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SPECTRUM OF GERMLINE CANCER SUSCEPTIBILITY GENE MUTATIONS IN BREAST AND OVARIAN CANCER PATIENTS IN THE BLACK SEA REGION OF TURKEY: SINGLE CENTER EXPERIENCE

KARADENİZ BÖLGESİNDEKİ MEME VE YUMURTALIK KANSERİ HASTALARINDA GERMLİNE KANSER YATKINLIK GENLERİNİN MUTASYON SPEKTRUMU: TEK MERKEZ DENEYİMİ

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ABSTRACT

Introduction: Breast cancer consists huge amount of the cancer-related death in population. Ovarian cancer is the second most frequent seen type of gynecological cancer and has the highest mortality among gynecological cancers since most cases are detected late. The current study intended to determine the prevalence of oncogene mutations, especially BRCA1 and BRCA2, in high-risk patients diagnosed with ovarian and breast cancer in the Black Sea region of our country.

Material and method: Between August 2017 and January 2022, a total of 223 individuals who applied to our center and met the genetic test criteria were included in the study. Next-generation sequencing (NGS) was used to detect germ-line deleterious variants in genes included in the oncogenetic panel of patients (34 genes).

Results: Among the 223 patients analyzed within the scope of the study, 195 had breast cancer, and 28 had ovarian cancer, resulting in the detection of 15 different pathogenic variants of BRCA1 (%4,9) and BRCA2 (%6,7) genes in 26 (11.6%) patients. In the analysis of 32 oncogenes other than BRCA1 and BRCA2 genes, 26 different pathogenic (P) or likely pathogenic (LP) variants were detected in a total of 35 patients (15.7%). Based on the analysis of 223 breast/ovarian cancer patients together, 41 different pathogenic (P) or likely pathogenic (LP) variants were found in 61 patients (27.3%). Furthermore, 65 different VUSs (Variant of Uncertain Significance) were detected in 73 patients (32.7%).

Conclusion: This is the first study to be conducted in our region in a single center located in the Black Sea region. The study was conducted in a single center within the Black Sea region and, to our knowledge, provides the first data in this region in terms of cancer genes other than BRCAs. To appreciate of the genetic susceptibility spectrum of hereditary breast and/or ovarian cancer better, it is imperative to clarify the risks associated with genes other than BRCAs, which carry a high risk for other breast and ovarian cancers, as well as BRCA1 and BRCA2. Therefore, patients in the risk group must undergo multigene panel testing in addition to routine BRCA1 and BRCA2 gene testing. We detected two novel variants in the BRCA2 gene and five novel variants other than BRCA oncogenes. Furthermore, the results of this study contributed to the development of our country's specific variant pool.

Keywords: Breast/Ovarian cancer, NGS, BRCA1, CHEK2

ÖZET

Giriş: Meme kanseri kadınlarda en sık görülen kanserdir ve kansere bağlı ölümlerin en sık nedenlerinden biridir. Over kanseri, jinekolojik kanserler arasında ikinci en sık kanser türüdür ve bu hastaların çoğu geç tanı aldığından dolayı mortalitesi en yüksek jinekolojik kanser olarak bilinir. Bu çalışmada ülkemiz karadeniz bölgesinde Meme ve Over kanseri tanısı almış yüksek risk grubundaki hastalarda BRCA1 ve BRCA2 başta olmak üzere sorumlu olabilecek onkogen mutasyonlarının prevalansı ve bölge populasyonumuza özgü varyantları belirlemeyi amaçladık.

Materyal ve metod: Çalışmada Ağustos 2017–0cak 2022 aralığında merkezimize başvuran ve genetik test kriterlerini karşılayan 223 hasta analiz edilmiştir. Çalışmaya alınan hastalarda onkogenetik panel (34 gen) kapsamındaki genlerde germ-line zararlı varyantları tanımlamak için (Next generation sequencing (NGS)) yeni nesil dizileme kullanıldı. Bulgular: Çalışma kapsamında analiz edilen 195 Meme kanserli ve 28 Over kanserli olmak üzere toplam 223 hastanın BRCA1 (%4,9) ve BRCA2 (6,7) genlerinde toplam 26 (%11,6) hastada 15 farklı Patojenik varyant saptanmıştır. BRCA1 ve BRCA2 genleri dışındaki diğer 32 onkogenin analizinde ise toplam 35 hastada (%15,7) 26 farklı Patojenik (P) veya Likely Patojenik (LP) varyant tespit edilmiştir. Analiz edilen 223 meme/over kanserli hasta beraber düşünüldüğünde ise 61 hastada (%27.3) 41 farklı Patojenik (P) veya Likely Patojenik (LP) variant tespit edilmiştir. Bunlara ek olarak Çalışmaki 73 hastada (%32.7) ise 65 farklı VUS (Variant of Uncertain Significance = Önemi Belirsiz Varyant) saptanmıştır.

Sonuç: Çalışma karadeniz bölgesindeki üçüncü basamak bir hastanede yürütülmüş olup bildiğimiz kadarıyla BRCA genleri dışındaki kanser genleri açısından bölgemize dair ilk verileri ortaya koymaktadır. Kalıtsal meme/over kanseri genetik yatkınlık spektrumunun daha fazla anlaşılması için BRCA1 ve BRCA2 genlerinin yanı sıra diğer meme/over ca açısından yüksek risk taşıyan BRCA1 ve BRCA2 genleri dışındaki kanser genlerine özgü risklerin de aydınlatılması gerekmektedir. Bundan dolayı risk grubundaki hastalara rutin BRCA1 ve BRCA2 genlerine ek olarak çoklu gen panel testine ihtiyaç vardır. Çalışmada BRCA2 geninde 2 novel variant saptanırken BRCA genleri dışındaki onkogenlerde 5 novel variant tespit edildi. Ek olarak çalışmamız sonucunda ülkemize spesifik varyant havuzuna da katkı sağlandı.

Anahtar kelimeler: NGS, BRCA1, CHEK2, Meme/Over kanseri

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INTRODUCTION

Breast Cancer (BC) is the most seen type of cancer and consists huge amount of the cancer-related death in women (1). The second common type of cancer among gynecological cancers is ovarian cancer and since most of these patients are diagnosed late, it is known as gynecological cancer with the highest mortality (2). Breast/ovarian cancer are divided into two groups as hereditary and sporadic. Almost 5-10% of all breast cancer cases and more than 23% of all ovarian cancers are thought to be hereditary (3, 4). It is thought that this hereditary condition develops due to highly effective germ-line mutations in oncogenes that predispose to breast cancer.

BRCA1 and BRCA2 genes were identified as high-penetration tenderness genetic factors for inherited ovarian and breast cancers (5, 6). Mutations in BRCA1 and BRCA2 genes are responsible for 25% of inherited breast cancers (HBCs), approximately (7). Individuals who carry BRCA1 and BRCA2 mutation have an 83% risk of future breast or 76% risk of ovarian cancer, respectively (8). Moreover, these patients also have an increased risk of developing other types of cancer such as pancreatic, prostate, stomach, colorectal, and malignant melanoma (8, 9). To test BRCA gene mutations is essential to agree prophylactic salpingo-oophorectomy and mastectomy to diminish the risk of ovarian and breast cancers in high-risk groups (10-12) The spectrum of mutation for BRCA1 and BRCA2 shows alterations amongst populations from different tertiaries and ethnic groups. Although initiator mutations have been shown in the literature in definite ethnic communities, most of the mutations recognized in BRCA1 and BRCA2 are dynasty dependent (13). The main risk factor in inherited ovarian and breast cancer cases is defects in BRCA1 and BRCA2 genes, however mutations in other cancer susceptibility genes including PTEN, TP53, CHEK2, ATM, PALB2, and STK11, which are less common, are also blamed (14). The prevalence and risk ratios of pathogenic variants in oncogenes predisposing to breast/ovarian cancer other than BRCA1 and BRCA2 genes may differ between populations (15). Oncogenetic testing facilitates a sound risk valuation and influences choices about preventive approachs, survivor, and management choices for both affected individuals and their families at risk (16).

Today, when breast/ovarian cancer is mentioned, mutations of BRCA1 and BRCA2 genes are generally thought of, and genetic analyzes may be limited to these genes. Clinical testing of inherited cancer susceptibility by concurrent sequencing of multiple target oncogenes is more available through in developments in Next Generation Sequencing (NGS) technology (17). By the introduction of next-generation sequencing into routine diagnosis, germ-line oncogenetic inherited testing for breast ca/over ca has moved beyond the analysis of the BRCA1 and BRCA2 genes (18). With the widespread use of next-generation sequencing (NGS)-based inherited ca panels, high-throughput sequencing became possible, databases of different populations were created, and the spectrum of cancer susceptibility genes was provided (19). While distinctive populations's data obtained from genetic databases and reported in academic studies continue to increase, most of the studies involve the cancer susceptibility genes mutation spectrum in various populations from all over the world (19, 20). Therefore, determining the oncogene mutation spectrum of different regions in our country will be a significant step in estimating the cancer risk of the population. Furthermore, it is very important to identify the spectrum of damaging variants in genes that predispose to oncogenetic and to identify common founder mutations to develop national health strategies for cancer screening. In this study, patients in the high-risk group diagnosed with Breast cancer (BC) and Ovarian cancer (OC) in the Black Sea region of our country were analyzed using a comprehensive oncogenetic panel (34 genes). The prevalence of germline mutations of oncogenes that may be responsible, especially BRCA1 and BRCA2, and the breast and ovarian ca risk associated with them were investigated.

MATERIALS AND METHODS

Study population

The study was performed in the Department of Medical Genetics of a single center in the Black Sea Region between August 2017 and January 2022. A total of 223 patients, 195 of them were diagnosed with Breast Ca and 28 with Ovarian Ca, who were analyzed to detect and identify genetic mutations that may cause the disease were evaluated retrospectively. Only individuals who have ca history were included in the study. In addition, individuals who were analyzed due to their high-risk family history were not included in the study. The local ethics committee approved the study before it was conducted (Tokat Gaziosmanpaşa University Clinical Research Ethics Committee/18.03.2021/21-KAEK-081). Before testing isolated DNA samples for research purposes, all participants provided written informed consent. All data such as sex, age at diagnosis, histopathology reports… of all patients included in the study were obtained from the hospital automation system. The study also recorded detailed demographic and clinical characteristics, including pedigree analyses and family cancer histories. The kinship statuses were revealed. Family histories of all patients were recorded from mothers and fathers. In addition, none of the patients included in the study were consanguineous. Based on National Comprehensive Cancer Network (NCCN) guidelines, a genetic risk assessment for cancer susceptibility was conducted.

Genetic analysis

The DNA isolation process was executed by following the instructions of manufacturer using a DNA isolation kit for 5 cc EDTA blood samples taken from the patients (21). The Hereditary Cancer Panel (Celemics) was sequenced on the Illumina system using next-generation sequencing (NGS). MiSeq platform (Illumina, San Diego, California, United States) was used for DNA sequencing of the products. VarSome, Franklin, InterVar, Illumina BaseSpace Variant Interpreter, ClinVar, PubMed, OMIM, and in silico methods (Mutation Taster, PolyPhen-2, SIFT) were utilized to annotate the obtained variations.

Genes (34 genes) included in the hereditary cancer panel in the study (Celemics): BRCA1, BRCA2, MLH1, MSH2, MSH6, VHL, BRIP1, BARD1, PALB2, CDH1, EPCAM, RAD50, NBN, PMS2, RB1, TP53, ATM, SLX4, MRE11, STK11, RAD51C, MEN1, SMAD4, CTNNB1, BLM, NF1, PTEN, BMPR1A, MUTYH, CHEK2, APC, CDKN2A, RAD51D

Sanger confirmation method was implemented for de novo variants, homopolymer regions, insertions and deletions, and splice site changes.

Variant Classification

"The American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG-AMP)" guidelines was followed for the classification of pathogenicity of the variations detected in the study (22). Pathogenic variants (P) have disease-causing DNA changes that are well-known in the literature.

Likely pathogenic (LP) variants are considered the probable cause of the disease or the effect on protein function is predicted to be possibly deleterious (>90% probability of causing disease). VUS (Variant of Uncertain Significance) changes are genetic variants that have an unknown or suspected effect on the disease. These variants are typically scarce and predicted to be harmful.

RESULTS

DNA sequence analysis was conducted in this study on peripheral blood samples taken from 223 patients, 195 with breast cancer and 28 with ovarian cancer, using NGS technology.

Of the 223 patients analyzed, five different pathogenic variants were detected in the BRCA1 gene in 11 (4.9%) patients and ten different pathogenic (P) variants in the BRCA2 gene in 15 (6.7%) patients. When the BRCA1 and BRCA2 genes were evaluated, 15 pathogenic variants were detected in 26 patients (11.6%) (Table 1). In 26 patients with pathogenic variants in the BRCA1 and BRCA2 genes, the average age was 42.7 years when breast/ ovarian CA was diagnosed. A total of 15 pathogenic variants were detected, of which 9 were frame-shift variants (in 10 patients), 6 were nonsense variants (in 15 patients), and 1 was a splice site defect (noncoding).

In 223 patients with breast/ovarian cancer, excluding the BRCA1 and BRCA2 genes, and in the analysis of another 32 genes within the scope of panels, 26 different pathogenic (P) or likely pathogenic (LP) variants were identified. The average age of the 35 patients in this group was 41.3 years. The distribution of pathogenic (P) or likely pathogenic (LP) variants in this group was as follows: CHEK2 gene 14 (6.2%) in patients, multigene 6 (2.7%) in patients, ATM gene 4 (1.8%) patients, MRE11 gene 2 (%) 0.9) patient, SLX4 gene 2 (0.9%) patient, MSH6 gene 1 (0.4%) patient, MLH1 1 (0.4%) patient, NBN gene in 1 (0.4%) patient, BRIP1 gene 1 (0.4%) patient MSH2 gene 1 (0.4%) patient, TP53 gene 1 (0.4%) patient, and RAD50 gene 1 (0.4) patient (Table 2). The spectrum of these 26 different pathogenic and likely pathogenic variants detected includes 14 missense mutations in 19 patients, one synonymous mutation in 2 patients, three frame-shift mutations in 3 patients, four nonsense mutations in 6 patients, and four splice site mutations in 4 patients (noncoding) (in 4 patients), and 1 of them was found as an in-frame variant (in 1 patient). Among the 223 breast/ovarian cancer patients analyzed, 41 different pathogenic (P) or likely pathogenic (LP) variants were identified in 61 patients (27.3%) according to a total of 34 gene analyses. Moreover, the mean age at diagnosis (61 patients) for all patients with P and LP variation was 41.8 years.

In our study (34 gene panel analyses), 64 different VUS (Variant of Uncertain Significance) changes were detected in 73 patients (32.7%), except for the pathogenic (P) or likely pathogenic (LP) variants. The mean age of the patients with VUS at diagnosis was 44.4 years. The distribution of these VUSs detected in patients according to genes is as follows: CHEK2 gene in 13 (5.8%) patients, ATM gene in 11 (4.9%) patients, MRE11 gene in 7 (3.1%) patients, BRCA2 gene in 6 (2.7%) patients, BRIP1 gene in 6 (2.7%) patients, and CDH1 gene in 3 (%) patients. 1,3) patients, MSH2 gene in 3 (1.3%) patients, MSH6 gene in 3 (1.3%) patients, RAD50 gene in 3 (1.3%) patients, SLX4 gene in 3 (1.3%) patients, APC gene in 3 (1.3%) patients, NF1 gene in 2 (0.9%) patients, PMS gene in 2 (0.9%) patients, BARD1 gene in 1 (0.4%) patient, BLM gene in 1 (0.4%) patient, BRCA1 gene in 1 (0.4%) patient, CDK4 gene in 1 (0.4%) patient, MLH gene in 1 (0.4%) patient, NBN gene in 1 (0.4%) patient, PALB2 gene in 1 (0%) patient. 4) The MUTYH gene was detected in 1 (0.4%) patient (Table 3). Among the 64 different VUS variants detected, 55 were missense variants (in 64 patients), 5 were synonymous variants (5 patients), and 4 were noncoding variants (4 patients).

DISCUSSION

In the current study, we assessed the spectrum and prevalence of pathogenic/likely pathogenic and VUS variants in 34 cancer susceptibility genes in 223 patients,

Table 1: *BRCA1 and BRCA2* Gene variant spectrum in patients with Breast/ Ovarian Ca

	Gen	Transcript	cDna Change	Protein Change	dbsnp	Zigosite	Consequence	Variant Type	n
Breast Cancer									
1	BRCA1	NM_007294	c.445G>T	p.Glu149Ter	rs876658381	Heterozygote	Non sense	Pathogenic	3
2	BRCA1	NM_007294	c.1961delA	p.Lys654SerfsTer47	rs80357522	Heterozygote	Frameshift	Pathogenic	1
3	BRCA1	NM_007300	c.843_846delCTCA	p.Ser282TyrfsTer15	rs80357919	Heterozygote	Frameshift	Pathogenic	1
4	BRCA2	NM_000059	c.8414dupT	p.Leu2805PhefsTer7	-	Heterozygote	Frameshift	Pathogenic	2
5	BRCA2	NM_000059	c.5073dupA	p.Trp1692MetfsTer3	rs80359479	Heterozygote	Frameshift	Pathogenic	1
6	BRCA2	NM_000059	c.2835delA	p.Asp946IlefsTer14	rs80359356	Heterozygote	Frameshift	Pathogenic	1
7	BRCA2	NM_000059	c.5975C>T	p.Ser1992Ter	rs80358830	Heterozygote	Non sense	Pathogenic	1
8	BRCA2	NM_000059	c.2808_2811delACAA	p.Ala938ProfsTer21	rs80359351	Heterozygote	Frameshift	Pathogenic	1
9	BRCA2	NM_000059	c.1670T>G	p.Leu557Ter	rs80358452	Heterozygote	Non sense	Pathogenic	2
10	BRCA2	NM_000059	c.7018G>T	p.Glu2340Ter	-	Heterozygote	Non sense	Pathogenic	3
11	BRCA2	NM_000059	c.378dupA	p.Ala127SerfTer3	rs879255321	Heterozygote	Frameshift	Pathogenic	1
12	BRCA2	NM_000059	c.5952dup	p.Ser1985IlefsTer18	rs397507814	Heterozygote	Frameshift	Pathogenic	1
13	BRCA2	NM_000059	c.3465_3466del	p.Ser1156Ter	rs397507671	Heterozygote	Non sense	Pathogenic	1
Ovarian Cancer									
14	BRCA1	NM_007294	c.445G>T	p.Glu149Ter	rs876658381	Heterozygote	Non sense	Pathogenic	4
15	BRCA1	NM_007300.3	c.2197_2201delGAGAA	p.Glu733ThrfsTer5	rs80357507	Heterozygote	Frameshift	Pathogenic	1
16	BRCA1	NM_007294.4	c.5194-12G>A	intronic	rs80358079	Heterozygote	Non coding	Pathogenic	1
17	BRCA2	NM_000059	c.7018G>T	p.Glu2340Ter	-	Heterozygote	Non sense	Pathogenic	1

Table 2: Spectrum of Pathogenic or Likely Pathogenic variants detected outside of *BRCA1 and BRCA2* Genes in patients with Breast/ Ovarian Cancer

	Gen	Transcript	cDNA Change	Protein Change	dbsnp	Zigosite	Consequence	Variant Type	n
Breast Cancer									
1	ATM	NM_000051	c.6679C>T	p.Arg2227Cys	rs564652222	Heterozygote	missense	Pathogenic	2
2	ATM	NM_000051	c.3576G>A	p.Lys1192=	rs587776551	Heterozygote	synonymous	Pathogenic	2
3	BRIP1	NM_032043	c.2947delA	p.Ile983LeufsTer2	rs774684620	Heterozygote	Frameshift	Pathogenic	1
4	CHEK2	NM_007194	c.291G>A	p.Trp97Ter	-	Heterozygote	Non sense	Pathogenic	3
5	CHEK2	NM_001005735	c.1309G>A	p.Glu437Lys	rs587780169	Heterozygote	missense	L.Pathogenic	1
6	CHEK2	NM_001005735	c.628G>A	p.Gly210Arg	rs72552322	Heterozygote	missense	L.Pathogenic	1
7	CHEK2	NM_007194	c.701T>G	p.Val234Gly	-	Heterozygote	missense	L.Pathogenic	1
8	CHEK2	NM_007194	c.967A>C	p.Thr323Pro	rs750984976	Heterozygote	missense	L.Pathogenic	1
9	CHEK2	NM_007194	c.592+3A>T	intronic	rs587782849	Heterozygote	Non coding	L.Pathogenic	1
10	CHEK2	NM_001005735	c.678G>C	p.Leu226Phe	rs745646057	Heterozygote	missense	L.Pathogenic	4
11	CHEK2	NM_007194	c.592+3A>T	intronic	rs587782849	Heterozygote	Non coding	L.Pathogenic	1
12	CHEK2	NM_007194	c.470T>G	p.Ile157Ser	rs17879961	Heterozygote	missense	L.Pathogenic	1
13	MLH1	NM_000249	c.883A>C	p.Ser295Arg	rs63751598	Heterozygote	missense	Pathogenic	1
14	MRE11	NM_005591	c.1500+1G>A	intronic	-	Heterozygote	Non coding	L.Pathogenic	1
15	MRE11	NM_005591	c.196A>T	p.Lys66Ter	-	Heterozygote	Non sense	Pathogenic	1
16	MSH2	NM_000251	c.274C>G	p.Leu92Val	rs587779154	Heterozygote	Missense	L.Pathogenic	1
17	MSH6	NM_000179	c.2515G>A	p.Asp839Asn	rs1553413868	Heterozygote	Missense.	L.Pathogenic	1
18	MUTYH	NM_001128425	c.934-2A>G	intronic	rs77542170	Heterozygote	Non coding	LPathogenic	1
19	MUTYH	NM_001128425	c.1187G>A	p.Gly396Asp	rs36053993	Heterozygote	missense	Pathogenic	2
20	MUTYH	NM_012222	c.875C>T	p.Pro292Leu	rs374950566	Homozygote	missense	Pathogenic	1
21	NBN	NM_002485	c.1071dupA	p.Val358SerfsTer9	-	Heterozygote	frameshift	Pathogenic	1
22	SLX4	NM_032444	c.634C>T	p.Arg212Ter	rs1395992833	Heterozygote	Non sense	L.Pathogenic	1
23	SLX4	NM_032444	c.2808_2809DelAG	p.Ala938ThrfsTer7	rs767631456	Homozygote	frameshift	Pathogenic	1
24	TP53	NM_000546	c.733G>A	P.Gly245Ser	rs28934575	Heterozygote	missense	Pathogenic	1
Ovarian Cancer									
25	RAD50	NM_005732.3	c.3229C>T	p.Arg1077Ter	rs368980595	Heterozygote	Non sense	Pathogenic	1
26	MUTYH	NM_001128425	c.1437_1439delGGA	p.Glu480del	rs587778541	Heterozygote	In frame	Pathogenic	1
27	MUTYH	NM_012222.2	c.875C>T	p.Pro292Leu	rs374950566	Homozygote	missense	Pathogenic	1

L.Pathogenic: Likely Pathogenic

including 195 breast cancer patients and 28 ovarian cancer patients in a single center in the Black Sea region, which presents the first data about our region. Using a comprehensive hereditary cancer panel (34 genes) in high-risk women with breast/ovarian cancer diagnosed at our center, we report for the first time the germ-line pathogenic/ likely pathogenic variation frequencies of oncogenes, especially BRCA1 and BRCA2.

In recent years, numerous studies have been conducted in Turkey regarding the BRCA1 and BRCA2 genes and hereditary breast/ovarian cancer. The article published by Bisgin A et al in 2022 regarding the BRCA1 and BRCA2 gene mutation spectrum in Turkey was conducted with the largest number of patients. In that study, germ-line analysis results of BRCA1 and BRCA2 genes from seven different regions of Turkiye were presented. The authors stated that 20.66% of cancer patients had BRCA1/2 variants associated with the disease. Of participants who had no disease, 22.61% were identified with potentially pathogenic variants. According to geographical distribution in their findings, the highest rates of potentially pathogenic variants were seen in the Aegean (43.39%) and Central Anatolia (46.49%) regions, while the lowest rates were seen in Eastern Anatolia (8.06%) and Mediterranean (8.05%), respectively. In the Black Sea region, this rate was reported to be 9.3% (23).

Regarding the BRCA1 and BRCA2 gene mutation spectrum, Bahsi T et al. published 1419 disease studies in 2019. This study found a 9.7% rate of pathogenic (9.4%) and likely pathogenic (0.3%) variants in the BRCA1 and BRCA2 genes. In the same study, VUS was found in the BRCA1 and BRCA2 genes with a rate of 6.4% (24). Our study did not detect any likely pathogenic variants in the BRCA1 and BRCA2 genes, and all variants we identified were classified as pathogenic. The rate of pathogenic variants in these genes was 11.6%, which was higher than the rate reported by Bahsi T et al. The rate of VUS in the BRCA1 and BRCA2 genes in our study was 2.7%. While we detected more pathogenic mutations than in Bahsi T et al, the number of VUS changes we detected was almost half. This may be an incidental finding or related to regional localization. This is because the study was conducted only in a single center in the Black Sea region and covers a relatively closed gene pool.

A study conducted by Solmaz et al. in 2020 examined the BRCA1 and BRCA2 genes in 910 patients at high risk for breast/ovarian cancer. In this study, pathogenic and likely pathogenic variants were detected in the BRCA1 and BRCA2 genes at a rate of 9.34% (in 85 patients), whereas VUS was detected in 6 patients (0.66%) (25).
In another study conducted in 2020 by Demir S et al. in the Thrace region of Turkey, a total of 493 participants were analyzed, of which 442 were diagnosed with breast/ovarian cancer, and 51 were not diagnosed with any clinical cancer but had a family history. Among all participants in this study, the frequency of pathogenic/likely pathogenic mutations in the BRCA1 and BRCA2 genes was 17.84%, while the frequency in participants with breast/ovarian cancer was 19.23% (85/442) (26).

In a study published by Gezdirici A et al. in 2021, BRCA1 and BRCA2 genes in hereditary breast/ovarian cancers were analyzed in 149 patients. The ACMG guidelines were used

Table 3: VUSs detected in patients with Breast/ Ovarian Cancer

	Gen	Transcript	cDNA Change	Protein Change	dbsnp	Zigosite	Consequence	n
Breast Cancer								
1	APC	NM_000038	c.220+7T>A	intronic	-	Heterozygote	Non coding	1
2	APC	NM_000038	c.317G>A	p.Arg106His	rs201764637	Heterozygote	missense	1
3	ATM	NM_000051	c.5051C>G	p.Ser1684Cys	rs1565473651	Heterozygote	missense	3
4	ATM	NM_000051	c.6109G>A	p.Glu2037Lys	rs1448711296	Heterozygote	missense	1
5	ATM	NM_000051	c.4396C>G	p.Arg1466Gly	rs730881369	Heterozygote	missense	1
6	ATM	NM_000051	c.8288G>A	p.Arg2763Gln	rs551411717	Heterozygote	missense	1
7	ATM	NM_000051	c.2251-4A>G	intronic	rs786202935	Heterozygote	Non coding	1
8	ATM	NM_000051	c.1272T>C	p.Pro424=	rs35578748	Heterozygote	synonymous	1
9	ATM	NM_000051	c.162T>C	p.Tyr54=	rs3218690	Heterozygote	synonymous	1
10	BARD1	NM_000465	c.697G>A	p.Glu233Lys	-	Heterozygote	missense	1
11	BLM	NM_000057	c.1979A>G	p.His660Arg	-	Heterozygote	missense	1
12	BRCA1	NM_007294	c.4054G>C	p.Glu1352Gln	rs80357202	Heterozygote	missense	1
13	BRCA2	NM_000059	c.10078A>G	p.Lys3360Glu	rs1593202262	Heterozygote	missense	1
14	BRCA2	NM_000059	c.4599A>C	p.Lys1533Asn	rs80358694	Heterozygote	missense	1
15	BRCA2	NM_000059	c.707A>G	p.His236Arg	rs80358938	Heterozygote	Missense.	1
16	BRCA2	NM_000059	c.1106A>C	(p.Asn369Thr)	rs876660966	Heterozygote	missense	1
17	BRCA2	NM_000059	c.6058G>A	p.Glu2020Lys	rs80358842	Heterozygote	missense	1
18	BRIP1	NM_032043	c.3459T>C	p.Asp1153=	rs4987050	Heterozygote	synonymous	1
19	BRIP1	NM_032043	c.415T>G	p.Ser139Ala	rs202072866	Heterozygote	missense	1
20	BRIP1	NM_032043	c.1883G>A	p.Gly628Asp	rs1064794907	Heterozygote	missense	1
21	BRIP1	NM_032043	c.2741T>C	p.Leu914Ser	rs886053215	Heterozygote	missense	1
22	BRIP1	NM_032043	c.3020C>A	p.Ser1007Tyr	rs886053214	Heterozygote	missense	1
23	BRIP1	NM_032043	c.1255C>T	p.Arg419Trp	rs150624408	Heterozygote	missense	1
24	CDH1	NM_004360	c.1793G>A	p.Arg598Gln	rs780759537	Heterozygote	missense	1
25	CDH1	NM_004360	c.160A>G	p.Arg54Gly	rs587781329	Heterozygote	missense	1
26	CDH1	NM_004360	c.1036C>G	p.Gln346Glu	rs878854676	Heterozygote	missense	1
27	CDK4	NM_000075	c.385G>A	p.Asp129Asn	rs876660606	Heterozygote	missense	1
28	CHEK2	NM_001005735	c.1166G>A	p.Arg389His	rs730881688	Heterozygote	missense	1
29	CHEK2	NM_001005735	c.721+3A>T	intronic	rs587782849	Homozygote	Non coding	1
30	CHEK2	NM_001005735	c.1196C>T	p.Ser399Leu	rs121908703	Heterozygote	Missense	3
31	CHEK2	NM_007194	c.967A>G	p.Thr323Ala	rs750984976	Heterozygote	missense	1
32	CHEK2	NM_001005735	c.1355A>T	p.Asp452Val	rs761095543	Heterozygote	missense	1
33	CHEK2	NM_001005735	c.678G>C	p.Leu226Phe	rs745646057	Heterozygote	missense	4
34	CHEK2	NM_001005735	c.678G>C	p.Leu226Phe	rs745646057	Homozygote	missense	1
35	CHEK2	NM_007194	c.549G>C	p.Leu183Phe	rs745646057	Heterozygote	Missense	1
36	MLH1	NM_000249	c.1876T>C	p.Phe626Leu	rs377241633	Heterozygote	Missense	1
37	MRE11	NM_005591	c.1475C>A	p.Ala492Asp	rs61749249	Heterozygote	missense	2
38	MRE11	NM_005591	c.1051C>T	p.Arg351Cys	rs757492041	Heterozygote	missense	1
39	MRE11	NM_005591	c.1123C>T	p.Pro375Ser	-	Heterozygote	missense	1
40	MRE11	NM_005591	c.358A>G	p.ile120val	rs372131911	Heterozygote	missense	1
41	MRE11	NM_005591	c.845+4_845+5delAG	intronic	-	Heterozygote	Non coding	1
42	MSH2	NM_000251	c.2606C>A	p.Ala869Glu	rs730881772	Heterozygote	missense	1
43	MSH2	NM_000251	c.1774A>G	p.Met592Val	rs371614039	Heterozygote	missense	1
44	MSH2	NM_000251	c.1004C>T	p.Thr335Ile	rs63750602	Heterozygote	missense	1
45	MSH6	NM_000179	c.898C>G	p.Arg300Gly	-	Heterozygote	missense	1
46	MSH6	NM_000179	c.3727A>T	p.Thr1243Ser	rs147453999	Heterozygote	missense	1
47	MSH6	NM_000179	c.2515G>A	p.Asp839Asn	rs1553413868	Heterozygote	missense	1
48	MUTYH	NM_001128425	c.715G>A	p.Val239Ile	rs759295912	Heterozygote	missense	1
49	NBN	NM_002485.5	c.628G>T	p.Val210Phe	rs61754796	Heterozygote	missense	1
50	NF1	NM_001042492	c.5401T>C	p.Leu1801=	-	Heterozygote	synonymous	1
51	NF1	NM_001042492	c.6581G>A	p.Arg2194Lys	-	Heterozygote	missense	1
52	PALB2	NM_024675	c.1696C>T	p.Arg566Cys	rs746582620	Heterozygote	missense	1
53	PMS2	NM_000535	c.2012C>T	p.Thr671Met	rs587780046	Heterozygote	missense	1
54	PMS2	NM_000535	c.2068A>C	p.Lys690Gln	rs587781909	Heterozygote	missense	1
55	RAD50	NM_005732	c.2003A>G	p.Gln668Arg	rs876660716	Heterozygote	missense	1
56	RAD50	NM_005732	c.2991A>G	p.Ile997Met	-	Heterozygote	missense	1
57	RAD50	NM_005732	c.980G>A	p.Arg327His	rs28903091	Heterozygote	missense	1
58	SLX4	NM_032444	c.5096C>G	p.Ser1699Cys	-	Heterozygote	missense	1
59	SLX4	NM_032444	c.2023G>A	p.Gly675Arg	rs767473953	Heterozygote	missense	1
60	SLX4	NM_032444	c.708G>A	p.Ala236=	rs765742613	Heterozygote	synonymous	1
Ovarian Cancer								
61	APC	NM_000038	c.6151A>G	(p.Lys2051Glu)	-	Heterozygote	missense	1
62	ATM	NM_000051	c.3630G>A	(p.Met1210Ile	rs587778073	Heterozygote	missense	1
63	ATM	NM_000051	c.5890A>G	p.Lys1964Glu	rs201963507	Heterozygote	missense	1
64	BRCA2	NM_000059	c.707A>G	p.His236Arg	rs80358938	Heterozygote	missense	1
65	MRE11	NM_005591	c.529G>A	(p.Ala177Thr)	142996063	Heterozygote	missense	1

in this study to classify the variants found in the study. Ten different pathogenic variants were detected in 12 patients in the BRCA1 gene, while five were detected in 6 patients in the BRCA2 gene. Additionally, four variants of uncertain clinical significance (VUS) were detected in five patients in the BRCA1 gene, while two different VUSs were detected in two patients in the BRCA2 gene. Considering the BRCA1 and BRCA2 genes, 15 different pathogenic variants have been reported in 18 patients, while 6 VUSs have been reported in 7 patients. Two of these reported variants were novel: a pathogenic variant and a VUS variant. The likely pathogenic variant was not reported in any patients in the current study (27).

BRCA1 and BRCA2 mutations prevelance was studied in high-risk breast ca individuals in Jordan in 2020. Based on a study of 517 patients, the rate of pathogenic and likely pathogenic variants was 13.9% (72 patients), with BRCA1 4.6% (24 patients) and BRCA2 9.3% (48 patients). The incidence of VUS was reported to be 10.3% (53 patients) (28).

In a study published in 2022 by Gerik Çelebi HB et al, routine molecular genetic analyses of the BRCA1 and BRCA2 genes were conducted on 120 hereditary breast and ovarian cancer patients. A targeted multigene panel analysis was also performed on genes other than BRCAs. It was reported that the rate of a pathogenic variant in BRCA1 and BRCA2 was 12.5%, the likely pathogenic variant rate was 0.83%, and the rate of VUS was 3.3%, resulting in an overall rate of 16.6% (29). These rates were closer to the variation in BRCA1 and BRCA2 that we found in our study. There may be variations in the BRCA1 and BRCA2 mutations frequencies in different ethnicities and tertiaries. The results of studies in our country show a wide range between 5% and 26.1% (24, 27, 29, 30). In our study, we observed similar BRCA1 and BRCA2 variation rates to those observed in previous studies in our country.

It is becoming increasingly common to screen hereditary breast/ovarian cancer patients using multigene panel analyses in addition to BRCA1 and BRCA2 (31). In the study of Gerik Çelebi HB et al, in addition to the BRCA1 and BRCA2 genes, multigene panel analysis was performed with NGS, and 12 genes were analyzed, including CHEK2, RAD51D, ATM, MSH6, RAD50, STK11, SDHA, RB1, CDH1, CDKN2, POLD1, and SMAD4. This study identified 21 variants in 20 patients (17.5%), including three pathogenic variants, five likely pathogenic variants, and 13 VUS variants. In this study, ATM gene variations were reported to be the most common among genes other than BRCAs (5%) (29). Based on our study, the most common variation other than of BRCA1 and BRCA2 was observed in the CHEK2 gene, while the ATM gene was the second

most common.

According to the study of Jan Hauke et al., published in 2018 as a German consortium and involving 5589 patients with hereditary breast and ovarian cancers with negative BRCA1/2 mutations, eight genes were analyzed, namely, ATM, CDH1, CHEK2, NBN, PALB2, RAD51C, RAD51D, and TP53. This study reported pathogenic and likely pathogenic variation in 6.1% (339/5589) of patients. The most significant prevalence of these deleterious variants was detected in the CHEK2 gene (138 carriers, 2.5%), followed by ATM (81 carriers, 1.4%) and PALB2 (68 carriers, 1.2%). In the same study, the prevalence of deleterious variants in the CDH1, NBN, RAD51C, RAD51D, and TP53 genes was reported as 0.3% or less for each gene (32). According to our study, the most common pathogenic/L pathogenic variation outside BRCA1 and BRCA2 was found in the CHEK2 gene (6.2%), which is consistent with Jan Hauke et al. (CHEK2 2.5%), and our rate is higher. According to Jan Hauke et al., the variation rates in the ATM gene (1.4%), which is the second most common pathogenic/L pathogenic variation in their study, were very similar to our findings (ATM gene (1.8%). The PALB2 gene, described by Jan Hauke et al. as the third most common pathogenic/L pathogenic variation in their study, was not detected in our study.

In our study, we observed 2.7% of pathogenic/L pathogenic variations in the MUTYH gene, which was not present in Jan Hauke et al.'s study. It is, therefore, not possible to make such a comment. Although the concordance and differences between studies are partially compatible with the gene selection and the number of genes studied in the panels, we think that the mentioned differences may primarily be due to the sample size or may be an incidental finding, as well as a result of social diversity or ethnicity differences.

Jan Hauke et al. detected mutations in 339 (6.1%) cases by analyzing eight genes other than BRCA1 and BRCA2. Nevertheless, in our study, mutations were found in 35 (15.7%) of the 32 genes other than BRCAs included in the panel. Unlike the studies of Jan Hauke et al., we selected 32 genes for analysis in our study. The number of genes we have analyzed is four times greater than that of Jan Huke and colleagues. We believe this situation contributes significantly to the higher mutation rates we observed in our study. Fifty-eight VUSs were detected in 66 (29.6%) patients in 32 genes other than BRCA1 and BRCA2 in our study. Most of the VUSs had missense mutations, with 49 (84.5%). Five VUSs were synonymous (8.6%), and 4 VUSs were splice defects (non-coding) (6.9%). Based on a study of the German consortium of Jan Hauke et al., 421 different VUS were observed in 827 (14.8%) of 5589 index patients, and the majority of the VUS (94.1%) were missense mutations. Although they found almost half as many VUSs as we did in our study, the most common missense defect they detected was consistent with what we found. The proportional difference between the two studies could be due to sample size or an incidental finding, or it could be related to social diversity or differences in ethnicity. Our data should be supported by studies involving a larger number of patients in our country. Moreover, more studies are needed regarding the pathogenicity of the VUSs found in this study.

In our study, the most common BRCA1/2 variant in patients with breast and ovarian cancer was c.445G>T (p. Glu149Ter) pathogenic variant in the BRCA1 gene, with a total rate of 3.1%. The most common variant in the BRCA2 gene was the pathogenic variant c.7018G>T (p. Glu2340Ter), which was detected as the novel variant, with a rate of 1.79% (4 patients). In addition to this novel variant, the other novel variant detected in the BRCA2 gene in our study was BRCA2 c.8414 dupT (p. Leu2805PhefsTer) with a rate of only 0.90% (in two patients). In the BRCA1/BRCA2 analysis, two different variants were detected in the brca2 gene, but no new variants were detected in the brca1 gene. A total of 2.69% of novel variants were detected (in 6 patients). We detected 26 different pathogenic or likely pathogenic variants in 12 genes, excluding BRCA1 and BRCA2, in 35 patients, 32 of whom had breast cancer and three of whom had ovarian cancer. Five were identified as novel variants, three of which were pathogenic, and two were likely to be pathogenic. A total of two novel mutations were found in the CHEK2 gene, two in the MRE11 gene, and one in the NBN gene (Table 2). Except for the BRCA1 and BRCA2 genes, the most common mutation was detected in the CHEK2 gene with the number 14 (6.2%). Next, the MUTYH gene 6 (2.7%), ATM gene 4 (1.8%), MRE11 gene 2 (0.9%), SLX4 gene 2 (0.9%), and single cases of the BRIP1, TP53, NBN, RAD50, MSH2, MSH6, APC genes were identified.

Our study confirmed the utility of multigene testing for the assessment of risk in Breast Cancer and Ovarian Cancer patients/ families. In our study, if only BRCA1 and BRCA2 genes were sequenced in routine molecular diagnosis for breast/ovarian cancer, information on approximately 35 patients with pathogenic or likely pathogenic variants could not be obtained. Therefore, BRCA1 and BRCA2 analysis alone may not reveal underlying molecular gene defects for many patients. Thus, some potential treatment options, potential risks of primary tumors developing in organs related to the current patient, and information concerning the involvement of the next generation will be left in the dark. Furthermore, early identification of mutations provides an advantage in surgical treatment options. By identifying mutations in patients with breast cancer, genetic counseling can be provided that will improve the chances of

the patient's blood relatives being aware of early diagnosis and treatment and taking precautions before they become sick.

Study limitation

MLPA (Multiplex ligation-dependent probe amplification) could have been used to detect possible deletions and duplications in the genes analyzed within the scope of the study, but we were unable to do so due to a lack of resources in our center. Additionally, our study involved a relatively small sample size. This finding should be supported by studies involving more patients. Furthermore, the genes we analyzed are limited by the panel (celemix) we use in an optimized manner at our center. A study using a kit that can analyze more genes will be able to provide more information.

CONCLUSION

Our study was conducted in a single center in the Black Sea region. To the best of our knowledge, this is the first study reporting the germ-line pathogenic/likely pathogenic/VUS variation frequencies of related oncogenes, particularly BRCA1 and BRCA2, in high-risk breast and ovarian cancer patients. In our study, we suggest that sequencing other oncogenes with high risk for breast and ovarian cancer and the BRCA1/BRCA2 genes is an appropriate approach for individuals at risk of developing oncogene-associated cancer. In our study, we discovered a high prevalence of VUS, which is one of the main disadvantages of using the multigene test as a routine diagnostic tool. As part of our study, we also identified two different pathogenic variants in the BRCA2 gene (6 patients), two different pathogenic/L pathogenic variants in the CHEK2 gene (4 patients), two different pathogenic/L pathogenic variants in the MRE11 gene (2 patients) and one pathogenic variant in the NBN gene (1 patient). A total of seven novel pathogenic/L pathogenic variants have been identified.

Ethics Committee Approval: The local ethics committee approved the study before it was conducted (Tokat Gaziosmanpaşa University Clinical Research Ethics Committee/18.03.2021/21-KAEK-081).

Informed Consent: Informed consent was provided from all patients who wanted participated in the study.

Authorship Contributions:

Idea/Concept: ŞÇ, YD, Design: ŞÇ, EA, KK, Supervision: ŞÇ, YD, KK, Data Collection or Processing: ŞÇ, YD, EA, KK, Analysis or Interpretation: ŞÇ, KK, Literature Search: ŞÇ, Writing: ŞÇ, Critical Review: ŞŞÇ, YD, EA, KK, References And Fundings: -, Materials: ŞÇ, YD, EA, KK.

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REFERENCES

1. Arbyn M, Weiderpass E, Bruni L, et al. Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis. Lancet Global Health 2020;8:e191-e203.
2. Ueland FR. A perspective on ovarian cancer biomarkers: past, present and yet-to-come. Diagnostics 2017;7:14.
3. Larsen MJ, Thomassen M, Gerdes A-M, Kruse. Hereditary breast cancer: clinical, pathological and molecular characteristics. Breast Cancer 2014;8:145-55.
4. Toss A, Tomasello C, Razzaboni E, et al. Hereditary ovarian cancer: not only BRCA 1 and 2 genes. BioMed Res Int 2015;2015:341723
5. Miki Y, Swensen J, Shattuck-Eidens D, et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 1994;266:66-71.
6. Wooster R, Bignell G, Lancaster J, et al. Identification of the breast cancer susceptibility gene BRCA2. Nature 1995;378:789-92.
7. Mavaddat N, Antoniou AC, Easton DF, Garcia-Closas M. Genetic susceptibility to breast cancer. Mol Oncol 2010;4:174-91.
8. Brose MS, Rebbeck TR, Calzone KA, Stopfer JE, Nathanson KL, Weber BL. Cancer risk estimates for BRCA1 mutation carriers identified in a risk evaluation program. J Nat Cancer Inst 2002;94:1365-72.
9. Breast Cancer Linkage Consortium. Cancer risks in BRCA2 mutation carriers. J Nat Cancer Inst 1999;91:1310-6.
10. Burki TK. Oophorectomy in women with BRCA1 or BRCA2 mutations. Lancet Oncol 2014;15:e156.
11. Lostumbo L, Carbine NE, Wallace J. Prophylactic mastectomy for the prevention of breast cancer. Cochrane Database Syst Rev 2010;11:CD002748.
12. Kang E, Seong M-W, Park SK, et al. The prevalence and spectrum of BRCA1 and BRCA2 mutations in Korean population: recent update of the Korean Hereditary Breast Cancer (KOHBRA) study. Breast Cancer Res Treat 2015;151:157-68.
13. Wang F, Fang Q, Ge Z, Yu N, Xu S, Fan X. Common BRCA1 and BRCA2 mutations in breast cancer families: a meta-analysis from systematic review. Mol Biol Rep 2012;39:2109-18.
14. Economopoulou P, Dimitriadis G, Psyrri A. Beyond BRCA: new hereditary breast cancer susceptibility genes. Cancer Treat Rev 2015;41:1-8.
15. Neben CL, Zimmer AD, Stedden W, et al. Multi-gene panel testing of 23,179 individuals for

hereditary cancer risk identifies pathogenic variant carriers missed by current genetic testing guidelines. J Mol Diagn 2019;21:646-57.

16. Akcay IM, Celik E, Agaoglu NB, et al. Germline pathogenic variant spectrum in 25 cancer susceptibility genes in Turkish breast and colorectal cancer patients and elderly controls. Int J Cancer 2021;148:285-95.

17. LaDuca H, Stuenkel A, Dolinsky JS, et al. Utilization of multigene panels in hereditary cancer predisposition testing: analysis of more than 2,000 patients. Genet Med 2014;16:830-7.

18. Easton DF, Pharoah PD, Antoniou AC, et al. Gene-panel sequencing and the prediction of breast-cancer risk. N Eng J Med 2015;372:2243-57.

19. LaDuca H, Polley EC, Yussuf A, et al. A clinical guide to hereditary cancer panel testing: evaluation of gene-specific cancer associations and sensitivity of genetic testing criteria in a cohort of 165,000 high-risk patients. Genet Med 2020;22:407-15.

20. Susswein LR, Marshall ML, Nusbaum R, et al. Pathogenic and likely pathogenic variant prevalence among the first 10,000 patients referred for next-generation cancer panel testing. Genet Med 2016;18:823-32.

21. Taşçanov MB, Çitli Ş. Akut Koroner Sendromlu Genç Hastalarda Monosit Kemoatraktan Protein-1 ve CC Kemokin Reseptörü Tip 2 Gen Polimorfizmlerinin Klinik Önemi. Harran Üniversitesi Tıp Fakültesi Dergisi 2022;19:272-6.

22. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405-23.

23. Bisgin A, Sag SO, Dogan ME, et al. Germline landscape of BRCAs by 7-site collaborations as a BRCA consortium in Turkey. The Breast 2022;65:15-22.

24. Bahsi T, Erdem HB. Spectrum of BRCA1/BRCA2 variants in 1419 Turkish breast and ovarian cancer patients: a single center study. Turk J Biochem 2020;45:83-90.

25. Solmaz AE, Onay H, Yeniay L, et al. BRCA1-BRCA2 mutation analysis results in 910 individuals: Mutation distribution and 8 novel mutations. Cancer Genetics 2020;241:20-4.

26. Demir S, Tozkir H, Gurkan H, et al. Genetic screening results of individuals with high risk BRCA-related breast/ovarian cancer in Trakya region of Turkey. J BUON 2020;25:1337-47.

27. Gezdirici A, İli EG, Değirmenci B, et al. Hereditary Breast-Ovarian Cancer and BRCA1/BRCA2 Variants: A Single Center Experience. Acta Oncologica Turcica 2021;54:264-72

28. Abdel-Razeq H, Abujamous L, Jadaan D. Patterns and prevalence of germline BRCA1 and BRCA2 mutations among high-risk breast cancer patients in Jordan: a study of 500 patients. J Oncology 2020; 2020: 8362179.

29. Çelebi HBG, Bolat H. BRCA and non-BRCA Variants Detected by Next Generation Sequencing in Patients with Hereditary Breast and/or Ovarian Cancer Syndrome. Acta Oncologica Turcica 2022;55:77-84

30. Yazici H, Bitisik O, Akisik E, et al. BRCA1 and BRCA2 mutations in Turkish breast/ovarian families and young breast cancer patients. Br J Cancer 2000;83:737-42.

31. Kraus C, Hoyer J, Vasileiou G, et al. Gene panel sequencing in familial breast/ovarian cancer patients identifies multiple novel mutations also in genes others than BRCA1/2. Int J Cancer 2017;140:95-102.

32. Hauke J, Horvath J, Groß E, et al. Gene panel testing of 5589 BRCA 1/2□negative index patients with breast cancer in a routine diagnostic setting: results of the German Consortium for Hereditary Breast and Ovarian Cancer. Cancer Med 2018;7:1349-58.