Original Article

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Frequency of Rearrangements Versus Small Indels Mutations in *BRCA1* and *BRCA2* Genes in Turkish Patients with High Risk Breast and Ovarian Cancer

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ABSTRACT

Objective: The current rearrangement ratio of *BRCA1* and *BRCA2* genes is not known in the Turkish population. Rearrangements are not routinely investigated in many Turkish laboratories. This creates problems and contradictions between clinics. Therefore, the aim of this study was to evaluate the distribution and frequency of rearrangements in BRCA1 and BRCA2 genes in high-risk families and to clarify the limits of *BRCA1* and *BRCA2* testing in Turkey.

Materials and Methods: The study included 1809 patients at high risk of breast cancer or ovarian cancer. All patients were investigated for both small indels and rearrangements of BRCA genes using DNA sequencing and multiplex ligation-dependent probe amplification (MLPA) analysis.

Results: The overall frequency of rearrangements was 2% (25/1262). The frequency of rearrangements was 1.7% (18/1086) and 4% (9/206) in patients with breast cancer and ovarian cancer, respectively. The frequency of rearrangements was 3.7% (8/215) in patients with triple-negative breast cancer. The rearrangement rate was 7.7% (2/26) in patients with both breast and ovarian cancer.

Conclusions: Rearrangements were found with high rates and were strongly associated with bilateral and triple-negative status of patients with breast cancer, which are signs of high risk for breast and ovarian cancer. Analysis of rearrangements should definitely be included in routine clinical practice in Turkey for high-risk families and also for improved cancer risk prediction for families.

Keywords: High-risk breast and ovarian carcinoma, *BRCA1* and *BRCA2* genes, rearrangements, Turkish population

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Introduction

Breast cancer is the most common cancer among women worldwide and causes significant morbidity and mortality (1). According to the 2009 statistics of the Turkish Statistical Institute (TUIK), the leading cancer and the seventh-most-frequent cancer among women were breast and ovarian carcinoma, respectively. Among Turkish women, the incidence of breast cancer and ovarian carcinoma is 23% and 3.9%, respectively. Therefore, breast and ovarian carcinoma are important health problems for Turkish society. Furthermore, consanguineous marriages, especially among first cousins, are quite common in Turkey. This may lead to higher cancer risks, especially in families with cancer histories. It is very important to detect hereditary cancer risk using genetic testing for individuals in high-risk families as well as genetic testing, if applied correctly. Hence it is very important to determine the limits and content of genetic tests.

Several factors increase the risk of breast cancer such as family history, reproductive history, diet, hormone use, radiation exposure, obesity, sedentary lifestyle, lack of breast-feeding, and exogenous hormone replacement therapy (1). Among these, a family history with breast and ovarian cancer in several generations is present in about 15–20% of all cases (2). Germline mutations of two major tumor suppressor genes, *BRCA1* and *BRCA2*, are inherited in an autosomal dominant pattern and have links to breast and ovarian cancer (3). These two mutated genes increase the risk of breast cancer by 87% and 44% for ovarian cancer over the lifetime of female patients (4, 5). *BRCA1* and *BRCA2* participate in cellular functions such as cell growth, cell division, and genetic instability.

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Several syndromes are known to be involved in the development of breast and ovarian tumors such as hereditary breast and ovarian cancer syndrome, Li-Fraumeni syndrome, Cowden disease, hereditary non polyposis colon carcinoma (HNPCC), and Peutz-Jeghers syndrome (6). Nowadays, all cancer predisposing syndromes can be tested genetically, requested by either physicians or licensed genetic counselors. Clinical identification of these syndromes is beneficial in reducing the risk of cancer in mutation-carrying individuals. Affected persons can take preventative precautions such as screening, chemoprevention, or prophylactic surgery for the organ or tissue. Detected at early stages, prophylactic measures can be used for definitive cancer prevention (5). Genetic testing, genetic counseling, and the quality and ability of laboratories to test genes are significant factors.

In our study, we evaluated the rate of rearrangements of the genes *BRCA1* and *BRCA2* in 1809 patients at high risk for breast and ovarian carcinoma, as the current rearrangement rate is not known in the Turkish population. Rearrangements are not investigated in many Turkish laboratories in routine BRCA testing. We conducted the study to emphasize the importance of examining rearrangements while conducting *BRCA1* and *BRCA2* tests, and to determine the content and limits of the tests.

Material and Methods

General features of patient group: The Oncology Institute Breast and Ovarian Cancer patient cohort compromises high-risk patients having strong family history of cancer, early age of cancer diagnosis, triple negativity, bilateral breast cancer, multifocal localizations of tumor, mixed types of histology results, case of male breast cancer in family from every geographic region of Turkey between 1994 and 2016. 1809 cases were referred to our center from all geographic regions. The diagnoses of 1809 patients were confirmed with their histopathology reports before a genetic counseling session. Patients who agreed to *BRCA1* and *BRCA2* genetic testing were asked to complete a questionnaire regarding their family histories. High-risk patients were selected in accordance with the National Comprehensive Cancer Network (NCCN) criteria for breast/ovarian cancer. Informed consent was obtained from all participants in the study. The control group was included 125 healthy adults who have no family history on cancer and matched age, gender and ethnicity according to patients group. The study was approved by the Ethics Committee of Istanbul Medical Faculty at Istanbul University (2011/1425-681). The number of patients and patients' diagnoses in the study subgroups are given in Table 1. This work was supported by Istanbul University, Research Fund, Grant No: 21952 and GP-7/08122004 and Government Planning Organization of Turkey, Grant No: 97K121700.

Mutation analysis: Genomic DNA was isolated from 5 mL of peripheral blood lymphocytes using a QIAamp mini DNA extraction Kit (Qiagen, Inc.). All coding exons and adjacent intronic splice junction regions of *BRCA1* and *BRCA2* genes were screened for mutations in fragments between 197 to 823 bp length for Sanger Sequencing and about 450 bp length for Next Generation Sequencing (NGS) using a Multiplicome BRCA MASTR Dx Kit, which has a CE-IVD certificate in the MiSeq Illimuna Platform. A reference sequence of NM_007294.3 was used for the *BRCA1* gene, and NM_000059.3

Table 1. The frequency of rearrangements and overall mutations in all patients according to diagnosis in high risk breast and ovarian cancer cases in Turkey

Diagnosis of Patients	Number of Patients (n)	Rearrangements n(%)	Small Indel Mutations n(%)	Overall Mutation Rate n(%)
Overall Breast Cancer and Ovarian Cancer Cases	1809	25/1262(2%)	268/1785(15%)	293/1785(17%)
All Breast Cancer Cases	1473	18/1086(1.70%)	204/1473(13.8%)	222/1473(15.5%)
Unilateral Breast Cancer	1273	11/924(1.2%)	155/1273(12.2%)	166/1273(13.4%)
Unilateral Breast Cancer and Ovarian Cancer	33	2/26(7.7%)	16/33(48.5%)	18/33(56.2%)
Unilateral Breast Cancer and Other Type of Cancer	34	0/29(0.0%)	7/34(20.6%)	7/34(20.6%)
Bilateral Breast Cancer	90	5/85(5.9%)	20/90(22.2%)	25/90(28.1%)
Bilateral Breast Cancer and Other Type of Cancer	2	0/2(0%)	0/2(0%)	0/2(0%)
Male Breast Cancer	39	0/17(0%)	6/39(15.4%)	6/39(15.4%)
Male Breast Cancer and Other Type of Cancer	1	0/1(0%)	0/1(0%)	0/1(0%)
Bilateral Breast Cancer and Ovarian cancer	1	0/1(0%)	0/1(0%)	0/1(0%)
Triple Negative Breast Cancer	272	8/215(3.7%)	67/272(24.5%)	75/272(28.2%)
Patients having positivity in ER,PR,ErbB2(at least one)	971	5/741(0.7%)	108/971(11.1%)	113/971(11.8%)
All Ovarian Cancer Cases	370	9/206(4%)	81/370(22%)	90/370(24%)
Ovarian Cancer	326	7/170(4%)	65/326(19%)	72/326(23%)
Ovarian Cancer and Unilateral Breast Cancer	33	2/26(7.7%)	16/33(48.5%)	18/33(56.2%)
Ovarian Cancer and Endometrium Cancer	7	0/6(0%)	0/7(0%)	0/7(0%)
Ovarian Cancer and Other type of Cancer	3	0/3(0%)	0/3(0%)	0/3(0%)
Ovarian Cancer and Bilateral Breast cancer	1	0/1(0%)	0/1(0%)	0/1(0%)

was used for the *BRCA2* gene. All DNA sequencing results were read according to the hg19 genomic sequence. All patients and controls were tested for the presence of small indel mutations and rearrangements. 1809 probands, diagnosed breast and ovarian cancer, and 125 healthy controls were sequenced for the full exons of *BRCA1*and *BRCA2* genes with Sanger Sequencing using Dye terminator Cycle sequencing (DTCS) kit (Beckman Coulter, CEQ8000 and GXL, USA) and BigDye Terminator (Applied Bioscience Inc., USA) systems. A total of 741 probands were analyzed using a Multiplicom BRCA MASTR Dx kit on an Illimuna MiSeq platform. All bioinformatic analyses were executed using Sophia Genetics. The analysis took into account the variants with a coverage ratio ≥ 300X and "Allele Variant/ Coverage" ≥0.2.

The data from NGS analysis was evaluated by using different types of bioinformatics software which were Variant Studio, Sophia Genetics and Genomize to classify the mutations in 5 different categories. The categorized alterations were checked in different databases which were HGMD (Human Genome Mutation Database), dbSNP (The singe nucleotide polymorphism database), ClinVar (Public archive of interpretations of clinically relevant variants) and Alamut (Interactive biosoftware) for clinical importance after classification.

Multiplex ligation-dependent probe amplification (mlpa) and copy number variation (cnv) analysis: We evaluated rearrangements using both the MiSeq NGS platform and MLPA analysis. To calculate CNVs, 300X coverage was used. MLPA analysis was also used to confirm the CNV results from the MiSeq Illumina. MLPA analysis was performed using MRC-Holland probe sets for *BRCA1* (P087/P002) and *BRCA2* (P045/ P077) genes. The manufacturer's instructions were followed. At least one negative and three normal controls were run in each experimental batch, including DNA molecular weight markers. Amplified DNA was run on a Beckman Coulter DNA sequencer (Beckman Coulter, CEQ8000 and GXL, USA) for fragment analysis. Row data of fragment analyses were analyzed using Coffalyser analysis software and peak areas were calculated using a Coffalyser algorithm. All experiments per patient were performed using four probe sets for both *BRCA1* and *BRCA2* genes to avoid false-negative and positive results, and to confirm deletions and duplications.

Positive results for pathogenic mutations were repeated with two independent experiments using two probe sets for each gene. Confirmation analysis of rearrangement results from NGS data was replicated by using MLPA analysis with two probe sets for each gene. Confirmation analysis of rearrangement results from MLPA analysis was repeated using MLPA analysis in two independent experiments using both normal and confirmation probe sets for each gene. All positive results were confirmed at least five times in our data set.

All genetic tests were run in the laboratory of Cancer Genetics Department in Oncology Institute. The laboratory is a reference center for BRCA testing in Turkey for both genotyping and genetic counseling.

Statistical analysis

Statistical analysis was performed with Statistical Packages for the Sıcial Sciences (SPSS) version 20 (IBM Corp.; Armonk, NY, USA)**.** Demographic and clinical features of 1809 patients in our cohort were compared with BRCA mutation status using Chi-square tests. The rearrangements prevalence was calculated for the cohort defined by age and family history.

Results

We searched for patients at high-risk of breast and ovarian cancer across seven different regions of Turkey in order to evaluate the prevalence and spectrum of rearrangements of *BRCA1* and *BRCA2* genes. We also aimed to emphasize the importance of examining rearrangements while conducting *BRCA1* and *BRCA2* tests, and to determine the content and limits of the tests.

Families were selected according to the NCCN criteria for breast/ovarian cancer. In the cohort, the patients with breast and ovarian cancer have family histories with breast, ovarian and other types of cancer at first and second-degree relatives mostly. All patients were investigated for both small indels and rearrangements of BRCA genes using DNA sequencing and MLPA analysis. Both CNVs and MLPA assays were used to detect the rearrangements of *BRCA1* and *BRCA2* genes. The study included 1809 patients, who were identified and confirmed through the cancer genetics clinic in our institution by a genetic counselor and a physician according to NCCN criteria.

The number of patients and the distribution of patients according to their diagnoses are given in Table 1. The mean age at diagnosis was 41.9±9.9 years for BRCA non-carriers and 40.6±9.7 years for carriers with *BRCA1* rearrangements in the cohort. Rearrangements in *BRCA1* were observed in 25 of 1809 (1.4%) patients with breast and ovarian cancer who had a high-risk family history. All rearrangements in our study population were found in the *BRCA1* gene. No *BRCA2* rearrangements were found among the 1809 patients. However, four BRCA mutations (3.2%) were found in the healthy controls.

The overall frequency of mutations (small indels and rearrangements) in *BRCA1* and *BRCA2* genes of patients at high-risk for breast and ovarian cancer was 17% in the cohort. In patients with a high risk of breast cancer, the total frequency of all mutations and rearrangements in *BRCA1/2* genes was 15.5% (222/1473) and 1.70% (18/1086), respectively. The highest frequency of rearrangements among patients with breast cancer was 7.7% (2/26) in patients who had ovarian carcinoma as a secondary tumor. The frequency of rearrangements was also high in patients with triple-negative breast cancer (3.7%, 8/215). Rearrangements were found in 5.9% (5/85) of patients with bilateral breast cancer. No rearrangements were detected in Turkish patients with male breast cancer although the overall *BRCA1* and *BRCA2* gene mutation rate was 15.4% (6/39) in that subgroup (Table 1). A total of 293 mutations were identified in the 1809 patients with breast/ovarian cancer (Table 2). Of these, 189 patients had frameshift mutations with a frequency of 63.5%. The frequency of nonsense mutations was 16%. The percentages of missense and splice error mutations were 5.8% and 6.2%, respectively (Table 2).

The overall mutation frequency of patients with ovarian cancer was 24% (90/370) for both small indels and rearrangements. The frequency of rearrangements in Turkish patients with ovarian cancer was found as 4% (9/206). The rearrangements percentage was 4% (7/170) in patients who had ovarian tumors only. The subgroups of patients with ovarian cancer and other types secondary tumors revealed no rearrangements.

A total of 25 rearrangements in *BRCA1* were identified among the 1809 patients. We found that 2% (25/1262) of Turkish patients with a family history of breast and ovarian cancer had rearrangements in the *BRCA1* gene. Sixteen rearrangements were observed in patients with breast cancer with a frequency of 64% (16/25). Nine of the detected

BRCA1 gene rearrangements were in ovarian cancer (36%, 9/25) and eight were in triple-negative breast cancer (62%, 8/13) (Table 3).

Table 2. The types of overall mutations and their percentages found in our study group

Types of mutations	BRCA1/BRCA2 mutation positive cases n(%)
Frameshift	186 (63.5%)
Nonsense	47 (16%)
Missense	17 (5.8%)
Rearrangement	25 (8.5%)
Splice error	18 (6.2%)
Total mutation	293

Table 3. Distribution of rearrangements according to diagnosis

Distributions of rearrangements according to diagnosis	
Diagnosis	**Number of rearrangements (%)**
Breast Cancer Cases	(16/25)(64%)
Ovarian Cancer Cases	(9/25)(36%)
Triple Negative Breast Cancer Cases	(8/13)(62%)
Total	25

Table 4. Types of rearrangements and their percentages found in our study group

Distribution of the different types of rearrangements in the Cohort	
Types of rearrangements	**Numbers of rearrangements (%)**
All Deletions	21(84%)
All Duplications	4(16%)
Exon 1-2 Deletion	1 (4%)
Exon 1-3 Deletion	1 (4%)
Exon 1-21 Deletion	3 (12%)
Exon 10-24 Deletion	1 (4%)
Exon 18-19 Deletion	10 (40%)
Exon 21-22 Deletion	1 (4%)
Exon 24 Deletion	2 (8%)
Exon 1-15 Deletion	1 (4%)
Exon 14 Deletion	1 (4%)
Exon 3-8 Duplication	1 (4%)
Exon 5-9 Duplication	1 (4%)
Exon 10-12 Duplication	2 (8%)
Total	25

Twenty-five *BRCA1* gene rearrangements were detected in our cohort (details are given in Table 4). Overall, 84% (21/25) of deletions and 16% (4/25) of duplications were detected among the rearrangements (Table 4). The most common alteration (10/25) was exon 18-19 deletion (Table 4) (Figure 1). The frequency of exon 18-19 deletion was 40% (10/25) in patients with a family history of breast and ovarian cancer, and all patients with mutations lived in the Black Sea region of Turkey. The second most common rearrangement was exon 1-21 deletion, which was seen with a frequency of 12% (3/25) in our cohort. The remaining thirteen different mutations were detected with frequencies of 4–8%.

The average age at diagnosis, histopathology, and family histories of patients among carriers of *BRCA1* gene rearrangements are given in Table 5. The mean age at diagnosis was 40.6±9.7 years for *BRCA1* rearrangement carriers. Of 18 patients, 16 patients with breast cancer had invasive ductal carcinoma (IDC), one had invasive lobular carcinoma (ILC) and one had ductal in situ carcinoma (DCIS). With the exception of one patient, all patients with ovarian cancer had serous histopathology.

Deletions of both exons 1-21 and 18-19 were found frequently in our study group. All patients who carried *BRCA1* gene exon 1-21 deletions had a strong history of breast cancer. In addition to four cases of breast

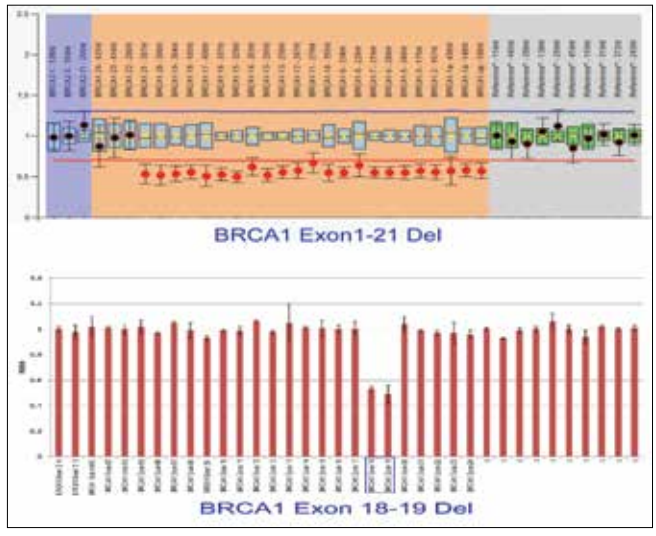

Figure 1. Results of MLPA analysis for *BRCA1* gene. (Upper): Patient DNA with the deletion of exon 1-21 region of *BRCA1* gene; (Bottom): Patient DNA with the deletion of exon 18-19 region of *BRCA1* gene

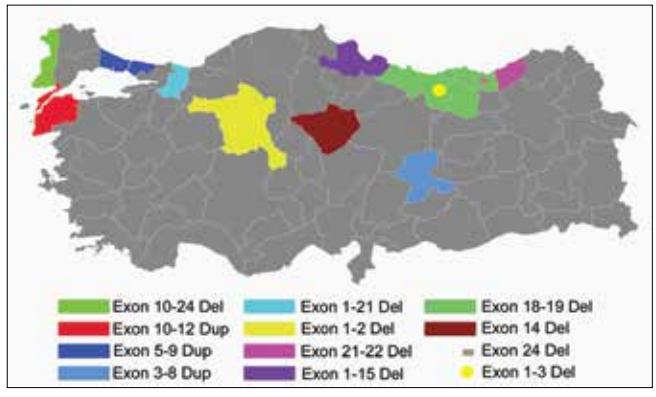

Figure 2. Distribution of *BRCA1* LGR mutations according to geographic regions of Turkey

Table 5. The family histories, age at diagnosis, clinical and histopathologic features of carriers with the rearrangements

The rearrangements of BRCA1 gene in Turkish High-Risk Breast and Ovarian Cancer Cases					
Patients	**Rearrangements**	**Diagnosis**	**Histopathology**	**Age at Diagnosis**	**Family History**
BR1487	Deletion of Exon 1-21	Breast Carcinoma	ILC	33	4BC+2OC+4OTC
BR1500	Deletion of Exon 1-21	Breast Cancer and Ovarian Cancer	IDC + Serous	42	4BC+1OC+1OTC
BR1589	Deletion of Exon 1-21	Breast Carcinoma	IDC	51	4BC+1OC+4OTC
BR1428	Deletion of Exon 18-19	Breast Carcinoma	IDC	50	1BC+4OC+5OTC
BR1679	Deletion of Exon 18-19	Breast Carcinoma	IDC	51	5BC+3OTC
BR1745	Deletion of Exon 18-19	Breast Carcinoma	IDC	30	2BC+1OTC
BR1903	Deletion of Exon 18-19	Breast Carcinoma	IDC	42	2BC+1OC+8OTC
BR1753	Deletion of Exon 18-19	Breast Carcinoma	DCIS	31	2BC+2OC+5OTC
BR1462	Deletion of Exon 18-19	Breast Cancer and Ovarian Cancer	IDC + Serous	41	3BC+1OC+2OTC
BR1508	Deletion of Exon 18-19	Ovarian Carcinoma	Serous	34	4OC+7OTC
BR1509	Deletion of Exon 18-19	Ovarian Carcinoma	Serous	49	3OC+5OTC
BR1592	Deletion of Exon 18-19	Ovarian Carcinoma	Serous	51	1BC+5OC+5OTC
BR1609	Deletion of Exon 18-19	Ovarian Carcinoma	Adenocarcinoma	36	2OC+ 1OTC
BR2064	Deletion of Exon 1-2	Bilateral Breast Carcinoma	IDC	46	1BC+1OC+2OTC
BR0527	Deletion of Exon 1-3	Bilateral Breast Carcinoma	IDC	35	1BC+3OTC
BR1488	Deletion of Exon 10-24	Ovarian Carcinoma	Serous	55	1BC+1OC+8OTC
BR1291	Deletion of Exon 21-22	Breast Carcinoma	IDC	25	4OTC
BR2231	Deletion of Exon 1-15	Breast Carcinoma	IDC	34	1BC+3OTC
BR1667	Deletion of Exon 24	Bilateral Breast Carcinoma	IDC	33	1OC+3OTC
BR1839	Deletion of Exon 24	Ovarian Carcinoma	Serous	64	3BC+2OC
BR2474	Deletion of Exon 14	Bilateral Breast Carcinoma	IDC	38	1OC+1OTC
BR2451	Duplication of Exon 10-12	Bilateral Breast Carcinoma	IDC	42	1BC+1OTC
BR1814	Duplication of Exon 10-12	Breast Carcinoma	IDC	31	2OTC
BR2037	Duplication of Exon 3-8	Breast Carcinoma	IDC	27	1BC+1OC+3OTC
BR1556	Duplication of Exon 5-9	Ovarian Carcinoma	Serous	45	4OC+1OTC

IDC: invasive ductal carcinoma; ILC: invasive lobular carcinoma; DCIS: ductal carcinoma in situ; BC: breast carcinoma; OC: ovarian carcinoma; OTC: other types of cancer

cancer in all of these families, there was at least one case of ovarian cancer, and also other types of cancer in all patients with mutated exon 1-21 deletions who lived in the Marmara region. When we examined the family history of patients with exon 18-19 deletions, there was at least one case of other cancers in the majority of families. In addition, cases of breast cancer and many ovarian cancers were observed. It was determined that all patients who carried exon 18-19 deletions were born and lived in the Black Sea region (Figure 2). There were only four large duplications found in patients with breast cancer and ovarian cancer.

The distribution of patients with carriers of rearrangement according to geographic regions of Turkey were 59.1% in the Black Sea region, 27.3% in the Marmara region, 4.5% in the Eastern Anatolia region, and 9.1% in the Central Anatolia region (Figure 2). Even though there were small indel mutations in the remaining three regions, no rearrangements were found.

Discussion and Conclusions

Three previous studies have detected rearrangements in the Turkish population. The first study was based on with 667 unselected patients with ovarian cancer and 27 rearrangements were found with a frequency of 4%. Most (25/27) rearrangements were found in patients with hereditary ovarian cancer (7). The rearrangement ratio (40.9%) given by Aktaş et al. (7) for patients with ovarian cancer who had family histories was very high according to the international literature (4, 7-17). The second study investigated the rearrangement ratio in patients with hereditary breast cancer, but with a small sample size. In the study, only 16 patients with hereditary breast cancer were investigated for rearrangements and none was observed. Manguoğlu et al. (18) suggested that the rearrangement percentage could have been low because of the small sample. The last study was performed by Aydın et al. (19) who tested 211 unselected patients with breast cancer who lived in the Black Sea region. Their rearrangement frequency was

1.9% and their findings gave no information about the rest of the country and hereditary ovarian cancer. All authors suggested that comprehensive studies should be performed in the Turkish population.

Hence there are no clear results about rearrangement ratios in the Turkish population. Consequently, the rearrangement of *BRCA1* and *BRCA2* genes are not routinely investigated in most clinical genetics laboratories in Turkey. This leads to conflicts between clinics and institutional laboratories, and it also affects the correct management of patients. *BRCA1* and *BRCA2* mutation screening is becoming more important in clinical practice for treatment options such as PARP inhibitors. The effective management of patients at high risk for breast and ovarian cancer depends on the identification of all mutations such as small indels and rearrangements, which can be screened using different molecular techniques or deep coverage. The knowledge of mutations could be used for risk reduction and chemoprevention as well as treatment options in patients and their relatives. Therefore, this study's goal was to identify the percentage of rearrangements in Turkish patients at high risk for breast and ovarian cancer within a large cohort and to ensure compatibility between laboratories in Turkey.

Many studies have revealed rearrangement frequencies with wide variations for different populations around the world. Judkins et al. (4) found that the rearrangement percentage was 6–10% for all mutations in *BRCA1* and *BRCA2* genes. Palma et al. (11)reported rearrangements a frequency of 18% in a specific population. Arnold et al. (20) found that rearrangements accounted for 12.7% in an admixture American population. Kwong et al. (17) showed that the rearrangement rate was 8.7% in the Chinese population. French and Czech population frequencies were 6–7.7%, and a high frequency of *BRCA2* gene rearrangements was determined in the French population (15). Rearrangement frequencies were between 3–3.7% in Australian and Korean populations (9, 13). Gutierrez-Enriquez et al. (10) detected 1.5% rearrangements in the Spanish population. The rearrangement rate was 0–1% in Chilean, Sri Lankan, and Finnish populations (12, 14, 16). However, there are still no clear data for many specific populations and laboratories that perform BRCA testing using only DNA sequencing or both DNA sequencing and rearrangement testing, which poses problems in terms of the selective use of treatments such as risk reduction surgery, preventive medicine, chemoprevention, and specific drugs such as PARP inhibitors.

In our study, the rearrangement of *BRCA1* and *BRCA2* genes were investigated using CNV analysis with next-generation sequencing and MLPA analysis in 1809 Turkish patients at high risk for breast and ovarian cancer. Among the 1809 patients, we detected only 25 *BRCA1* gene rearrangements with a frequency of 2% (25/1262) versus 15% (268/1785) small indel mutations. Our findings indicate that it would be beneficial to test patients with high-risk family histories to better estimate the probability of mutations.

We found that all rearrangements were located on the *BRCA1* gene in our cohort. Our results confirmed the higher prevalence of rearrangements in the *BRCA1* gene versus the *BRCA2* gene documented in previous reports (21-25).

In our study group, the rearrangement rate was high in patients with ovarian cancer (4%, 9/206), triple-negative breast cancer (3.7%, 8/215), bilateral breast cancer (5.9%, 5/85), and patients with breast and ovarian cancer (7.7%, 2/26). Therefore, in high-risk patients, rearrangement testing should be included in standard *BRCA1* and *BRCA2* gene tests. Furthermore, it was determined that the frequency of rearrangements differed across various geographic regions in Turkey.

In our study, exon 18-19 deletion was the most common rearrangement and all mutation carriers were born and lived in the Black Sea region. Aktaş et al. (7) and Aydın et al. (19) reported the same mutation with a low percentage in a small group of patients from the same region Therefore, we think that exon 18-19 deletion could be a regional alteration specific to the Black Sea region. However, exon 18-19 deletions (40%, 10/25) were the most frequent rearrangements in our cohort. Exon 1-2 deletions (27.8%) were the most common rearrangements in the study by Aktaş et al. (7) in a Turkish population. However, their study group was very small, with 61 patients at high risk for ovarian cancer. In our study, half of the exon 18-19 deletion carriers were diagnosed as having breast cancer, the other half had ovarian cancer. When we examined the family history of patients with exon 18-19 deletions, there was at least one case of other cancers in the majority of families. In addition, there were breast and ovarian cancers.

The second most common mutation was the exon 1-21 deletion (12%, 3/25), which was found in patients living in the Marmara region. All exon 1-21 deletion carriers had breast cancer, and at least 4 cases of breast cancer and one case of ovarian cancer, and other types of cancer were seen in their families.

The cohort included 39 male patients with breast cancer. No rearrangements were found in this subgroup, although the percentage of small indel mutations was 15.4% (6/39). The studies performed by Manguoğlu et al. (18) and Falchetti et al. (26) also showed that there were no rearrangements in breast cancer in Turkish and Italian men, respectively. Another study in a Brazilian population showed that the rearrangement rates in men with breast cancer were less than 1% (27).

In conclusion, rearrangements found in the *BRCA1* gene were present in a considerable proportion of the mutations detected among women who were being treated at a cancer genetics clinic for breast and ovarian cancer risk assessment. Some rearrangements are more common in specific regions of Turkey. Patients at high risk for ovarian cancer, triple-negative breast cancer, and bilateral breast cancer, and patients with breast and ovarian cancer should be tested for rearrangements. Furthermore, the analysis of rearrangements should be part of *BRCA1* and *BRCA2* testing and a standard application for Turkish patients at high risk for breast and ovarian cancer.

According to our results, there is no longer any doubt as to whether rearrangements should be tested in patients at high risk for breast and ovarian cancer in Turkey. Rearrangement testing should include *BRCA1* and *BRCA2* analyses in all routine genetic tests in Turkey. We think that our results have clarified the limits and contents of *BRCA1* and *BRCA2* testing in Turkey.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Istanbul University, İstanbul School of Medicine (2011/1425-681).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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