Original Article

BRCA and non-BRCA Variants Detected by Next Generation Sequencing in Patients with Hereditary Breast and/or Ovarian Cancer Syndrome

Kalıtsal Meme ve/veya Over Kanseri Sendromlu Olgularda Yeni Nesil Dizileme ile Saptanan BRCA ve non-BRCA Varyantlar

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ABSTRACT

Introduction: Breast cancer is the most frequently diagnosed female cancer according to the 2020 data of the World Health Organization. It is mostly sporadic, 10-15% of which occur on the basis of genetic predisposition. In this study, we aimed to detect mutations in *BRCA* and *non-BRCA* genes in patients admitted with the diagnosis of breast cancer and/or ovarian cancer, and to identify mutations with increased frequency and variants specific to the Turkish population.

Materials and methods: Between January 2019 and August 2021, 120 patients who applied to our clinic with hereditary Breast and/or Ovarian Cancer meeting the genetic test study criteria were included in the study. First, *BRCA1/2* genes next-generation sequencing were performed on these patients, respectively. *BRCA1* and *BRCA2* gene deletion duplication analysis and/or multiple gene panel associated with cancer susceptibility were studied from patients with no mutations.

Results: In this molecular genetic susceptibility study associated with Hereditary Breast and/or Ovarian Cancer, 33.3 % positive variants were found in BRCA and non-BRCA genes. *BRCA1/2* mutations were detected in 20 patients. In addition, *non-BRCA* mutations (*ATM, CHEK2, RAD50, RAD51D, STK11, SDHA, RB1, POLD1, SMAD4, CDH1* and *CDKN22* genes) were detected in 20 patients. We identified a total of 7 new variants in the *BRCA2, ATM, RAD50, RAD51D, STK11* and *POLD1* genes.

Discussion: Clarification of risks specific to non-BRCA genes is necessary for a better understanding of the Hereditary Breast and/or Ovarian Cancer Syndrome (HBOC) genetic susceptibility spectrum. Therefore, multi-gene panel testing is needed after routine *BRCA* genes. In our study, most of the novel mutations were detected in *non-BRCA* genes. In addition, two novel *BRCA* variants have been reported. It also contributed to the identification of mutations specific to the Turkish population.

Keywords: BRCA1/2, non-BRCA, HBOC, Turkish population, Multi-gene panel test

ÖZET

Giriş ve Amaç: Meme kanseri Dünya Sağlık Örgütü'nün 2020 yılı verilerine göre en sık tanı alan kadın kanseridir. Çoğunlukla sporadik olmakla birlikte %10-15 oranında genetik yatkınlık zemininde ortaya çıkar. Meme kanseri ve/veya over kanseri tanısıyla başvuran hastalardaki genetik yatkınlığı araştırdığımız bu çalışmamızda amacımız *BRCA* ve *non-BRCA* genlerinde saptadığımız varyantları sunmak ve Türk popülasyonuna özgü mutasyonları belirmektir.

Yöntem ve Gereçler: Ocak 2019-Agustos 2021 tarihleri arasında klinigimize kalitsal Meme ve/veya Yumurtalik Kanseri ile basvuran, genetik test calisma kriterlerini karsilayan 120 hasta calismaya dahil edildi. Öncelikle sırasıyla *BRCA1* ve *BRCA2* geni dizi analizi çalışıldı. *BRCA1/2* tüm gen dizi analizi yöntemiyle mutasyon tespit edilmeyen hastalardan *BRCA1* ve *BRCA2* geni delesyon dublikasyon analizi ve/ veya kansere yatkınlıkla ilişkilendirilen çoklu gen paneli çalışıldı.

Bulgular: Herediter Meme ve/veya Over Kanseri ile ilişkili genetik etyolojiyi araştırdığımız bu çalışmada *BRCA* ve *non-BRCA* genlerde %33.3 pozitif varyant saptandı. 20 hastada *BRCA1* ve *BRCA2* genlerinde, 20 hastada ise *non-BRCA* genlerinde (*ATM, CHEK2, RAD50, RAD51D, STK11, MSH6,*

SDHA, RB1, POLD1, SMAD4, CDH1 ve *CDKN22*) mutasyon tespit edildi. *BRCA2, ATM, RAD50, RAD51D, STK11* ve *POLD1* genlerinde olmak üzere toplam 7 novel varyant tanımlandı.

Tartışma ve Sonuç: Herediter Meme ve/veya Over Kanseri genetik duyarlılık spektrumunun daha iyi anlaşılması için *non-BRCA* genlerine özgü risklerin aydınlatılması gerekmektedir. Bu nedenle rutin *BRCA* genlerinden sonra çoklu gen panel testine ihtiyaç vardır. Çalışmamızdaki, yeni mutasyonların çoğu *non-BRCA* genlerinde tespit edildi. Ek olarak, iki yeni *BRCA* varyantı raporlandı. Ayrıca bu çalışma ile Türk popülasyonuna özgü mutasyonların belirlenmesine de katkı sağlandı.

Anahtar Kelimeler: BRCA1/2, non-BRCA, HBOC, Türk popülasyonuna, Çoklu gen panel test

Introduction

Breast cancer is the most common type of cancer in both genders and all ages, according to the 2020 data of the World Health Organization-International Agency for Research on Cancer (IARC) [1,2]. Ovarian cancer is the most lethal gynecologic cancer in women [3]. Breast cancer is mostly sporadic, and genetic factors play an important role in 10-15% of cases [4].

Hereditary breast and/or ovarian cancer syndrome (HBOC) is characterized by a familial predisposition to cancers such as female and male breast cancer, ovarian cancer and less frequently pancreatic cancer, prostate cancer and melanoma [5]. Mostly, mutations in the *BRCA1* and *BRCA2* genes are associated with HBOC syndrome [6]. These genes are tumor suppressor genes that play critical roles in repair of DNA double strand breaks, homologous recombination and transcription [7,8]. *BRCA1* DNA repair-associated protein (*BRCA1* [MIM no:113705]) gene is located in the 17q21.31 chromosomal region. *BRCA1* gene consists of 24 exons and 23 coding exons, according to the Ensembl ENST00000471181.7 transcript. BRCA2 DNA repair-associated protein (BRCA2 [MIM no:600185]) gene map to 13q13.1. According to the ENST000000380152.8 ensembl transcript, 27 exons consist of 26 coding exons. The 11th exon of the *BRCA2* gene is the most mutated and largest coding part [9].

Mutations in the BRCA1/2 genes are responsible for approximately 25% of HBOC syndrome [10]. More than 50% of HBOC is thought to be caused by mutations in non-*BRCA* genes such *as CHEK2, CDH1, MLH1, MSH2, MSH6, PMS2, PALB2, RAD50, RAD51C, RAD51D, PTEN, STK11 and ATM* [11,12]. Most of these genes are tumor suppressor genes that play a role in DNA damage response by similar mechanisms [13]. It is estimated that the risks of developing breast and ovarian cancer are 87% and 44%, respectively, in individuals carrying *BRCA1/2* gene mutations until the age of 70 [14].

Since breast and ovarian cancers occur on the background of genetic predisposition, genetic analysis plays an important role in the treatment of cancer, screening for other cancer types with increased risk in their lives, and genetic counseling to other family members [15,16]. In conclusion, the genetic susceptibility spectrum needs to be better understood by elucidating the risks specific to *non-BRCA* genes beyond *BRCA1/2*, taking into account regional and ethnic differences. At the same time, the amount of targeted multigene panels has increased in standard clinical practise of HBOC cases with the rapid development of technology, reduced cost and increased accessibility in next generation sequencing (NGS) analyses.

In this study, we aimed to provide new information to the literature of our country by means of targeted multigene panels, where there is not enough data on *non-BRCA* genes yet, by performing molecular genetic analysis in accordance with international guidelines in cases applying for HBOC.

Materials and Methods

Patients

In the present study, 120 female patients who applied to Balıkesir Atatürk City Hospital Medical Genetics Department between

01.01.2019 and 01.08.2021 due to HBOC syndrome were included in the study according to criteria of the National Comprehensive Cancer Network (NCCN) [17]. The criteria used to access the test are: individual who have a pathogenic/probably pathogenic variant in their cancer susceptibility genes in any of their relatives, breast cancer diagnosed ≤45 years, breast and ovarian cancer, multiple primary breast cancers either in one or both breasts, male breast cancer triple negative (estrogen receptor-negative, progesterone receptor-negative, and HER2/neu-negative breast cancer diagnosed <60 years, bilateral breast cancer with the first diagnosed <50 years, personal history of breast cancer and one close blood relative with breast cancer <50 or ovarian cancer or pancreatic cancer. The mean age of the patients was 45 years (32-71 years). This study was conducted in Balikesir University Medical Faculty Health Sciences Ethics Committee dated 24.11.2021 and numbered 2021/260. The study was evaluated as a research file and it was decided that it was scientifically and ethically appropriate.

Genetic Analysis

Sequence analysis of the *BRCA1* and *BRCA2* genes, respectively, was studied from the patients. Then, *BRCA1* and *BRCA2* gene deletion duplication analysis and/or multi-gene panel associated with cancer susceptibility were studied from patients in whom no mutation was detected. The results of familial segregation analysis of patients with mutations were not included in this study.

DNA extraction

Approximately 2 ml of venous blood was collected from all patients in an EDTA tube. DNA was isolated from 200 μl peripheral blood samples from patients using QIAamp DNA Blood Mini Kit (Qiagen Inc.).

BRCA1/2 gene next-generation sequencing

All the coding exons, with flanking intron regions, of the *BRCA1/2* gene were amplified by the polymerase chain reaction and sequenced using next-generation sequencing (NGS). After library enrichment (MiSeq Reagent Kit v2, MS-102-2003) and quality control, the samples were sequenced using the MiSeq platform (Illumina, San Diego, California, United States). Raw reads were quality trimmed with Trimmomatic and were mapped to reference human genome (hg19) with using BWA (Burrows-Wheeler Alignment Tool). Duplicates were removed using SAMTools and realignment across indels and base quality recalibration were performed with GATK. Annotation of detected variants were performed using Illumina BaseSpace Variant Interpreter, InterVar, Franklin, VarSome, ClinVar, OMIM, and Pubmed. Variants with a frequency higher than 0.5% were filtered out. dbNSFP (contains SIFT, PolyPhen-2, LRT, Mutation Taster) was used to predict the pathogenicity about the deleteriousness of variants. Rare variants were classified according to the ACMG/AMP variant interpretation framework.

BRCA1/2 gene Deletion/Duplication Analysis

BRCA1 gene exon deletion/duplication was investigated by multiplex ligation-dependent probe amplification (MLPA) analysis. SALSA® Probemix P002 *BRCA1* MLPA® kit was used to analyze all the coding exons as described by manufacturer's recommendation (MRCHolland, Amsterdam, Netherlands). The reactions were run and analyzed on an ABI Prism 3130xl DNA Sequencer (Applied Biosystems, Foster City, CA, USA) and Coffalyser.Net data analysis software.

BRCA2 gene exon deletion/duplication was investigated by multiplex ligation-dependent probe amplification (MLPA) analysis. SALSA® Probemix P090 *BRCA2* MLPA® kit was used to analyze all the coding exons as described by manufacturer's recommendation (MRCHolland, Amsterdam, Netherlands). The reactions were run and analyzed on an ABI Prism 3130xl DNA Sequencer (Applied Biosystems, Foster City, CA, USA) and Coffalyser.Net data analysis software.

Targeted multigene panel analysis

A custom target enrichment panel was designed to capture 19 genes (*TP53, RAD51D, SMAD4, PTEN, ATM, RB1, PALB2, CDKN2A, RAD50, STK11, RAD51C, MSH2, MSH6, PMS2, CDH1, BLM, CHEK2, MEN1, MUTYH*) related with hereditary cancer. All exons, the 25 base pairs of intronic flanking region and 5′ and 3′ untranslated region of each gene were sequenced. After library enrichment and quality control, the samples were sequenced using the MiSeq platform (Illumina, San Diego, California, USA). Raw reads were trimmed with Trimmomatic and were mapped to reference human genome (hg19) with using the Burrows-Wheeler Aligner (BWA). Duplicates were removed using SAMTools and realignment across indels and base quality recalibration were performed with Genome Analysis Toolkit (GATK). Variants with a frequency higher than 0.5% were filtered out. Annotation of detected variants were performed using, Franklin (https://franklin.genoox.com/ clinical-db/home), VarSome (https://varsome.com/), ClinVar (https:// www. ncbi.nlm. nih.gov/ clinvar/), Online Mendelian Inheritance in Man (OMIM) and Pubmed. Rare variants were classified according to the ACMG/AMP variant interpretation framework [18]. The following public databases were used for the interpretation of the variants: ClinVar, LOVD (https://databases.lovd.nl/shared/genes), the Human Genome Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/ac/index.php), and BRCA Exchange database (https:// brcaexchange. org/). Mutations in all patients were classified according to ACMG criteria into three categories: pathogenic, likely pathogenic, and uncertain significance. Likely benign and benign variants according to ACMG have not been reported.

Results

In our study, BRCA1/2 mutations including 15 pathogenic, one likely pathogenic and four uncertain significant mutations were detected in 20 (16.66%) patients. At the same time, three pathogenic, five likely pathogenic, and 13 uncertain significance mutations in *non-BRCA* genes in 20 (16.66%) patients. *BRCA1* mutations in four (3.33%) patients, *BRCA2* mutations in 11 (9.16%) patients, *ATM* mutations in two (1.66%) patients, and *POLD1* mutation in one (0.83%) patients, a total of 18 (15%) patients pathogenic variant was detected. In addition, one (0.83%) *BRCA2* gene, two (1.66%) *CHEK2* gene, one (0.83%) *ATM* gene, one (0.83%) *RB1* gene and one (0.83%) *CDKN2* gene, a total of six (5%) likely pathogenic variants were detected. Totally, 17 (14.16%) uncertain significance variants were detected, four (3.33%) *BRCA2,* three (2,5%) *ATM*, two (1.66%) *CHEK2*, two (1.66%) *RAD50*, two (1.66%) *SMAD4* and one each in the *RAD51D, STK11, SDHA, CDH1* genes. this study (Table1, Table 2).

Double mutation carriers in different non-*BRCA* genes were found in one case. A 45-year-old patient with breast cancer was found to be a carrier of double mutations, c.670C>T likely pathogenic in the *CHEK2* gene and c.7475T>G mutation of uncertain significance in the ATM gene. In the patient with invasive ductal cancer type, ER 98%, PR 50%, Ki 67 40%, cerb-B2 +3 strong membranous positive staining was found. Diffuse staining was observed with E-cadherin. The patient had relatives with breast cancer and all three daughters suffered from fibroadenomas beginning in the second decade.

Discussion

In this study, routine *BRCA1/2* molecular genetic analyzes and Targeted Multigene Panel analysis for *non-BRCA* genes recommended in international guidelines were performed on hereditary breast and/or ovarian cancer cases. *BRCA1/2* mutation carrier was detected in 20 (16.66%) patients, with 12.5% pathogenic, 0.83% likely pathogenic, and 3.33% uncertain significance. In the study of Bahsi et al., which included the largest number of patients (1419 cases) in the Turkish population, 9.4% pathogenic, 0.3% likely pathogenic, 6.4% uncertain significance BRCA1/2 mutation was detected [5]. The frequency of BRCA1/2 mutations, which varies between different populations and different ethnic groups, is approximately between 5% and

Table 1**:** *BRCA1/2* mutations and their classification according to ACMG

The gene and transcript	Number	dbSNP ID	Mutation	HGVS protein reference	Variant type	ACMG Classification
BRCA1 gene: NM_007300.4	3	rs80357906	c.5329dup	p.Gln1777ProfsTer74	Insertion	Pathogenic
	1	rs80357626	c.2019del	p.Glu673AspfsTer28	Deletion	Pathogenic
BRCA2 gene: NM_000059.4	1	rs397507683	c.3751dup	p.Thr1251AsnfsTer14	Insertion	Pathogenic
	1	rs80359732	c.8940del	p.Glu2981LysfsTer7	Deletion	Pathogenic
	1	rs80359672	c.7673_7674del	p.Glu2558ValfsTer7	Deletion	Pathogenic
	1	rs80359212	c.9382C>T	p.Arg3128Ter	Nonsense	Pathogenic
	1	rs276174813	c.1796_1800del	p.Ser599Ter	Deletion	Pathogenic
	1	rs398122761	c.3302A>G	p.His1101Arg	Missense	Uncertain significance
	1	Novel	c.2117C>T	p.Ala706Val	Missense	Uncertain significance
	1	rs587778119	c.2892A>T	p.Lys964Asn	Missense	Uncertain significance
	1	rs397507639	c.2765dup	p.Lys923GlnfsTer13	Insertion	Pathogenic
	1	Novel	c.682A>C	p.Asn228His	Missense	Likely pathogenic
	1	rs397507639	c.2765dup	p.Lys923GlnfsTer13	Insertion	Pathogenic
	2	rs80359502	c.5270_5286del	p.Tyr1757SerfsTer5	Deletion	Pathogenic
	1	rs876658951	c.8494G>T	p.Glu2832Ter	Nonsense	Pathogenic
	1	rs80359460	c.4631dup	p.Asn1544LysfsTer4	Insertion	Pathogenic
	1	rs786201837	c.2779A>G	p.Met927Val	Missense	Uncertain significance

Table2. Non-*BRCA* mutations and their classification according to ACMG

The gene and transcript	Number	dbSNP ID	Mutation	HGVS protein reference	Variant type	ACMG Classification
ATM gene: NM_000051.4	1	Novel	c.658G>A	p.Ala220Thr	Missense	Uncertain significance
	1	rs1591475608	c.487C>T	p.Gln163Ter	Missense	Pathogenic
	1	rs56399857	c.7475T>G	p.Leu2492Arg	Missense	Uncertain significance
	1	rs567060474	c.6820G>A	p.Ala2274Thr	Missense	Likely pathogenic
	1	rs730881329	c.8762C>T	p.Thr2921Met	Missense	Uncertain significance
	1	rs762083530	c.4852C>T	p.Arg1618Ter	Nonsense	Pathogenic
CHEK2 gene: NM_001005735.2	1	rs137853010	c.670C>T	p.Arg224Cys	Missense	Likely pathogenic
	1	rs587782268	c.1182G>T	p.Glu394Asp	Missense	Uncertain significance
	1	rs531398630	c.1240C>T	p.His414Tyr	Missense	Uncertain significance
	1	rs200050883	c.1441G>T	p.Asp481Tyr	Missense	Likely pathogenic
RAD50 gene: NM_005732.4	1	Novel	c.89C>T	p.Pro30Leu	Missense	Uncertain significance
	1	rs757043253	c.2204T>A	p.Met735Lys	Missense	Uncertain significance
RAD51D gene: NM_002878.4	1	Novel	c.208G>C	p.Asp70His	Missense	Uncertain significance
STK11 gene: NM_000455.5	1	Novel	c.263T>C	p.Ile88Thr	Missense	Uncertain significance
SDHA gene: NM_004168.4	1	rs112307877	c.1945_1946del	p.Leu649GlufsTer4	Deletion	Uncertain significance
RB1 gene: NM_000321.3	1	rs138201027	c.1546T>G	p.Trp516Gly	Missense	Likely pathogenic
POLD1 gene:NM_001256849.1	1	Novel	c.2820+2T>C	-	Splicing	Pathogenic
SMAD4 gene: NM_005359.6	1	rs1568205010	c.512C>T	p.Ser171Leu	Missense	Uncertain significance
	1	rs1599204052	c.1321C>T	p.Arg441Cys	Missense	Uncertain significance
CDH1 gene: NM_004360.5	1	rs1182000968	c.2312A>G	p.Gln771Arg	Missense	Uncertain significance
CDKN2 gene: NM_000077.5	1	rs587782797	c.335G>A	p.Arg112His	Missense	Likely pathogenic

26.1% in Turkish population [5,19-21]. Our study is similar to other publications in terms of BRCA1/2 mutation carriers in Turkish population. The most common c.5329dup (2.5%) mutation was found in the *BRCA1* gene. This result was similar to large studies in the Turkish population [5, 20]. However, there was no *BRCA2* mutation with increasing frequency. At the same time, two novel variants of c.682A>C likely pathogenic and unceratin significance c.2117C>T in the *BRCA2* gene were detected.

Targeted multigene panel analysis is increasingly used to screening for patients with HBOC [22]. We also identified 21 mutations in 20 (17.5%) patients in 12 different *non-BRCA* genes, including *ATM, CHEK2, RAD50, RAD51D, STK11, MSH6, SDHA, RB1, POLD1, SMAD4, CDH1* and *CDKN2*. We reported five novel mutations in different genes, including *ATM, RAD50, RAD51D, STK11* and *POLD1*. In our study, the most common mutation in the *ATM* gene (5%) was found among the *non-BRCA* genes. The prevalence of moderate-penetrance gene *ATM* mutation carriers in breast cancer is 2-4% [23, 24].

We identified 24 (20%) pathogenic or likely-pathogenic variants (PV) in 24 of 120 (20%) participants, including seven insertions, six deletions and seven missense, three nonsense, one splice variants among: *BRCA1, BRCA2, ATM, CHEK2, RB1, POLD1* and *CDKN2* genes. To date, approximately 3000 *BRCA1*, 3500 *BRCA2* pathogenic mutations have been identified in the Clinvar database [25]. It is now very difficult to find novel mutations in *BRCA1/2* genes. While no novel *BRCA1/2* mutations were reported in the report by Bahsi et al., which includes the largest Turkish population (1419 patients), we detected two novel *BRCA2* variants in our study. We found 17 (14.16%) uncertain significance mutations, including 16 missense-type and one deletion-type. The uncertain significance mutations reported here will also contribute to future studies in larger patient groups.

BRCA mutations are responsible for one quarter of HBOC syndrome, yet there is not enough information about the *non-BRCA* genes responsible for the vast majority. Clarification of risks specific to *non-BRCA* genes is necessary for a better understanding of the HBOC genetic susceptibility spectrum. In conclusion, a better understanding of the genetic susceptibility spectrum and clarification of risks specific to *non-BRCA* genes are required. This report supports studies to identify mutations specific to Turkish people.

REFERENCES

1.Marmolejo DH, Wong MYZ, Bajalica-Lagercrantz S, et al. Overview of hereditary breast and ovarian cancer (HBOC) guidelines across Europe. European Journal of Medical Genetics. 2021: 104350.

2.World Health Organization International Agency for Research on Cancer. The global cancer observatory. 2020 statistics. Available from: https:// gco.iarc.fr /today/ online-analysis-multi-bars.

3.Mallen AR, Townsend MK, Tworoger SS. Risk factors for ovarian carcinoma. Hematology/Oncology Clinics. 2018;32: 891-902.

4.Mittal A, Deo SVS, Gogia A, et al. Profile of pathogenic mutations and evaluation of germline genetic testing criteria in consecutive breast cancer patients treated at a North Indian tertiary care center. Annals of Surgical Oncology. 2021: 1-10.

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

6. Judkins T, Rosenthal E, Arnell C, et al. Clinical significance of large rearrangements in BRCA1 and BRCA2. Cancer. 2012;118: 5210-5216.

7. Miramar MD, Calvo MT, Rodriguez A, et al. Genetic analysis of BRCA1 and BRCA2 in breast/ovarian cancer families from Aragon (Spain): two novel truncating mutations and a large genomic deletion in BRCA1. Breast Cancer Research and Treatment. 2008;112: 353-358.

8. Thirthagiri E, Lee SY, Kang P, et al. Evaluation of BRCA1 and BRCA2 mutations and risk-prediction models in a typical

Asian country (Malaysia) with a relatively low incidence of breast cancer. Breast Cancer Research. 2008;10: 1-12.

9. Karami F., Mehdipour P. A comprehensive focus on global spectrum of BRCA1 and BRCA2 mutations in breast cancer. BioMed research international. 2013.

10.Teker ND, Eyerci N. Double Heterozygous Mutations in the BRCA2 and ATM Genes: A Case Report and Review of the Literature. Breast Care. 2021;16: 412-417.

11.Tedaldi G, Tebaldi M, Zampiga V, et al. Multiple-gene panel analysis in a case series of 255 women with hereditary breast and ovarian cancer. Oncotarget. 2017;8: 47064-47075

12.Pinto P, Paulo P, Santos C, et al. Implementation of next-generation sequencing for molecular diagnosis of hereditary breast and ovarian cancer highlights its genetic hetero-geneity. Breast cancer research and treatment. 2016; 159: 245-256.

13.Tung N, Lin NU, Kidd J, et al. Frequency of germline mutations in 25 cancer susceptibility genes in a sequential series of patients with breast cancer. Journal of Clinical Oncology. 2016;34: 1433-1455.

14.Hall MJ, Reid JE, Burbidge LA, et al. BRCA1 and BRCA2 mutations in women of different ethnicities undergoing testing for hereditary breast-ovarian cancer. Cancer, 2009;115: 2222-2233.

15.LaDuca H, Stuenkel AJ, Dolinsky JS, et al. Utilization of multigene panels in hereditary cancer predisposition testing: analysis of more than 2,000 patients. Genetics in medicine. 2014;16: 830-837.

16.Pashayan N, Antoniou AC, Ivanus U, et al. Personalized early detection and prevention of breast cancer: ENVISION consensus statement. Nature Reviews Clinical Oncology. 2020;17: 687-705.

17. Goetz MP, Gradishar WJ, Anderson BO, et al. NCCN Guidelines Insights: Breast Cancer, Version 3.2018. J Natl Compr Canc Netw. 2019;17(2):118-126.

18.Richards S, Aziz N, Bale S, et al. ACMG Laboratory Quality Assurance Committee. 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: 405–24

19.Yazici H, Bitisik O, Akisik E, et al. BRCA1 and BRCA2 mutations in Turkish breast/ ovarian families and young breast cancer patients. British Journal of Cancer. 2000;83: 737-742.

20.Cecener G, Takanlou LB, Takanlou MS, et al. Clinicopathologic Features and Genetic Characteristics of the BRCA1/2 Mutation in Turkish Breast Cancer Patients. Journal Pre-proof. S2210-7762 (19)30335-7.

21. Gezdirici A, Gökpınar İli E, Değirmenci B, et al. Hereditary Breast-Ovarian Cancer and BRCA1/BRCA2 variants: a single center experience. Acta Oncologica Turcica, 54(3), 264-272.

22. 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. International journal of cancer. 2017;140: 95-102.

23. Marabelli M, Cheng SC, Parmigiani G. Penetrance of ATM gene mutations in breast cancer: a meta-analysis of different measures of risk. Genetic epidemiology, 2016;40: 425-431.

24.Tung N, Lin NU, Kidd J, et al. Frequency of germline mutations in 25 cancer susceptibility genes in a sequential series of patients with breast cancer. Journal of Clinical Oncology. 2016;34: 1460.

25. ClinVar: https://www.ncbi.nlm.nih.gov/clinvar. Accessed 7 Sept 2020.

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