisation
SNP: Single Nucleotide Polymorphism
TP53: Transformation-related protein 53
EOC : Epithelial ovarian carcinomas
SC: Serous carcinoma
HGSC: High-grade serous carcinoma
LGSC: Low-grade serous carcinoma
MC: Musinous Carcinoma
MOGCT : Malignant germ cell tumor types
SCST : Malignant sex cord-stromal tumors
FOXL2: Forkhead Box L2
TR : Telomerase RNA
DNA: Deoxyribonucleic acid
TEP1: Telomerase associated protein1
hTERT: Human TERT
UTUC: Upper Tract Urinary Carcinoma thyroid cancer
HCC: Hepatocellular carcinoma
GABPA : GA Binding Protein Transcription Factor Subunit Alpha
GABPB1: GA-Binding Protein Subunit Beta-1
CADD: Combined Annotation Dependent Depletion

ABSTRACT

Capar Gorali, B. Mutation Analysis of the TERT Gene in Ovarian Cancer Patients of Turkish Population by Next Generation Sequencing Method. Yeditepe University, Institute of Health Sciences, Department of Molecular Medicine. Doctoral Thesis. Istanbul, 2022

Ovarian cancer is the most common gynecological cancer after uterine cancer in our country. Although the frequency of the disease varies among different racial and ethnic groups, it is higher in developed countries than in developing countries. It affects women of all ages. Epithelial tumors constitute the majority of malignant ovarian tumors. Various risk factors are known to be associated with an increased risk of ovarian cancer. Among these risk factors, especially genetic factors, are responsible for the increased risk of developing ovarian cancer. Mutations are inherited changes in genetic material and are important because they can cause cancer. Most of the mutations in TERT, ATRX, MGMT, and IDH genes are associated with the risk of multiple cancer types. The Next Generation Sequencing approach is used in cancer studies, including ovarian cancer, facilitating the identification of existing mutations as well as new discoveries. In our study, 33 female patients between the ages of 18 and 76 who were diagnosed with ovarian cancer at Yeditepe University hospital and had demographically identical characteristics were included. With their consent, DNA was isolated from the blood samples collected from the patients, and significant and nonsense mutations in the IDH1, IDH2, TERT, MGMT, and ATRX genes were detected using the Next Generation Sequencing method. A bioinformatics analysis study was carried out with the Illumina Data Analysis Program. 7 different variants of the TERT gene were observed in 24 to 33 patients. Mutations were seen in both exonic and intronic regions. In our study, in the TERT gene, heterozygous in 7 patients and homozygous in 1 patient, c.2850-3039C>T; (p.H950H, p.H1013H) mutation, heterozygous in 2 patients c.2517G>A;(p.T839T) mutation, heterozygous in 1 patient, c.2031C>T; (p.G677G) mutation, heterozygous in 3 patients c.835G>A (p.A279T) mutation, heterozygous in 1 patient c.1392C>T; (p.F464F) mutation, heterozygous in 1 patient c.2995-3184G>A; (p.A1062T, p.A999T) mutation and heterozygous in 16 patients and homozygous in 3 patients c.915G>A; (p.A305A) mutations have been detected. Among the 7 different variants seen in the study, variants of unknown clinical significance were detected. For the other genes included in our study,

5 variants in the ATRX gene, 3 variants in the IDH1 gene, 1 variant in the IDH2 gene, and 6 variants in the MGMT gene were detected. A c.699-3delC deletion was observed in one patient in the intronic region of the IDH1 gene, and the c.532G > A (p.V178I) mutation seen in 3 patients was evaluated as possibly harmful. In addition, *novel* variant c.881A > G ; c.995A > G was detected in the ATRX gene. The heterozygous novel variant detected in the ATRX gene was confirmed to be heterozygous by Sanger sequencing.The variants of TERT, IDH1, IDH2, MGMT, and ATRX genes we found in our study were evaluated by comparing them with existing databases and literature. The variants we found were not previously associated with ovarian cancer and were evaluated as a new marker candidate for ovarian cancer susceptibility. If our results are confirmed by future studies with larger cohorts or functional studies, our findings will contribute significantly to elucidating the molecular mechanisms of ovarian cancer.

Key Words: Over cancer, Tert, polymorphisms

ABSTRACT (Turkish)

Capar Gorali, B. Türk Popülasyonunda Over Kanseri Hastalarında TERT Geninin Yeni Nesil Dizileme Yöntemiyle Mutasyon Analizi. Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Moleküler Tıp Anabilim Dalı. Doktora Tezi. İstanbul, 2022

Yumurtalık kanseri, ülkemizde rahim ağzı ve rahim kanserinden sonra en sık görülen jinekolojik kanserdir. Hastalığın sıklığı, farklı ırk ve etnik gruplar arasında değişiklik göstermekle birlikte, gelişmiş ülkelerde, gelişmekte olan ülkelere göre daha yüksektir. Her yaştan kadını etkiler. Kötü huylu yumurtalık tümörlerinin büyük çoğunluğunu Epitelyal tümörler oluşturur. Çeşitli risk faktörlerinin yumurtalık kanseri riskinin artmasıyla ilişkili olduğu bilinmektedir. Bu risk faktörleri arasında özellikle genetik faktörler yumurtalık kanseri gelişme riskinin artmasından sorumludur. Mutasyon genetik materyaldeki kalıtsal değişikliklerdir ve kansere neden olabildiği için önemlidir. TERT, ATRX, MGMT, IDH genlerindeki mutasyonların birçoğu, birden çok kanser türü riski ile ilişkilendirilmektedir. Yeni Nesil Dizileme yaklaşımı, yumurtalık kanseri dahil olmak üzere kanser çalışmalarında kullanılarak, mevcut mutasyonların tanımlanmasının yanında, yeni keşiflerin de yapılmasını kolaylaştırmaktadır. Çalışmamızda Yeditepe Üniversitesi hastanesinde Over kanseri tanısı almış, demografik açıdan özdeş niteliklere sahip 18 – 76 yaş arası 33 kadın hasta dahil edilmiştir. Hastalardan rızaları dahilinde toplanan kan örneklerinden DNA izolasyonu yapılıp, Yeni Nesil Dizileme yöntemi kullanılarak IDH1, IDH2, TERT, MGMT ve ATRX genlerindeki anlamlı ve anlamsız mutasyonlar tespit edilmiştir. Biyoinformatik analiz çalışması Illumina Data Analysis Program ile gerçekleştirilmiştir. Toplam 33 hastanın 24'nde TERT genine ait 7 farklı varyant gözlenmiştir. Mutasyonlar hem ekzonik hem de intronik bölgelerde görülmüştür. Çalışmamızda TERT geninde, 7 hastada heterozigot ve 1 hastada homozigot c.2850-3039C>T; (p.H950H, p.H1013H) mutasyonu, 2 hastada heterozigot c.2517G>A; (p.T839T) mutasyonu, 1 hastada heterozigot, c.2031C>T; (p.G677G) mutasyonu, 3 hastada heterozigot c.835G>A (p.A279T) mutasyonu, 1 hastada heterozigot c.1392C>T; (p.F464F) mutasyonu, 1 hastada heterozigot c.2995-3184G>A; (p.A1062T, p.A999T) mutasyonu, 16 hastada heterozigot ve 3 hastada homozigot c.915G>A; (p.A305A) mutasyonları tespit edilmiştir. Çalışmada görülen 7 farklı varyant içerisinde klinik önemi bilinmeyen varyantlar saptanmıştır. Çalışmamıza dahil ettiğimiz diğer genler için ATRX geninde 5 varyant, IDH1 geninde 3 varyant, IDH2 geninde 1 varyant ve MGMT geninde

6 varyant tespit edilmiştir. IDH1 geninde intronik bölgede bir hastada c.699-3delC delesyon gözlenmiş olup, 3 hastada görülen c.532G>A (p.V178I) mutasyonun muhtemel zararlı olarak değerlendirilmiştir. Ayrıca ATRX geninde heterozigot *Novel* varyant; c.881A>G c.995A>G (p.D332G; p.D294G) tespit edilmiştir. ATRX geninde tespit edilen *Novel* varyant, Sanger dizileme ile heterozigot olarak doğrulanmıştır. Çalışmamızda bulduğumuz TERT,IDH1,IDH2,MGMT ve ATRX genlerine ait varyantlar mevcut veri tabanları ve literatürle karşılaştırılmıştır. Bulduğumuz varyantlar daha önce yumurtalık kanseriyle ilişkilendirilmemiş olup, yumurtalık kanserine yatkınlık için yeni bir belirteç adayı olarak değerlendirilmiştir. Sonuçlarımızın, ileride daha büyük kohortlar ile gerçekleştirilecek çalışmalar veya fonksiyonel çalışmalarla doğrulanması durumunda, bulgularımız, yumurtalık kanserinin moleküler mekanizmalarının aydınlatılmasına önemli katkı sağlayacaktır.

Anahtar Kelimeler: Over cancer, Tert, polymorphisms

1. INTRODUCTION AND PURPOSE

Cancer is a term that causes a number of diseases that affect various cell groups in the body (1). Ovarian cancer (OC) occupies the seventh place between malignant tumors and the eighth-place as a cause of death from cancer in women in the world. In addition, to be one of the most common gynecologic cancers, they have the highest mortality rate between them and occupy the third place in mortality, only after cervical and uterine cancer (2). Despite the developments in recent years, ovarian cancer remains one of the cancer types with the highest mortality rates among gynecological malignancies. Although the prevalence of ovarian cancer differs among different racial and ethnic groups, the incidence of ovarian cancer in developed countries is higher than in developing countries. Ovarian cancer's incidence and death rate increase with age. Most cases of ovarian cancer occur in women over the age of 50, but it can develop at any age, including infancy. (3). A number of risk factors are known to be associated with an increased risk of ovarian cancer. These risk factors are demographic, reproductive, gynecological, hormonal, genetic, and lifestyle-related (4). Non-genetic risk factors for epithelial ovarian cancer include growing age, postmenopausal hormone therapy (especially over five years), obesity or weight gain, and a family history of epithelial ovarian cancer. There is a history of ovarian cancer (5,6,7). Among the genetic factors associated with an increased risk of ovarian cancer, mutations are the most common. Mutations are inherited changes in genetic material and are important because they can cause cancer. Most mutations in TERT, ATRX, MGMT, and IDH genes are associated with an increased risk of multiple cancers. TERT is a single-copy gene with 16 exons and 15 introns found only in humans. The TERT gene, which makes the catalytic subunit of telomerase, controls how much telomerase is made. This helps keep telomere length stable by adding "TTAGG" repeats to the ends of the chromosomes.(8) Mutation in TERT reactivates telomerase; therefore, it increases the transition to the process of carcinogenesis. He associated single nucleotide polymorphisms (SNPs) in the TERT gene with telomere length and telomerase reactivation (9).

The IDH mutation is shown as a common mutation among primary and recurrent tumors. Cancer-associated IDH mutations block normal cellular differentiation and promote tumorigenesis through aberrant production of the oncometabolite 2-

HG(10). Tumor cells are often characterized by global changes in epigenetic patterns, and genes encoding epigenome regulators are frequently mutated in cancer. The ATRX gene, a component of the SWI/SNF chromatin remodeling complex, is repeatedly mutated in a number of human cancers (11). On the other hand, the MGMT gene is a central DNA repair mechanism that plays an important role in ameliorating DNA damage caused by alkylating agents and inhibiting human oncogenesis. MGMT is extensively methylated at the promoter region in various carcinomas, including ovarian cancer. It directly regulates the damage-reversal repair pathway, preventing DNA damage and potential cancer development (12). The Next Generation Sequencing approach can be used in other cancer studies, especially in ovarian cancer, making it easier to make new discoveries as well as identify existing mutations (13).

In our study, we aimed to identify significant and nonsense mutations in TERT, ATRX, MGMT, IDH1, and IDH2 genes in ovarian cancer patients by Next Generation Sequencing. We observed the effect of these mutations on ovarian cancer. Since TERT and other genes that we examined in ovarian cancer have many different functions, we anticipate that our study will add to the literature.

2. LITERATURE REVIEW

2.1.Cancer

Cells continue and terminate their lives with management and control mechanisms such as growth, division, and apoptosis. When these mechanisms are disabled, cancer is seen. Cancer is a complex disease that is under the influence of genetic and environmental conditions, including the entire process of uncontrolled cell proliferation, invasion of surrounding tissues, and metastasis to distant organs. Cancer is a group of more than 100 diseases involving different cell groups in the body (14).

Mutations that cause cancer transformation are classified as activation of proto-onco genes that trigger growth and cell proliferation and inactivation of tumor suppressor genes, DNA repair enzymes, and apoptosis (15). Oncogene activations can occur as a result of point mutations, chromosome translocations, and gene amplification. Activated oncogenes begin to encode proteins that trigger cell proliferation. The proteins in question are those that affect every stage, from the binding of growth factors to the receptors on the cell membrane to the transcription of genetic material (16). Tumor suppressor genes, DNA repair enzymes, and apoptosis inactivation occur as a result of physical and environmental factors, lifestyle factors such as dietary habits, and a risk of mutation on the genetic material. Mutations that occur are determined and neutralized by some genes and the proteins encoded by these genes. The task of these genes, which are responsible for the control of genetic material, is to eliminate mutations that may cause excessive and uncontrolled cell proliferation and to preserve the integrity of the genome (17). If the DNA damage is repairable, DNA repair enzymes are activated, the damaged part is removed and re-synthesized according to the correct sequence. If irreparable damage has occurred, cell division is canceled and its life is terminated by activating apoptosis (18). Tumor cells develop mechanisms to escape apoptosis. They keep living and growing even though they have changed their DNA (19).

2.1.1.Ovarian cancer

Globally, ovarian cancer affects 239.000 patients and causes 152.000 deaths every year. Despite the developments in recent years, ovarian cancer is still a cancer type with a high mortality rate among gynecological cancers (20).

2.1.2.Ovarian cancer epidemiology

Ovarian cancer is one of the gynecological cancers seen in women and has a high mortality rate. It is the third deadliest after cervical and uterine cancer (21). Globally, 225,500 new cases of ovarian cancer are diagnosed each year, resulting in 140,200 cancer-specific deaths (22). Although the incidence of ovarian cancer varies among different racial and ethnic groups, it is higher in developed countries than in developing countries. It affects women of all ages (23). Ovarian cancer incidence and death rates increase with age. Most cases of ovarian cancer occur in women over the age of 50, but it can be diagnosed at any age, including infancy. Compared with other cancers, ovarian cancer does not account for a significant number of deaths compared with lung cancer, colorectum cancer, or stomach cancer (Table 1) (24).

Table 1: Ovarian Cancer Incidence and Mortality Rates Information

Age-Related Incidence and Mortality of Ovarian Cancer*

AGE	INCİDENCE	MORTALİTY
Younger than 20 years	0.7	0.04
20 to 49 years	6.6	2.0
50 to 64 years	26.9	16.0
65 to 74 years	48.6	36.1
75 years and older	55.6	55.2

2.1.3.Ovarian Cancer Risk Factors

Various risk factors have been identified, and it is recognized that these factors are linked with an increased incidence of ovarian cancer. These risk factors can be divided into demographic, reproductive, gynecological, hormonal, genetic and lifestyle (Table 2) (25).

Table 2: Correlations with Ovarian Cancer Development.

Factors		Protective	Predisposing	Controversial
Demographic	Age		✓	
Reproductive	Menstrual-related factors		✓	
	Age of menarche and menopause			✓
	Parity	✓		
	Pregnancy characteristics			✓
	Higher age of childbirth	✓		
Gynecologic	Pelvic inflammatory disease			✓
	Endometriosis		✓	
Hormonal	Contraceptive methods	✓		
	Hormone Replacement Therapy (HRT)			✓
	Infertility treatments			✓
Genetic	Family history		✓	
	BRCA mutations		✓	
	Lynch syndrome		✓	
Lifestyle	Nutrition and Diet			✓
	Obesity and physical activity			✓
	Alcohol, caffeine and cigarettes			✓
Other	Lactation	✓		
	Lower socioeconomic status		✓	

2.1.3.1.Genetic Factors:

Many genetic variables are linked to an increased risk of ovarian cancer development. Inheritance of a genetic abnormality from one's parents or grandparents is the most potent known cause of ovarian cancer (Table 3) (4,26). The BRCA1 and BRCA2 mutations are the most common genetic risk factors for ovarian cancer, and they are present in 17% of patients (27). In addition to BRCA1 and BRCA2, additional germline mutations in DNA repair genes, such as RAD51C, RAD51D, BRIP1, BARD1, and PALB2, may increase the chance of developing ovarian cancer. These include the CHEK2, MRE11A, ATM, RAD50, and TP53 (28).

Ovarian cancer risk may also be increased by inherited abnormalities in other genes that play a role in DNA repair (29). Ovarian cancer is more common in women who have certain genetic abnormalities. These malignancies are more likely to occur in women with hereditary breast and ovarian syndrome. Ovarian cancer risk may be increased by other genetic illnesses, such as Lynch syndrome. Besides colorectal, endometrial, and ovarian cancers, Lynch syndrome has been linked to malignancies of the urinary system, stomach, small intestines, and biliary tract (29,30).

Table 3: Functions of Commonly Mutated Inherited Genes Associated with Increased Risk of Ovarian Cancer (1)

<table>
<tr><th>Gene</th><th>Protein</th><th>Protein function</th></tr>
<tr><td>BRCA1</td><td>Breast cancer type 1 susceptibility protein</td><td rowspan="2">• Crucially involved in the repair of double-strand breaks by homologous recombination
• Serves as a scaffold for other proteins involved in double-strand DNA repair, mostly through defective homologous recombination
• Stabilizes RAD51-ssDNA complexes</td></tr>
<tr><td>BRCA2</td><td>Breast cancer type 2 susceptibility protein</td></tr>
<tr><td>BARD1</td><td>BRCA1-associated RING domain protein 1</td><td>• Forms a heterodimer with BRCA1
• The BRCA1-BARD1 complex is essential for mutual stability</td></tr>
<tr><td>BRIP1</td><td>BRCA1-interacting protein 1 (also known as Fanconi</td><td>• Binds to BRCA1
• The BRCA1-BRIP1 complex is required</td></tr>
<tr><td></td><td>anaemia group J protein)</td><td>• for S phase checkpoint activation</td></tr>
<tr><td>PALB2</td><td>Partner and localizer of BRCA2</td><td>• A bridging protein that connects BRCA1 and BRCA2 at sites of DNA damage
• Helps load RAD51 onto ssDNA</td></tr>
</table>

Table 3: Functions of Commonly Mutated Inherited Genes Associated with Increased Risk of Ovarian Cancer (2)

<table>
<tr><td>RAD51C</td><td>DNA repair protein RAD51 homologue 3</td><td rowspan="2">• Strand exchange proteins that bind to ssDNA breaks to form nucleoprotein filaments and initiate DNA repair</td></tr>
<tr><td>RAD51D</td><td>DNA repair protein RAD51 homologue 4</td></tr>
<tr><td>MSH2</td><td>MutS protein homologue 2</td><td rowspan="4">• Mismatch repair proteins that recognize and repair base-pairing errors occurring during DNA replication
• Mutations in mismatch repair genes are associated with Lynch syndrome</td></tr>
<tr><td>MLH1</td><td>MutL protein homologue 1</td></tr>
<tr><td>MSH6</td><td>MutS protein homologue 6</td></tr>
<tr><td>PMS2</td><td>Mismatch repair endonuclease PMS2</td></tr>
</table>

2.1.3.2.Hormone Replacement Therapy (HRT)

Hormone replacement therapy has been shown to increase the risk of developing ovarian cancer in postmenopausal women; Estrogen-only therapy increased the risk of ovarian cancer by 22%, while combined estrogen and progesterone therapy increased the risk by 10% (31,32).

2.1.3.3.Others Factors

Most women with ovarian cancer don't have a relevant family history since only 10% to 12% of cases have a hereditary foundation. Age, postmenopausal hormone treatment (particularly over five years), and obesity or weight increase are all known risk factors for epithelial ovarian malignancies (33).

Overweight postmenopausal women may have an increased chance of developing ovarian cancer, as shown by a meta-analysis in which women who gained

less than 5 kg on hormone replacement treatment were found to have an elevated ovarian cancer risk (34).

Dietary variables are linked to an increased risk of ovarian cancer in the general population, according to a number of studies Even while one research found an inverse link between skim milk and lactose intake and the chance of having ovarian cancer in maturity, the overall risk of acquiring ovarian cancer is low. Lactose consumption was shown to have an inverse connection to the risk of endometrial cancer in this investigation, as well (35).

For women with chronic depression, defined as meeting the current and previous surveys' definitions of depression, there is an increased risk of ovarian cancer, according to data from the Nurses' Health Study (NHS) (36,37).

2.1.4.Classification of Ovarian Tumors

2014 World Health Organization (WHO), according to tumor morphology, ovarian tumors; It is divided into 3 categories as Epithelial (60%), Germ Cell (30%) and Sex-Cord Stromal tumors (8%). However, the vast majority of malignant ovarian tumors (80-85%) are classified as epithelial tumors (38).

2.1.4.1.Epithelial Ovarian Cancer

Epithelial ovarian cancer includes a heterogeneous group of tumors. The World Health Organization (WHO) classifies epithelial ovarian carcinomas (EOC) into several morphological categories based on cell type. The four most common subtypes are serous, endometrioid, clear cell, and mucinous carcinoma. Less common are transitional cell carcinoma and malignant Brenner tumor carcinoma. These subtypes differ in etiology, morphology, molecular biology, and prognosis, but are treated as a single entity (39,40).

2.1.4.1.1.Serous Carcinoma

Serous carcinoma (SC) accounts for 75-80% of epithelial carcinomas and is subdivided into High-grade serous carcinoma (HGSC) and Low-grade serous carcinoma

(LGSC). Histologically, HGSC and LGSC show some phenotypic similarities but evolve from different molecular pathways (Figure 1) (Figure 2) (41,42,43).

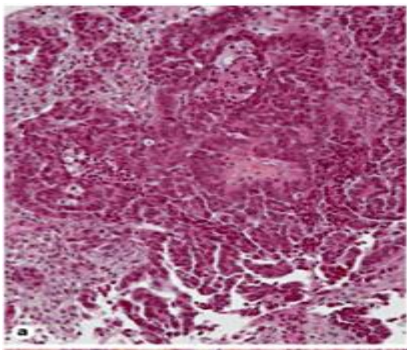


Figure 1: High-grade serous carcinoma (41).

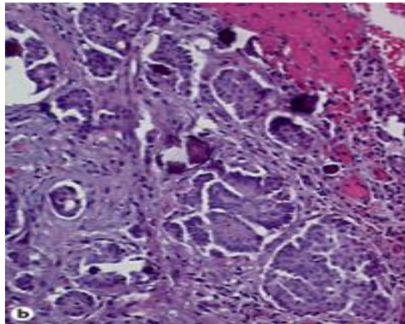


Figure 2: Low grade serous carcinoma (41).

High-grade serous carcinoma accounts for 85-90% of serous carcinomas. Homologous recombinations are defective in approximately 50% of high-grade serous carcinomas. Low-grade serous carcinomas are rare, representing 10-15% of serous carcinomas and 2% of all ovarian carcinomas (44,45).

2.1.4.1.2.Mucinous Carcinoma

Musinous Carcinoma (MC), previously called intestinal type carcinoma, is rare and accounts for only 2-3% of ovarian carcinomas. Morphologically, they consist of cysts and glands of varying sizes with a unified pattern and successive glands. The cells are long, columnar and stratified with a large mucin-containing cytoplasm (Figure 3) (45,46,47).

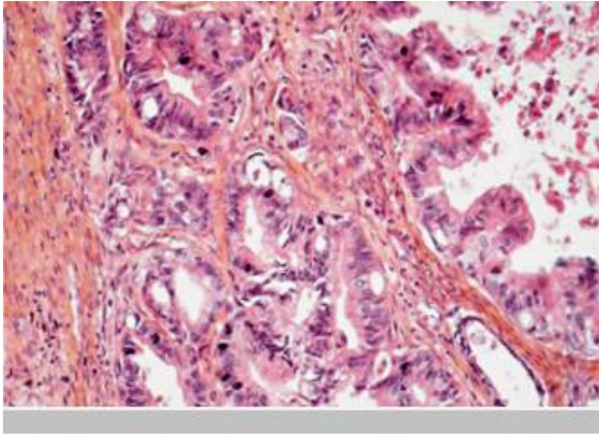


Figure 3: Mucinous carcinoma (45).

2.1.4.1.3.Endometrioid Carcinoma

Endometrioid Carcinoma (EC) accounts for 10% of all ovarian carcinomas and are usually unilateral solid masses. These tumors are low grade and associated with a good prognosis. They consist of glands that histologically resemble endometrial epithelium (Figure 4) (48,49).

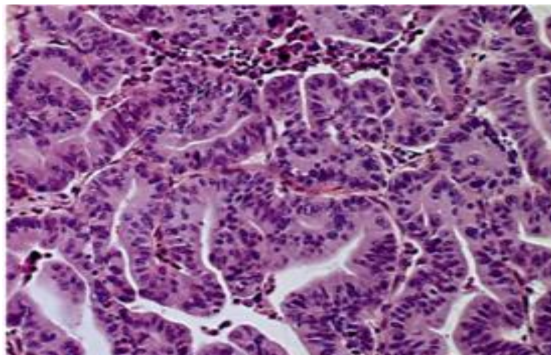


Figure 4: Endometrioid carcinoma (48).

2.1.4.1.4.Clear cell (mesonephroid) carcinoma

Clear cell (mesoneproid) carcinoma has been defined as a new subtype of Endometrioid Carcinoma (50). Clear cell (mesoneproid) carcinomas are rare and constitute 3.7-12.1% of all Endometrioid Carcinomas. Some studies have shown that Clear cell (mesoneproid) carcinoma has the worst prognosis of all Endometrioid

carcinomas (Figure 5) (51). Clear cell (mesoneproid) carcinoma is found at a younger age than serous carcinomas and has a clear association with endometriosis (52,53,54). Histologically, Clear cell (mezonephroid) carcinoma consists of large, cubic-shaped, pointed or flattened clear cells loaded with glycogen (55).

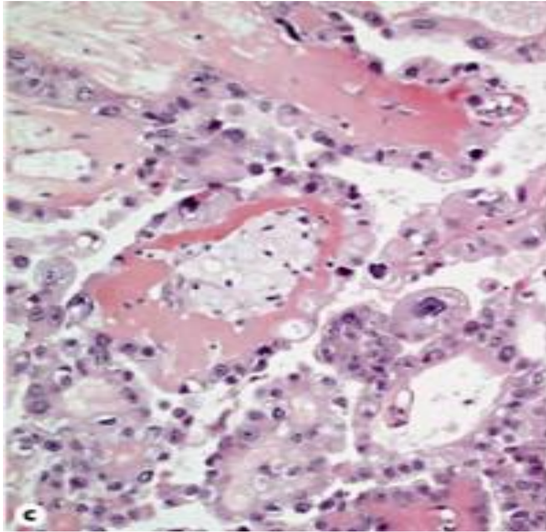


Figure 5: Clear cell carcinomas (51).

2.1.4.1.5.Brenner's tumor

Brenner tumors arise from the ovarian epithelium, accounting for approximately 5% of benign ovarian epithelial tumors. World Health Organization (WHO) Brenner tumors; benign, borderline and malignant divided into 3 classes (56,57). Most of these tumors (95%) are seen as benign, 5% of them are borderline and less than 1% are malignant (Figure 6) (58,59,60).

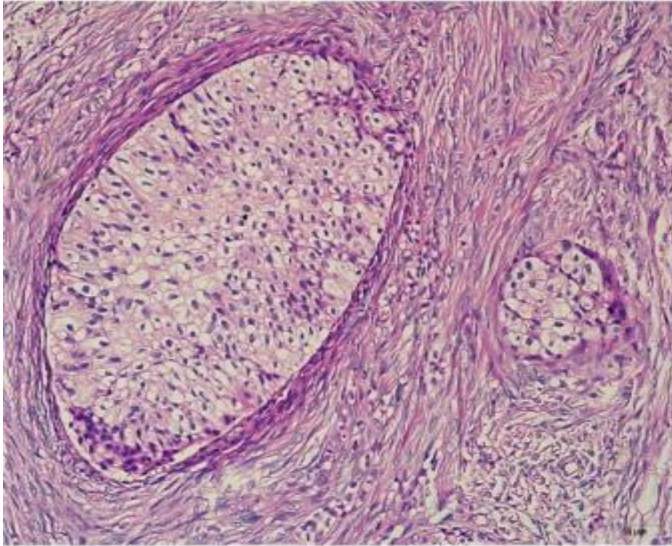


Figure 6: Brenner's tumor consisting of transitional epithelial cells arrayed as well-demarcated solid islands in the fibrous stroma (58).

2.1.4.2.Germ Cell Tumors

Ovarian germ cell tumors constitute approximately 15-20% of all ovarian tumors. They are more common in young women, adolescents, and young adults. (61,62). Malignant germ cell tumors account for 90%t of all cases (63).

2.1.4.3.Sex Cord Stromal Tumors

Sex Cord Stromal Tumors (SCST) consists of two types: those arising from sex cord cells and those arising from cells that surround and support the gonads, such as granulosa and theca cells and fibroblasts. SCSTs are very rare and mostly affect women aged 40 and 50 (64,65). More than 70% of all female cord-stromal cancers in most series were granulosa cell tumors, the most common subtype (66,67). The second most common type of tumor is called a Sertoli-Leydig tumor. Although rare in adolescent granulosa cell carcinoma, it has also been seen in 50% of granulosa theca cell carcinomas. In addition, adult granulosa cell cancers are often associated with TERT mutations. A mutation in the TERT promoter (TERT c.-124C>T) observed in up to 40% of patients was associated with more aggressive disease and shorter overall survival. (68).

2.2.Structure of Telomeres

Eukaryotic chromosomes are linear and terminate in nucleoprotein complexes called telomeres. Telomeres are nucleoprotein structures located at the end of the each chromosome arm and are responsible for maintaining genome stability (69). Telomere DNA consists of simple tandem repeats rich in CA and GT. Although the structure of the telomere is basically the same in different organisms, there are differences in length and repeat sequences. Telomere-Associated Proteins are different proteins that function by binding to the double and single branches of telomeres. Some of these proteins protect the telomere from degradation by wrapping the telomere single and double branch ends and aiding in t-loop formation. Some of them control telomere length by ensuring that the telomerase enzyme binds to the telomere. Although the structure and functions of all these proteins are basically the same, they differ in different species (70). These proteins can be investigated as single and double-branched proteins. Shelterin complex proteins, among others, are structured into a looping structure known as the T-ring. The T-ring is connected to particular proteins, including the proteins that make up the Shelterin complex. The looping structure is created by nucleolytic activity at the extremities of telomeric DNA, which results in the formation of a single-stranded G-rich overhang (Figure 7) (71,72,73). That means that the nucleoprotein structure turns in the opposite direction and moves into the double-stranded telomere tube. This allows free DNA ends to be housed inside the structure of the nucleoprotein (74).

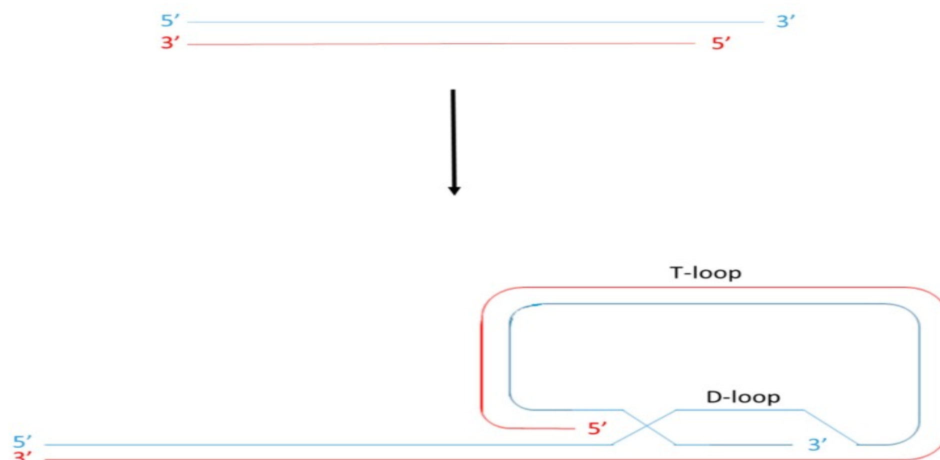


Figure 7: The telomere structure of T loop and D loop (71).

Interactions between members of the shelterin complex and the DNA sequence of the telomere stabilize telomere structure and regulate access to proteins involved in DNA repair and elongation. (75,76). Besides having formed a cover at the chromosome ends, this specialized nucleoprotein structure serves two primary functions: First, it protects the ends of the chromosome arms from unsuitable DNA repair mechanisms, which would otherwise identify loose DNA strands as double-strand breaks. Second, this envelope structure prevents the degradation of genes near the ends of chromosome arms caused by insufficient DNA replication (77).

2.3.Functions of Telomeres

Telomeres are involved in completing the last part of the linear chromosomal DNA molecule during replication. It protects the last part of the chromosome against abnormal conditions such as recombination, destruction, and fusion. It ensures the integrity and stability of chromosomes. It allows the chromosomes to hold a certain position by attaching to the nuclear membrane (78). In the absence of functional telomeres, the freed DNA ends cannot be stable, and random repair of DNA breaks results in impaired cellular functions. Broken chromosomes are cut by nucleases and their ends are randomly fused. (78,79).

2.3.1.Telomerase

Telomerase is a ribonucleoprotein reverse transcriptase and a large enzyme complex that catalyzes the addition of telomeric repeats to the telomeres of eukaryotic chromosomes (80,81).

Telomerase activity is required for the maintenance of telomeres. This is because DNA polymerases cannot fully replicate straight DNA ends. Telomerase activity is generally suppressed in normal somatic cells, except in some self-renewing tissues with high reproliferation potential. So, telomerase activity is very important to keep the length of telomeres and the growth of cancer cells (82).

2.3.2.Telomerase Components

Telomerase consists minimally of telomerase reverse transcriptase (TERT) and telomerase RNA (TR) (83).

Telomerase RNA is TERC

Telomerase proteins (TERT)

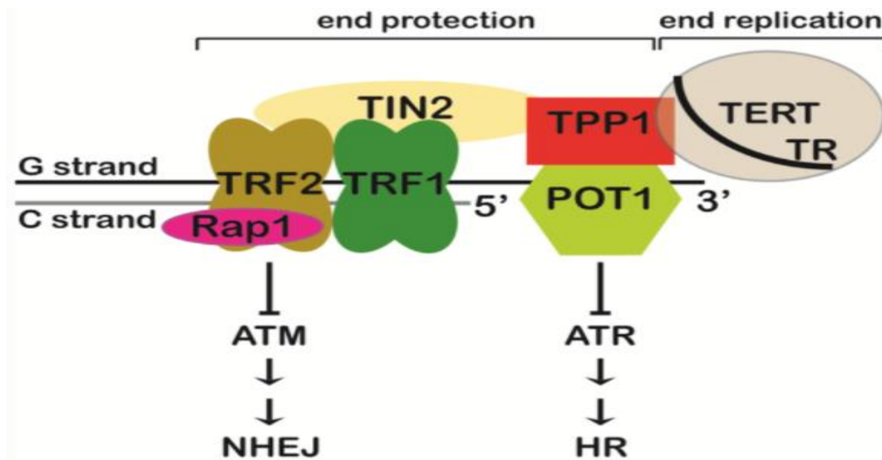
2.3.2.1.Telomerase RNA (TERC)

The activity of telomerase depends on the RNA subunit in its structure. All of these RNAs contain a sequence that extends on the palm of the protein portion and is complementary to approximately 1.5 telomere repeats. This sequence is 5'UAACCUAA3' in humans (84).

2.3.2.2.Other Telomerase-Associated Proteins

Several accessory proteins associated with the active telomerase RNP complex have been identified. The molecular chaperones p23 and Hsp90 bind to human TERT (hTERT), and chemical inhibition of Hsp90 reduces telomerase activity (113). These two adenosine triphosphatase enzymes, Reptin and Pontin, help bind human TR and hTERT to the catalytically active telomerase enzyme. With pontine degradation (KD), telomerase activity and hTR levels decrease (86).

Telomerase, an enzyme specifically designed to prevent DNA degradation, creates new telomeric repeats at the ends of human chromosomes (Figure 8) (87). Catalytic reverse transcriptase domains are found in TERT, the telomerase protein component used to synthesize new DNA (88,89). The telomerase RNA component (TERC or TR) containing the telomeric reslicing template is a subunit of telomerase. (90, 91).



. **Figure 8:** Schematic showing the composition of complexes involved in chromosome end protection and end replication (87).

2.3.2.3.Telomerase Reverse Transcriptase

The second major component of telomerase RNP is the reverse transcriptase TERT. There are four conserved domains in TERT, which are as follows: the telomerase key N-terminal domain (TEN), the TERT RNA-binding domain (TRBD), the reverse transcriptase domain (RT), and the thumb domain, also known as the C-terminal extension (CTE) (Figure 9) (92,93).

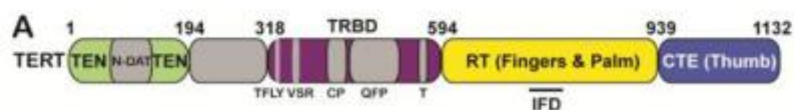


Figure 9: Crystal structures of telomerase domains and related interfaces (92).

In the model proposed regarding the structure of TERT, which constitutes the protein part of the telomerase enzyme; TERT is likened to a half-open right hand. Therefore, the protein consists of the thumb-palm-other fingers parts. The part of the RNA carried by the enzyme complex, which is complementary to the 1.5 telomeric repeats in the palm, is involved in the elongation of the G3' end of the DNA (94). In human cells without telomerase activity in in vitro studies, expression of the hTERT protein enabled telomerase to become active again (95).

2.4.hTERT Regulation in Healthy and Cancerous Cells

TERT Expression and Regulation in Normal Human Cells Comprehensive knowledge of the control mechanism for TERT transcription in a physiological setting greatly aids in a better understanding of TERT gene repression during oncogenesis. Telomerase is silenced as a consequence of transcriptional repression of the TERT gene in the majority of human differentiated cells (8,9). Cells with strong proliferative capacity, such as the stem cells and activated lymphocytes, express TERT (1,8,96). Transcriptional factors from the MYC network and WNT/-catenin signaling have been implicated in activating the TERT gene and telomerase in these cells (97,98). Some cells that express the estrogen receptor, such as lymphocytes, endometrial epithelial cells, and vascular endothelial cells, have estrogen response elements in their TERT promoters. Cells might not grow or change the way they should after TERT induction. This could make an organ or tissue ineffective(99,100).

Normal cells' telomerase activity is suppressed by the transcriptional control of the TERT gene. To activate telomerase during oncogenesis, it is required to induce TERT expression by inhibiting this gene. Oncogenic signal acquisition and/or loss, tumor virus-derived exogenous oncoproteins, epigenetics and/or tumor suppression function loss are a few of the methods used to boost malignant cell TERT transcription and expression. It has now been shown that a wide range of methods that change or modify genetic material are used (101,102). Figure 10 shows these methods (101).

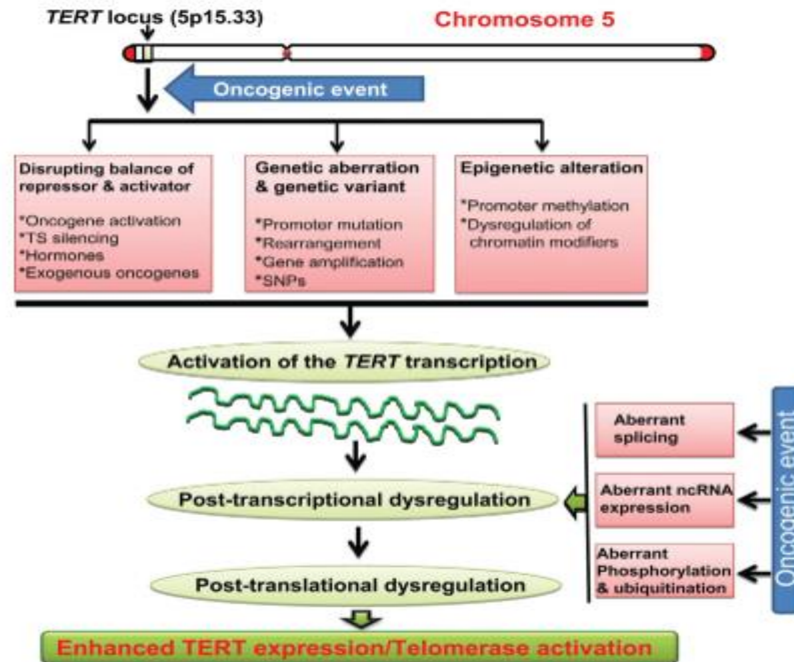


Figure 10: The activation of the TERT gene transcription and telomerase mediated by oncogenic events during oncogenesis (101).

Inhibition of cancer-specific TERT induction has been demonstrated to be mediated by recurrent tumor-specific TERT promoter mutations, localized TERT locus rearrangements/amplification, and oncoviral DNA insertion, among other factors (10). In addition, specific Single Nucleotide Polymorphisms (SNPs) in the TERT gene are associated with the regulation of TERT transcription and telomere length, thereby influencing cancer susceptibility or initiation, as well as cancer growth and metastasis. (11,12).

2.4.1.TERT Promoter Mutations

According to an analysis of all TCGA cohorts with available mutation data, the most common noncoding regulatory region mutations in cancer, C228T and C250T, were discovered in 27% of tumors. The highest mutation rates are found in malignant melanoma, glioblastoma, urothelial bladder cancer, myxoid liposarcoma, different

forms of skin cancer, and medulloblastoma. (103.104). Thyroid cancer, hepatocellular carcinoma (HCC), head and neck cancer, and ovarian clear cell carcinoma, among many others, have mutation rates range from 15% to 50%. Lung, breast, stomach, prostate, and kidney cancers, as well as blood and bone marrow malignancies, have mutation rates of less than 10% (105,106).

TERT and telomerase activity tend to be elevated in primary tumors with TERT promoter mutations. As a result of the de novo ETS transcription factor binding motif generated by the C228T or C250T mutation, upregulation of TERT is induced (107).

2.5. ATP-dependent helicase (ATRX) Gene

ATRX is a member of the key and sucrose nonfermentable (SWI-SNF) protein family. SWI-SNF proteins are involved in numerous biological processes, such as DNA recombination and repair, transcriptional regulation, and nucleosome modification. The ATRX and DAXX (death domain associated protein) genes encode proteins that interact to produce a histone chaperone complex essential for the accumulation of histone variant H3.3 at telomeres and pericentric heterochromatin areas. This protein regulates chromatin remodeling and transcriptional control. It is necessary for the effective replication of a subset of genomic loci and enhances DNA replication in many cell types. (108, 109, 110)

ATRX gene expression has been detected in at least 15 types of cancer, including neuroblastoma, glioma, pancreatic neuroendocrine tumors, and pediatric osteosarcoma, and has recently emerged as a crucial marker for molecular World Health Organization (WHO) classification of nervous system tumors, such as central cancer glioma. (111,112,113).

2.6. O6-Methylguanine-DNA Methyltransferase (MGMT) Gene

MGMT is a protein with the remarkable ability to repair DNA adducts stoichiometrically and to deactivate itself. It is located at 10q26 on chromosome 10 and consists of 5 exons and 4 introns spanning more than 300 kb. Its MGMT protein is comprised of 207 amino acids and has been conserved throughout evolution (114).

The MGMT gene is a crucial tumor suppressor gene, and abnormal promoter methylation has been observed in a variety of malignancies. Multiple investigations have established that MGMT levels are higher in tumors than in normal tissue. The expression of MGMT is extremely diverse, not just amongst individuals but also in tumor tissues. MGMT expression silencing has been identified in a range of tumor types (115). In contrast, elevated MGMT levels have been seen in colorectal, pancreatic, breast, and lung carcinomas, non-lymphoma Hodgkin's and myeloma, and gliomas. The promoter region of MGMT is frequently methylated in numerous carcinomas, including ovarian cancer (116).

2.7.Isocitrate dehydrogenase (IDH)

Isocitrate dehydrogenase (IDH) genes are mutated in a wide variety of tumor forms, including glioma, chondrogenic tumors, leukemia, and other proliferative bone marrow malignancies. IDH1 and IDH2 mutations are seen in more than 70% of low-grade gliomas and leukemias (levels II and III) (117,118). The most significant genetic alteration in glioma is the IDH mutation. The mutation occurs in a single amino acid's isocitric acid binding site. Clinical investigations have demonstrated that IDH1 mutations are related with a younger age, secondary GBMs, glioma, and secondary glioblastoma, and play a critical role in the creation, development, and evolution of glioma (119,120).

3.MATERİALS AND METHODS

3.1.The Study Population and Protocol

This study was carried out at Yeditepe University Faculty of Medicine, Department of Molecular Medicine. Our study, which was found ethically appropriate with the decision number 1431 at the meeting of the Yeditepe University Faculty of Medicine Clinical Research Ethics Committee dated April 21, 2021, was carried out with the voluntary participation of the patients.

The samples included in the study consisted of the ovarian cancer patient group (n = 33). The patient group in the study consisted of patients diagnosed with ovarian cancer who applied to Yeditepe University Hospital, Department of Obstetrics and Gynecology. Clinical evaluations and sampling of ovarian cancer patients included in the study were performed by the relevant clinic. Blood samples from selected cases were taken into separate purple-capped ethylene diamine tetraacetic acid EDTA tubes. This is how we got DNA samples from blood leukocytes: We put the leukocytes in EDTA tubes and used an iPrep TM Purification Instrument from Invitrogen and Thermo Fisher Scientific.

3.1.1. Genomic DNA Isolation from Blood

Peripheral venous blood samples taken from the patient in 5 ml EDTA tubes were kept in a +4 C° refrigerator until DNA isolation. DNA isolation of the samples was performed using a DNA isolation robot (iPrep TM Purification Instrument, Invitrogen, Thermo Fisher Scientific Inc.) and an iPrep genomic DNA isolation kit from blood (iPrepTM PureLinkTM gDNA Blood Kit). This system is capable of isolating DNA from 13 samples in one run, using 350µl of peripheral blood for each sample. The procedure in the kit was performed in accordance with the Invitrogen iPrep Isolator. The iPrepTM Isolator uses ChargeSwitch® Technology (CST®).This system is a magnetic bead-based technology that depends on the surface charge that can be changed by the pH of the buffer in the environment. Cartridges are shaken for a while in order for the magnetic beads to bind to DNA in the most effective and efficient way. From the individuals included in the study, 350 µl of peripheral blood collected in EDTA tubes is withdrawn, transferred to 1.5 ml eppendorf tubes, and placed in the sample section of the iPrep isolation cartridge. In order to put the diluted DNA into the reaction, it is

placed in the eppendorf elution section of 1.5 ml. The pipette tips necessary for pipetting during isolation are placed in their special compartment. In the low pH condition, CST beads have a positive charge that binds to the negatively charged nucleic acid backbone.

Therefore, proteins and other contaminants cannot bind and are washed with liquid wash buffer. To clean nucleic acids, the charge of the bead surface is neutralized by raising the pH to 8.5 using a low-salt wash buffer (elution). The isolated nucleic acid is immediately transferred to the wash buffer and is ready for use in applications. The DNA isolation process was carried out within 45 minutes in this closed system, and approximately 150 µl of DNA was obtained at the end of this process. Finally, the purified and diluted DNA samples were placed in Eppendorf tubes and refrigerated at +4 C.

3.1.2. DNA Purity and Concentration Measurements

Determination of the Concentration and Quality of the Obtained DNA Spectrophotometer (NanoDrop 2000, Thermo Scientific Inc) device was used to determine the concentrations and quality of the DNA samples obtained. This instrument measures high concentrations (2 ng/µL – 15000 ng/µL dsDNA) without the need for dilution in less than 5 seconds by simply pipetting the sample. It is assumed that the double-stranded DNA content of 50 µg/ml gives an optical density (OD) of 1 at a wavelength of 260 nm.

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

Qiaseq DNA targeted libraries using Illumina NGS systems need a specific primer sequence. The panel was built for genes and areas of interest where 10-40 ng Fresh DNA may be used to enhance the sample.

This method goes through four stages:

1. Fragmentation: A regulated multi-enzyme process fragments the genomic DNA sample, end-repair is performed, and an A-tail is introduced. The sequence platform

connects ready-to-use DNA fragments with adapters that can be used at each of the five ends of the fragment (such as the UMI and sample index).

2.UMI assignment: Before the target enrichment and library construction steps, each DNA molecule is given a unique sequence or index known as the UMI. In order to complete this task, a segment of DNA was ligated to a 12-base random sequence adaptor . As a result of this procedure, each adaptor has a total of 412 unique marks, and each DNA molecule in the sample has its own UMI sequence. In addition, the attached adapter has the first sample index.

3.Target enrichment and final library creation: DNA molecules containing UMI are properly enriched during the target enrichment step, which is followed by the production of the final library. Targeted PCR is used to enrich UMI-linked DNA molecules using an adaptor complementer 1 primer and a universal primer.

The final step is to employ Universal PCR for library expansion and the addition of platform-specific adaptor sequences and additional sample indices (Figure 11).

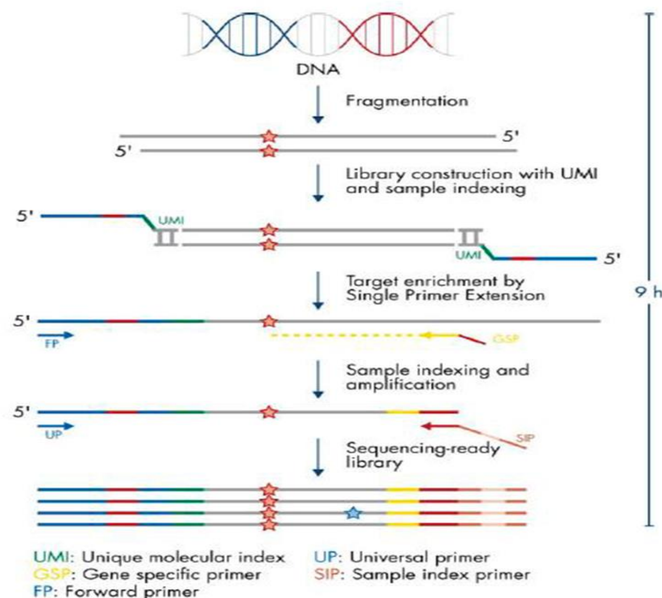


Figure 11: Qiaseq Targeted DNA panel procedure schematic summary.

3.1.3.1. Fragmentation, End-Repair, and Addition:

Fragmentation, end-repair, and A-addition mixing, the Thermocycler was preheated to 4 degrees, and the following mix was prepared according to the table below and pipetted 7-8 times. There was a brief centrifugation. Ice blocks were being worked on.2.5 µl of Fragmentation enzyme Mix was added to each reaction and mixed by pipetting 7-8 times. Care was taken to ensure that the tubes were on the cold block during the reaction setup. They were organized according to the following PCR program. The PCR program was started, and paused when it reached 4 C°.The device was loaded with samples, and the application was started. The samples were taken on the ice after the program was completed and the block had reached 4 C°. The adapter ligation phase was started immediately (Table 4).

Table 4: Fragmentation, End-Repair, and Addition

	Standard DNA, FFPE DNA, cfDNA
Contents	Volume **(µL)**
DNA	Various
Fragmantation Buffer, 10x	2,5
Nuclease- Free Water	Various
FERA solution	0,75
TOTAL	20

3.1.3.2.Adapter Ligation of Samples:

The reaction mixture was prepared in accordance with the following table. All of the ingredients were well blended using a soft-tip pipette applied 10-12 times. Despite its viscosity, the ligation solution was added to each subsequent reaction following the one that did not contain it. A scraper was used to remove any remaining ligation solution from the pipette tip. Thermocycler was set to 20 C°.and incubated for 15 minutes. After the reaction was finished, it was placed on ice and the QIAseq Beads cleaning process began (Table 5).

Table 5. Adapter Ligation of Samples

	Standard DNA	**FFPE DNA**	**cfDNA**
Content	Volume **(µL)**	Volume **(µL)**	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	50	50

3.1.3.3. Clearance of Adapter-bound DNA with QIAseq Beads:

The ligation process resulted in the transfer of a 50 µl DNA sample to a 1.5 ml LoBind tube. The volume was increased from 50 ml to 100 ml by adding nuclease-free water. The DNA study used 100 l of QIAseq beads. Pipetting the mixture several times ensured that it was thoroughly blended. After 5 minutes of incubation at room temperature, it was removed and examined. Beads were removed from the supernatant after 10 minutes on a magnetic rack. The supernatant was carefully removed and discarded after the solution became clear. Target DNA beads were avoided. Then the

magnetic rack was filled with 200 µl of freshly produced ethanol with 80% ethanol concentration. The beads were cleaned by turning the tube in the sink. The supernatant (extracellular matrix) was carefully removed and discarded. Ether alcohol was washed several times. At this point ETOH should be removed from the equation. The remaining liquid was drained with a 200 µl pipette and the remaining liquid with a 10 µl pipette. After removing the ethanol from the magnetic rack, the beads were allowed to dry completely for 10 minutes. DNA was eluted into target beads outside the magnetic rack using 52 µl of nuclease-free water. The solution was re-pipetted and placed back on the magnetic rack until clear. PCR plate wells or LoBind tubes with a capacity of 1.5 ml 50 µl of the supernatant was collected. Quantities of QiAseq beads were used: 70 µl for DNA was added to a volume of DNA solution 50 µl by volume. The mixture was thoroughly mixed by repeatedly pipetting. It was kept at room temperature for 5 minutes before being tested. Beads were removed from the supernatant after 5 minutes on a magnetic rack. After the solution was crystal clear, the supernatant was carefully removed and discarded. The magnetic rack contained 200 µl of freshly produced 80% ethanol. The beads were washed by rotating the tube or shifting the plate. The supernatant (extracellular matrix) was carefully removed and discarded. Ethanol washing step was repeated once more. To complete the ethanol extraction, a 200 µl pipette tip was used first, followed by a 10 µl tip. On the magnetic rack, the beads were dried for 15 minutes. Make sure the beads are completely dry before elution. If ethanol is present, the efficiency of the Target Enrichment PCR will be reduced. The DNA target beads were eluted with 12 µl of nuclease-free water after being removed from the magnetic rack. To ensure that everything was well combined, pipetting was used. The solution was left on the rack until it turned clear, at which point it was discarded. The target enrichment PCR setup had to be started by adding 9.4 µl of supernatant from the PCR tube or well to an empty PCR tube or plate well.

3.1.3.4.Target enrichment PCR:

The reaction was prepared according to the following table. After a brief centrifugation, it was pipetted 7-8 times and centrifuged again (Table 6).

Table 6. 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

3.1.3.5.Clearance of the target enrichment PCR with QIAseq Beads:

PCR product was transferred to a 1.5ml LoBind tube at a volume of 20µl. 80 µl of nuclease-free water was added to bring the total volume to 100 µl. A total of 70 µl of nuclease-free water was added to the 20 µl of sample for the DNA sample, and the final volume was 90 µl. PCR solution was diluted to 100µl with 100µl of QIAseq Beads (1.0x volume). The mixture was well blended by repeatedly pipetting it. We let it sit for 5 minutes at room temperature before analyzing it. Using the magnetic rack, we collected a sample and let it sit for around 5 minutes while the beads separated from the supernatant. After the solution had become crystal clear, the supernatant was carefully removed and discarded. 200 mL of newly made 80% ethanol were added (while still on the magnetic rack). Turning the tube or sliding the plate was used to wash the beads. The extracellular matrix (supernatant) was carefully removed and discarded. The washing step with ethanol was done a further time. The ethanol was completely extracted by first using a 200µl pipette tip and then a 10µl pipette tip. Ten minutes of drying time was given to the beads while they were still on the magnetic rack. A total of 16µl of nuclease-free water was used to elute the DNA beads. Pipetting was used to

ensure that everything was well combined. Once the solution became clear, it was removed from the rack and discarded. The Universal PCR amplification setup was started using 13.4 µl of supernatant in a clean PCR tube.

3.1.3.6.Universal PCR Amplification:

The universal PCR primers were designed using the table below. It was centrifuged briefly and pipetted 7-8 times (Table 7).

Table 7. Universal PCR Amplification

GRADIENT	Volume (µl)
Enriched DNA	13.4
UPCR Buffer, 5x	4
Hot Start Taq DNA polimeraz	1
Nuclease Free Water	1.6
TOTAL	20

3.1.3.7.Clearance of the Universal PCR with QIAseq Beads:

20 µl of the PCR reaction was added to 1-1.5ml of the LoBind tube. It was diluted with 80 liters of Nuclease-free water (to a final volume of 100 liters). 100 µl of PCR solution was mixed with 100 µl of QIAseq Beads (1.0x volume).It was thoroughly blended by pipetting it several times. At room temperature, a 5-minute incubation period was carried out. Three minutes were spent in a magnetic rack separating the beads from the supernatant. Following that, the supernatant was carefully removed and discarded as waste. 200 mL of freshly produced ethanol (80%) were added (while still on the magnetic rack). You'd either turn the tube or move the plate when washing beads. The supernatant was extracted and disposed of with care. After the first ethyl alcohol

wash, a second wash was performed. Ethanol has been removed. After being pulled with a 200 µl pipette and the remainder with a 10 µl pipette, these beads were dried for 10 minutes (on the magnetic rack). The DNA library beads were eluted with 30 µl of nuclease-free water outside the magnetic rack. Pipetting the solution and re-placing it on the magnetic rack ensured that it was completely clear. The first tube contained 28 µl of supernatant, which was then transferred to the second tube, which was a 1.5ml LoBind or PCR tube.

3.1.3.8.Qiaseq Quant Assay Protocol

The ILMN DNA standard was thawed in an ice bath. The ILMN DNA standard was serially diluted five times as shown in the table below (Table 8). Five tubes were made for the ILMN standard dilution. Each tube was given 45 µl of dilution buffer. Pipetting was used to combine 5 µl of ILMN standard stock in the first tube. 5 µl of solution from the first tube was added to the second tube. The same method was used for the remaining tubes and the results were the same as before. Samples were diluted three times (first dilution: 1:50, second dilution: 1:5,000 and third dilution: 1:50,000). The purpose of the first dilution is to prepare samples for subsequent dilutions. Preparation of the PCR mix,

SYBR Green ROX FAST Mastermix was centrifuged for 15 seconds. Three copies of each of the six standards and an NTC were made. As a result, standards and NTC will be placed in the first 18 RotorGene tubes. RotorGene tubes were filled with a copy of the dilution of each sample.

Then we set up the RotorGene real-time PCR system. Results were obtained after 30 cycles of fluorescent irradiation. As soon as the PCR cycle was made, the relevant data were taken from the samples and transferred to the "Illumina Data Analysis Program".

Table 8. Qiaseq Quant Assay Protocol

Standard	Ion Torrent or Illumina DNA Standard	Dilution buffer
Std1	5 µl undiluted	45 µl
Std2	5 µl Std1	45 µl
Std3	5 µl Std2	45 µl
Std4	5 µl Std3	45 µl
Std5	5 µl Std4	45 µl

3.1.3.9.QiaSeq Upload Protocol

Prior to beginning the dilution, the SampleSheet was created using the Illumina Experiment Manager. Section 2 of the Reagent Cartridge Barcode begins with the character MSxx. The area on the right was labeled "custom primer for read 1." We diluted the 1N stock NaOH to 0.2N using 800 milliliters of nuclease-free water. Quant assay procedure findings showed that all of the samples were 4 molars. The 0.2 µl PCR tube was filled to the appropriate capacity depending on the number of primers from each library in order to create a mega pool. A total of 7. 5 ml was transferred from the mega pool tube to a new tube. The mixture was then diluted with 5 µl of 0.2N NaOH. It was gently spun and vortexed for 5 minutes at room temperature to denaturate it, and then spun down. Meanwhile, 990 µl of the yellow-capped Hyb buffer solution was poured into the 1.5 ml tube labeled "20 pM pool" from the cartridge. After 5 minutes, we transferred 10 µl of the NaOH solution and our sample to the 1.5ml tube containing 99 µl Hyb, labeled as the "20 pM pool." The dilution rate of the samples was kept constant using the cartridge and library provided. Another 1.5 cc vial of "Forward primer" was labeled. This tube is now filled to the brim with 597 µl of HT1. Primer 1 was added to the tube with 3 µl of primer labeled "Qiaseq A Read1 Custom Primer1" from the index box. The tubes were stored on a cold block until they were loaded. Before the sequencing begins, the MiSeq Control Software turns the instrument off. The device was expected to turn on after being turned off for a period of time and then turn back on using the rear-mounted button. As illustrated in the figure below, we poured 600 µl of our concentrated samples into well 17. The liquid was drained from well number 18 and 600 µl of the substance was extracted from the tube labeled "Forward primer." After loading the flow cell and reagents, evaluate the run settings and perform a pre-run check prior to starting the run. During the run, the Sequencing screen displays

the runner's progress, intensities, and quality scores. Before continuing, we can view the results of our work on the sequencing page. We washed our hands following the run. The sequencing screen displays various run statistics following each run.

3.1.3.10.Bioinformatics Analysis

Data were loaded and analyzed using QIAGEN Clinical Insight (QCI) Interpret software. The QIAGEN Clinical Insight (QCI) Interpretation is a bioinformatics tool available in clinical laboratories for accurate, efficient and standardized variant interpretation used to detect relevant emerging mutations, based on the AMP/ASCO/CAP classification system.

3.1.4. Validation of Mutations by Sanger Sequencing

Target-specific primers were designed and synthesized for the Sanger sequencing section of the study in order to confirm the variant detected in patients 15 and 32.

3.1.4.1.Designing Primers for Sanger Sequencing

Designing primers for Sanger sequencing, it is critical to consider the absence of previously identified single-nucleotide polymorphisms in the dbSNP database in the selected primers. Furthermore, these regions should not contain repetitive sequences, and the binding temperatures of the primers in the regions for which they are designed should not be too close to each other. Purines and pyrimidines were distributed as evenly as possible in the primers. Table 9 shows the sequenced primers and information obtained using NCBI Blast.

Table 9:. Designed Primer Sequences

Gene	**Forward primer sequence (5'-3')**	**Reverse primer sequence (5'-3')**
ATRX	GCATTCTACGCAACCTTGGTCGAA AGG	ACTGGAGTTCATGTTGGCTGTG GTC

3.1.4.2.Polymerase Chain Reaction (PCR)

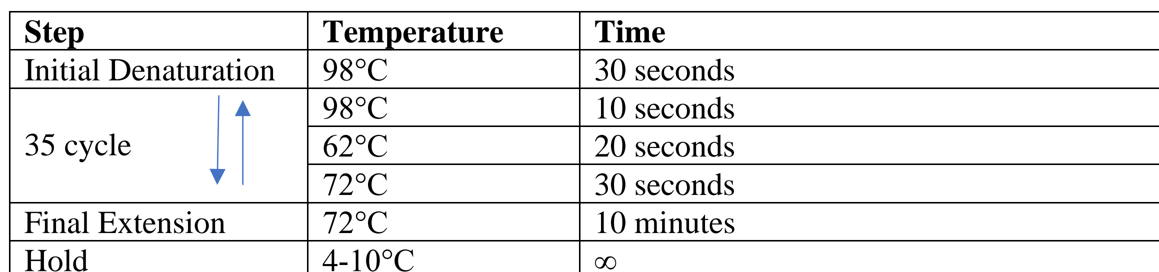
The *novel* variant in the ATRX gene was amplified by Polymerase Chain Reaction using site-specific primer sequences. Polymerase Chain Reaction was performed in a final volume of 50 µL, and PCR mixtures for each sample were prepared as in Table 10.

Table 10: The Reaction Mix Prepared for Each Sample.

Component	**1x Reaction**
5X Phusion HF buffer	10 ul
10 mM dNTPs	1 ul
Forward Primer	2.5 ul
Reverse Primer	2.5 ul
Phusion DNA Polymerase	0.5 ul
ddH_2O	30.5 ul
Total	~47

Each prepared sample obtained 47 µl of the mixture that was subsequently dispensed into sterile PCR tubes and bearing numbers. Then, 3 µl of DNA sample was placed on it and placed in the device, and amplification was performed in the heat cycler with the PCR technique. The PCR program was applied as in Table 11.

Table 11 : Thermal Cycle Program Used in Replicating Target Regions



Step	**Temperature**	**Time**
Initial Denaturation	98°C	30 seconds
35 cycle	98°C	10 seconds
	62°C	20 seconds
	72°C	30 seconds
Final Extension	72°C	10 minutes
Hold	4-10°C	∞

3.1.4.3.Sequence Analysis

The PCR amplification products were obtained by using the "Bigdye Terminator v3.1 Cycle Sequencing kit" (Applied Biosystems, Foster City, CA, USA) containing the labeled dideoxynucleotides, using the "Cycle Sequence" PCR with site-specific primers.

The reaction products were then electrophoresed on the "ABI PRISM 3130XL Genetic Analyzer" (Applied Biosystems, Foster City, CA, USA) automated DNA sequence analysis device, and DNA sequence analysis data in the form of chromatograms were obtained. Chromatography is a mechanism that provides waveform analysis of the color/base relationship for each reaction, allowing for the recording and storage of sequence analysis data in secure environments. The peak lengths of the chromatogram waves were compared after the sequence analysis, and any necessary corrections were made. For chromatographic data analysis, CLUSTALX and GENEDOC multiple sequence analysis programs were used. Both chains were aligned by facing each other in CLUSTAL X (version 1.83). Using the GENDOC (version 2.6.002) program, the ends of the sequences that were too well aligned were cut off and the final consensus sequence was written down.

4.RESULTS

4.1. Demographics of the Subjects

A mutation analysis study was carried out in Yeditepe University Molecular Medicine Department using by next generation sequencing technology on 33 patients diagnosed with ovarian cancer who applied to the Department of Obstetrics and Gynecology at Yeditepe University and showed similar demographic data and disease stages. The patients were between the ages of 18 and 76, and the mean age was calculated as 53.36±12.79 (n = 33). Table 12, Age, weight, and body mass index values of the patients are shown.

Table 12: Age, weight, and body mass index results are shown as Mean±SD (n = 33)

	Mean±SD	**Median**	**Min.-Maks.**
Age (years)	53,36±12,79	54	18-76
Weight (kg)	74,24±12,22	73	52-100
Height (cm)	157,84±6,70	157	150-170
Body mass index (kg/m2)	29,79±4,54	29,68	23,11-38,3

When the patients were divided into postmenopausal and premenopausal, 7 (21.2%) of 33 patients were premenopausal and 26 (78.8%) were postmenopausal. There was one (3.03%) alcoholic drinker and six (18.1%) smokers.

When the stage evaluation was made, 13 (39.3%) patients were found to be in Stage 3. It was observed that 8 (24.2%) patients were in stage 1, 5 (15.1%) patients were in stage 2, and 7 (21.2%) patients were in stage 4 (**Figure 12**).

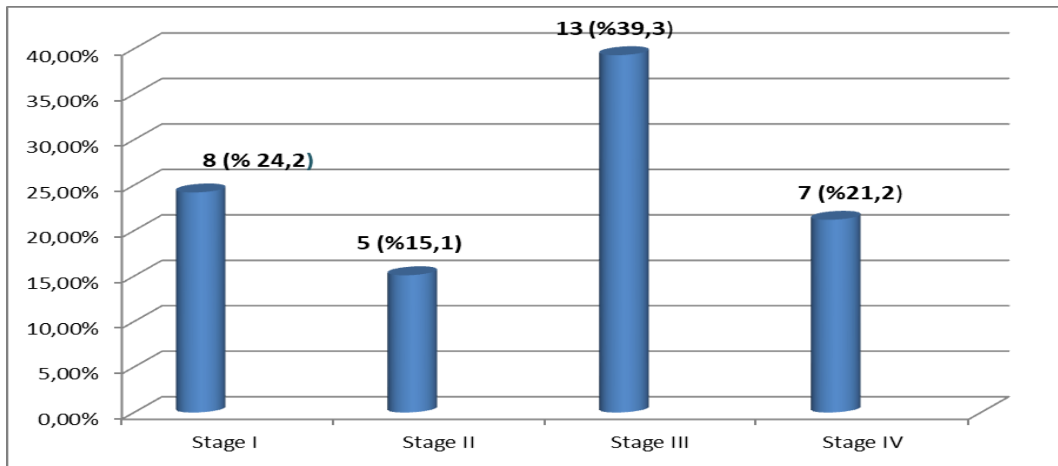


Figure 12: Distribution of the ovarian cancer patient group by stages (n,%).

28 (84.9%) patients had epithelial cell ovarian cancer, 2 (6.1%) patients had sex cord stromal type ovarian cancer, and 3 (9.1%) patients had germ cell ovarian cancer. (**Figure 13**).

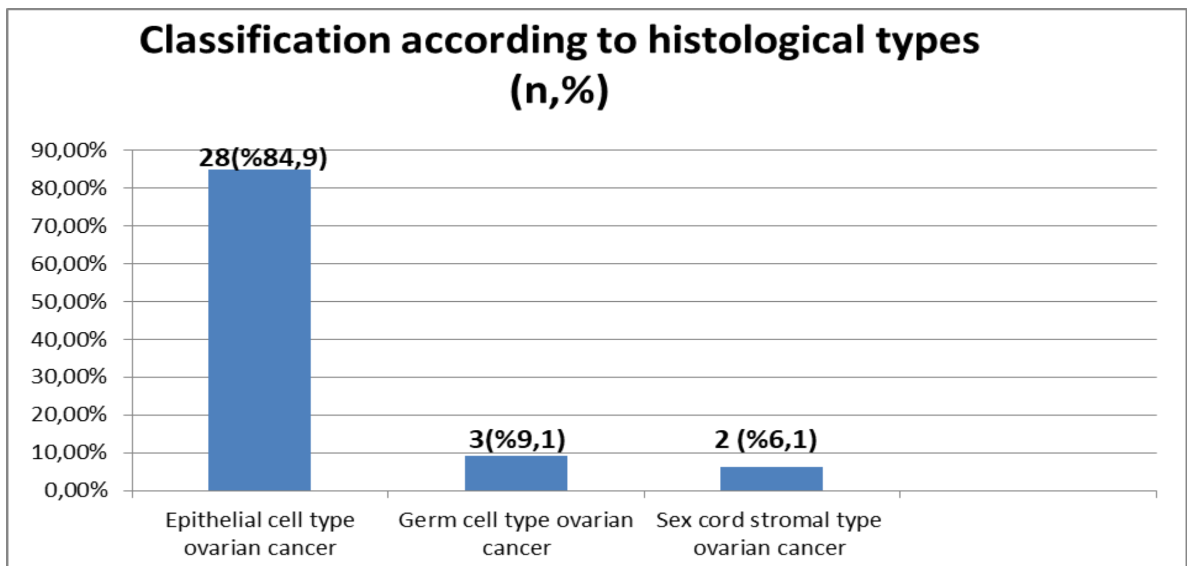


Figure 13: Classification of ovarian cancer patient group according to histological types (n,%).

4.2.Next Generation Sequencing (NGS) Analysis

TERT gene mutation were not detected in 9 (27.7%) of the 33 patients who underwent TERT gene sequencing, while mutations in the TERT gene were detected in the other 24 patients (72.8%). A total of 7 variants of the TERT gene were found. Mutations were observed in both exonic and intronic regions in these variants. In our study, in the TERT gene, heterozygous in 7 patients and homozygous in 1 patient, c.2850-3039C>T; (p.H950H, p.H1013H) mutation, heterozygous in 2 patients c.2517G>A; (p.T839T) mutation, heterozygous in 1 patient, c.2031C>T; (p.G677G) mutation, heterozygous c.835G>A (p.A279T) mutation in 3 patients, heterozygous in 1 patient c.1392C>T; (p.F464F) mutation, heterozygous in 1 patient c.2995-3184G>A; (p.A1062T, p.A999T) mutation and heterozygous in 16 patients and homozygous in 3 patients c.915G>A; (p.A305A) mutations have been detected. The variants and features of the TERT gene are given in Table 13.

Population databases were used primarily to make sense of the changes in our study findings. The gnomAD, ExAC, NHLBI , ESP Frequency and 1000Genomes databases were used to see the population frequencies of the variants we found in our study. According to these databases, 2 of the 7 variants detected (rs186596886 and rs140124989) were shown as less common variants than the other variants. Variants and allele frequencies in our study are given in Table 14.

In our study, in which we studied a total of 33 patient samples and detected variants in 24 patients, the distribution of variants according to patients, classification of variants, translation effect and the results of various in silico prediction programs are shown in Table 15. Classification of variants was seen as Benign, Likely Benign. The translation effect was seen as missense in 27,28,29th patients, and the pathogenicity effect was evaluated as tolerated/benign according to the SIFT Function Prediction and PolyPhen-2 Function Prediction databases.

Multiple calculation evidence shows no effect on the gene or gene product as the CADD score (Combined Annotation Dependent Depletion) is CADD < 10 in allvariants

Table 13: Variants of the tert gene found in patients and their characteristics

Position	Reference Allele	Sample Allele	Exon Region	Zygosity	Transcript Variant	Protein Variant
1254594	C	T	Exon14	heterozygous	c.2995G>A;n.2856G>A; c.3184G>A; n.2892G>A	p.A1062T; p.A999T
1255520	G	A	Exon 13	heterozygous/ homozygous	c.2850C>T; n.2711C>T; c.3039C>T; n.2747C>T	p.H950H; p.H1013H
1268700	C	T	Exon 9	heterozygous	n.2414G>A; n.2378G>A; c.2517G>A	p.T839T
1279505	G	A	Exon 5	heterozygous	c.2031C>T; n.2110C>T	p.G677G
1293609	G	A	Exon 2	heterozygous	c.1392C>T; n.1471C>T	p.F464F
1294086	C	T	Exon 2	heterozygous/ homozygous	c.915G>A; n.994G>A	p.A305A
1294166	C	T	Exon 2	heterozygous	n.914G>A; c.835G>A	p.A279T

Table 14: Variants and allele frequencies

Chromosome	Position	Transcript Variant	Transcript ID	1000 Genomes Frequency	NHLBI ESP Frequency	ExAC Frequency	gnomAD Frequency	dbSNP ID
5	1254594	c.2995G>A; n.2856G>A; c.3184G>A; n.2892G>A	NR_149163.2; ENST00000334602.10 ;	0.519	1.587	1.335	1.246	35719940
5	1255520	c.2850C>T; n.2711C>T; c.3039C>T; n.2747C>T	NR_149163.2; ENST00000334602.10 ;	11.781	7.381	12.388	12.529	33954691
5	1268700	n.2414G>A; n.2378G>A; c.2517G>A	NR_149163.2; ENST00000334602.10 ;	0.220	0.154	0.284	0.268	140124989
5	1279505	c.2031C>T; n.2110C>T	NR_149163.2; ENST00000334602.10 ;	1.717	2.076	1.827	1.189	33956095
5	1293609	c.1392C>T; n.1471C>T	NR_149163.2; ENST00000334602.10 ;	0.100	-	0.256	0.102	186596886
5	1294086	c.915G>A; n.994G>A	NR_149163.2; ENST00000334602.10 ;	26.558	20.753	40.447	27.537	2736098
5	1294166	n.914G>A; c.835G>A	NR_149163.2; ENST00000334602.10 ;	0.958	2.123	3.611	2.314	61748181

Table 15: Variant Distribution According to Patients, Classification of Variants, Translation Effect (1)

Patient No	dbSNP ID	Classification	Translation Impact	SIFT Function Prediction	PolyPhen-2 Function Prediction	CADD Score	Conservation phyloP p-value
1	Rs2736098	Benign	synonymous			< 10	
2	Rs2736098	Benign	synonymous			< 10	
3							
4	Rs2736098	Benign	synonymous			< 10	
	Rs33954691	Benign	synonymous			< 10	1,90E-04
5	Rs2736098	Benign	synonymous			< 10	
8	Rs2736098	Benign	synonymous			< 10	
	Rs33954691	Benign	synonymous			< 10	1,90E-04
9	Rs140124989	Likely Benign	synonymous			< 10	5,16E-02
10	Rs2736098	Benign	synonymous			< 10	
11	Rs2736098	Benign	synonymous			< 10	
13	Rs33954691	Benign	synonymous			< 10	1,90E-04
14	Rs2736098	Benign	synonymous			< 10	
15	Rs2736098	Benign	synonymous			< 10	
16	Rs2736098	Benign	synonymous			< 10	
	Rs140124989	Likely Benign	synonymous			< 10	5,16E-02
18	Rs2736098	Benign	synonymous			< 10	

Table 15: Variant Distribution According to Patients, Classification of Variants, Translation Effect (2)

Patient No	dbSNP ID	Classification	Translation Impact	SIFT Function Prediction	PolyPhen-2 Function Prediction	CADD Score	Conservation phyloP p-value
21	**Rs2736098**	Benign	synonymous			< 10	
22	**Rs33954691**	Benign	synonymous			< 10	**1,90E-04**
23	**Rs2736098**	Benign	synonymous			< 10	
	Rs33954691	Benign	synonymous			< 10	**1,90E-04**
	Rs186596886	Likely Benign	synonymous			< 10	**1,65E-05**
24	**Rs2736098**	Benign	synonymous			< 10	
26	**Rs2736098**	Benign	synonymous			< 10	
27	**Rs2736098**	Benign	synonymous			< 10	
	Rs61748181	Benign	missense	Tolerated	Benign	< 10	
	Rs35719940	Benign	missense	Tolerated	Benign	< 10	
28	**Rs61748181**	Benign	missense	Tolerated	Benign	< 10	
	Rs33956095	Benign	synonymous			< 10	**9,95E-02**
29	**Rs61748181**	Benign	missense	Tolerated	Benign	< 10	
30	**Rs2736098**	Benign	synonymous			< 10	
	Rs33954691	Benign	synonymous			< 10	**1,90E-04**
31	**Rs2736098**	Benign	synonymous			< 10	
	Rs33954691	Benign	synonymous			< 10	**1,90E-04**

Figure 14 : TERT variants detected in Next Generation Sequencing system

In addition to the TERT gene, IDH1, IDH2, MGMT and ATRX genes were also examined in our study to strengthen them. Five variants of the ATRX gene, three variants of the IDH1 gene, one variant of the IDH2 gene and six variants of the MGMT gene were detected. A novel variant c.881A>G in the ATRX gene; c.995A>G (p.D332G; p.D294G) was detected. Table 16 shows the variant and its features.

A deletion (c.699-3delC) in the IDH1 gene was detected in the 21st patient, and its significance was shown as unknown in variant classification. c.478G>A in the MGMT gene in the 32th patient; In the c.571G>A (p.G160R; p.G191R) variant and in the 32th and 15th patients, c.881A>G in the ATRX gene; The c.995A>G (p.D332G; p.D294G) variants were shown as unknown in variant classification. The c.532G>A (p.V178I)(rs 34218846) variant of the IDH1 gene has been shown to be tolerated/ Probably Damaging according to the SIFT Function Prediction and PolyPhen-2 Function Prediction databases in 11,12,24 th patients. (Table 17) For variants with a CADD score of CADD < 10, there is no effect on the gene or gene product, while higher values indicate a detrimental effect on the gene or gene product. Variants and allele frequencies in our study are given in Table 18.

Table 16: Variant and Features

Chromosome	dbSNP ID	Gene Symbol	Transcript Variant	Protein Variant
2	-	IDH1	c.699-3delC	
2	34218846	IDH1	c.532G>A	p.V178I
2	11554137	IDH1	c.315C>T	p.G105G
10	16906252	MGMT	c.66C>T; c.-28C>T	p.R22R
10	1803965	MGMT	c.159C>T; c.252C>T	p.L84L; p.L53L
10	12917	MGMT	c.250C>T; c.343C>T	p.L115F; p.L84F
10	2308321	MGMT	c.427A>G; c.520A>G	p.I143V; p.I174V
10	2308318	MGMT	c.478G>A; c.571G>A	p.G160R; p.G191R
10	2308327	MGMT	c.626A>G; c.533A>G	p.K178R; p.K209R
15	61737003	IDH2	c.996C>T; c.840C>T; c.606C>T	p.S280S; p.S332S; p.S202S
X	45439799	ATRX	c.5465A>G; c.5579A>G	p.N1822S; p.N1860S
X	3088074	ATRX	c.2785C>G; c.2671C>G; c.2785G>C *; c.2671G>C *	p.Q891E; p.Q929E; p.E929Q; p.E891Q
X	143413618	ATRX	c.2606G>A; c.2720G>A	p.R869Q; p.R907Q
X	61752455	ATRX	c.2481C>G; c.2595C>G	p.H865Q; p.H827Q
X	-	ATRX	c.881A>G; c.995A>G	p.D332G; p.D294G

Table 17: Classification of Variants and Translation Effect

Gene Symbol	Translation Impact	Classification	SIFT Function Prediction	PolyPhen-2 Function Prediction	CADD Score
IDH1		Uncertain Significance			7.379
IDH1	missense	Benign	Tolerated	Probably Damaging	25.000
IDH1	synonymous	Benign			13.260
MGMT	synonymous	Benign			15.700
MGMT	synonymous	Benign			< 10
MGMT	missense	Benign			12.340
MGMT	missense	Benign			12.520
MGMT	missense	Uncertain Significance			24.100
MGMT	missense	Benign			12.650
IDH2	synonymous	Benign			< 10
ATRX	missense	Likely Benign	Tolerated	Benign	11.720
ATRX	missense	Benign	Tolerated		< 10
ATRX	missense	Likely Benign	Tolerated	Benign	< 10
ATRX	missense	Likely Benign	Tolerated	Benign	< 10
ATRX	missense	Uncertain Significance	Tolerated	Benign	22.400

Table 18: Population Frequencies of Variants

Gene Symbol	dbSNP ID	1000 Genomes Frequency	NHLBI ESP Frequency	ExAC Frequency	gnomAD Frequency
IDH1	533101765	0.08	-	0.14	0.09
IDH1	34218846	4.932	6.251	5.003	4.951
IDH1	11554137	5.691	7.104	5.240	5.211
MGMT	16906252	2.456	4.369	6.857	5.605
MGMT	1803965	14.716	13.471	13.149	13.203
MGMT	12917	14.836	13.471	14.204	14.308
MGMT	2308321	5.132	9.251	9.880	9.171
MGMT	2308318	0.160	0.138	0.064	0.067
MGMT	2308327	5.132	9.250	9.473	9.264
IDH2	61737003	7.867	7.608	4.916	3.523
ATRX	45439799	0.291	0.635	0.630	0.668
ATRX	3088074	55.232	50.833	39.236	38.534
ATRX	143413618	0.053	0.028	0.054	0.043
ATRX	61752455	0.185	0.947	0.861	0.818
ATRX	-	-	-	-	-

Figure 15 : ATRX, MGMT, IDH1 and IDH2 variants detected in Next Generation Sequencing system (1)

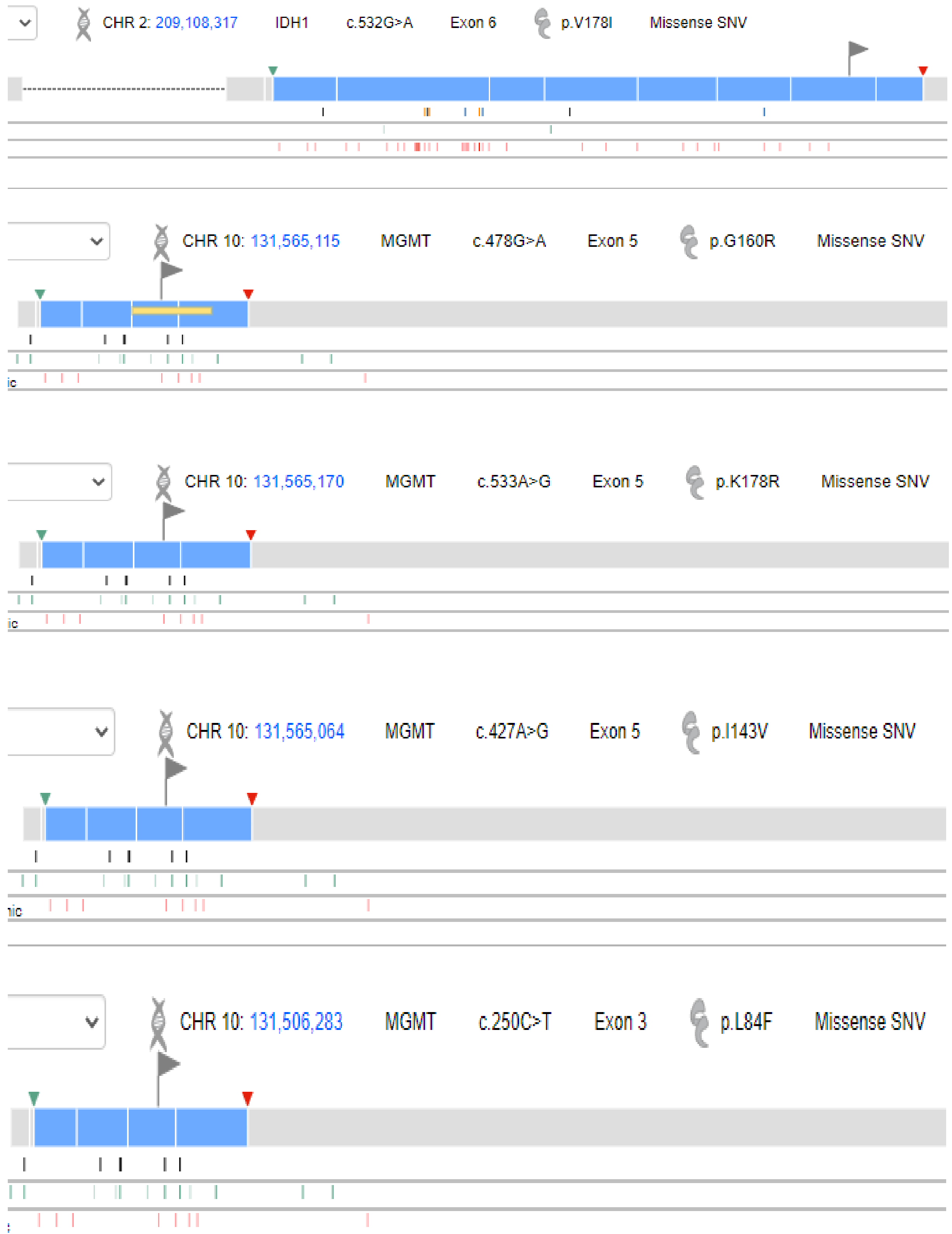


Figure 15 : ATRX, MGMT, IDH1 and IDH2 variants detected in Next Generation Sequencing system (2)

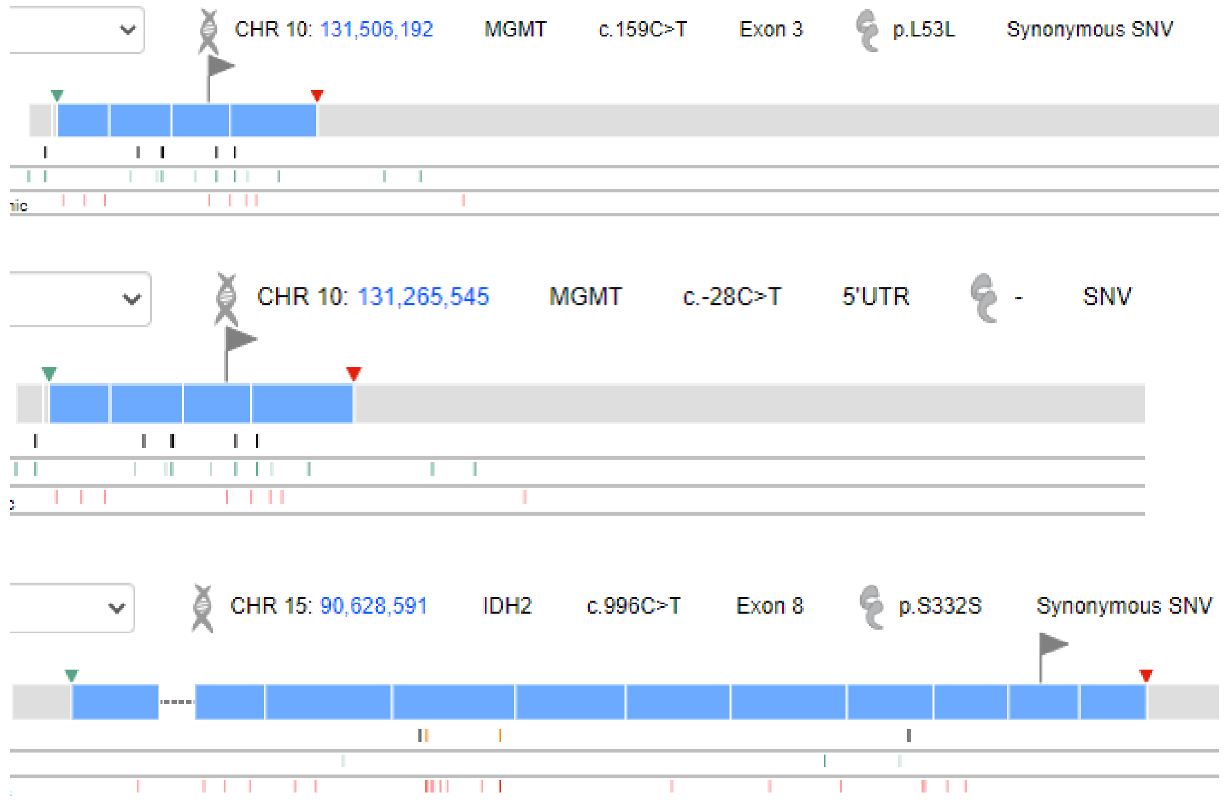


Figure 15 : ATRX, MGMT, IDH1 and IDH2 variants detected in Next Generation Sequencing system (3)

4.3. Validation of Mutations by Sanger Sequencing

DNA sequence change detected by NGS was confirmed by Sanger sequencing using primer sequences. The sequencing of the ATRX mutation in chromosome X was performed using the ABI 3130XL Genetic Analyzer (Life Technologies) by designing a primer suitable for the region. As a result of the studies, c.881A > G , c.995A > G heterozygous *novel* variant was detected in the ATRX gene in patients numbered 15 and 32. The variant we detected was Sanger sequencing and it was confirmed that the cases were heterozygous. Forward and reverse images of the detected variant (ATRX: c.995A>G (NM_000489) = c.881A>G(NM_138270)) confirmed by sanger sequencing are shown in figure 16 and figure 17.

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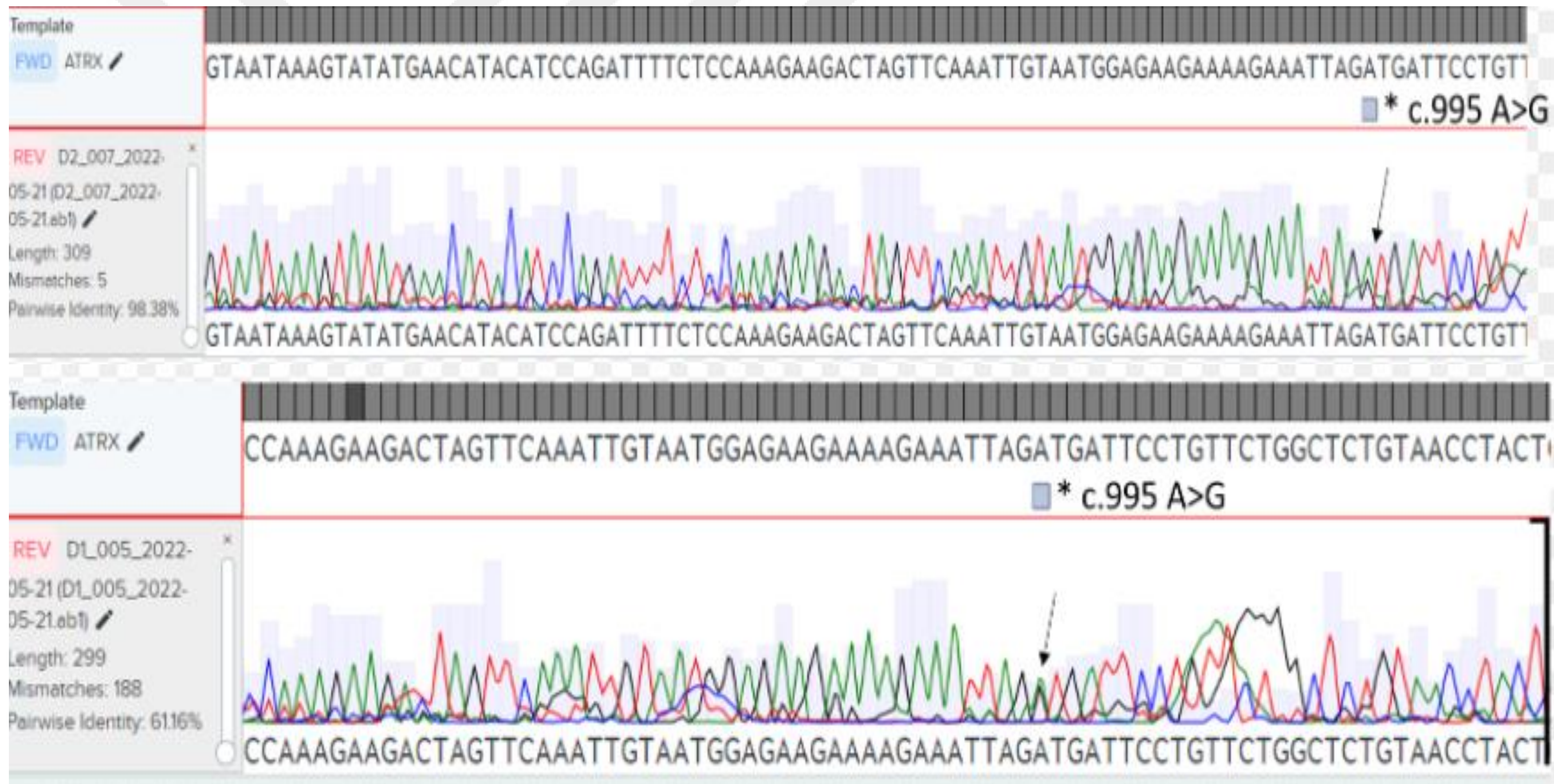

Figure 16: ATRX: c.995A>G (NM_000489) = c.881A>G (NM_138270) Forward İmage and Confirmation by Sanger Sequencing

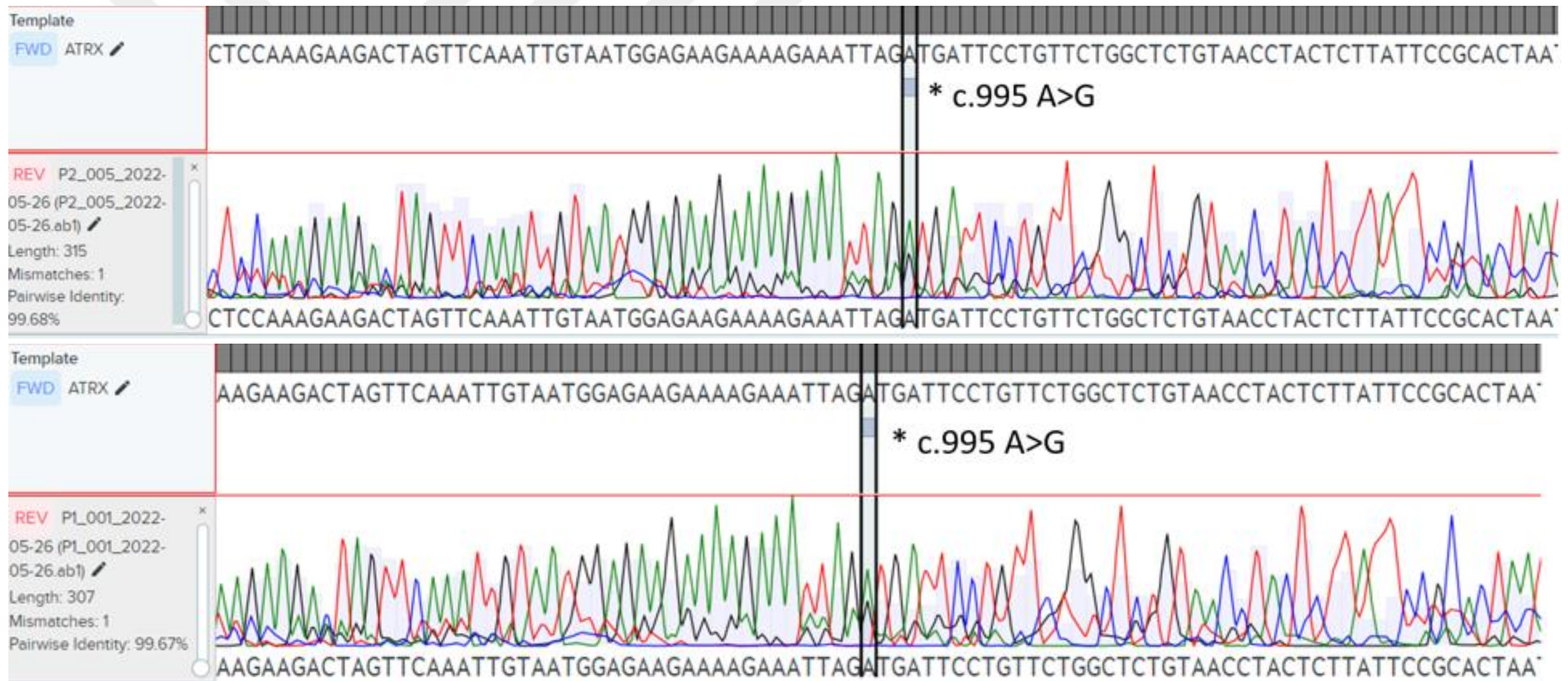

Figure 17: ATRX; c.995A>G (NM_000489) = c.881A>G (NM_138270) Reverse Image and Confirmation by Sanger Sequencing

5. DISCUSSION AND CONCLUSION

Today, the incidence and prevalence of cancers are increasing in parallel with the prolongation of human life due to changing living conditions. For this reason, studies on genetic and environmental factors that are thought to be effective against different types of cancer are gaining momentum.

Ovarian cancer is one of the most common types of gynecological cancer in women, with a high mortality rate. Despite recent advances, ovarian cancer remains a cancer type with a high mortality rate when compared to other gynecological cancers (20). It is the third most lethal type of cancer, behind cervical and uterine cancer (21). While the incidence of ovarian cancer varies by race and ethnic origin, it is significantly higher in developed countries than in developing countries. It is prevalent in women of all ages. The incidence and mortality rates of ovarian cancer increase with age. Although the majority of women diagnosed with ovarian cancer are over the age of 50, it can occur at any age, including infants.

The purpose of this study was to determine the effect of the TERT gene mutation on ovarian cancer. Additionally, we performed mutation analyses on the ATRX, IDH1, IDH2, and MGMT genes, which we believe are associated with various types of cancer. Bioinformatics methods were used to analyze the variants detected in TERT and other genes in these individuals diagnosed with ovarian cancer, as well as the relationship between these variants and the disease. The TERT, ATRX, IDH1, IDH2, and MGMT genes were all found to have 22 distinct types of mutations in this study. The ACMG classification system is used to classify these variants. There were no pathogenic variants identified. The fact that our study discovered variants in the TERT, ATRX, IDH1, IDH2, and MGMT genes that were not previously associated with ovarian cancer suggests that there may be additional variants associated with these genes and ovarian cancer.

In various studies, single nucleotide polymorphisms (SNPs) in the TERT gene have been linked to the telomere length and telomerase reactivation. TERT mutations reactivate telomerase, accelerating the transition to cancer. As a result, it contributes to the sensitivity, development and growth of cancer. It has been demonstrated that it affects TERT transcription and telomerase activity, increasing a person's risk of

developing cancer, resistance to cancer treatment, and longevity. As a result, studies to determine whether there is a link between TERT genetic mutations and cancer risk are of greater interest (8,9). Recurrent TERT promoter mutations, focal TERT locus rearrangement and amplification, and oncoviral DNA inserts have all been implicated in cancer-specific TERT induction (10). Previous research was reviewed to ascertain an association between the TERT gene variants discovered in our patients and ovarian cancer. Certain variants in the Telomerase reverse transcriptase (TERT) gene have been associated with a significantly increased risk of serous ovarian cancer in previous studies. TERT has been linked to genomic imbalance and tumor cell proliferation in cancer cells (121). Additionally, previous studies sequenced the genome to look for TERT promoter mutations in adult granulosa cell tumors of the ovary and identified a TERT C228T promoter mutation. There was a TERT C228T mutation in 50% of relapsed tumors but not in 50% of the original tumors (122).

The variants we found in our study were not known to be associated with ovarian cancer in previous studies. In 19 out of 24 patients with variant detection, the variant rs2736098 in the TERT gene was observed. The rs2736098 (G > A) polymorphism, one of the most studied variants in TERT, has been linked to an increased risk of cancer in a variety of tumor types (121). In a comprehensive study, the case and 82 articles, including control a total of 103 studies were reached. They focused on the two polymorphisms of rs2736100 and rs2736098 in TERT, which were themost commonly studied polymorphisms associatedwith multiple types of cancers. Variant rs2736098 has been associated with cancer risk in all populations analysis based on cancer type, rs2736098 was observed to increase the risk of bladder cancer and lung cancer (123). On the other hand, established the genetic determinants of lung cancer in order to confirm them. He performed a meta-analysis of 1018 publications, analyzing 2910 genetic variants in 754 distinct genes or chromosome loci. The TERT rs2736098 polymorphism was found to be a big factor in lung cancer risk (124,125).Across all demographic groups, the TERT rs2736098 polymorphism has been linked to an increased risk of cancer. According to the subgroup analysis, various cancer types have distinct cancer-promoting properties. The TERT rs2736098 (G > A) polymorphism increased cancer susceptibility in five genetic models of lung cancer. Another study found that the homozygous TERT rs2736098 AA genotype was associated with an increased risk of lung cancer in smokers and nonsmokers in a hospital-based study of

patients with and without cancer (126). In addition, other research conducted an assignment and subset-based association analysis between various cancer types and identified multiple independent risk loci in the TERT region. In their research, genome-wide association studies (GWAS) mapped risk alleles for at least ten different types of cancer to a small region on the chromosome. The TERT genes are located in this area. They classified the variant rs2736098 as a risk variant for lung, bladder, prostate, ovarian, and breast cancers (127).

The rs61748181 variant observed in patients 27, 28, and 29 was previously associated with lung cancer risk by researchers in a sensitive mapping analysis of the 5p15.33 region. The risk variant rs61748181 in the TERT gene was found to have a stronger association with adenocarcinoma risk. The risk variant rs61748181 in the TERT gene was found to have a stronger association with adenocarcinoma risk. The associations observed for adenocarcinoma suggest that the TERT gene is most likely the gene involved in the pathogenesis of cancer in this region(128).

The other TERT variant, rs35719940, was the most frequently occurring in patients with acute myeloid leukemia (AML), with a threefold increase in allele frequency in patients compared to controls (129). Other researchers investigated the possible association between TERT and germline variants in 13 genes and susceptibility to papillary thyroid cancer. They discovered a link between TERT rs33954691 and an increased risk of papillary thyroid cancer. According to research rs33954691 is associated with longevity. Apart from this, little is known about this variant of TERT (130).

Other TERT gene variants, Rs140124989 and Rs186596886, were recently identified by reputable sources who described them as benign but provided insufficient evidence for the laboratory to make an independent assessment. Previously conducted studies were unable to establish a link between both variants and cancer. Numerous computational analyses indicate that CADD 10 has no effect on the gene or gene product in either variant. The allele frequency of the variant rs140124989 is higher than expected for the disorder [expected maximum frequency: 0.100 percent; gnomAD frequency: 0.735 percent].Furthermore, variant rs186596886 has a higher allele frequency than would be expected for a disorder (Expected Maximum Frequency: 0.100%; gnomAD Frequency: 0.813 percent).

We investigated whether mutations in the ATRX, MGMT, IDH1, and IDH2 genes are associated with ovarian cancer, as these genes are frequently mutated in other types of cancer. 5 ATRX variants, 3 IDH1 variants, 1 IDH2 variant, and 6 MGMT variants were identified. IDH1 and IDH2 gene mutations Previous research has not established a link between sporadic ovarian sex cord-stromal tumors. In contrast to the association between IDH mutations and gliomas and AML, ovarian tumors lacked IDH1 or IDH2 mutations (123). Our study identified a total of four IDH gene variants. Variant c.532G > A (p.V1788I) (rs34218846) in the IDH1 gene was identified as possibly harmful by PolyPhen-2 Prediction of Function databases in patients with numbers of 11, 12, and 24. Additionally, c.699-3delC deletion in the IDH1 gene's intronic region was observed in one patient. Additionally, both univariate and multivariate analyses revealed that the IDH1 rs11554137 variant is a poor prognostic factor for overall survival in acute myeloid leukemia (131,132,133).

Our research included the MGMT gene. We looked into patient differences and how they might be linked to ovarian cancer. Previous studies have not linked the rs2308318 c.478G > A, c.571G > A (p.G160R, p.G191R) variants of the MGMT gene, a DNA repair protein, to ovarian cancer. Our study's high CADD score (CADD = 24.1), on the other hand, supports multiple computational evidence for a gene or gene product's deleterious effect. However, its significance for cancers and tumors is still debatable. In a 2010 study, the relationship between MGMT polymorphisms and head and neck squamous cell carcinoma was investigated. The variant rs2308318 was discovered, but its significance was unknown (133). In 3,9,10,15,19,22 patients, the rs16906252 variant was discovered. In glioma patients, Kuo-Chen Wei et al. (2017) looked into the rs16906252 variant, the relationship between MGMT methylation status and OST survival time. Patients with LGG who have the C allele and rs16906252 have a significantly higher overall survival (OST).Finally, people who had LGG low-grade glioma who had the rs16906252 T allele and had a hypo-methylated MGMT promoter had a worse chance of survival (134). In our study, we detected variants of rs2308327 rs2308321 , rs12917. In a previous meta-analysis rs12917 was associated with a higher prostate cancer risk.The MGMT gene polymorphisms rs2308327 and rs2308321 were not linked to prostate cancer susceptibility(135).

The ATRX gene contains *novel* variants c.881A > G, c.995A > G (p.D332G ,p.D294G), but their clinical significance is unknown. It may have a negative effect on the gene or gene product, according to computational evidence (CADD = 22.4). This variant was found in exon 8 and had a 0% prevalence in the general population. In the ATRX gene, determined c.881A > G; c.995A > G; a heterozygous novel variant. We used Sanger sequencing to confirm the heterozygous variant observed in patients 15 and 32. Sanger sequencing confirmed that the detected variation was heterozygous. We think that this *novel* variant, which we verified, may be a new candidate variant for ovarian cancer.

To our knowledge, it is the only study showing the association of variants of TERT, ATRX, MGMT, IDH1 and IDH2 genes with ovarian cancer. There is a need to increase the patient population in order to obtain more detailed results in the treatment of ovarian cancer and to determine the effects of these variants. The limitations of this study are the small number of patients, the absence of a control group, and the fact that the samples were studied from blood tissue. We think that including people with and without ovarian cancer will further strengthen the link between the variants we found and ovarian cancer.

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7. APPENDİCES

7.1.ETHICAL APPROVAL

T.C. YEDİTEPE ÜNİVERSİTESİ

Sayı : 37068608-6100-15- 2135 **22/04/2021**
Konu: Klinik Araştırmalar
Etik Kurul Başvurusu hk.

İlgili Makama (Betül Çapar)

Yeditepe Üniversitesi Moleküler Tıp AD. Prof. Dr. Turgay İsbir'in proje koordinatörü olduğu, Kadın Hastalıkları ve Doğum AD. Prof. Dr. Rukset Attar'ın sorumlu araştırmacı olduğu **"Türk Popülasyonunda Over Kanseri Hastalarında TERT Geninin Yeni Nesil Dizileme Yöntemiyle Mutasyon Analizi"** isimli araştırma projesine ait Klinik Araştırmalar Etik Kurulu (KAEK) Başvuru Dosyası **(2163)** kayıtlı Numaralı KAEK Başvuru Dosyası, Yeditepe Üniversitesi Klinik Araştırmalar Etik Kurulu tarafından **21.04.2021** tarihli toplantıda incelenmiştir.

Kurul tarafından yapılan inceleme sonucu, yukarıdaki isim belirtilen çalışmanın yapılmasının etik ve bilimsel açıdan uygun olduğuna karar verilmiştir **(KAEK Karar No: 1431)**

7.2. CIRRUCULUM VITAE

Personal Informations

Name	Betül	**Surname**	ÇAPAR GORALI

Educational Informations

	Name of Institution	**Year**
Doctorate	Yeditepe University Health Institute Molecular Medicine	**2018- 2022**
Master Degree	Demiroğlu Bilim Universty Health Institute Medical Genetics	**2013-2015**
Medical School	Hitit Universty Faculty of Science Biology	**2008-2012**

Work Experience

Biologist	Bahçeci IVF Centre	2012-2018

Language	**Reading***	**Speaking***	**Writing***	**KPDS/UDS/YDS/ YÖKDİL Score**	**(109) Score**
English	Good	Good	Good	70	

*Excellent**,** Very Good, Good, Basic

Computer skills

Program	**Ability to use**
Microsoft Office	Good