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ORIGINAL ARTICLE

Genetic screening results of individuals with high risk BRCA-related breast/ovarian cancer in Trakya region of Turkey

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Summary

Purpose: *Pathogenic/likely pathogenic (P/LP) germline variations in BRCA1 and BRCA2 genes are responsible for the majority of hereditary breast and ovarian cancers. This study presents the BRCA1/BRCA2 sequencing and deletion duplication analyses results of of 493 participants (485 women, 8 men) selected based on the National Comprehensive Cancer Network (NCCN) guidelines.*

Methods: *Next generation sequencing (NGS) and multiplex ligation-dependent probe amplification methods (MLPA) were used to define germline BRCA1/BRCA2 positivity.*

Results: *Overall, the P/LP frequency of the participants was 17.8%. Five of the likely pathogenic variants were novel. The 5266dupC pathogenic variation, which is a founder mutation in the Ashkenazi Jewish population, was the most common variation among the patients, with a frequency of 5.47%. The pathogenic/likely pathogenic variation frequency was significantly higher (p=0.01) among clinically diagnosed familial cancer patisents than those participants without personal history of cancer but enrolled for BRCA1 testing due to familial risk. BRCA1/BRCA mutation positivity was significantly higher (p=0.000) among those who had at least one first- or second-degree relative with breast/ovarian cancer from patients who had no family history. BRCA1/BRCA2 mutation positivity was 69.23% between the patients who had personal history of both breast and ovarian cancer.*

Conclusion: *Based on our findings, we suggest that sequencing all of the coding regions of the BRCA1/BRCA2 genes using NGS is a feasible approach for individuals who are at risk of developing BRCA-related cancer according to NCCN guidelines. The 5266dupC pathogenic variation, as the most common pathogenic variation in the Trakya region of Turkey, should be included if a targeted mutatin screening is planned.*

Key words: *HBOC, BRCA1, BRCA2, NGS*

Introduction

BRCA1 (OMIM* 113705) and *BRCA2* (OMIM* 600185) genes are tumor supressors that play important roles in DNA repair, chromosomal stability, and cell-cycle control. Pathogenic/likely pathogenic (P/LP) germline variations in *BRCA1* and *BRCA2* genes are responsible for the majority of hereditary breast and ovarian cancers (HBOCs). HBOC (OMIM #604370 and OMIM #612555) may also be related to prostate cancer, pancreatic cancer and melanoma in some patients [1,2].

Women diagnosed with breast cancer may have 5-10% germline mutation in their *BRCA1* and *BRCA2* genes. P/LP variation carriers have a lifetime risk of 40-85% of developing breast cancer

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and a 30-50% risk of developing ovarian cancer [3,4]. The risk of developing contralateral breast cancer significantly increased for women diagnosed as having a *BRCA1* or *BRCA2* P/LP variation [5].

In the ClinVar database, there are a total of 246 pathogenic and 6,263 likely pathogenic *BRCA1* and *BRCA2* gene variants, including point mutations, small deletions/insertions and complex rearrangements. The BRCA Exchange database contains 4,826 records for pathogenic BRCA1/*BRCA2* variations [6,7] The prevalance of *BRCA1*/*BRCA2* mutation carriers is 1/400 and 1/800, respectively. This prevalence varies between ethnic groups. The prevalence of *BRCA1* and *BRCA2* germline pathogenic variants varies depending on the technology used for variant screening, the population size, and the extent to which the genes are tested [8,9].

Classical Sanger sequencing is difficult and time-consuming for mutation screening due to the large size of *BRCA1* and *BRCA2* genes. However, next generation sequencing (NGS) is cheaper and more feasible for the sequencing of *BRCA1* and *BRCA2* genes . Nevertheless, the disadvantage of both Sanger sequencing and NGS lies in their inability to define complex rearrengements that are responsible for some *BRCA1*- and *BRCA2*-related cancers. Thus, the need for multiplex ligation-dependent probe amplification (MLPA) persists for analyzing such genomic rearrengements [10,11].

In this study, we aimed to report on the four years of results of the NGS and MLPA analyses for *BRCA1* and *BRCA2* screening in high-risk individuals in the Trakya region of Turkey. We also aimed to report five novel deleterious variations. Given the importance of diagnosis, risk percentages, and the management of breast/ovarian cancer, quantifying the extent to which these variations prevail in the Trakya region is important.

Methods

Subjects

The present study presents the results of sequencing and deletion duplication analyses of 493 unrelated individuals (485 women, 8 men; mean age: 46.51 years) who were directed to Trakya University, Medical Faculty Department of Medical Genetics, Department for BRCA1/*BRCA2* testing, based on the National Comprehensive Cancer Network 2019 (NCCN) guidelines between May 2014 and June 2019 (Table 1). The study has been approved by the Research Ethics Boards of Trakya University's Faculty of Medicine. Each sample was screened using NGS for point mutations and small deletions/insertions. The MLPA method was used for large genomic rearrengements.

DNA isolation

Genomic DNA was isolated from peripheral blood samples using EZ1 DNA Investigator Kit (Qiagen, Hilden, Germany). Primary quality control of the isolated DNA samples was ensured using NanoDrop (Thermo Fisher Scientific, Waltham, MA), and samples that had A260/280 values of between 1.8-2.0 were used.

Next generation sequencing

Two different benchtop next generation sequencers were used to sequence all of the coding regions of *BRCA1* and *BRCA2* genes (Figure 1). The first sequencer was the Ion Torrent Personal Genome Machine (PGM) (Life Technologies Corporation, Carlsbad, CA, USA), and the second was the Illumina MiSeq (Illumina Inc., San Diego, CA, USA). NM_007294.3 and NM_000059.3 transcripts were accepted as references for *BRCA1* and *BRCA2*, respectively.

Table 1. Sample groups according to NCCN criteria

NCCN criteria	*Samples*
Personal history of breast cancer diagnosed ≤ 45 y	213
Personal history of breast cancer diagnosed 46-50 y with an additional breast cancer primary at any age	21
Personal history of breast cancer diagnosed 46-50 y with ≥1 close blood relatives with breast cancer at any age	21
Personal history of breast cancer diagnosed 46-50 y with an unknown or limited family history	24
Diagnosed ≤ 60 y with triple-negative breast cancer	26
Diagnosed at any age with ≥1 close blood relative with/breast cancer diagnosed ≤ 50 y/ovarian carcinoma/ or male breast cancer/metastatic prostate cancer/ pancreatic cancer	163
Personal history of ovarian carcinoma	44
Personal history of male breast cancer	8
Personal history of metastatic prostate cancer	1
An individual who does not meet the other criteria but with ≥1 first -or second-degree blood relative meeting any of the above criteria.The significant limitations of interpreting test results for an unaffected individual should be discussed	51

For Ion Torrent, the amino acid coding regions of the *BRCA1* and *BRCA2* genes were amplified using primers designed by the Ion AmpliSeq Designer (Life Technologies Corporation, Carlsbad, CA, USA). Libraries were amplified using Ion Xpress (Thermo Fisher Scientific, Waltham, MA, USA). Barcoded libraries were equalized using the Ion Library Equalizer Kit (Thermo Fisher Scientific, Waltham, MA, USA). Enriched, template-positive ion sphere particles were prepared on an Ion OneTouch 2 System (Thermo Fisher Scientific, Waltham, MA, USA) and using the Ion One Touch ES Instrument (Thermo Fisher Scientific, Waltham, MA, USA) using 200 bp chemistry, following the manufacturer's manual. The sequencing of enriched particles was performed on the PGM (Thermo Fisher Scientific, Waltham, MA, USA) with 314 chips and according to the Ion PGM Sequencing 200 Kit v2 (Thermo Fisher Scientific, Waltham, MA, USA) user guide. Raw data were processed and aligned to the hg19 human reference genome (Genome Reference Consortium GRCh37) using Torrent Suite version 5 (Thermo Fisher Scientific, Waltham, MA, USA). A coverage analysis plugin was used for each sample in order to define the total reads at the target bases. A minimum of 20x coverage for all bases in the targeted region was accepted as a reliable variant calling [12,13]. Ion Reporter version 4.0 (Thermo Fisher Scientific, Waltham, MA, USA) was used to annotate the variants. The Integrated Genomics Viewer (http://software.broadinstitute.org/software/ igv/) was used to conduct a visual assessment of the aligned amplicons.

Two different target enrichement methods, Nextera (Illumina, San Diego, CA) and QIAseq Targeted DNA Panel (Qiagen, Hilden, Germany) were used for sequencing the coding regions of the *BRCA1* and *BRCA2* on MiSeq System (Illumina, San Diego, CA) For Nextera (Illumina Inc., San Diego, CA, USA), polymerase chain reactions (PCRs) were performed using a commercial kit (Multigen FAST-BRCA® NGS Sequencing Kit, Multigen, Izmir, Turkey) and included multiplexed primers for all coding regions of the *BRCA1* and *BRCA2* genes. All of the amplicons were then purified using the Agencourt AMPure XP system (Beckman Coulter, Pasadena, CA, USA), and the starting DNA library was quantified using the Qubit dsDNA BR Assay kit (Invitrogen, Carlsbad, CA, USA). Sequencing library construction was performed according to the Nextera XT preparation guide (Illumina, San Diego, CA, USA), which uses transposome to fragment the ends and simultaneously adds adapter and barcoding sequences. The pooled and barcoded libraries were subsequently sequenced using on the MiSeq sequencer (Illumina Inc., San Diego, CA, USA). Variant calling and analysis was performed on Genomize Seq Software (Genomize, Turkey). For OIAseq Targeted DNA Panel (Qiagen, Hilden, Germany), libraries covering the *BRCA1* and *BRCA2* genes were prepared according to the QIAseq Targeted DNA Panel (DHS-001Z germline ILM v1.0: QIAseq Human Breast Cancer Panel ILM) protocol. Following the target enrichment process, libraries were sequenced using the MiSeq System (Illumina, San Diego, CA, USA). Variations were defined via the QIAGEN Clinical Insight Analyze software suite (Qiagen, Hilden, Germany).

Multiplex ligation dependent probe amplification

MLPA was applied to all samples using MRC-Holland commercial kits for *BRCA1* (SALSA MLPA P002-D1) and *BRCA2* (SALSA MLPA P045-B3) (MRC-Holland, Amsterdam, the Netherlands) according to the manufacturer's instructions. Fragments were separated using capillary gel electrophoresis in an Applied Biosystems 3130xl Genetic Analyzer (Applied Biosystems, Carlsbad, USA). The fragments were analysed using Coffalyser software (MRC-Holland, Amsterdam, the Netherlands) [14].

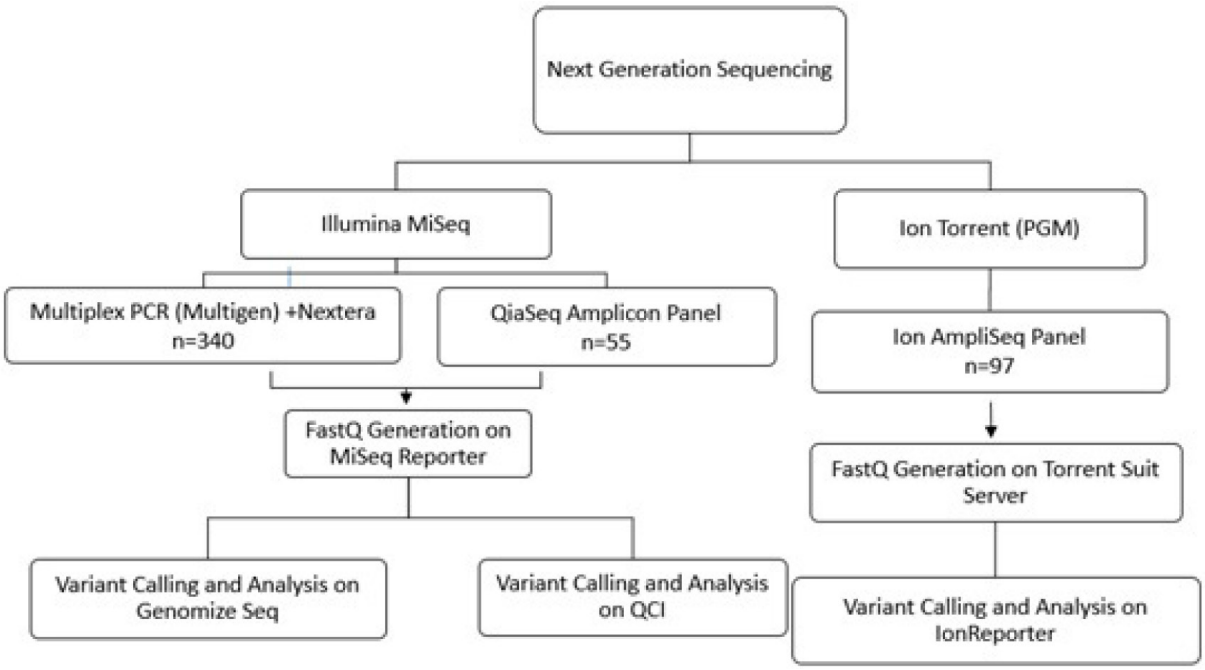

Figure 1. Next generation sequencing workflow.

Sanger sequencing

Sanger sequencing with in-house designed primer sets was used for the segregation analysis and to confirm the pathogenic variants found in the NGS. PCR was performed with intronic primers for the indicated exons of the BRCA1/*BRCA2* genes. Sequencing reaction was performed with the BigDye Terminator v3.1 Cycle Sequencing Kit (PE Applied Biosystems, Foster City, CA, USA). Dideoxy-terminated products were analyzed using the ABI-PRISM 3130 Genetic Analyzer (Applied Biosystems, Carlsbad, USA) according to the manufacturer's instructions.

Classifying the variants

Recommendations of the Human Genome Variation Society [15] were followed to describe the novel variants, and ACMG's 2015 [16] guidelines were followed for the classification of the variants.ClinVar [7], ENIGMA and literatures are considered for collecting the information about known variations [17].

Statistics

Statistical analyses were performed with IBM SPSS software, version 20.0. All the categorical variables were determined using Pearson's x^2 test. Two-sided p values <0.05 were considered as statistically significant.

Results

Of 493 participants 442 had cancer. The remaining 51 individuals were not clinically diagnosed with any type of cancer but were tested because of their family history (Table 2). In total 5 novel and 34 known P/LP variations have been determined (Table 3). BRCA1/*BRCA2* P/LP variation frequency was 17.84% among all the participants, but the frequency was 19.23% (85/442) in those individuals diagnosed with cancer. P/LP variation frequency was significantly higher (p=0.039) in individuals who were diagnosed with cancer than those without a cancer diagnosis who were tested because they met other NCCN criteria (Table 4). P/LP variations were mostly small insertions/deletions followed by point mutations. Splice site changes and missense mutations were equal. Only one patient had a gross deletion encompasssing the region between exon 3 and exon 8 of the *BRCA1* gene (Figure 2). Among all participants, patients with a personal history of both breast and ovarian cancer had the highest *BRCA1*/*BRCA2* P/LP variation frequency (69.23%) (Table 5). *BRCA1*/*BRCA2*

Table 2. Cancer types among participants

Samples	*n*
Breast cancer	370
Ovarian cancer	44
Breast and ovarian cancer	13
Other cancers	15
Participants without a cancer diagnosis but in risk group according to NCCN	51
Total	493

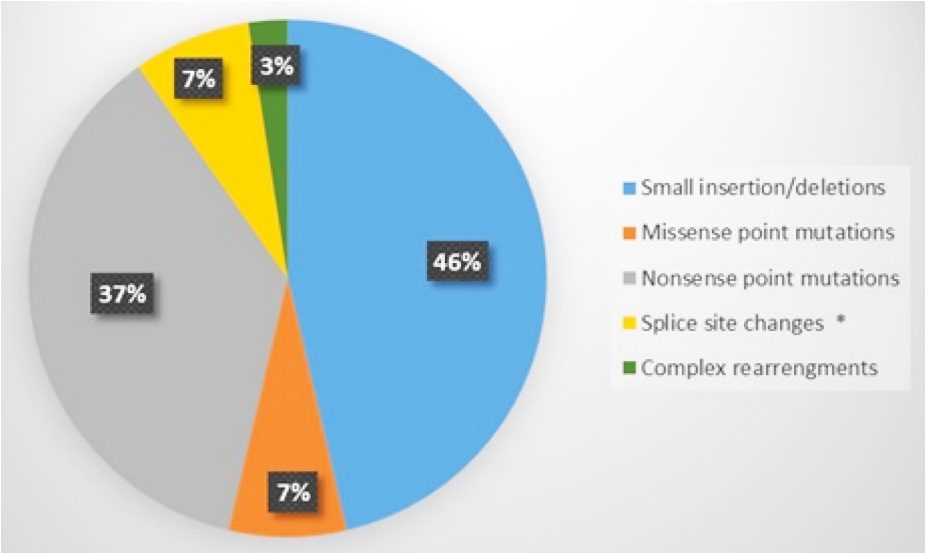

Figure 2. The distribution of mutation types determined in participants.

Table 3. Novel and known pathogenic /likely pathogenic variations defined with NGS analysis in participants

Variation according to HGVS nomenclature	*Sample ID*	*Age*	*Gender*	*Primary diagnosis*	*Family history*	*Variant classification according to ClinVar*	*Variant classification according to ACMG 2015*	*Variant classification according to ENIGMA (ENIGMA BRCA1/2 Classification Criteria (2015)*
NM_007294.3(BRCA1):c.5266dupC	S81	24	F	Breast cancer	F	pathogenic	pathogenic	pathogenic
(p.Gln1756Profs)	S122	54	F	Breast cancer	NA			
	S132	72	F	Breast and ovarian cancer	F			
	S164	50	F	Breast and ovarian cancer	F			
	S173	60	F	Ovarian cancer	S			
	S197	29	F	Breast cancer	F			
	S244	75	F	Ovarian cancer	F			
	S278	51	F	Breast and ovarian cancer	F			
	S306	52	F	Ovarian cancer	F			
	S316	34	F	breast cancer	F			
	S325	68	F	Breast and ovarian cancer	F			
	S328	47	F	Ovarian cancer	F			
	S425	39	F	Ovarian cancer	F			
	S445	48	F	Breast cancer	F			
	S497	25	F	Breast cancer	S			
	S539	42	F	Breast cancer	F			
	S548	28	F	Breast cancer	F			
	S625	42	F	Breast cancer	F			
	S643	54	F	Ovarian cancer	F			
	S644	48	F	Breast cancer	F			
	S652	46	F	Ovarian cancer	F			
	S784	45	F	Ovarian cancer	S			
	S806	49	F	Ovarian cancer	S			
	S822	37	F	Ovarian cancer	F			
	S838	44	F	Ovarian cancer	F			
	S562	47	F	Breast cancer	NA			
	S839	39	F	Ovarian cancer	F			

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Variation according to HGVS nomenclature	*Sample ID*	*Age*	*Gender*	*Primary diagnosis*	*Family history*	*Variant classification according to ClinVar*	*Variant classification according to ACMG 2015*	*Variant classification according to ENIGMA (ENIGMA BRCA1/2 Classification Criteria (2015)*
NM_000059.3(BRCA2):c.67+1G>A	S148	47	F	Breast cancer	F	pathogenic	pathogenic	NA
	S169	29	F	Breast cancer	F			
	S211	41	F	Breast cancer	F			
	S229	29	F	Breast cancer	F			
	S719	64	F	Tuba carcinoma	F			
	S774	55	F	Breast cancer	F			
NM_000059.3(BRCA2):c.9097dupA	S38	42	F	Breast cancer	F	pathogenic	pathogenic	pathogenic
(p.Thr3033Asnfs)	S240	52	F	Breast cancer	F			
	S598	59	F	Breast and stomach cancer	F			
	S695	57	F	Breast cancer	S			
NM_007294.3(BRCA1):c.3700_3704de	S233	73	F	Breast and ovarian cancer	F	pathogenic	pathogenic	pathogenic
lGTAAA (p.Val1234Glnfs)	S692	41	F	Breast cancer	NA			
	S242	37	F	Breast cancer	F			
	S538	42	F	Breast cancer	F			
	S561	43	F	Breast cancer	S			
NM_000059.3(BRCA2):c.9682delA	S255	56	F	Breast and ovarian cancer	F	pathogenic	pathogenic	pathogenic
(p.Ser3228Valfs)	S518	35	F	Breast cancer	f			
	S409	27	F	Breast cancer	F			
NM_007294.3(BRCA1):c.181T>G	S218	45	F	Breast cancer	F	pathogenic	pathogenic	pathogenic
(p.Cys61Gly)	S580	46	F	Breast cancer	NA			
	S638	32	F	Breast and ovarian cancer	F			
NM_007294.3(BRCA1):c.1789G>T	S424	54	F	Breast cancer	NA	pathogenic	pathogenic	pathogenic
(p.Glu597Ter)	S789	36	F	Breast cancer	S			
NM_007294.3 (BRCA1):c.4566C>A	S135	44	F	Breast cancer	F	pathogenic	pathogenic	pathogenic
(p.Tyr1522Ter)	S471	40	F	Breast cancer	S			
NM_007294.3(BRCA1):c.397delC	S515	48	F	Breast cancer	F	pathogenic	pathogenic	pathogenic
(p.Arg133Valfs)	S660	56	F	endometrium and breast	S			
NM_007294.3(BRCA1):c.5209A>T	S648	38	F	Breast cancer	F	pathogenic	pathogenic	pathogenic
(p.Agr1737Ter)	S389	55	F	Breast and ovarian cancer	F			

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Variation according to HGVS nomenclature	*Sample ID*	*Age*	*Gender*	*Primary diagnosis*	*Family history*	*Variant classification according to ClinVar*	*Variant classification according to ACMG 2015*	*Variant classification according to ENIGMA (ENIGMA BRCA1/2 Classification Criteria (2015)*
NM_000059.3(BRCA2):c.8002A>T	S296	32	F	Breast cancer	F	pathogenic	pathogenic	pathogenic
(p.Arg2668Ter)	S870	47	M	Breast cancer	F			
NM_000059.3(BRCA2):c.537dup	S303	52	F	Breast cancer	F	pathogenic	pathogenic	pathogenic
(p.Ile180Tyrfs*3)	S142	33	F	Healthy	F			
NM_000059.3(BRCA2):c.721A>T (p.Lys241Ter)	S136	22	F	Breast cancer	NA	pathogenic	pathogenic	pathogenic
NM_000059.3(BRCA2):c.7069_7070del (p.Leu2357fs)	S139	54	F	Ovarian cancer	F	pathogenic	pathogenic	pathogenic
NM_000059.3(BRCA2):c.6482_6485del ACAA (p.Lys2162fs)	S160	41	F	Breast cancer	S	pathogenic	pathogenic	pathogenic
NM_007294.3(BRCA1):c.4035del (p.Glu1346fs)	S257	53	F	Breast and ovarian cancer	F	pathogenic	pathogenic	pathogenic
NM_007294.3(BRCA1):c.1885A>T (p.Arg629Term)	S298	43	F	Breast cancer	F	pathogenic	pathogenic	pathogenic
NM_007294.3(BRCA1):c.4524G>A (p.Trp1508Ter)	S391	33	F	Breast cancer	NA	pathogenic	pathogenic	pathogenic
NM_000059.3(BRCA2):c.8023A>G (p.Ile2675Val)	S412	59	F	Breast cancer	F	conflicting (pathogenic/likely pathogenic)	likely pathogenic	NA
NM_000059.3(BRCA2):c.2471T>G (p.Leu824Ter)	S453	22	F	Breast cancer	F	pathogenic	pathogenic	pathogenic
NM_007294.3(BRCA1):c.788dupG (p.Ser264Terfs)	S510	32	F	Ovarian cancer	F	pathogenic	pathogenic	pathogenic
NM_007294.3(BRCA1):c.2952del (p.Ile986fs)	S524	28	F	Breast cancer	S	pathogenic	pathogenic	pathogenic
NM_000059.3(BRCA2):c.6462_6463TC[2] (p.Ser2156fs)	S617	49	F	endometr	F	pathogenic	pathogenic	pathogenic
NM_007294.3(BRCA1):c.843_846del (p.Ser282fs)	S688	61	F	Ovarian cancer	F	pathogenic	pathogenic	pathogenic

Continued on the next page

Variation according to HGVS nomenclature	*Sample ID*	*Age*	*Gender*	*Primary diagnosis*	*Family history*	*Variant classification according to ClinVar*	*Variant classification according to ACMG 2015*	*Variant classification according to ENIGMA (ENIGMA BRCA1/2 Classification Criteria (2015)*
NM_007294.3(BRCA1):c.3607C>T (p.Arg1203Ter)	S730	43	F	Ovarian cancer	F	pathogenic	pathogenic	pathogenic
NM_007294.3(BRCA1):c.2800C>T (p.Gln934Ter)	S762	37	F	Breast cancer	F	pathogenic	pathogenic	pathogenic
NM_000059.3(BRCA2):c.6478C>T (p.Gln2160Ter)	S775	42	F	Breast cancer	F	pathogenic	pathogenic	pathogenic
NM_000059.3(BRCA2):c.3545_3546del (p.Phe1182fs)	S792	42	F	Breast cancer	S	pathogenic	pathogenic	pathogenic
NM_000059.3(BRCA2):c.2490_2491insT (p.Val831fs)	S831	83	F	Breast cancer	F	pathogenic	pathogenic	pathogenic
NM_000059.3 (BRCA2): c.7007G>A (p.Arg2336His)	S858	45	F	Breast cancer	S	pathogenic	pathogenic	NA
NM_007294.3(BRCA1):c.135-2A>G	S863	50	F	Ovarian cancer	NA	pathogenic	pathogenic	NA
NM_007294.3(BRCA1):c.5030_5033del (p.Thr1677fs)	S876	42	F	Breast cancer	S	pathogenic	pathogenic	pathogenic
NM_007294.3(BRCA1):c.5136G>A (p.Trp1712Ter)	S247	50	F	Healthy	F	pathogenic	pathogenic	pathogenic
NM_000059.3(BRCA2):c.1769_1772TTAT[1] (p.Ile591fs)	S87	36	F	Healthy	F	pathogenic	pathogenic	pathogenic
NM_007294.3(BRCA1):c.129_134+2delTTGCAAGT	S559	55	F	Ovarian cancer	S	novel	likely pathogenic	NA
NM_007294.3(BRCA1):c.3884delT(p.Leu1295CysfsTer12)	S800	43	F	Breast cancer	F	novel	likely pathogenic	NA
NM_000059.3 (BRCA2):c.469A>T (p.Lys157Ter)	S856	50	F	Breast cancer	F	novel	likely pathogenic	NA
NM_007294.3(BRCA1):c.3626T>A (p.Leu1209Ter)	S241	45	F	Tuba carcinoma	F	novel	likely pathogenic	NA
NM_000059.3(BRCA2) :c.8833C>T (p.Gln2945Ter)	S894	40	F	Breast cancer	S	novel	likely pathogenic	NA

F: Familial, S: Spoardic NA: Not avaliable. HGVS: Human Genome Variation Society

Table 4. Pathogenic/likely pathogenic variation frequency comparison between cancer patients and asymptomatic familial participants

	BRCA1/BRCA2 pathogenic variation positives n (%)	
Indexes with a cancer diagnosis (n=442)	85 (19.23)	p=0.039 (95% CI 1.95)
Indexes without a cancer diagnosis (n=51)	3 (5.88)	
Total (n=493)	88 (17.84)	

Table 5. The results of indexes who had a cancer diagnosis

Diagnosis	*BRCA1/2 pathogenic variant positivity n (%)*	*BRCA1 positivity n (%)*	*BRCA2 positivity n (%)*
Breast (370)	52 (14.5)	29 (7.83)	23 (6.21)
Ovarian (44)	19 (43.1)	18 (40.9)	1 (2.27)
Breast and ovarian (13)	9 (69.23)	8 (61.53)	1 (7.69)
Others (15)	5 (33.33)	2(13.33)	3 (20)
Total (442)	85 (19.23)	57 (12.89)	28 (6.33)

Table 6. Comparing the positivity rates between hereditary and sporadic cases

Hereditary/Sporadic	*BRCA1/BRCA2 pathogenic variation positive cases n (%)*	
Hereditary	16 (7.5)	p= 0.0489* (95% CI 1.96)
Sporadic	61 (29.9)	

*Pearson's two-sided x^2 test

P/LP variation positivity was significantly higher among patients who had at least one first- or second-degree relative with breast/ovarian cancer, compared with patients who had no family history (Table 6)

Discussion

This is the first study to report on the P/LP variation frequencies of *BRCA1/BRCA2* genes among individuals in the Trakya region of Turkey who are at high risk of developing BRCA-related cancer. Overall, the pathogenic variation frequency was 17.8%, with five novel mutations. Pathogenic variation frequency was 19.2% in participants who had been previously diagnosed with cancer. With an overall frequency of 5.47%, the *5266dupC* pathogenic variation, which was originally described as a founder mutation in the Ashkenazi Jewish population, was the most common pathogenic variation among the patients in this region.

The frequency of pathogenic variations of *BRCA1/BRCA2* genes may vary due to different methods used and different populations included in the studies. The P/LP *BRCA1/BRCA2* variation frequency in cases of breast/ovarian cancer included in our study according to NCCN criteria was 19.2%. The frequency of P/LP variation in hereditary breast/ovarian cancer (HBOC) cases was reported as 19.8% in a Brazilian population [18] . The frequency of P/LP variants (class 4 and class 5 variants were reported) was 19.4% in a study permormed with 206 unrelated breast/ovarian cancer patients who met NCCN guidelines for genetic testing [19]. Cock-

Rada et al reported the P/LP variation frequency as 17.6% in a study of 85 women who met the testing criteria for HBOC [20]. The results of these studies are similar to the current study, where we reported that the *BRCA1/BRCA2* P/LP variant frequency was 17.84%. Although they were performed on different populations, the similarities in the results of these studies may be explained by the comparable methodologies, risk definiton criteria, and variant classification criteria used.

P/LP variation frequencies reported in studies performed on the Turkish population were conflicting because of the different methodologies used [21-31]. Geredeli et al performed a study on 99 patients with breast/ovarian cancer and reported a P/LP variation frequency of 19% [22]. In a study performed on 1,809 patients with breast/ovarian cancer and 125 healthy control individuals, the P/LP variation frequency was reported as 17% [24]. Our findings are thus supported by the results of these studies.

Considering the family history of BRCA-related cancer cases, the incidence of pathogenic variation increases to 29.9%. Cipriano et al reported incidence of *BRCA1* and *BRCA2* pathogenic variations in 26.2% of patients at high risk of developing HBOC [32]. Meisel et al reported that the frequency of pathogenic variations of *BRCA1/BRCA2* was 23.1% [33]. In our study, the frequency of *BRCA1* and *BRCA2* mutations in familial cases (29.9%) was similar to these studies.

The detection of BRCA mutations in ovarian cancers is becoming increasingly important, both in terms of diagnosis and treatment. In recent studies it has been reported that the response to PARP inhibitors is higher in ovarian cancer patients who carry BRCA mutations [34-36]. In our study, the frequency of *BRCA1/BRCA2* pathogenic variations in ovarian cancer cases was 43.1% compatible with the results of Helpman et al, who reported in their study of the Israeli population that the pathogenic variation in *BRCA1/BRCA2* was 49.6%.

The *5266dupC* pathogenic variation is a founder mutation with a frequency of approximately 1/400 in a healthy population of Ashkenazi Jews [37]. No data are available on the frequency of this variation in the healthy Turkish population. Manguoglu et al [27] reported that there was no *5266dupC* variation in breast/ovarian cancers in their study of the Turkish population, but Yazici et al reported that the frequency of *5266dupc* was 2/105 and suggested that this pathogenic variation may be a possible founder mutation in the Turkish population [23]. Egeli et al reported that the *5266dupC* frequency was 2/63 in their study of 38 patients with breast/ovarian cancer. In our study, this pathogenic variation was identified as the most common (5.4%) variation [38]. This finding supports the view that this pathogenic variation may also be a founder mutation in the Turkish population.

To conclude, we suggest that sequencing all of the coding regions of *BRCA1/BRCA2* genes using NGS is a feasible approach for individuals who are at risk of developing BRCA-related cancer. The *5266dupC* pathogenic variation, as the most common pathogenic variation in the Trakya region of Turkey, should be included in targeted mutation screenings. As indicated in the NCCN guidelines, we recommend beginning the analysis with individuals who have a personal history of cancer.

Conflict of interests

The authors declare no conflict of interests.

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