Original Article

Hereditary Breast-Ovarian Cancer and BRCA1/BRCA2 Variants: A Single Center Experience

Herediter Meme-Over Kanseri ve BRCA1/BRCA2 Varyantları: Tek Merkez Deneyimi

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ABSTRACT

Objective: In this study, it was aimed to determine the frequency of BRCA1 and BRCA2 variants in patients admitted to our clinic with hereditary breast-ovarian cancer and / or family history and to evaluate them in the light of the literature.

Materials and Methods: All patients in our study were selected according to the current NCCN guideline test criteria. The Ion Torrent ™ Oncomine ™ BRCA Research Assay was used to sequence the coding regions of the BRCA1 and BRCA2 genes in our patients. In addition, all patients with copy number changes were confirmed with SALSA® MLPA® Probemix P002 BRCA1 and Probemix P090 BRCA2 (MRC Holland).

Results: Variants (pathogenic, likely pathogenic, variants of uncertain clinical significance, and copy number variations) were detected in 39 of the 149 patients included in the study. Novel variants that were not previously described in the literature were detected in two patients, one of the BRCA1 and one of the BRCA2 gene, respectively.

Conclusion: In our study, the incidence of BRCA1 and BRCA2 variants was found to be 26.1%. This rate was higher than previous studies conducted in Turkey. Further studies are needed to identify common variants in the Turkish population and to evaluate the pathogenity of variants of uncertain clinical significance.

Keywords: Hereditary cancer, breast cancer, ovarian cancer, BRCA1, BRCA2

ÖZET

Amaç: Bu çalışmada kliniğimize herediter meme-over kanseri ve/veya aile öyküsü nedeniyle başvuran hastalardaki BRCA1 ve BRCA2 varyantlarının sıklığının tespiti ve literatür eşliğinde değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntem: Çalışmamızdaki tüm hastalar güncel NCCN rehberi test kriterleri doğrultusunda seçilerek dahil edilmiştir. Hastalarımızda BRCA1 ve BRCA2 genlerinin kodlayıcı bölgelerini dizilemek için Ion Torrent™ Oncomine™ BRCA Research Assay kullanılmıştır. Ayrıca kopya sayısı değişiklikleri tespit edilen tüm hastalar SALSA® MLPA® Probemix P002 BRCA1 ve Probemix P090 BRCA2 (MRC Holland) ile konfirme edildi.

Bulgular: Çalışmaya dahil edilen toplam 149 hastanın 39'unda varyantlar (patojenik, muhtemel patojenik, klinik önemi belirsiz varyantlar ve kopya sayısı değişiklikleri) tespit edilmiştir. İki hastamızda (Biri BRCA1 geninde, biri BRCA2 geninde) daha önce literatürde tanımlanmamış yeni varyantlar tespit edilmiştir.

Sonuç: Çalışmamızda BRCA1 ve BRCA2 varyantlarının görülme sıklığı %26,1 olarak belirlendi. Bu oran Türkiye'de yapılan önceki çalışmalara göre daha yüksek bulundu. Türk toplumundaki sık varyantların ve özellikle klinik önemi belirsiz varyantların patojenitesinin daha net değerlendirilebilmesi için daha fazla çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: Herediter kanser, meme kanseri, over kanseri, BRCA1, BRCA2

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Introduction

According to the WHO 2020 records, more than 2,250,000 individuals have been diagnosed with breast cancer and the breast cancer has become the most common type of cancer in the world. More than 300,000 women were also diagnosed with ovarian cancer[1]. Many molecular pathways, both genetically and epigenetically, play role in the etiopathogenesis of breast cancer and ovarian cancer. Both cancers show genetic heterogeneity in terms of clinical and biological features. Most cancer cases are considered to be sporadic appearing tumors because there is no clear family history, but cancer syndromes with a known genetic cause or hereditary predisposition to cancer have also been identified [2]. Individuals carrying an inherited genetic mutation and epigenetic abnormalities in tumor suppressor genes have an increased risk of developing cancer throughout their lifetime. Germline mutations in cancer susceptibility genes cause cancer if the normal allele is lost or inactivated. Breast and ovarian cancers (5-10%) can be inherited and occur with cancer-prone syndromes [3].

There are many genes that can increase the risk of developing breast and/or ovarian cancer. In the early 1990s, the BRCA1 and BRCA2 genes were identified as associated with breast and ovarian cancer [4, 5]. Hereditary breast and ovarian cancer (HBOC) caused by pathogenic variants in the BRCA1 and BRCA2 genes is the best known and most common form. It occurs in all ethnic populations. The prevalence of BRCA1/2 pathogenic variants in the population is estimated to be 1/400 to 1/500 [6]. International guidelines such as NCCN state that patients with suspected hereditary breast cancer and all women with epithelial ovarian cancer should seek genetic counseling and comprehensive genetic testing should be recommended. In centers with suitable conditions, patients should be directed to genetic counselors. Before genetic tests, a comprehensive risk assessment should be done to patients and their relatives.

HBOC is a well-known cancer syndrome in which BRCA pathogenic variants are responsible for up to 80% [7]. In addition, high risk gene variants (PALB2, TP53, PTEN) or intermediate risk gene variants (ATM, CHEK2) are also associated with HBOC syndrome [8]. According to a metaanalysis study, individuals with HBOC syndrome have a lifetime risk of developing ovarian cancer (40% for BRCA1 variants and 18% for BRCA2 variants by the age of 70) and/or breast cancer (57% for BRCA1 variants and 49% for BRCA2 variants by the age of 70) [9]. Genetic testing is now widely recommended in cancer diagnosis all around the world and may have the potential to influence treatment decisions. For example, current guidelines recommend the use of poly ADP-Ribose polymerase inhibitors (PARPi) in treatment protocols for patients with BRCA 1/2-related cancer [10]. Therefore, it is very important to determine cancer-related genetic etiology in patients. Identifying pathogenic variant carriers and individuals at risk may reduce morbidity and mortality from cancer. Identifying pathogenic variants in at-risk individuals, it may significantly influence disease course by giving individuals the opportunity to evaluate risk-reducing strategies, such as enhanced surveillance, variant-specific next-generation treatments or surgical interventions.

The National Comprehensive Cancer Network (NCCN) has published recommendations to assist clinicians in identifying individuals with hereditary cancer syndrome, and these recommendations are frequently updated. In the presence of any of the following criteria, there is an indication for genetic testing for hereditary breast, ovarian and pancreatic cancer (Table-1) [11].

After genetic counseling and a comprehensive risk assessment, there may be differences in

Table 1. Testing criteria for breast and/or ovarian cancer susceptibility genes

1.	Individuals with any blood relative with a known Pathogenic/Likely pathogenic variant in a cancer susceptibility gene
2.	Individuals meeting the criteria below but tested negative with previous limited testing (eg, single gene and/or absent deletion duplication analysis) interested in pursuing multigene testing
3.	Personal history of cancer • Breast cancer with at least one of the following ➢ Diagnosed at age ≤45 y; or ➢ Diagnosed at age 46-50 y with ▪ Unknown or limited family history; or ▪ A second breast cancer diagnosed at any age; or ▪ ≥1 close blood relative with breast, ovarian, pancreatic or prostate cancer at any age ➢ Diagnosed at age ≤60 y with triple-negative breast cancer ➢ Diagnosed at any age with; ▪ Ashkenazi Jewish ancestry; or ▪ ≥1 close blood relative with breast cancer at age ≤50 y ovarian, pancreatic, metastatic, intraductal/cribriform histology or high- or very-high-risk group prostate cancer at any age; or ▪ ≥3 total diagnoses of breast cancer in patient and/or close blood relatives ➢ Diagnosed at any age with male breast cancer • Epithelial ovarian cancer (including fallopian tube cancer or peritoneal cancer) at any age • Exocrine pancreatic cancer at any age • Prostate cancer at any age with ➢ metastatic, intraductal/cribriform histology or high- or very-high-risk group ➢ Any NCCN risk group with the following family history: ▪ Ashkenazi Jewish ancestry; or ▪ ≥1 close blood relative with breast cancer at age ≤50 y ovarian, pancreatic, metastatic or intraductal/cribriform prostate cancer at any age; or ▪ ≥2 close relative with either breast or prostate cancer (any grade) at any age • A mutation identified on tumor genomic testing that has clinical implications if also identified in the germline • Individuals who meet Li-Fraumeni syndrome testing criteria or Cowden syndrome/PTEN hamartoma tumor syndrome testing criteria • To aid in systemic therapy decision-making, such as for HER2-negative metastatic breast cancer
4.	Family history of cancer • An affected or unaffected individual with a first- or second-degree blood relative meeting any of the criteria listed above ➢ If the affected relative has pancreatic cancer or prostate cancer, only first-degree relatives should be offered testing unless indicated for other relatives based on additional family history • An affected or unaffected individual who otherwise does not meet the criteria above but has a probability ≥5% of a *BRCA1/2* pathogenic variant based on prior probability models.

choosing the appropriate test for individuals. Preferably, the analysis of BRCA1 and BRCA2 genes, which are known to be associated with hereditary breast and ovarian cancer, is generally a suitable option. However, with the advancing new technologies in recent years, multiple gene panels including BRCA1 and BRCA2 genes can be preferred at the first stage [12].

Material-Method

A total of 149 patients who applied to Başakşehir Çam and Sakura City Hospital, Medical Genetics Department between May 2020 and April 2021 were included in our study. Our study was conducted in accordance with the Declaration of Helsinki and was approved by ethics committee of Basaksehir Çam and Sakura City Hospital (KAEK/ 2021.04.57).

All included patients were selected according to the current NCCN genetic testing criteria in relation to hereditary breast-ovarian cancer syndrome. We examined the variants in the BRCA1 and BRCA2 genes via the next generation sequencing. In addition, we performed CNV analyzes of these genes and also confirmed with MLPA in some of our patients.

Genomic DNA was isolated from peripheral blood of patients after the completion of a consent form. DNA isolations were made from sterile 2 ml EDTA peripheral blood samples using PureLink ™ Genomic DNA Mini Kit. After DNA isolation, the densities of the DNA samples to be included in the study were measured with the Qubit dsDNA HS Assay Kit with the fluorometric method (Qubit® 4.0 Fluorometer), and the final DNA concentrations were ensured to be in the desired range (5-10 ng/µl) for next generation sequencing. Library preparation, BRCA1 and BRCA2 genes were amplified using Oncomine BRCA panel pools using Ion AmpliSeq Library Kit Plus (Life Technologies) in accordance with company protocols. Library products were created in 200 bp fragments. Library products were barcoded with the IonXpress Barcode Adapters Kit (Life Technologies). In order to remove other materials and enzymes from the barcoded samples, the unproduced library products were enzymatically purified by FuPa. At this stage, the application of emulsion PCR and enrichment processes to the normalized library products and the purification and sequencing of the enriched PCRs, the clonal reproduction of the library products containing the target regions by creating oil-water emulsions and loading them on the reading chips were performed on the Ion Chef ™ device. Ion AmpliSeq ™ Library Kit Plus was used for sequencing. In accordance with company protocols; for reading libraries on the Ion GeneStudio S5 Plus sequencer, the enriched products were loaded into the chip (Ion 520 ™ Chip) on the Ion Chef ™ instrument. After sequencing, the BAM files belonging to the data transferred to the Torrent server software were transferred to the Ion Reporter software for analysis. Each patient was analyzed with the programs Ion Reporter™ 5.16.0.3 and The Integrative Genomics Viewer (IGV) with this flowchart. The variants were classified according to the open access databases and ACMG guidelines, and their pathogenicity was determined. Sanger validation was performed for: homo-polymer regions, low quality variants, insertions and/or deletions, splice site alterations and novel variants. Since the reading depths of the samples are at least 300x, CNV (copy number variation) analysis for each sample was also analyzed with Ion Reporter ™ 5.16.0.3 program.

All CNVs detected via the analysis of next generation sequencing data were confirmed by MLPA. SALSA® MLPA® Probemix P002 BRCA1 (MRC Holland) and SALSA® MLPA® Probemix P090 BRCA2 (MRC Holland) were used in combination with a

SALSA MLPA reagent kit according to manufacturer's guidelines. CNV analysis was performed using Coffalyser.Net data analysis software.

Results

A total of 149 patients with breast and / or ovarian cancer or family history selected in line with the NCCN guidelines were included in our study. Variants (Pathogenic, likely pathogenic, VUS and CNVs) in BRCA1 and BRCA2 genes were detected in (26,1%) 39 of 149 patients. While a total of 14 different BRCA1 variants (single nucleotide variations and small indels) were detected in 17 patients, a total of 7 different BRCA2 variants were detected in 8 patients. When these 14 different BRCA1 variants were classified according to the ACMG guideline, 10 of these were considered pathogenic and four of these were considered variant of uncertain significance (VUS). When BRCA2 variants were evaluated according to the ACMG guideline, five of these were considered pathogenic and two of these were considered VUS. To the best of our knowledge, two novel variants were detected which have not been reported in the literature and public databases previously, one in the BRCA1 gene and one in the BRCA2 gene.

When the patients are evaluated according to the application reason, BRCA1 variants were found in 7 of 15 patients and BRCA2 variants were found in 8 of 15 patients tested for breast cancer. 9 patients were tested because of the ovarian cancer. BRCA1 variants were found in 7 of these patients and BRCA2 variants were found in two of these patients. The remaining five patients were evaluated because of their family history of cancer. BRCA1 variants were detected in four and BRCA2 variant in one of these patients.

In addition, as a result of CNV analysis, various deletions and duplications containing one or more exons in the BRCA1 and BRCA2 genes were detected in 11 patients. CNVs were detected in the BRCA1 gene in 7 individuals from five families. Also, CNV was detected in the BRCA2 gene in four individuals from the same family. All of these CNVs were confirmed by MLPA analysis. Detailed information about the patients detected variants are summarized in Table-2 and 3.

Discussion

In the literature, there are many studies conducted in many ethnic groups related to hereditary breast and ovarian cancer and BRCA1/ BRCA2 genes. The rate of detecting genetic variants in these studies varies according to the characteristics of the patients analyzed. For example, in a study of 517 patients in Jordan, pathogenic or likely pathogenic BRCA1 or BRCA2 variants were detected in 72 (13.9%) patients in the whole group in the BRCA1 (n=24, 4.6%) and BRCA2 (n = 48, 9.3%) genes, while VUS was reported in 53 (10.3%) patients [13].

Pathogenic BRCA1/ 2 variants were detected in 13 of 65 patients in a single-center study conducted in Japan in which individuals with triple negative breast cancer were examined. The reason for the small number of patients in the study was emphasized as that the BRCA1/ 2 genetic tests are not under the guarantee of the national health system [14].

There are also several studies conducted in different centers in Turkey associated with the BRCA1 and BRCA2 genes and HBOC recently. In the study of Solmaz et al. published in 2020, variants were detected in 85 of 910 (9.34%) patients selected according to the genetic test criteria in line with the NCCN guidelines. They have determined 31 different variants of the BRCA1 gene in 41 patients and 37 different variants of BRCA2 genes in 44 patients [15]. In another study in published in 2020 conducted in the Thrace region of Turkey, 39 different variants were identified in (17.8%) 88 out of a total of 493

Table 2. *BRCA1* and *BRCA2* sequence variants and pathogenicity classifications of our patients

Patient No	Age	Family History	Reason for application	Gene	Transcript	Location	cDNA change	Protein change	Varyant type	dbSNP	ACMG Classification
1	63	(-)	Ovarian cancer	BRCA1	NM_007300.4	Exon 4	c.181T>G	(p.Cys61Gly)	SNV	rs28897672	Pathogenic (PM1,PM2,PM5,PP3,PP5,BP1)
2	39	(-)	Breast Cancer	BRCA1	NM_007300.4	Exon 10	c.1259A>G	p.Asp420Gly	SNV	rs730881442	VUS (PM2)
3	39	(+)	Multiple cancer history in the family	BRCA1	NM_007300.4	Exon 10	c.1286T>C	p.Ile429Thr	SNV	rs775869160	VUS (PM2,PM3,BP1)
4	38	(+)	Multiple cancer history in the family	BRCA1	NM_007300.4	Exon 10	c.1286T>C	p.Ile429Thr	SNV	rs775869160	VUS (PM2,PM3,BP1)
5	37	(+)	Ovarian cancer history in the family	BRCA1	NM_007300.4	Exon 10	c.1504_1508delTTAAA	p.Leu502AlafsTer2	Deletion	rs80357888	Pathogenic (PVS1, PM2, PP3, PP5).
6	48	(-)	Ovarian cancer	BRCA1	NM_007300.4	Exon 10	c.2131_2132delAA	p.Lys711ValfsTer6	Deletion	rs398122653	Pathogenic (PVS1,PM2,PP5)
7	57	(+)	Breast Cancer	BRCA1	NM_007300.4	Exon 10	c.2666 C>T	p.Ser889Phe	SNV	rs769712441	VUS (PM2,BP1)
8	39	(+)	Ovarian cancer	BRCA1	NM_007300.4	Exon 10	c.3333delA	p.Glu1112AsnfsTer5	Deletion	rs80357966	Pathogenic (PVS1,PM2,PP5)
9	51	(+)	Ovarian cancer	BRCA1	NM_007300.4	Exon 10	c.3477_3480delAAAG	p.Ile1159MetfsTer50	Deletion	rs80357781	Pathogenic (PVS1,PM2,PP3,PP5)
10	60	(+)	Ovarian cancer	BRCA1	NM_007300.4	Exon 10	c.4036delG	p.Glu1346LysfsTer20	Deletion	rs886040189	Pathogenic (PVS1,PM2,PP3,PP5)
11	24	(-)	Breast Cancer	BRCA1	NM_007300.4	Exon 12	c.4246G>C	p.Ala1416Pro	SNV	**Novel**	VUS(PM2,PP3,BP1)
12	72	(+)	Breast Cancer	BRCA1	NM_007300.4	Exon 18	c.5159G>A	p.Arg1720Gln	SNV	rs41293459	Pathogenic (PM1,PM2,PM5,PP3,PP5,BP1)
13	43	(+)	Breast Cancer	BRCA1	NM_007300.4	Intron 19	c.5256+1G>A	----------	SNV	rs80358004	Pathogenic (PVS1,PM2,PP3,PP5)
14	47	(+)	Breast Cancer	BRCA1	NM_007300.4	Exon 20	c.5329dupC	p.Gln1777ProfsTer74	Insertion	rs80357906	Pathogenic (PVS1,PS3,PM2,PP3,PP5)
15	42	(+)	Ovarian cancer	BRCA1	NM_007300.4	Exon 20	c.5329dupC	p.Gln1777ProfsTer74	Insertion	rs80357906	Pathogenic (PVS1,PS3,PM2,PP3,PP5)
16	45	(-)	Breast Cancer	BRCA1	NM_007300.4	Exon 20	c.5329dupC	p.Gln1777ProfsTer74	Insertion	rs80357906	Pathogenic (PVS1,PS3,PM2,PP3,PP5)
17	45	(+)	Multiple cancer history in the family	BRCA1	NM_007300.4	Exon 23	c.5507G>A	p.Trp1836Ter	SNV	rs80356962	Pathogenic (PVS1, PM2, PP2, PP3, PP5)
Patient No	**Age**	**Family History**	**Reason for application**	**Gene**	**Transcript**	**Location**	**cDNA change**	**Protein change**	**Varyant type**	**dbSNP**	**ACMG Classification**
1	37	(+)	Breast Cancer	BRCA2	NM_000059.4	Exon 5	c.469A>T	p.Lys157Ter	SNV	rs1593886887	Pathogenic (PVS1,PM2,PP3,PP5)
2	81	(-)	Breast Cancer + Pancreatic cancer	BRCA2	NM_000059.4	Exon 11	c.3318C>G	p.Ser1106Arg	SNV	rs1298550035	VUS (PM2,PP3,BP1)
3	49	(-)	Ovarian cancer	BRCA2	NM_000059.4	Exon 11	c.3751dupA	p.Thr1251AsnfsTer14	Insertion	rs397507683	Pathogenic (PVS1,PM2,PP3,PP5)
4	41	(+)	Multiple cancer history in the family	BRCA2	NM_000059.4	Exon 11	c.3751dupA	p.Thr1251AsnfsTer14	Insertion	rs397507683	Pathogenic (PVS1,PM2,PP3,PP5)
5	40	(+)	Breast Cancer	BRCA2	NM_000059.4	Exon 11	c.5578A>T	p.Lys1860Ter	SNV	rs431825332	Pathogenic (PVS1,PM2,PP5,BP4)
6	57	(+)	Ovarian cancer	BRCA2	NM_000059.4	Exon 11	c.6054_6058delTAACG	p.Ser2018ArgfsTer29	Deletion	**Novel**	Pathogenic (PVS1,PM2,PP3)
7	31	(-)	Breast Cancer	BRCA2	NM_000059.4	Exon 11	c.6562A>G	p.Lys2188Glu	SNV	rs1135401833	VUS (PM2)
8	33	(+)	Breast Cancer	BRCA2	NM_000059.4	Exon 19	c.8395delA	p.Arg2799AspfsTer22	Deletion	rs80359709	Pathogenic (PVS1,PM2,PP5)

Table 3. *BRCA1* and *BRCA2* copy number variations of our patients

Patient No	Age	Famiy History	Reason for application	Gene	Transcript	Exon	CNV type
1	43	(-)	Breast Cancer	BRCA1	NM_007300.4	3-5-6-7-8	Duplication
2	21	(+)	Family history of cancer	BRCA1	NM_007300.4	11	Deletion
3	18	(+)	Family history of cancer	BRCA1	NM_007300.4	11	Deletion
4	54	(-)	Ovarian cancer	BRCA1	NM_007300.4	11	Deletion
5	36	(+)	Family history of cancer	BRCA1	NM_007300.4	18-19	Deletion
6	65	(+)	Breast Cancer	BRCA1	NM_007300.4	18-19	Deletion
7	19	(+)	Family history of cancer	BRCA1	NM_007300.4	24	Deletion
Patient No	**Age**	**Famiy History**	**Reason for application**	**Gene**	**Transcript**	**Exon**	**CNV type**
1	23	(+)	Family history of cancer	BRCA2	NM_000059.4	3	Deletion
2	56	(+)	Family history of cancer	BRCA2	NM_000059.4	3	Deletion
3	53	(+)	Family history of cancer	BRCA2	NM_000059.4	3	Deletion
4	43	(+)	Family history of cancer	BRCA2	NM_000059.4	3	Deletion

individuals selected in line with the NCCN guidelines. The c.5266dupC (p.Gln1756Profs) variant in the BRCA1 gene, which is particularly common in the Ashkenazi population, was identified as the most common variant in this study at a rate of 5.47% [16]. This pathogenic BRCA1 variant was the most common (in three different patients) in our study too. Furthermore, in a large study of 1419 patients from Turkey in 2020, pathogenic variants were identified in (9.4%) 134 patients and likely pathogenic variants in (0.3%) five patients. BRCA1 variants were detected in 58 of these patients and BRCA2 variants were detected in 64 of them. Also, variants of uncertain significance were detected in (6.4%) 91 patients [17]. Additionally, less than breast and ovarian cancers, there is also an increased risk of developing other types of cancer at solid organs including prostate cancer, melanoma, and pancreatic cancer. Recent years, BRCA variant spectrum of pancreatic cancer has been determined both nationally and worldwide [18, 19].

In our study, the incidence of BRCA1 and BRCA2 variants in our cohort was 26.1%, which was higher than in previous studies conducted in Turkey. This result may be due to relatively lower number of patients in our study. It may have also resulted from the fact that a more well-selected group was tested.

When the distribution of variants on the gene was examined, it was seen that the variants in the BRCA1 gene were mostly (9/18, 50%) on the 10th exon. It was observed that the variants in the BRCA2 gene were mostly (6/9, 66%) on the 11th exon. When the literature, ClinVar and HGMD were examined, it was seen that these regions were hot-spot regions for these genes.

According to the literature, some BRCA1 and BRCA2 variants are common in certain populations. For example; 3 different variants (BRCA1 c.68_69delAG, BRCA1 c.5266dupC and BRCA2 c.5946delT) are seen in the majority of cases in Ashkenazi Jewish patients, which is also a testing criterion in the NCCN guidelines. When we look at the studies conducted in Turkish society, we see that the variants do not cluster but generally show a distribution. We also detected one novel variant each in BRCA1 and BRCA2

genes. The patient with a novel variant in the BRCA1 gene was a female diagnosed with breast cancer at 24 years old. Her family history was unremarkable. The detected BRCA1 variant (NM_007300.4:c.4246G>C: p.Ala1416Pro) was evaluated as uncertain clinical significance (VUS) according to the ACMG guidelines. The other patient, in whom we detected a novel variant in the BRCA2 gene, was admitted with the diagnosis of ovarian cancer at the age of 57. In the family story, her sister had ovarian cancer and her grandmother had a history of breast cancer. The detected BRCA2 variant (NM_000059.4:c.6054_6058del:p.Ser2018ArgfsTer29) was evaluated as pathogenic according to the ACMG guidelines.

Large deletions / duplications in the BRCA1 and BRCA2 genes differ between populations. It is estimated to be around 10 percent on average. Methods such as MLPA are used to detect large CNVs in the BRCA1 and BRCA2 genes. However, the multistep approach with CNV analysis followed by sequencing may be both expensive and time consuming. Recent developments with the NGS technology now allow simultaneous detection of CNVs and single nucleotide variations/small indels using different bioinformatic pipelines. We used Oncomine™ BRCA assay in combination with Ion Reporter ™ 5.16.0.3 software for this purpose. CNVs were found in %7.3 of cases in our cohort and all cases were confirmed by MLPA. Germani et. al (2018) reported %100 concordance between NGS results and MLPA/multiple amplicon quantification (MAQ) results using the same approach [20]. In another study, various assays analyzed with Sophia DDM platform and SeqNext software were compared with conventional methods [21]. Sensitivity of BRCA Tumor and BRCA HC assays analyzed with both Sophia DDM platform and SeqNext software were reported 100%. The specificity was highest (100%) for BRCA HC Assay-Sophia DDM platform combination and lowest (99.489%) for BRCA HC assay-SeqNext software combination. These data suggest that NGS-CNV detection algorithms show promise for a more efficient approach instead of multi-step testing. However, it is important to validate the assays and bioinformatic pipelines.

Conclusion

In this study, our experience of one year in our center is summarized in the light of scientific literature. We detected two novel variants in our cohort that were not previously described in the literature. For patients whose BRCA1 and BRCA2 variants could not be detected and who meet the NCCN hereditary breast and ovarian cancer syndrome criteria, multigene panels have been recommended to examine additional genetic causes. Further studies are needed in our country in order to evaluate the pathogenicity of BRCA1 and BRCA2 genes, especially variants of uncertain clinical significance.

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