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Research Article

Importance of Diagnosis in Breast Cancer with Non-BRCA Pathogenic Germline Variants of Cancer Susceptibility Genes using High-Throughput Sequencing Analysis

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Abstract

Objectives: The aim was to point out the importance of the diagnosis rate of breast cancer (BC) by analyzing the cancer predisposition genes except *BRCA1/2* with multigene testing.

Methods: In this study, 232 non-BRCA cases with BC and/or BC family history (FH) were analyzed using the next-generation sequencing method.

Results: Twenty-two different pathogenic/likely pathogenic variants were determined in 24 (10.34%) of cases, and these variants were detected in the *CHEK2* (7/24, 29.1%), *ATM* (5/24, 20.8%), *MUTYH* (3/24, 12.5%), *BLM* (2/24, 8.3%), *WRN* (2/24, 8.3%), *TP53* (1/24, 4.1%), *BRIP1* (1/24, 4.1%), *MSH2* (1/24, 4.1%), *NBN* (1/24, 4.1%), and *PTEN* (1/24, 4.1%) genes including three novel variants which were identified in the *BLM, ATM*, and *MSH2* (3/22, 13.6%) genes. Fourteen of 24 (58.3%) cases had BC diagnosis, and 10 of 24 (41.6%) cases had a FH of BC.

Conclusion: Among non-BRCA BC and/or BC FH cases, cancer susceptibility gene frequency was 10.34% in this study. *CHEK2* and *ATM* genes had relatively high mutation rates.

Keywords: Breast cancer, Cancer susceptibility, Non-*BRCA1/2*, Targeted gene analysis

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Epidemiological studies have shown that family history (FH) is the most important risk factor in breast cancers (BCs).[1] Although the majority of BC is sporadic cases, familial BC occurs at a rate of 5%–10% with hereditary causes.[2] Hereditary BCs occur 5–15 years earlier than sporadic cases. Many genes are involved in the development of BC, but mutations of some genes that are responsible for hereditary BCs, especially those that function in the maintenance of genome stability, have been shown.[3] *BRCA1* and *BRCA2* genes have been found as susceptibility genes for BC with high penetration, which are observed in hereditary BC.[4] Women who include germ cell mutations in these genes have a high risk of developing BC at some time in their lives. Germ cell mutations in the *BRCA1* and *BRCA2* genes have been shown in many studies as high-risk factors for BC.[5] These two genes, which still carry the most severe mutations for familial BC, are at the forefront of mutation analysis in BC risk determination. Apart from these two genes, it is known that there are other genes that cause breast and ovarian cancer.[6] The new genes detected in

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these studies have also been associated with BC and have been added to clinical BC research as new mutations. First of all, *CDH1, PTEN, STK11*, and *TP53* gene mutations were among these genes, and later genes such as *ATM, BARD1, CHEK2*, and *PALB2* with functions similar to *BRCA1* and *BRCA2* were started to be analyzed. In addition to all these, candidate genes thought to play a role in BC (e.g., *CDKN2A, MEN1, MLH1, MSH2, MSH6*, and *MUTYH*) were added to increase the number of mutations examined.[7]

With rapidly developing technology in recent years, new generation devices have been produced, and many panel-based genes have begun to be analyzed simultaneously with the next-generation sequencing (NGS) method. The ultimate goal of panel-based genetic tests is to provide the highest level of care and treatment approaches that can be given to cancer patients and their relatives.[8] This situation aims to prevent cancer formation among unaffected family members, especially in the evaluation of contralateral BC risk and evaluation of other cancers with a high probability of occurrence (e.g., ovarian, colorectal, pancreatic cancers).

To date, however, the prevalence of germline pathogenic/likely pathogenic variants in non-BRCA genes is partially investigated in breast/ovarian cancer, and available data about these genetic risk factors in cancer are still poor. The aim of the current study was to present the prevalence of non-*BRCA1/2* genes in breast/ovarian cancer cases from Istanbul, Turkey, and evaluate the clinical utility of multigene panels.

Methods

Study Population

The patient files of 254 cases with breast/over cancer and/or hereditary breast/over cancer history (from October 15, 2018, to December 31, 2020) were reviewed in the medical genetics department. Twenty-two cases had a *BRCA1/BRCA2* pathogenic variant and were excluded. The remaining 232 patients were included in our study. Clinical information was obtained through the patient's clinical chart. For the FH, data were obtained from pedigrees. The genetic testing was applied according to the American National Comprehensive Cancer Network (NCCN) guidelines.

The cases were evaluated in two different clinical definitions: (1) BC history and (2) BC FH. If the case had not any diagnosis of breast/over cancer, but has one or more first- or second-degree relatives with breast/over cancer, then this case was called "positive FH"). These cases were tested in a medical genetics clinic and had the analysis of germline cancer predisposition genes. After examining file records, FH was reviewed for each case. NCCN guidelines were used to predict the prognosis of a case carrying a germline mutation of cancer predisposition genes.

This study is approved by the Ethical Committee of our university with decision number 168/2021 and performed in consonance with the principles of the Declaration of Helsinki. The written informed consent forms were obtained from the cases and/or families.

Targeted NGS Panel and NGS Data Analysis

Two 2 mL of peripheral blood samples of patients were collected to EDTA-containing tubes. Genomic DNA was isolated using MagPurix® Blood DNA Extraction Kit (Zinexts, New Taipei, Taiwan). Quality control of the isolated DNA samples was checked using SpectraMax i3x (Molecular Devices, California, USA). Samples that have an A260/280 value between 1.8 and 2.0 were included. Low-quality samples were reextracted from stored blood samples.

Fastq generation was performed on Illumina Nextseq 500 platform (Illumina, Inc., San Diego, CA, USA). Libraries covering the target genes were prepared according to the TruSight Cancer Panel protocol (Illumina, Inc., San Diego, CA, USA). Following the target enrichment process, libraries were sequenced on the Illumina Nextseq 500 platform (Illumina, Inc., San Diego, CA, USA).

TruSight® Cancer Sequencing Panel (Illumina, Inc., San Diego, CA, USA) and a custom panel (23 genes) were used according to the manufacturer's instructions for NGS. Targeted gene panel 1 included *ATM, BLM, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM, FANCC, MEN1, MLH1, MSH2, MSH6, MUTYH, NBN, PALB2, PMS2, PTEN, RAD51C, RAD51D, STK11, TP53*, and *XRCC2* (23 genes) genes, and panel 2 included AIP, *ALK, APC, ATM, BAP1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, BUB1B, CDC73, CDH1, CDK4, CDKN1C, CDKN2A, CEBPA, CEP57, CHEK2, CYLD, DDB2, DICER1, DIS3L2, EGFR, EPCAM, ERCC2, ERCC3, ERCC4, ERCC5, EXT1, EXT2, EZH2, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, FH, FLCN, GATA2, GPC3, HNF1A, HRAS, KIT, MAX, MEN1, MET, MLH1, MSH2, MSH6, MUTYH, NBN, NF1, NF2, NSD1, PALB2, PHOX2B, PMS1, PMS2, PRF1, PRKAR1A, PTCH1, PTEN, RAD51C, RAD51D, RB1, RECQL4, RET, RHBDF2, RUNX1, SBDS, SDHAF2, SDHB, SDHC, SDHD, SLX4, SMAD4, SMARCB1, STK11, SUFU, TMEM127, TP53, TSC1, TSC2, VHL, WRN, WT1, XPA,* and *XPC* (94 genes) genes.

All variants classified according to our pipeline as likely pathogenic or pathogenic were confirmed by conventional capillary Sanger sequencing. For this, the genomic DNAs were amplified by PCR, purified with the enzyme Exosap-IT (USB) and Big Dye X terminator kit (Applied Biosystems), and sequenced bidirectionally using the 3500XL platform (Applied Biosystems).

NGS Data Analysis

Alignment to the reference genomes (hg19 for humans) was performed using Burrows-Wheeler Aligner. The identified variants were functionally annotated using ANNOVAR. Variants were visually examined using Integrative Genomics Viewer 2.8.13 (https://software.broadinstitute.org/software/igv/). Recommendations of the Human Genome Variation Society[9] were followed to describe the novel variants, and ACMG's 2015[10] guidelines were followed for the classification of all the variants. ClinVar[11] and literature studies are considered for collecting information about known variations.

Results

A total of 232 cases with breast/ovarian cancer and/or breast/ovarian cancer FH, who satisfied the NCCN testing criteria for the multigene panel and excluded *BRCA1/2* pathogenic/likely pathogenic variants, were included in this study. Among these 232 patients with breast and/or ovarian cancer, 44.82% (104/232) had their primary cancer diagnosis at age 45 years or younger. Of these 232 patients, 122 (52.58%) had at least one first-degree relative affected with breast or ovarian cancer. Most of the tested individuals were female, comprising 99.1% (230/232) of the total. The majority of the breast tumors were invasive ductal carcinomas with a range of 83.1% (193/232). HER2, estrogen, and progesterone receptor status were available for a subgroup of 127/232 (54.7%) of BC patients, of which 27/148 (18.2%) had triple-negative BC (TNBC). Among the mutation-positive cases, 62.5% (15/24) had been evaluated for BC history, 37.5% (9/24) had been evaluated for having BC FH.

The median age at diagnosis was 39 years (range 27–70 years) among 24 cases who had germline mutations. Among these 24 patients (24/232, 10.34%) with 22 different pathogenic/likely pathogenic germline variants, the major mutant non-BRCA genes were *CHEK2* (n=7), *ATM* (n=5), and *MUTYH* (n=3). Other pathogenic/likely pathogenic variants were in the *BLM* (n=2), *WRN* (n=2 sisters), *TP53* (n=1), *BRIP1* (n=1), *MSH2* (n=1), *NBN* (n=1), and *PTEN* (n=1) genes. We identified three novel pathogenic/likely pathogenic variants that were never reported before, including *BLM* c.572_573delGA, *ATM* c.7629+1G>T, and *MSH2* c.908A>G (Table 1). Cases 20 and 23, who have the same variant in *CHEK2* gene, were not related. Fourteen of 24 (58.3%) cases had BC diagnosis, 10 of 24 (41.6%) cases had a FH of BC. *CHEK2* mutated four patients had BC diagnosis, and 3 cases had a FH of BC. ATM mutated 4 patients had BC diagnosis and 1 case had a FH of BC. MUTYH mutated 1 patient had BC diagnosis and 2 cases had a FH of BC. The distribution of BC diagnosis and positive FH of BC were not significantly different in gene mutations.

Besides breast/ovarian cancer, lung cancer (n=3, three families), colon cancer (n=2, two families), brain cancer (n=3, three families), osteosarcoma (n=1, 1 family), thyroid cancer (n=1, 1 family), gastric cancer (n=1, 1 family), bladder cancer (n=1, 1 family), liver cancer (n=1, 1 family), leukemia (n=1, 1 family) lymphoma (n=1, 1 family), and endometrium cancer (n=1, 1 family) were also observed.

Discussion

The present study demonstrated that about 10.3% of Turkish breast/over cancer patients who were previously tested BRCA-negative could have been diagnosed as mutated with multigene testing. Our study contributed also to the knowledge of pathogenic/likely pathogenic germline variants in multiple cancer susceptibility genes in the Turkish population. In total, 232 consecutive individuals with personal or FH of breast and/or ovarian without pathogenic/likely pathogenic variants in *BRCA1* and *BRCA2* genes were analyzed (Table 2).

BC is the most common type of cancer and the most common mortal malign disease in women, and its incidence increases with age. It is in the first place among cancers seen in women with a rate of 24.1%.[12] Positive FH is an important risk factor for BC. It is stated that a person with a first-degree relative with BC has a 1.8-fold risk of developing BC, and in the presence of two first-degree relatives, this risk increases 2.9-fold. If the relative with BC is diagnosed before the age of 30 years, the risk increases 2.9 times, and if diagnosed after the age of 60 years, the risk increases 1.5 times.[13] In the current study, 54.16% (13/24) had BC FH, and 37.5% (9/24) had other cancer family histories of our cases. The incidence of BC under 40 years of age in Turkey is reported as 20%.[14] A study from Turkey reported that 31% of BC is seen in women between the ages of 40 and 50 years and 20.2% in women under the age of 40 years.[14] In our study, the percentage of primary cancer diagnosis under 45 years was 44.82% and under 40 years was 59%. A high percentage of our cases had been diagnosed at a younger age. In an invasive BC cohort study, including 54 555 cases, the mean age was 49.5 years for patients with a single primary breast tumor.[15]

In hereditary BC, *BRCA1* and *BRCA2* genes, which encode proteins involved in maintaining genome continuity and DNA repair mechanisms, are indicated as susceptibility genes to BC with high penetration. It is stated that the risk of developing BC varies between 45% and 65% in cases with germline mutations in these genes by the age of 70 years.[16] It has been known that there are BC susceptibility genes except *BRCA1/2*. In the current study, we identified non-

Table 1. Clinical findings, family histories of the cases, and the pathogenic/likely pathogenic variants detected in the current study

Case/gender/age	Test indication	Affected family member	Gene	Variant	Protein	Pathogenicity	dbSNP	Gene panel*
1/F/31	Unilateral BC (right)	Mother – lung cancer; father –colon cancer; two sisters – breast cancer	WRN	(NM_000553.6):c.3493C>T	p.(Gln1165Ter)	Pathogenic (PVS1, PM2, PP3, PP5)	rs121908447	2
2/F/34	Positive FH	Mother – lung cancer; father – colon cancer; three sisters – breast cancer	WRN	(NM_000553.6):c.3493C>T	p.(Gln1165Ter)	Pathogenic (PVS1, PM2, PP3, PP5)	rs121908447	2
3/F/29	Positive FH	Aunt – breast cancer	CHEK2	(NM_007194.4):c.422A>C	p.(Lys141Thr)	Likely pathogenic (PM1, PM2, PP2, PP3)	rs786203192	2
4/F/27	Unilateral BC (left)	Uncle – osteosarcoma; cousin – brain tumor	MUTYH	(NM_001128425.2):c.884C>T	p.(Pro295Leu)	Pathogenic (PM1, PM2, PP2, PP3, PP5)	rs374950566	2
5/F/38	BC	–	BLM	(NM_000057.4):c.1642C>T	p.(Gln548Ter)	Pathogenic (PVS1, PM2, PP3, PP5)	rs200389141	1
6/F/53	Positive FH	Aunt – breast cancer	NBN	(NM_002485.5):c.2140C>T	p.(Arg714Ter)	Pathogenic (PVS1, PM2, PP3, PP5)	rs730881864	1
7/F/70	BC	–	CHEK2	(NM_001005735.2):c.599T>C	p.(Ile200Thr)	Pathogenic (PS3, PM1, PM5, PP2, PP5)	rs17879961	1
8/F/49	Positive FH	Sister – breast cancer	MUTYH	(NM_001128425.2): c.1437_1439delGGA	p.(Glu480del)	Pathogenic (PS3, PM1, PM2, PM4, PP3, PP5)	rs587778541	1
9/F/36	BC	Aunt and aunt's daughter – breast cancer; grandmother – brain tumor	BLM	(NM_000057.4):c.572_573delGA	p.(Arg191LysfsTer4)	Pathogenic (PVS1, PM2, PP3)	Novel	1
10/F/38	BC	Sister – thyroid cancer; cousin – gastric cancer; aunt – lymphoma	ATM	(NM_000051.4):c.7629+1G>T	–	Pathogenic (PVS1, PM2, PP3)	Nnovel	1
11/F/38	Positive FH	Aunt – breast cancer	MUTYH	(NM_001128425.2):c.1187G>A	p.(Gly396Asp)	Pathogenic (PS3, PM1, PM5, PP2, PP3, PP5)	rs36053993	1
12/F/33	Positive FH	Aunt – breast cancer	ATM	(NM_000051.4): c.5986_5988delGAA	p.(Glu1996del)	Likely pathogenic (PM1, PM2, PM4, PP3)	rs1555111872	1
13/F/54	Positive FH	Mother – breast cancer	MSH2	(NM_000251.3):c.908A>G	p.(Asp303Gly)	Likely pathogenic (PM2,PP2,PP3)	Novel	1
14/M/38	BC, prostate cancer?	Mother's father – lung cancer	CHEK2	(NM_007194.4):c.1169A>C	p.(Tyr390Ser)	Likely pathogenic (PM1, PM2, PM5, PP2, PP3)	rs200928781	1
15/F/59	Positive FH	Cousin – breast cancer	CHEK2	(NM_007194.4):c.1049delC	p.(Pro350GlnfsTer15)	Pathogenic (PVS1, PM2, PP3, PP5)	rs1601727022	1
16/F/32	Unilateral BC	–	ATM	(NM_000051.4):c.6082C>T	p.(Gln2028Ter)	Pathogenic (PVS1, PM2, PP3, PP5)	rs876659454	1
17/F/34	BC	–	BRIP1	(NM_032043.3):c.3072delG	p.(Ser1025HisfsTer34)	Likely pathogenic (PVS1, PM2)	rs1342519012	1
18/F/29	Unilateral BC	Aunt – brain tumor; mother's mother – liver cancer; father – bladder cancer	ATM	(NM_000051.4):c.6154G>A	p.(Glu2052Lys)	Likely pathogenic (HGMD-CM1612882-disease causing)	rs202206540	2
19/F/42	Positive FH	Sister – breast cancer	CHEK2	(NM_007194.4):c.1232G>A	p.(Trp411Ter)	Pathogenic (PVS1, PM2, PP3, PP5)	rs371418985	1
20/F/40	BC	Mother's mother – breast cancer	CHEK2	(NM_007194.4):c.1427C>T	p.(Thr476Met)	Likely pathogenic (PM1, PM2, PM5, PP2, PP3, PP5)	rs142763740	2
21/F/45	BC	Sister – breast cancer	ATM	(NM_000051.4):c.6199-1G>T	–	Pathogenic (PVS1, PM2, PP3, PP5)	rs1591788932	1

Table 1. CONT.

Case/gender/age	Test indication	Affected family member	Gene	Variant	Protein	Pathogenicity	dbSNP	Gene panel*
22/F/39	Unilateral BC (left)	Father – leukemia	TP53	(NM_001276760.2):c.257C>T	p.(Thr86Met)	Pathogenic (PM1, PM2, PM5, PP2, PP3)	rs786201057	2
23/F/40	BC	Mother – ovarian cyst/cancer?	CHEK2	(NM_007194.4):c.1427C>T	p.(Thr476Met)	Likely pathogenic (PM1, PM2, PM5, PP2, PP3, PP5)	rs142763740	1
24/F/42	Bilateral BC	Brother – lymphoma; cousin – endometrium cancer	PTEN	(NM_000314.8):c.333G>A	p.(Trp111Ter)	Pathogenic (PVS1, PM2, PP3, PP5)	rs1554898097	1

*Panel 1: *ATM, BLM, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM, FANCC, MEN1, MLH1, MSH2, MSH6, MUTYH, NBN, PALB2, PMS2, PTEN, RAD51C, RAD51D, STK11, TP53, and XRCC2* (23 genes).
Panel 2: *AIP, ALK, APC, ATM, BAP1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, BUB1B, CDC73, CDH1, CDK4, CDKN1C, CDKN2A, CEBPA, CEP57, CHEK2, CYLD, DDB2, DICER1, DIS3L2, EGFR, EPCAM, ERCC2, ERCC3, ERCC4, ERCC5, EXT1, EXT2, EZH2, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, FH, FLCN, GATA2, GPC3, HNF1A, HRAS, KIT, MAX, MEN1, MET, MLH1, MSH2, MSH6, MUTYH, NBN, NF1, NF2, NSD1, PALB2, PHOX2B, PMS1, PMS2, PRF1, PRKAR1A, PTCH1, PTEN, RAD51C, RAD51D, RB1, RECQL4, RET, RHBDF2, RUNX1, SBDS, SDHAF2, SDHB, SDHC, SDHD, SLX4, SMAD4, SMARCB1, STK11, SUFU, TMEM127, TP53, TSC1, TSC2, VHL, WRN, WT1, XPA, and XPC* (94 genes).

BRCA1/2 twenty-one different pathogenic/likely pathogenic germline variants in cancer susceptibility genes. Similar studies reported different frequencies of non-*BRCA1/2* pathogenic germline variants as 10%,[6] 14%,[17] 12.3%,[18] 3.97%,[19] and 4.9%.[20] Another study including only under 40 years of age and non-*BRCA1/2* BC patients reported 11% pathogenic/likely pathogenic variants in the cancer susceptibility genes.[21] With the rate of 10.34%, our study showed a similar rate for non-*BRCA1/2* pathogenic variants compared with other populations.

We analyzed cancer susceptibility genes with two different targeted gene panels (23 genes and 94 genes panels). In a study, whole exome sequencing (WES) was applied to identify new breast and/or ovarian cancer predisposition genes in 52 non-BRCA1/BRCA2/TP53 mutation carrier women at high risk for hereditary breast and ovarian cancer.[22] The pathogenic variants were identified in *CHEK2, MUTYH, PMS2, RAD51C, FAN1, POLQ, RAD54L, DROSHA, and SLC34A2* genes.[22] The largest gene panel included 94 genes in our study and we identified the pathogenic variants in *CHECK2, ATM, MUTYH, BLM, WRN, TP53, BRIP1, MSH2, and NBN* genes with three novel variants. This result may be due to the relatively high case number of our study (232 cases) compared with this study (52 cases). The most frequent pathogenic variants were in CHEK2 gene in both our study and this WES study. With larger gene panels or with whole exome sequencing, new cancer susceptibility genes will be identifiable.

The most frequent non-*BRCA1/2* cancer susceptibility genes were *MUTYH* and *PTCH1* in China,[6] *CHEK2*, *ATM*, and *PALB2* in Germany,[23] *CHEK2* and *ATM* genes in the USA.[21] Our study, presenting Turkey population frequencies, identified the most frequent non-*BRCA1/2* pathogenic variants in *CHEK2*, *ATM*, and *MUTYH*, showing similarities and differences with these studies. A study from China evaluating germline variants of 16 DNA repair genes (*ATM, BLM, CHEK2, FANCC, MER11A, MLH1, MSH2, MSH6, MUTYH, NBN, PALB2, PMS2, RAD50, RAD51C, RAD51D, and TP53*) determined 3.4% frequency.[24] The most frequent mutations were in *PALB2*, *TP53*, *ATM*, and *RAD51D* genes[24] different from our study except ATM frequency. In Cyprus, the frequency of non-BRCA cancer susceptibility genes was reported as 4.9% in TNBC patients with the most frequent mutated gene *PALB2*.[20]

The most frequent pathogenic variants were in the *CHEK2* gene in our study similar to Felicio et al.'s study.[22] *CHEK2* protein is a serine/threonine kinase and is a transmission protein in the DNA damage checkpoint pathway.[25] DNA repair begins as a result of CHEK2 protein phosphorylating the BRCA1 protein serine 988 (S988) amino acid.

Table 2. Clinical findings, family histories of the cases except mutated patients detected in the current study

Case	Gender	Age	Test indication	Affected family member	Gene panel*
1002575	F	47	Familial breast cancer	No family history	1
1002274	F	38	Familial breast cancer	No family history	1
1004340	F	30	Familial breast cancer	No family history	1
1005248	F	55	Familial breast cancer	No family history	1
1003405	F	47	Familial breast cancer	No family history	1
1004810	F	46	Familial breast cancer	No family history	1
1008117	F	48	Familial breast cancer	No family history	1
1005127	F	57	Familial breast cancer	No family history	1
1004665	F	49	Familial breast cancer	No family history	1
1004666	F	51	Familial breast cancer	No family history	1
1004701	F	42	Familial breast cancer	No family history	1
1004888	F	36	Familial breast cancer	No family history	1
1004954	F	34	Familial breast cancer	No family history	1
1005459	F	40	Familial breast cancer	No family history	1
1005750	F	46	Familial breast cancer	Aunt and mother – breast and ovarian cancer	1
1008990	F	38	Breast cancer	No family history	1
1010675	F	34	Breast cancer	No family history	1
1014362	F	56	Familial breast cancer	No family history	1
1013429	F	34	Breast Mass	Mother and two aunts – breast cancer	1
1013430	F	60	Mass ın the left breast	Mother and sister – breast cancer	1
1013839	F	42	Familial breast cancer	No family history	1
1013875	F	22	Familial breast cancer	No family history	1
1013879	F	47	Left breast cancer	No family history	1
1013925	F	51	Breast cancer Story	No family history	1
1013931	F	50	Operated breast cancer	No family history	1
1014015	F	43	Breast cancer famıly story	No family history	1
1014144	F	41	Breast cancer	No family history	1
1013981	F	44	Familial breast cancer	No family history	1
1013982	F	46	Familial breast cancer	No family history	1
1015292	F	30	Familial breast cancer	No family history	1
1013329	F	53	Breast cancer	No family history	1
1015525	F	74	Familial breast cancer	No family history	1
1015526	F	54	Familial breast cancer	No family history	1
1015527	F	53	Familial breast cancer	No family history	1
1015528	F	47	Familial breast cancer	No family history	1
1015989	F	40	Familial breast cancer	No family history	1
1016045	F	48	Familial breast cancer	Mother and aunt – breast cancer	1
1016067	F	32	Familial breast cancer	No family history	1
1016400	F	33	Familial breast cancer	No family history	1
1016597	F	46	Familial breast cancer	Mother – breast cancer	1

Table 2. CONT.

Case	Gender	Age	Test indication	Affected family member	Gene panel*
1016784	F	50	Familial breast cancer	Mother and aunt – breast cancer	1
1018079	F	43	Risk of breast and ovarıan cancer	Mother and aunt – breast cancer	1
1018387	F	36	Hereditary breast cancer	No family history	1
1018580	F	34	Hereditary breast cancer	Mother breast cancer	1
1018746	F	37	Hereditary breast cancer	No family history	1
1018796	F	49	Hereditary breast cancer	Mother breast cancer	1
1018959	F	39	Hereditary breast cancer	Grandmother, mother, and aunt – breast cancer	1
1018854	F	38	Hereditary breast cancer	No family history	1
1018853	F	48	Hereditary breast cancer	Mother breast cancer	1
1018822	F	34	Hereditary breast cancer	Mother – breast cancer; uncle and daughter – breast cancer	1
1019193	F	43	Hereditary breast cancer	No family history	1
1019020	F	45	Hereditary breast cancer	Mother and aunt – breast cancer	1
1019071	F	35	Hereditary breast cancer	Sıster – breast cancer	1
1019333	F	46	Hereditary breast cancer	Mother – breast cancer	1
1019302	F	52	Hereditary breast cancer	Breast cancer in the family	1
1019440	F	65	Breast cancer	Mother – breast cancer	1
1019759	F	47	Familial breast cancer	Mother – breast cancer	1
1019910	F	43	Familial breast cancer	No family history	1
1020026	F	35	Familial breast cancer	No family history	1
1020087	F	30	Hereditary breast cancer	Aunt – breast cancer	1
1020476	F	36	Familial breast cancer	No family history	1
1020531	F	45	Malıgnant mass in right breast	No family history	1
1020928	F	24	Hereditary breast cancer	Grandmother – breast cancer	1
1020980	F	23	Hereditary breast cancer	Grandmother – breast cancer	1
1021409	F	32	Hereditary breast cancer	No family history	1
1022249	F	50	Familial breast cancer	Mother – breast cancer	1
1022251	F	24	Hereditary breast cancer	Breast cancer in the family	1
1022199	F	46	Familial breast cancer	Mother – breast cancer	1
1022659	F	60	Familial breast cancer	Sister – breast cancer	1
1022977	F	62	Breast cancer	No family history	1
1023033	F	33	Invasive ductal carcinoma	No family history	1
1023048	F	35	Familial breast cancer	Mother – breast cancer; father – renal transitional cell carcinoma	1
1023409	F	46	Familial breast cancer	Breast and uterine cancer in her aunt's daughter	1
1023418	F	47	Familial breast cancer – right breast cancer	Lung small cell cancer ın father; postmenopausal breast cancer in her uncle's daughter	1
1023480	F	32	Familial breast cancer – early breast cancer	No family history	1
1023486	F	58	Familial breast cancer	Brother – breast cancer	1
1023485	M	52	Familial breast cancer	No family history	1
1023888	M	47	Familial breast cancer	No family history	1
1024363	F	36	Familial breast cancer	Mother – breast cancer; mother's aunt breast cancer	1
1024404	F	32	Familial breast cancer	Mother – invasive breast cancer	1

Table 2. CONT.

Case	Gender	Age	Test indication	Affected family member	Gene panel*
1024442	F	56	Familial breast cancer	Sister and aunt's daughter – breast cancer	1
1025022	F	55	Familial breast cancer	Sıster – breast cancer; brother – lung cancer	1
1025374	F	41	Familial breast cancer	Aunt – breast cancer	1
1025381	F	47	Familial breast cancer	2 aunts, sister, and uncle's daughter – breast cancer	1
1025547	F	39	Familial breast cancer	Father – colon cancer; sister – breast cancer	1
1025569	F	50	Familial breast cancer	Mother – breast cancer	1
1025931	F	29	Familial breast cancer	Mother – breast cancer	1
1026059	F	28	Familial breast cancer	Mother and aunt – breast cancer	1
1026220	F	48	Familial breast cancer	Sister – breast cancer	1
1026599	F	41	Familial breast cancer	Grandfather and mother – breast cancer	1
1026732	F	47	Familial breast cancer/over cancer	No family history	1
1026986	F	34	Familial breast cancer	Grandfather, mother, and aunt – breast cancer	1
1027165	F	41	Familial breast cancer	No family history	1
1027631	F	47	Familial breast cancer	2 aunts – breast cancer	1
1028633	F	49	Familial breast cancer	Mother – breast cancer	1
1028683	F	50	Familial breast cancer	Breast cancer in daughter of his uncle's son; father – colon cancer; uncle – stomach cancer	1
1028745	F	32	Familial breast cancer	Aunt – breast cancer	1
1028858	F	44	Familial breast cancer	Mother – breast cancer	1
1028873	F	29	Familial breast cancer	Mother – breast cancer	1
1028933	F	47	Familial breast cancer	Mother – breast cancer	1
1029113	F	46	Familial breast cancer	No family history	1
1029965	F	51	Familial breast cancer	Mother's sister and father's sıster – breast cancer	1
1030065	F	45	Familial breast cancer	No family history	1
1029487	F	40	Familial breast cancer	Mother – breast cancer	1
1029709	F	50	Familial breast cancer	Mother – breast cancer	1
1029803	F	49	Familial breast cancer	Breast cancer in two aunt's daughters	1
1030130	F	32	Familial breast cancer	Aunt – breast cancer	1
1030217	F	39	Familial breast cancer	Aunt – breast cancer	1
1030505	F	51	Familial breast cancer	Mother, aunt, and father's sister – breast cancer	1
1030564	F	48	Familial breast cancer	No family history	1
1030800	F	50	Familial breast cancer	No family history	1
1031034	F	45	Familial breast cancer	Family history of cancer	1
1031239	F	32	Familial breast cancer	Aunt – breast cancer	1
1031246	F	50	Familial breast cancer	No family history	1
1031249	F	59	Familial breast cancer	No family history	1
1031320	F	42	Familial breast cancer	Mother – breast cancer	1
1031563	F	40	Familial breast cancer	Aunt – breast cancer	1
1031696	F	35	Familial breast cancer	Aunt's daughter – breast cancer	1
1031784	F	48	Familial breast cancer	No family history	1
1032423	F	61	Familial breast cancer	Aunt's daughter – breast cancer	1

Table 2. CONT.

Case	Gender	Age	Test indication	Affected family member	Gene panel*
1032499	F	48	Familial breast cancer	Sister – breast cancer	1
1032519	F	50	Familial breast cancer	No family history	1
1032628	F	52	Familial breast cancer	Aunt – breast cancer	1
1032693	F	39	Familial breast cancer	No family history	1
1032711	F	46	Familial breast cancer	Grandfather – over cancer; mother – uterine cancer	1
1032825	F	51	Familial breast cancer	No family history	1
1032353	F	55	Familial breast cancer	No family history	1
1032303	F	55	Familial breast cancer	Aunts – breast cancer	1
1032257	F	41	Familial breast cancer	No family history	1
1032255	F	49	Familial breast cancer	No family history	1
1032242	F	42	Familial breast cancer	Aunt – breast cancer	1
1033249	F	63	Familial breast cancer	No family history	1
1033958	F	60	Familial breast cancer – breast cancer	No family history	1
1030342	F	50	Familial breast cancer – breast cancer	Sister – breast cancer; aunt's daughter – breast cancer	1
1032984	F	49	Familial breast cancer	No family history	1
1033012	F	46	Familial breast cancer	Daughter of father's uncle – breast cancer	1
1033291	F	49	Familial breast cancer	Aunt – breast cancer	1
1033356	F	50	Familial breast cancer	Aunt – breast cancer	1
1033430	F	48	Familial breast cancer	No family history	1
1033431	F	49	Familial breast cancer	Aunt – breast cancer	1
1033631	F	22	Familial breast cancer	Aunt and grandpa – breast cancer	1
1033642	F	56	Familial breast cancer	Sister – breast cancer	1
1033643	F	26	Familial breast cancer	Aunt – breast cancer	1
1038206	F	43	Mass in the breast	No family history	1
1034096	F	41	Familial breast cancer	Sister – breast cancer	1
1033852	F	48	Familial breast cancer – breast cancer	No family history	1
1034279	F	48	Familial breast cancer	Sister – breast cancer	1
1033889	F	55	Familial breast cancer	Two sisters – breast cancer	1
1033927	F	49	Familial breast cancer	Father – lung and larynx cancer	1
1034264	F	40	Familial breast cancer	No family history	1
1034315	F	29	Familial breast cancer – mass in the breast	Mother – breast cancer; father – lung cancer; brother – colon cancer; breast cancer suspect in daughter	1
1034440	F	44	Familial breast cancer	Sister – breast cancer	1
1034316	F	57	Familial breast cancer – mass in the breast	Mother – breast cancer; father – lung cancer; brother – colon cancer; breast cancer suspect in daughter	1
1034485	F	37	Familial breast cancer	No family history	1
1034685	F	46	Familial breast cancer	Mother – breast cancer	1
1034731	F	48	Familial breast cancer	Father – pancreas cancer; uncle – lung cancer; grandpa and uncle's daughter – breast cancer	1
1034847	F	54	Familial breast cancer	Sister – breast cancer	1
1038025	F	54	Familial breast cancer	Family history of cancer	1

Table 2. CONT.

Case	Gender	Age	Test indication	Affected family member	Gene panel*
1034959	F	45	Familial breast cancer	Mother and uncle's daughter – breast cancer	1
1034967	F	56	Familial breast cancer	Sister – breast cancer	1
1034968	F	54	Familial breast cancer	Sister and uncle's daughter – breast cancer	1
1035087	F	40	Familial breast cancer	Sister – breast cancer	1
1035137	F	43	Familial breast cancer	Mother – breast cancer; father – prostate cancer	1
1035224	F	65	Familial breast cancer	Mother and aunt – breast cancer	1
1035232	F	39	Familial breast cancer	Grandfather – breast cancer	1
1035235	F	53	Familial breast cancer	No family history	1
1036007	F	46	Familial breast cancer	Sister – breast cancer	1
1036184	F	36	Familial breast cancer	Mother's aunt – breast cancer	1
1036232	F	32	Familial breast cancer	Mother – breast cancer	1
1035697	F	58	Breast cancer	Mother, uncle's daughter, aunt's daughter – breast cancer	1
1036584	F	47	Familial breast cancer	Aunt – bladder cancer; uncle – lung cancer; another uncle – prostate cancer	1
1036965	F	47	Breast cancer	Father's uncle – prostate cancer; uncle's children – lung cancer	1
1036624	F	37	Familial breast cancer	Sister – breast cancer	1
1036659	F	36	Breast cancer	No family history	1
1036748	F	62	Familial breast cancer	Sister – breast cancer	1
1036752	F	40	Familial breast cancer	No family history	1
1036908	F	38	Familial breast cancer	Mother and father's aunt – breast cancer	1
1036959	F	44	Familial breast cancer	Father and uncle – lung cancer; aunt's daughter – breast cancer	1
1037002	F	33	Familial breast cancer	Aunt – breast cancer	1
1037266	F	37	Familial breast cancer	Aunt – breast cancer; father – colon cancer; aunt's daughter – over cancer	1
1037296	F	28	Familial breast cancer	Grandfather – uterus cancer; brother – lung cancer; sister – breast, uterus, lung, and liver cancer	1
1037428	F	43	Familial breast cancer	No family history	1
1037598	F	36	Familial breast cancer	No family history	1
1038354	F	44	Familial breast cancer	Grandfather – breast cancer; father – prostate cancer	1
1038943	F	26	Familial breast cancer	No family history	1
1038957	F	40	Familial breast cancer	No family history	1
1037848	F	41	breast cancer	Uncle – lung cancer	1
1037906	F	38	Familial breast cancer	Uncle – prostate cancer	1
1037941	F	27	Familial breast cancer	Sister and aunt – breast cancer	1
1039034	F	34	Early breast cancer	No family history	1
1039042	F	38	Familial breast cancer	Mother and aunt – breast cancer	1
1039215	F	46	Familial breast cancer	Mother – breast cancer	1
1039219	F	34	Familial breast cancer	Sister and aunt – breast cancer	1
1039266	F	56	Breast cancer	No family history	1
1039093	F	39	Familial breast cancer	Mother – breast cancer	1
1039340	F	42	Familial breast cancer	Familial breast cancer	1
1039563	F	44	Familial breast cancer	Mother – breast cancer	1
1039628	F	55	Breast cancer	Sister – breast cancer	1

Table 2. CONT.

Case	Gender	Age	Test indication	Affected family member	Gene panel*
1039859	F	46	Genetic breast cancer	No family history	1
1040055	F	33	Familial breast cancer	Sister, cousin, and mother – breast cancer	1
1040157	F	32	Familial breast cancer	Mother – breast cancer	1
1040160	F	47	Breast cancer	Father – stomach cancer; uncle – lung cancer	1
1040420	F	34	Breast cancer	No family history	1
1040486	F	40	Breast cancer	No family history	1
1040676	F	46	Breast cancer	No family history	1
1040814	F	52	Familial breast cancer	Sister – breast cancer; father – stomach cancer; mother – over cancer	1
1040825	F	36	Familial breast cancer	Aunt and mother – breast cancer	1
1003405	F	47	Familial breast cancer	No family history	1

*Panel 1: *ATM, BLM, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM, FANCC, MEN1, MLH1, MSH2, MSH6, MUTYH, NBN, PALB2, PMS2, PTEN, RAD51C, RAD51D, STK11, TP53, and XRCC2* (23 genes)

Panel 2: *AIP, ALK, APC, ATM, BAP1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, BUB1B, CDC73, CDH1, CDK4, CDKN1C, CDKN2A, CEBPA, CEP57, CHEK2, CYLD, DDB2, DICER1, DIS3L2, EGFR, EPCAM, ERCC2, ERCC3, ERCC4, ERCC5, EXT1, EXT2, EZH2, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, FH, FLCN, GATA2, GPC3, HNF1A, HRAS, KIT, MAX, MEN1, MET, MLH1, MSH2, MSH6, MUTYH, NBN, NF1, NF2, NSD1, PALB2, PHOX2B, PMS1, PMS2, PRF1, PRKAR1A, PTCH1, PTEN, RAD51C, RAD51D, RB1, RECQL4, RET, RHBDF2, RUNX1, SBDS, SDHAF2, SDHB, SDHC, SDHD, SLX4, SMAD4, SMARCB1, STK11, SUFU, TMEM127, TP53, TSC1, TSC2, VHL, WRN, WT1, XPA, and XPC* (94 genes)

CHEK2 protein also phosphorylates FOXM1 (forkhead box M1) which is a transcription factor, increasing its stability. FOXM1 transcription factor, on the other hand, increases the expression of BRCA2, which is involved in homologous recombination DNA repair mechanism, and X-ray repair cross-complementing protein 1 (XRCC1) genes, which are involved in the base (cutout) repair mechanism.[26] The most frequent pathogenic variants in CHEK2 gene should not be a surprise for BC in our study due to CHEK2 effects *BRCA1/2* mechanisms in different ways.

A rapid evolution occurred for genetic testing in hereditary cancer predisposition. In the past years, the recommended genetic tests were preferred and requested primarily according to the phenotype of the patient, while the current approach is to perform panel-test-based genetic tests.[27] Although the genetic test to be planned is shown as an indication for only one or two mutations based on the criteria specified in the guidelines, this also means testing the presence of many pathogenic variants in many different genes. Indeed, NCCN guidelines recommend a multigene panel assessment for efficiency and cost-effectiveness for individuals with negative BRCA1 and BRCA2 test results and suspected of having one or more inherited syndromes for cancer prevention, surveillance, and management. It should be kept in mind that the ultimate goal of all these expanded, panel-based genetic tests is to provide the highest level of care and treatment approaches that can be given to cancer patients and their relatives. This situation aims to prevent cancer formation among unaffected family members, especially in the evaluation of contralateral BC risk and evaluation of other cancers with a high probability of occurrence (e.g., ovarian, colorectal, pancreatic cancers). In recent years, genetic tests used in the diagnosis of BC have become an indispensable tool in the personalization of the treatment of the disease and in identifying and managing individuals at risk in their families. The multigene targeted panel testing is offered for being cost-effective.[28] In addition, with the correct interpretation of genetic tests and genetic counseling, an important contribution is made to specialists responsible for the treatment of individuals with BC.

Conclusion

In summary, the present study performed a characterization of germline variants identified in cancer susceptibility genes, using two targeted gene panels and bioinformatic analyses in Turkish non-BRCA1/BRCA2 mutation carrier cases with personal and/or familial BC history. Our results suggest that non-*BRCA1/2* genes such as *CHEK2, ATM, MUTYH, BLM, WRN, TP53, BRIP1, MSH2, and NBN* may have a role in BC. Three novel pathogenic/likely pathogenic variants in

BLM, ATM, and MSH2 genes were identified in the current study. This is a cross-sectional study from Istanbul, which is a city demonstrating general Turkey demographic features, which reports the non-*BRCA1/2* gene frequencies, and which suggests that targeted gene analysis increases the diagnosis rate in cases with personal and/or FH of BCs.

Disclosures

Ethics Committee Approval: The study was approved by the Local Ethics Committee.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Concept – A.A., S.Y.; Design – A.A., S.Y.; Supervision – A.A., S.S., F.C.G.; Materials – A.A., F.C.G., S.Y.; Data collection &/or processing – A.A., S.Y., S.S.; Analysis and/or interpretation – A.A., S.S.; Literature search – A.A., SY.; Writing – A.A., S.Y., S.S., F.C.G.; Critical review – A.A., S.Y., S.S., F.C.G.

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