**Original Article**

Eur J Breast Health 2023; 19(1): 55-69
*DOI: 10.4274/ejbh.galenos.2022.2022-7-2*

# Genetic, Surgical and Oncological Approach to Breast Cancer, with *BRCA1, BRCA2, CDH1, PALB2, PTEN* and *TP53* Variants

Aslı Subaşıoğlu[1], Zeynep Gülsüm Güç[2], Emine Özlem Gür[3], Mustafa Agah Tekindal[4], Murat Kemal Atahan[3]

[1]Department of Medical Genetics, İzmir Katip Çelebi University Faculty of Medicine, İzmir, Turkey
[2]Department of Medical Oncology, İzmir Katip Çelebi University Faculty of Medicine, İzmir, Turkey
[3]Department of General Surgery, İzmir Katip Çelebi University Faculty of Medicine, İzmir, Turkey
[4]Department of Biostatistics, İzmir Katip Çelebi University Faculty of Medicine, İzmir, Turkey

## ABSTRACT

**Objective:** The aim of this study was to determine the frequency of germline variants in *BRCA1, BRCA2, CDH1, PALB2, PTEN* and *TP53* in patients admitted to a medical genetics clinic with breast cancer and to assess these identified variants according to published genetic, surgical and oncological perspectives.

**Materials and Methods:** Medical history, and cancer diagnosis information for 195 independent probands with operated breast cancer were collected from requisition forms and medical records. The exonic regions and exon-intron junctions in *BRCA1, BRCA2, CDH1, PALB2, PTEN* and *TP53* genes were sequenced. Analysis of fastq files was performed on the Qiagen Clinical Insight-Analyse Universal with panel-specific pipeline and vcf files were interpreted clinically using Qiagen Clinical Insight-Interpret.

**Results:** Gene variants (pathogenic, likely pathogenic and variants of unknown significance) were detected in 53 (27.2%). Detailed information about the patients (age of diagnosis, family history, gender), cancer stage, tumour characteristics (ER, PR, human epidermal growth factor receptor 2 status) and all information related to the detected variants (gene, location, nucleotide and amino acid change, exon number, impact, mutation classification, dbSNP number and HGMD variant class) were assessed. In total, 58 mutations were identified including 14 novel, previously unreported variants.

**Conclusion:** Molecular characterization and identification of mutations have important implications for predictive, preventive, and personalized medicine, including genetic counseling and development of specific treatment protocols. We emphasize variants of unknown significance (VUS) as the clinical significance of VUS changes over time and variant classification is important for clinical molecular genetic testing and clinical guidance. This study may provide new insights into risk assessment for variants in *CDH1, PALB2, PTEN* and *TP53*, in addition to *BRCA1* and *BRCA2*, which may prove useful for clinical management of breast cancer patients. Further studies are needed to identify the common gene variants in the Turkish population and evaluate the pathogenity of VUS.

**Keywords:** *BRCA1/2*; breast cancer; *CDH1/PALB2*; genetic testing; *TP53/PTEN*

**Cite this article as:** Subaşıoğlu A, Güç ZG, Gür EÖ, Tekindal MA, Atahan MK. Genetic, Surgical and Oncological Approach to Breast Cancer, with *BRCA1, BRCA2, CDH1, PALB2, PTEN* and *TP53* Variants. Eur J Breast Health 2023; 19(1): 55-69

### Key Point

- The purpose of this study was to determine the frequency of germline variants in *BRCA1, BRCA2, CDH1, PALB2, PTEN* and *TP53* in a population of Turkish patients admitted to a medical genetics clinic with breast cancer. In addition, the identified variants were assessed in the light of published genetic, surgical and oncological perspectives.

**Corresponding Author:**
**Aslı Subaşıoğlu; aslisubasioglu@gmail.com**

Received: 19.07.2022
Accepted: 25.09.2022
Available Online Date: 01.01.2023

## Introduction

Breast cancer is the most prevalent cancer and the leading cause of death among women worldwide, accounting for approximately 12% of all new cancer cases diagnosed in recent years (1). The etiology of breast cancer is multifactorial and complex, and most cases are sporadic, although genetic factors play an important role. The most common cause of hereditary breast cancer is because of inherited germline mutations in the high-penetrant cancer predisposition genes *BRCA1* and *BRCA2*. Beside these genes, advances in DNA sequencing techniques, such as next generation sequencing, have helped to identify additional breast cancer susceptibility genes, including *TP53, CDH1, PALB2* and *PTEN* and various rare gene variants have also been reported to increase the risk of developing breast cancer. The prevalence of *BRCA1* and *BRCA2* pathogenic variants is estimated to be 1/400 to 1/500 in the general population and is increased in some populations due to the founder effect (2). It is estimated that the risk of developing breast cancer by age of 80 is 72% for *BRCA1* mutation carriers and 69% for *BRCA2* mutation carriers, respectively. According to the literature, the risk for contralateral breast cancer 20 years after first breast cancer diagnosis is 40% for *BRCA1* and 26% for *BRCA2* carriers (3).

Tumor protein p53 (*TP53*) is one of the most mutated genes in cancer, including breast cancer. *TP53* is a tumor suppressor gene that encodes tumor protein p53, a transcription factor. Germline *TP53* mutations are associated with Li-Fraumeni syndrome, a rare autosomal dominant genetic disorder. In Li-Fraumeni syndrome, in addition to breast cancer, brain tumors, adrenocortical carcinoma, leukemia, and germ cell tumors have been reported. Although germline *TP53* mutations are rare and seen in approximately 1% of all breast cancers, the lifetime risk of breast cancer in *TP53* mutation carriers is nearly 80–90%, considerably greater than for other genes (4).

The *CDH1* gene encodes E-cadherin, a calcium ion-dependent cell-cell adhesion protein, and it is known that germline *CDH1* pathogenic variants predispose the individuals to both diffuse gastric cancer and lobular breast cancer (LBC). Studies have shown that estimated cumulative risk of LBC for women is 42% by age 80 years (5, 6). Partner and localizer *BRCA2* (*PALB2*) is one of the important DNA repair genes that co-localizes with *BRCA2* in nuclear foci. *PALB2* has functions in homologous recombination, recombinational repair and checkpoint mechanisms. Several studies showed the increased risk of breast cancer in *PALB2* mutation carriers. In a large cohort, *PALB2* mutations were found in approximately 1% of the *BRCA1/2* negative patients with breast cancer and in 0.19% of healthy controls (7). Phosphatase and tensin homolog (*PTEN*) is one of the most common tumor suppressor genes. It is known that germline *PTEN* mutations predispose to the development of Cowden syndrome, an autosomal dominant inherited cancer syndrome characterized by multiple hamartomas and malignancies, including breast, thyroid, and endometrial cancer. Studies have shown that the prevalence of Cowden syndrome to about 1:200,000 patients and women with Cowden syndrome have a 20% to 50% lifetime risk of developing breast cancer (8).

Patients with suspected hereditary breast cancer should obtain genetic counseling and be informed about the risk factors. The National Comprehensive Cancer Network (NCCN) has published recommendations to guide healthcare providers in identifying individuals with hereditary cancer syndrome (Table 1) (9). In the presence of any of the criteria, there is an indication for genetic testing for hereditary breast cancer as there is high lifetime risk for mutation

Table 1. Testing criteria for breast cancer susceptibility genes (summarized from ref. 9)

| | | | |
|---|---|---|---|
| 1. | Individuals with any blood relative with a known pathogenic or likely pathogenic variant in a cancer susceptibility gene | | |
| 2. | Personal history of cancer | | |
| | a. Breast cancer with at least one of the following | *Diagnosed at age ≤45 y; or | |
| | | *Diagnosed at age 46-50 y with | • Unknown or limited family history,<br>• A second breast cancer diagnosed at any age,<br>• ≥1 close blood relative with breast, ovarian, pancreatic or prostate cancer at any age |
| | | *Diagnosed at age ≤60 y with triple-negative breast cancer | |
| | | *Diagnosed at any age with; | • Ashkenazi Jewish ancestry,<br>• ≥1 close blood relative with breast cancer at age ≤50 y ovarian, pancreatic, metastatic, intraductal/ cribriform histology or high-risk group prostate cancer at any age,<br>• ≥3 total diagnoses of breast cancer in patient and/or close blood relatives |
| | | *Diagnosed at any age with male breast cancer | |
| | b. A mutation identified on tumor genomic testing that has clinical implications if also identified in the germline | | |
| | c. Individuals who meet Li-Fraumeni syndrome testing criteria or Cowden syndrome/PTEN hamartoma tumor syndrome testing criteria | | |
| | d. To aid in systemic therapy decision-making, such as for HER2-negative metastatic breast cancer | | |
| 3. | Family history of cancer | • An individual with a first- or second-degree blood relative with any of these criteria<br>An individual who does not meet the criteria but has a probability ≥5% of a *BRCA1/2* pathogenic variant based on probability models | |

PTEN: phosphatase and tensin homolog; HER2: human epidermal growth factor receptor 2

carriers (Table 2) (10). Detection of germline mutations may lead to the improvement of diagnostics and selection of patients sensitive to targeted therapeutics. Therefore it may help the development of familial screening strategies and may also have important implications for development of specific treatment and prevention protocols in mutation carriers.

## Materials and Methods

A total of 195 operated breast cancer patients who attended a Medical Genetics Department between 2019 and 2022 were included. According to the NCCN guideline criteria, in the 195 patients selected for this study, the age and the family history of the patients were the main criteria (Table 1). The study was conducted in accordance with the Declaration of Helsinki and was approved by İzmir Katip Çelebi Non-Interventional Clinical Studies Institutional Review Board (0028/20.01.2022). Medical history was collected from requisition forms and medical records. Written informed consent forms were obtained from all patients. In this study, the exonic regions and exon-intron junctions in *BRCA1, BRCA2, CDH1, PALB2, PTEN* and *TP53* genes were analyzed. The workflow included sample extraction, library preparation, sequencing and bioinformatics steps. In the sample extraction step, DNA from blood samples was extracted using EZ1 DSP DNA Blood Kit (Qiagen, Hilden, Germany). Qubit dsDNA HS, Thermo kit with Qubit 3 Fluorometer were performed to measure and optimize DNA concentration. QIAseq Targeted DNA Human *BRCA1* and *BRCA2* Plus Panel, Qiagen (333505) were used according to manufacturer instructions for Illumina sequencers. The genomic DNA was fragmented and all fragmented DNA was barcoded with unique molecular indices to track the original DNA molecule; hence, high sensitivity detection was obtained. Additionally, targeted genes were amplified with single primer extension technology and a bead clean-up step was performed to discard unwanted fragments. The concentration optimization of libraries was performed using Qiaseq Quant Assay Kit (Qiagen), and all libraries were diluted to 4 nM. Libraries with different sample indices were combined in equimolar amounts in a final pool. As a next step, the final pool was sequenced in a Miseq System, (Illumina Inc., San Diego, CA, USA), according to manufacturer's guide. The secondary analysis of fastq files was performed using Qiagen Clinical Insight-Analyse Universal with a panel-specific pipeline. Finally, the vcf files were clinically interpreted using Qiagen Clinical Insight-Interpret. Pathogenic, likely pathogenic variants and VUS in *BRCA1, BRCA2, CDH1, PALB2, PTEN* and *TP53* genes were identified, reported and assessed in light of published evidence.

## Results

Gene variants (pathogenic, likely pathogenic and VUS) in *BRCA1, BRCA2, CDH1, PALB2, PTEN* and *TP53* genes were found in 53 (27.2%) of 195 patients. Of the cohort, 122 (62.6%) attended clinic with a positive family history. Furthermore, 121 (62.05%) were under 45 years of age and 48 (24.6%) met both family history and age criteria. In total, 58 mutations were identified with four patients having more than one mutation, while 14 novel previously unreported variants were detected. Of 53 patients, 20 had pathogenic variants, three had likely pathogenic variants and 35 had VUS.

Of the nine patient with *BRCA1* variants, eight had different variants, three had the same mutation and one had two *BRCA1* variants. Of the eight different *BRCA1* variants, four were considered pathogenic and four were VUS.

There were 26 different *BRCA2* variants identified in 32 patients, and of these 10 were pathogenic, one likely pathogenic and 15 were VUS.

Three different *TP53* variants were detected in three patients, of which two were pathogenic and one was VUS. Eight different *PALB2* variants were detected in eight patients, including two pathogenic, two likely pathogenic and four VUS. Five different *CDH1* variants were identified in five patients, all of which were VUS. In this cohort, no variant was found in the *PTEN* gene. Detailed information about the patients (age of diagnosis, family history, gender), cancer stage, tumour characteristics [ER, PR, and human epidermal growth factor receptor 2 (HER2) status] and all information related with the detected variants (gene, location, nucleotide and amino acid change, exon number, impact, mutation classification, dbSNP number and HGMD variant class) can be seen in Tables 3–7 and Figures 1–3.

Table 2. *BRCA1, BRCA2, TP53, CDH1, PALB2* and *PTEN* genes and lifetime risks for breast cancer (10)

| Gene and transcript | Cytogenetic location | Genomic coordinates (GRCh38) | Tumor age (years) | Risk (%) | Incidence (birth incidence of pathogenic variants) | Life expectancy |
|---|---|---|---|---|---|---|
| *BRCA1* NM_007294.4/ | CHR 17q21.31 | 17:43,044,294-43,125,363 | >18 | 50–90 | 1 in 400–800 | 62 years |
| *BRCA2* NM_000059.4/ | CHR 13q13.1 | 13:32,315,507-32,400,267 | >18 | 40–90 | 1 in 800 | 68 years |
| *TP53* NM_000546.6/ | CHR 17p13.1 | 17:7,668,420-7,687,489 | >18 | 80–95 | 1 in 5000 | Severely reduced |
| *CDH1* NM_004360.5/ | CHR 16q22.1, | 16:68,737,291-68,835,536 | >18 | 70–80 | Very rare | Reduced |
| *PALB2* NM_024675.4 | CHR 16p12.2 | 16:23,603,164-23,641,309 | >18 | 40–60 | <1 in 1000 | Normal |
| *PTEN* NM_000314.8 | CHR 10q23.31 | 10:87,863,624-87,971,929 | >18 | 60 | 1 in 10000–250000 | Reduced |

Table 3. Detailed information about the patients and detected variants in the *BRCA1* gene. In total, nine patient had eight different *BRCA1* mutations, three of them had the same mutation and one (P2) had two *BRCA1* variants

| | Analysis ID | Age of diagnosis | Family history | Gender | Gene/location | Nucleotide/ amino acid change | Exon | Impact | Mutation classification | dbSNP number | HGMD variant class/evidence |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | S133 | 38 | + | Female | BRCA1 NM_007294.4/ CHR 17: 41,276,047 | c.66dupA p.E23fs*18 | 2 | Frameshift | Pathogenic | rs80357783 | Disease causing mutation |
| | | | | | CDH1 NM_004360.5/ CHR 16: 68,847,232 | c.1154C>G p.P385R | 9 | Missense | Variant of uncertain significance | No dbSNP ID found | Not reported |
| 2 | S69 | 50 | + | Female | BRCA1 NM_007294.4/ CHR 17: 41,243,715 | c.3833A>C p.K1278T | 10 | Missense | Variant of uncertain significance | No dbSNP ID found | Not reported |
| | | | | | BRCA1 NM_007294.4/ CHR 17: 41,244,466 | c.3082C>T p.R1028C | 10 | Missense | Variant of uncertain significance | rs80357049 | Disease causing mutation? |
| 3 | S366 | 28 | + | Female | BRCA1 NM_007294.4/ CHR 17: 41,246,489 | c.1059G>A p.W353* | 10 | Stop gain | Pathogenic | rs80356935 | Disease causing mutation |
| 4 | S109 | 39 | | Female | BRCA1 NM_007294.4/ CHR 17: 41,244,748 | c.2800C>T p.Q934* | 10 | Stop gain | Pathogenic | rs80357223 | Disease causing mutation |
| 5 | S539 | 55 | + | Female | BRCA1 NM_007294.4/ CHR 17: 41,245,664 | c.1884T>G p.S628R | 10 | Missense | Variant of uncertain significance | rs80357495 | Not mentioned as a disease causing mutation |
| 6 | S1512 | 45 | | Female | BRCA1 NM_007294.4/ CHR 17: 41,228,634 | c.4358-3A>G | Intron 12 | Splicing | Variant of uncertain significance | No dbSNP ID found | Disease causing mutation? |
| 7 | S425 | 69 | + | Female | BRCA1 NM_007294.4/ CHR 17: 41,209,079 | c.5266dupC p.Q1756fs*74 | 19 | Frameshift | Pathogenic | rs80357906 | Disease causing mutation |
| 8 | S1408 | 49 | + | Female | BRCA1 NM_007294.4/ CHR 17: 41,209,079 | c.5266dupC p.Q1756fs*74 | 19 | Frameshift | Pathogenic | rs80357906 | Disease causing mutation |
| 9 | S809 | 65 | + | Female | BRCA1 NM_007294.4/ CHR 17: 41,209,079 | c.5266dupC p.Q1756fs*74 | 19 | Frameshift | Pathogenic | rs80357906 | Disease causing mutation |

Table 4. Detailed information about the patients and detected variants in the *BRCA2* gene. Twenty-six different *BRCA2* variants were detected in 31 patients; 10 were considered pathologic, 1 likely pathologic and 15 were considered variant of uncertain significance (VUS)

| | Analysis ID | Age of diagnosis | Family history | Gender | Gene/location | Nucleotide/amino acid change | Exon | Impact | Mutation classification | dbSNP number | HGMD variant class/evidence |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | S374 | 52 | | Female | BRCA2 NM_000059.4/ CHR 13: 32,899,249 | c.353G>A p.R118H | 4 | Missense | Variant of uncertain significance | rs80358603 | Disease causing mutation? |
| 2 | S1538 | 37 | | Female | BRCA2 NM_000059.4/ CHR 13: 32,905,066 | c.692G>C p.S231T | 9 | Missense | Variant of uncertain significance | rs1064793498 | Disease causing mutation |
| 3 | S2101 | 29 | | Female | BRCA2 NM_000059.4/ CHR 13: 32,906,669 | c.1055dupA p.Y352* | 10 | Frameshift Insertion | Pathogenic | rs886038060 | Not reported |
| 4 | S1012 | 53 | + | Female | BRCA2 NM_000059.4/ CHR 13: 32,907,376 | c.1763_1766delATAA p.N588fs*25 | 10 | Frameshift | Pathogenic | rs80359303 | Disease causing mutation |
| 5 | S231 | 42 | | Female | BRCA2 NM_000059.4/ CHR 13: 32,911,100 | c.2608A>G p.I870V | 11 | Missense | Variant of uncertain significance | No dbSNP ID found | Not reported |
| 6 | S1754 | 35 | | Female | BRCA2 NM_000059.4/ CHR 13: 32,911,418 | c.2926_2927delTCinsAT p.S976I | 11 | Missense | Variant of uncertain significance | No dbSNP ID found | Not mentioned as a disease causing mutation |
| 7 | S1579 | 39 | + | Female | BRCA2 NM_000059.4/ CHR 13: 32,911,802 | c.3310A>C p.T1104P | 11 | Missense | Variant of uncertain significance | rs80358577 | Disease causing mutation? |
| | | | | | BRCA2 NM_000059.4/ CHR 13: 32,911,995 | c.3503T>A p.M1168K | 11 | Missense | Variant of uncertain significance | rs80358598 | Not mentioned as a disease causing mutation |
| | | | | | CDH1 NM_004360.5/ CHR 16: 68,867,348 | c.2595G>C p.W865C | 16 | Missense | Variant of uncertain significance | rs778019174 | Not reported |
| 8 | S144 | 28 | + | Female | BRCA2 NM_000059.4/ CHR 13: 32,912,525 | c.4033G>T p.D1345Y | 11 | Missense | Variant of uncertain significance | No dbSNP ID found | Not mentioned as a disease causing mutation |

Table 4. continued

| | Analysis ID | Age of diagnosis | Family history | Gender | Gene/location | Nucleotide/amino acid change | Exon | Impact | Mutation classification | dbSNP number | HGMD variant class/evidence |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **9** | **S111** | **60** | **+** | **Male** | **BRCA2 NM_000059.4 / CHR 13: 32,912,962** | **c.4471_4474delCTGA p.L1491fs*12** | **11** | **Frameshift** | Pathogenic | rs80359451 | Disease causing mutation |
| **10** | **S183** | **39** | | **Female** | **BRCA2 NM_000059.4/ CHR 13: 32,913,118** | **c.4631dupA p.N1544fs*4** | **11** | **Frameshift** | Pathogenic | rs80359460 | Disease causing mutation |
| 11 | S2090 | 52 | + | Female | BRCA2 NM_000059.4/ CHR 13: 32,913,696 | c.5206_5208delCAA p.Q1736del | 11 | In-frame deletion | Likely Pathogenic | No dbSNP ID found | Not reported |
| **12** | **S1526** | **37** | | **Female** | **BRCA2NM_000059.4/ CHR 13:32,914,066** | **c.5576_5579delTTAA p.I1859fs*3** | **11** | **Frameshift** | Pathogenic | rs80359521 | Disease causing mutation |
| **13** | **S1727** | **75** | **+** | **Female** | **BRCA2 NM_000059.4/ CHR 13: 32,914,283** | **c.5791C>T p.Q1931*** | **11** | **Stop gain** | Pathogenic | rs80358807 | Disease causing mutation |
| **14** | **S125** | **28** | **+** | **Female** | **BRCA2 NM_000059.4/ CHR 13: 32,914,919** | **c.6427_6428delTCinsAT p.S2143I** | **11** | **Missense** | **Variant of uncertain significance** | No dbSNP ID found | Disease causing mutation? |
| **15** | **S89** | **38** | **+** | **Female** | **BRCA2 NM_000059.4/ CHR 13: 32,914,919** | **c.6427_6428delTCinsAT p.S2143I** | **11** | **Missense** | **Variant of uncertain significance** | No dbSNP ID found | Disease causing mutation? |
| **16** | **S429** | **33** | **+** | **Female** | **BRCA2 NM_000059.4/ CHR 13: 32,915,118** | **c.6626T>C p.I2209T** | **11** | **Missense** | **Variant of uncertain significance** | rs431825344 | Not mentioned as a disease causing mutation |
| **17** | **S1820** | **43** | **+** | **Female** | **BRCA2 NM_000059.4/ CHR 13: 32,921,033** | **c.7007G>A p.R2336H** | **13** | **Splicing** | Pathogenic | rs28897743 | Disease causing mutation |
| **18** | **S1038** | **80** | **+** | **Female** | **BRCA2 NM_000059.4/ CHR 13: 32,953,960** | **c.7481G>A p.R2494Q** | **15** | **Missense** | **Variant of uncertain significance** | rs80358973 | Disease causing mutation? |

Table 4. continued

| | Analysis ID | Age of diagnosis | Family history | Gender | Gene/location | Nucleotide/amino acid change | Exon | Impact | Mutation classification | dbSNP number | HGMD variant class/evidence |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 19 | S1547 | 45 | + | Female | BRCA2 NM_000059.4/ CHR 13: 32,944,552 | c.8345G>A p.S2782N | 19 | Missense | Variant of uncertain significance | No dbSNP ID found | Not mentioned as a disease causing mutation |
| 20 | S959 | 58 | + | Female | BRCA2 NM_000059.4/ CHR 13: 32,944,558 | c.8351G>A p.R2784Q | 19 | Missense | Pathogenic | rs80359076 | Disease causing mutation |
| | | | | | PALB2 NM_024675.4/ CHR 16: 23,614,913 | c.3428T>A p.L1143H | 13 | Missense | Variant of uncertain significance | No dbSNP ID found | Disease causing mutation |
| 21 | S2047 | 52 | + | Female | BRCA2 NM_000059.4/ CHR 13: 32,944,638 | c.8431G>A p.D2811N | 19 | Missense | Uncertain significance | No dbSNP ID found | Not reported |
| 22 | S956 | 40 | + | Female | BRCA2 NM_000059.4/ CHR 13: 32,945,189 | c.8585dupT p.E2863fs*6 | 20 | Frameshift | Pathogenic | rs80359720 | Disease causing mutation |
| 23 | S639 | 42 | + | Female | BRCA2 NM_000059.4/ CHR 13: 32,950,845 | c.8671A>G p.T2891A | 21 | Missense | Variant of uncertain significance | No dbSNP ID found | Not mentioned as a disease causing mutation |
| 24 | S143 | 39 | + | Female | BRCA2 NM_000059.4/ CHR 13: 32,950,845 | c.8671A>G p.T2891A | 21 | Missense | Variant of uncertain significance | No dbSNP ID found | Not mentioned as a disease causing mutation |
| 25 | S1751 | 52 | | Female | BRCA2 NM_000059.4/ CHR 13: 32,953,603 | c.8904C>T p.T2968T | 22 | Synonymous | Variant of uncertain significance | rs41293519 | Not reported |
| 26 | S1031 | 36 | + | Female | BRCA2 NM_000059.4/ CHR 13: 32,953,960 | c.9027delT p.H3010fs*18 | 23 | Frameshift | Pathogenic | rs80359742 | Disease causing mutation |

Table 4. continued

| | Analysis ID | Age of diagnosis | Family history | Gender | Gene/location | Nucleotide/amino acid change | Exon | Impact | Mutation classification | dbSNP number | HGMD variant class/evidence |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **27** | **S48** | **37** | **+** | **Female** | **BRCA2 NM_000059.4/ CHR 13: 32,972,626** | **c.9976A>T p.K3326*** | **27** | **Stop gain** | **Variant of uncertain significance** | rs11571833 | Disease-associated polymorphism with additional supporting functional evidence |
| **28** | **S56** | **33** | | **Female** | **BRCA2 NM_000059.4/ CHR 13: 32,972,626** | **c.9976A>T p.K3326*** | **27** | **Stop gain** | **Variant of uncertain significance** | rs11571833 | Disease-associated polymorphism with additional supporting functional evidence |
| **29** | **S60** | **48** | **+** | **Female** | **BRCA2 NM_000059.4/ CHR 13: 32,972,626** | **c.9976A>T p.K3326*** | **27** | **Stop gain** | **Variant of uncertain significance** | rs11571833 | Disease-associated polymorphism with additional supporting functional evidence |
| **30** | **S2003** | **44** | **+** | **Female** | **BRCA2 NM_000059.4/ CHR 13: 32,972,626** | **c.9976A>T p.K3326*** | **27** | **Stop gain** | **Variant of uncertain significance** | rs11571833 | Disease-associated polymorphism with additional supporting functional evidence |
| **31** | **S2054** | **29** | | **Female** | **BRCA2 NM_000059.4/ CHR 13: 32,972,626** | **c.9976A>T p.K3326*** | **27** | **Stop gain** | **Variant of uncertain significance** | rs11571833 | Disease-associated polymorphism with additional supporting functional evidence |

Table 5. Detailed information about the patients and detected variants in the TP53 gene. In total, three patients had three different TP53 mutations, one of which was previously unreported

| | Analysis ID | Age of diagnosis | Family history | Gender | Gene/location | Nucleotide/ aminoacid change | Exon | Impact | Mutation classification | dbSNP number | HGMD variant class/evidence |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | **S904** | **27** | | **Female** | **TP53 NM_000546.6/ CHR 17: 7,579,428** | **c.259C>T p.P87S** | **4** | **Missense** | **Variant of uncertain significance** | No dbSNP ID found | Not reported |
| **2** | **S154** | **39** | **+** | **Female** | **TP53 NM_000546.6/ CHR 17: 7,578,457** | **c.473G>A p.R158H** | **5** | **Missense** | Pathogenic | rs587782144 | Disease causing mutation |
| **3** | **S1196** | **47** | **+** | **Female** | **TP53 NM_000546.6/ 17: 7,577,093** | **c.845G>T p.R282L** | **8** | **Missense** | Pathogenic | rs730882008 | Disease causing mutation |

Table 6. Detailed information about the patients and detected variants in the *CDH1* gene. In total five patient had five different *CDH1* mutations; one had a *BRCA1* mutation and one other had two *BRCA2* mutations. Four of the *CDH1* mutations were previously unreported

| | Analysis ID | Age of diagnosis | Family history | Gender | Gene/location | Nucleotit/ aminoacid change | Exon | Impact | Mutation classification | dbSNP number | HGMD variant class |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | **S133** | **38** | **+** | **Female** | BRCA1 NM_007294.4/ CHR 17: 41,276,047 | c.66dupA p.E23fs*18 | 2 | Frameshift | Pathogenic | rs80357783 | Disease causing mutation |
| | | | | | **CDH1 NM_004360.5/ CHR 16: 68,847,232** | **c.1154C>G p.P385R** | **9** | **Missense** | **Variant of uncertain significance** | **No dbSNP ID found** | **Not reported** |
| **2** | **S1488** | **37** | **+** | **Female** | **CDH1 NM_004360.5/ CHR 16: 68,849,520** | **c.1423G>A p.V475M** | **10** | **Missense** | **Variant of uncertain significance** | **rs587782113** | **Disease causing mutation** |
| **3** | **S1602** | **59** | **+** | **Female** | **CDH1 NM_004360.5/ CHR 16: 68,855,982** | **c.1790C>G p.P597R** | **12** | **Missense** | **Variant of uncertain significance** | **No dbSNP ID found** | **Not reported** |
| **4** | **S2106** | **44** | | **Male** | **CDH1 NM_004360.5/ CHR 16: 68,867,313** | **c.2560G>A p.D854N** | **16** | **Missense** | **Variant of uncertain significance** | **No dbSNP ID found** | **Not reported** |
| **5** | **S1579** | **39** | **+** | **Female** | BRCA2 NM_000059.4/ CHR 13: 32,911,995 | c.3503T>A p.M1168K | 11 | Missense | Variant of uncertain significance | rs80358598 | Not mentioned as a disease causing mutation |
| | | | | | BRCA2 NM_000059.4/ CHR 13: 32,911,802 | c.3310A>C p.T1104P | 11 | Missense | Variant of uncertain significance | rs80358577 | Disease causing mutation? |
| | | | | | **CDH1 NM_004360.5/ CHR 16: 68,867,348** | **c.2595G>C p.W865C** | **16** | **Missense** | **Variant of uncertain significance** | **rs778019174** | **Not reported** |

Table 7. Detailed information about the patients and detected variants in the *PALB2* gene. In total eight patients had seven different *PALB2* mutations; two had the same mutation and one also had a *BRCA2* variant. Three of the mutations were previously unreported

| | Analysis ID | Age of diagnosis | Family history | Gender | Gene/location | Nucleotide/ amino acid change | Exon | Impact | Mutation classification | dbSNP number | HGMD variant class |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | S206 | 35 | + | Female | PALB2 NM_024675.4/CHR 16: 23,649,188 | c.194C>T p.P65L | 3 | Missense | Variant of uncertain significance | rs62625272 | Not mentioned as a disease causing mutation |
| 2 | S1914 | 49 | + | Female | PALB2 NM_024675.4/ CHR 16: 23,647,033 | c.833_834delTAinsAT p.L278H | 4 | Missense | Likely pathogenic | rs587778582 | Disease causing mutation? |
| 3 | S1202 | 39 | | Female | PALB2 NM_024675.4/ CHR 16: 23,641,218 | c.2257C>T p.R753* | 5 | Stop gain | Pathogenic | rs180177110 | Disease causing mutation |
| 4 | S807 | 53 | | Female | PALB2 NM_024675.4/ CHR 16: 23,641,218 | c.2257C>T p.R753* | 5 | Stop gain | Pathogenic | rs180177110 | Disease causing mutation |
| 5 | S434 | 55 | + | Female | PALB2 NM_024675.4/ CHR 16: 23,619,249 | c.3285dupT p.N1096* | 12 | Frameshift | Likely pathogenic | No dbSNP ID found | Not reported |
| 6 | S252 | 67 | + | Female | PALB2 NM_024675.4 CHR 16: 23,614,953 | c.3388G>C p.A1130P | 13 | Missense | Variant of uncertain significance | No dbSNP ID found | Not reported |
| 7 | S959 | 58 | + | Female | BRCA2 NM_000059.4/ CHR 13: 32,944,558 | c.8351G>A p.R2784Q | 19 | Missense | Pathogenic | rs80359076 | Disease causing mutation |
| | | | | | PALB2 NM_024675.4/ CHR 16: 23,614,913 | c.3428T>A p.L1143H | 13 | Missense | Variant of uncertain significance | No dbSNP ID found | Disease causing mutation |
| 8 | S1690 | 39 | | Female | PALB2 NM_024675.4/ CHR 16: 23,614,894 | c.3447C>A p.A1149A | 13 | Synonymous | Variant of uncertain significance | No dbSNP ID found | Not reported |

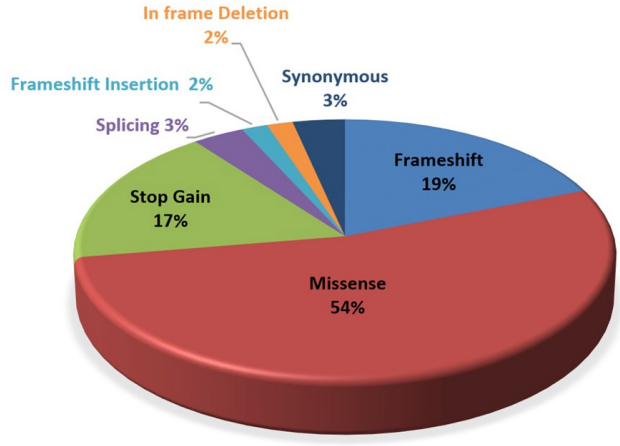


**Figure 1.** Detected variants' impacts

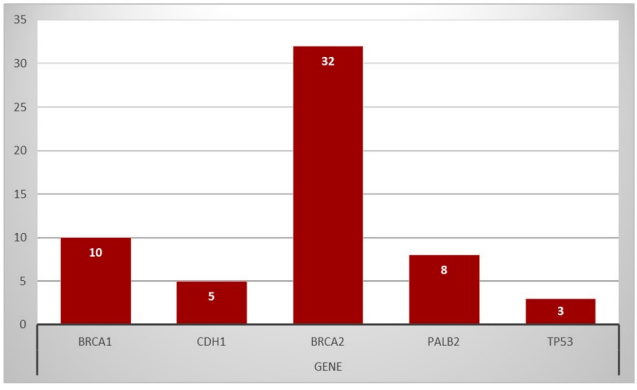


**Figure 2.** Distribution of variants by genes

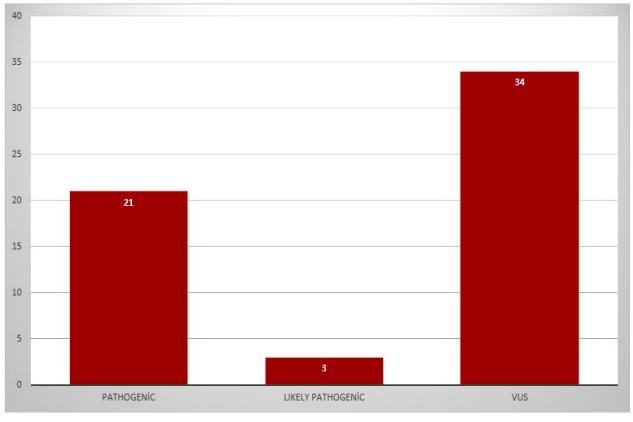


**Figure 3.** Distribution of variants by pathogenicity

## Discussion and Conclusion

*BRCA1* is predominantly a breast cancer gene and for carriers the estimated lifetime for breast cancer ranges between 50–85% (10). In the present study, eight different *BRCA1* variants were detected in nine patients, and four were considered pathogenic and four were VUS. Pathogenic variants were c.5266dupC (p.Q1756fs*74) and c.66dupA (p.E23fs*18) that are both frameshift mutations and c.1059G>A (p.W353*) and c.2800C>T (p.Q934*) that are both stop codon mutations. Frameshift mutations are particularly severe and cause changes in many bases downstream and can affect many of the amino acids in the protein. c.5266dupC (p.Q1756fs*74), which is one of the most common pathogenic variants in *BRCA1* gene, was found in three patients (1.54%) in this study. According to the previous studies the estimated prevalence of the mutation is between 2.5% and 7.1% in breast cancer patients (11). c.66dupA (p.E23fs*18), c.1059G>A (p.W353*) and c.2800C>T (p.Q934*) mutations have been reported previously, while the c.2800C>T (p.Q934*) mutation was previously recognized as a Japanese founder mutation (12). c.3833A>C (p.K1278T) and c.3082C>T (p.R1028C) mutations, which were both VUS and missense mutations, were found in the same patient. The c.3833A>C p.K1278T mutation has not been reported previously. In this case compound heterozygosity may be responsible for the disease. The c.1884T>G variant found in one patient, was not considered a disease-causing mutation in HGMD and was classified as VUS, and in ClinVar this variant was observed with hereditary breast and ovarian cancer syndrome (13). c.4358-3A>G, found in one patient in the present study, was a splicing mutation. The variant has no dbSNP ID yet and was previously identified in only two reports. One mentioned the susceptibility to breast and ovarian cancer and the other reported that it was observed associated with a cancer predisposition syndrome (14, 15). When we looked at the exons, exon 10 was remarkable. Out of the eight different mutations, five were in exon 10. In previous studies, similar data was given. Exon 10 was the most common location for *BRCA1* mutations and mutations were predominantly distributed around exon 10 (16).

Germline mutations in the *BRCA2* gene are highly penetrant for increased risks of breast and ovarian cancers, and male breast cancer, prostate cancer, pancreatic cancer, and melanoma (17). In this study 26 different *BRCA2* variants were detected in 32 patients with operated breast cancer and 10 were considered pathogenic, one likely pathogenic and 15 were considered VUS. Pathogenic variants were c.8585dupT (p.E2863fs*6), c.1763_1766delATAA (p.N588fs*25), c.9027delT (p.H3010fs*18), c.5576_5579delTTAA (p.I1859fs*3), c.4471_4474delCTGA (p.L1491fs*12), c.4631dupA (p.N1544fs*4), c.8351G>A (p.R2784Q), c.5791C>T (p.Q1931*), c.7007G>A (p.R2336H) and c.1055dupA (p.Y352*). Most of the pathogenic variants were frameshift mutations (6/10) while frameshift insertion, stop codon, missense and a splicing mutation were identified. In the present study five patients had c.9976A>T p.K3326* variant which was considered VUS. The variant was a stop codon mutation and in HGMD it was reproted that the variant was a disease-associated polymorphism with additional supporting functional evidence. For these patients further studies are needed to identify the additional findings. Two patients had c.6427_6428delTCinsAT (p.S2143I) variant, which was considered a VUS. The variant had no dbSNP ID at the time of writing and was previously reported in only two studies. Both studies mentioned that the variant was observed with hereditary risk breast/ovarian cancer (18, 19). Besides this variant, there were 11 more variants in exon 11 found in our study. Exon 11 is very important for the *BRCA2* gene, which comprises over 50% of the gene and encompasses half of the coding region. In our study, 12 mutations were found in exon 11 and four were pathogenic, one was likely pathogenic and seven were VUS. Out of four pathogenic variants, three were frameshift mutations and one was a stop codon mutation. We found c.4471_4474delCTGA (p.L1491fs*12) mutation in a male patient. This variant deletes four nucleotides in exon 11 of the gene and as a result, creates a frameshift and premature translation stop signal. Functional studies have not been reported for this variant but

is expected to result in an absent or non-functional protein product (20). The c.4033G>T (p.D1345Y) variant, considered a VUS, has no dbSNP ID and has not been mentioned as a disease-causing mutation. In the literature one report was found that related this variant with rectal adenocarcinoma (21). The c.2608A>G (p.I870V) variant found was considered a VUS, was not been previously reported and had no dbSNP ID, like the other c.5206_5208delCAA (p.Q1736del) variant that was considered likely pathogenic. The novel *BRCA2* mutation, c.5206_5208delCAA (p.Q1736del) was an in-frame 3 bp deletion that is predicted to result in the loss of one amino acid. A deletion is in-frame and if the reading frame of the gene is preserved and not disrupted, so a protein can be made and it may still be partially functional. These kinds of mutations are much rarer than the other types, such as stop, frameshift, and missense or splicing. In-frame deletions typically result in milder conditions but the functional impact and prognostic value of this novel in-frame deletion variant in *BRCA2* remains to be elucidated. The c.6626T>C (p.I2209T) variant, considered a VUS, was not mentioned as a disease-causing mutation in HGMD but in ClinVar, it was associated with hereditary breast and ovarian cancer syndrome (22). The c.2926_2927delTCinsAT (p.S976I) variant has no dbSNP ID at present and was previously reported in only two reports. Both mentioned that the variant was observed in association with breast cancer (23, 24). Two missense variants in the *BRCA2* gene, c.3503T>A (p.M1168K) and c.3310A>C (p.T1104P) and one variant in *CDH1* c.2595G>C (p.W865C), were found in one patient, and all of them are considered as VUS. c.3503T>A variant was not mentioned as a disease-causing mutation. The c.8671A>G (p.T2891A) variant that we identified, is considered a VUS, but was not mentioned as a disease-causing mutation. However, one report was found and mentioned that it was observed with breast cancer (25). In another case we identified the c.353G>A (p.R118H) variant, previously reported once, and the case was suffering from different types of cancer, including not only breast, but also endometrial and ovarian cancer (26). The c.1055dupA, p.Y352* was another variant that was not reported previously. This duplication of one nucleotide creates a nonsense variant, which changes a tyrosine to a premature stop codon. It was predicted to cause loss of normal protein function and was considered pathogenic because of the protein truncation or nonsense-mediated mRNA decay (27). In another case, we identified the c.7481G>A (p.R2494Q) variant and according to the literature there are some conflicting findings related to pathogenicity (28).

Germline mutations in the *TP53* gene cause a familial cancer predisposition, and carriers have a very high lifetime risk of malignancies, especially soft tissue sarcomas and breast cancer in women. The penetrance of breast cancer is very high and according to the National Cancer Institute Li-Fraumeni syndrome cohort cumulative incidence is 85% by age 60 years (29, 30). In the present study, three different variants were detected in three patients. All were missense mutation, one of which was a novel mutation, c.259C>T (p.P87S). The others had been reported previously and both were considered pathogenic.

Most recently, it was reported that 7% of all *CDH1* mutations are present in non-gastric tumors, especially being identified in cases with breast cancer. In our study, five *CDH1* gene variants were found, all of which were missense mutations and considered VUS. Four of them, c.1154C>G (p.P385R), c.2595G>C (p.W865C), c.1790C>G (p.P597R) and c.2560G>A (p.D854N) were all novel mutations and not previously reported. The patient with c.1154C>G variant also carried a pathogenic *BRCA1* variant, c.66dupA (p.E23fs*18). Another case who had the c.2595G>C (p.W865C) variant also had two *BRCA2* mutations, c.3503T>A (p.M1168K) and c.3310A>C (p.T1104P). The p.V475M variant (also known as c.1423G>A), results in a conservative amino acid change in the encoded protein sequence. This amino acid position is highly conserved. Although *in silico* tools predict a damaging effect on protein function, the data on variant occurrences in the general population are insufficient to allow any conclusion and the clinical significance of this alteration remains unclear (31, 32).

*PALB2* mutations are found in 0.6 to 3.9% of families with a history of breast cancer. In previous studies, the estimated mean risk of breast cancer for female *PALB2* mutation carriers by 70 years of age is 35% (33). In the present study, seven *PALB2* gene variants were identified in eight cases, three of which were novel mutations and not reported before. The c.3285dupT (p.N1096*) mutation was considered likely pathogenic but has not been reported previously. The c.3388G>C (p.A1130P) and c.3447C>A (p.A1149A) variants were also previously unreported, were classified as VUS, and have no dbSNP ID. Another missense mutation, c.3428T>A p.L1143H, was identified in a patient who also had the *BRCA2* pathogenic c.8351G>A (p.R2784Q) mutation. This variant in the *PALB2* gene had no dbSNP ID, and was mentioned in only a few reports in the literature. In a study based in the Turkish population, this was observed with breast cancer (34). The c.194C>T (p.P65L) variant, considered a VUS, was not mentioned as a disease-causing mutation, but in the ClinVar database it was reported that this amino acid position was poorly conserved and thus the predicted effect will be tolerated by *in silico* analysis. Since supporting evidence is limited, the clinical significance of this alteration remains unclear (35). The c.833_834delTAinsAT p.L278H variant, classified as a VUS, and as functional studies have not been performed for this variant, the available evidence is insufficient to determine the role in the protein. *BRCA1, BRCA2, TP53, CDH1, PALB2* and *PTEN* genes and lifetime risks for breast cancer are summarized in Table 6 (10).

Besides medical geneticists, breast surgeons and oncologists are well placed as a resource for patients who could benefit from genetic testing. The patients must be evaluated in the multidisciplinary councils and informed about the risks and benefits, and also discuss risk management strategies. The American Society of Clinical Oncology and the NCCN recommend considering genetic testing for breast cancer in patients, especially diagnosed at early age, with bilateral breast cancer, ER-/PR-/HER2- disease status, strong family history of breast and/or ovarian cancer, or a combination of these characteristics (9). The identification of an inherited pathogenic mutation predisposing to breast cancer in genetic testing does not mean that risk-reducing mastectomy is indicated. While risk-reducing mastectomy can be considered in *BRCA1, BRCA2, PTEN* and *TP53*, the situation can differ in other gene mutations. However, when combined with a significant family history of breast cancer, prophylactic surgery may be appropriate for patients with mutations in other genes (36) but the risks of the surgery should be discussed with the patient. In a multicenter study investigating the prevention of breast cancer with nipple-sparing mastectomy, prophylactic nipple-sparing mastectomy was performed in 346 patients who were carriers of *BRCA1* and *BRCA2*. None of them developed breast cancer at the 56-month follow-up. In the risk analysis conducted in the same study, it was reported that if mastectomy was not performed, breast cancer was predicted to have developed in 22 of the patients (37). In another study, follow-up and prophylactic mastectomy were compared in order to reduce the risk of breast cancer in *BRCA*-positive patients. It was shown that mastectomy significantly

reduced the risk of developing breast cancer, but had complications and risks (38). Prophylactic oophorectomy in premenopausal women with pathogenic variants in *BRCA2* has also been shown to reduce the risk of breast cancer by approximately 50%. This rate is lower in *BRCA1* patients who underwent risk-reducing oophorectomy (39). Advanced screening is recommended for patients with *ATM, CDH1, CHEK2, NBN, NF1, PALB2,* and *STK11* mutations, but currently the data are insufficient to support risk-reducing mastectomy in the absence of other factors such as a strong family history. Risk is modulated by age, family history, and in some cases, a specific mutation in a particular gene. The guidelines broadly support the consideration of contrast and non-contrast breast magnetic resonance imaging and tomosynthesis mammography for annual screening, due to the increased risk of breast cancer in individuals with this group of mutations (36). As also seen in our study, studies assessing mutations, which are commonly identified in the young patient population, as treatment targets have been underway for a long time. Besides evaluating mutations in terms of recurrence, secondary malignancy development or familial risk, they have an important role in both treatment and prognosis, again emphasizing the importance of genetic analysis.

Analysis of the relationship between mutations and the histopathological characteristics of tumors show that *BRCA1* mutations are associated with breast cancers that have a basal-like gene expression profile, high histological grade, negative estrogen receptor status, and HER2-negative (triple negative) status. *BRCA2*-associated breast cancers are usually high-grade, estrogen receptor-positive, and HER2-negative (40). The 5-year survival rate for patients with metastatic breast cancer is reported to be 27%, whereas for patients with metastatic triple-negative breast cancer associated with a *BRCA1* mutation, this rate is only 11%. Therefore, new treatments that provide lasting benefits are needed in patients with advanced breast cancer associated with a germline *BRCA* mutation. Breast cancers with *BRCA1* and *BRCA2* mutations, are deficient in homologous recombination and disrupt the ability of cancer cells to repair DNA damage, and also known to be sensitive to both poly (ADP-ribose) polymerase (PARP) inhibitors and platinum agents (41). PARP is an enzyme family responsible for the cellular activities involved in DNA repair, such as the base excision repair pathway and genetic stability. PARP plays a key role in the repair of single-strand DNA breaks, which is important for the survival of the cell. The inhibition of PARP leads to continued single-strand DNA breaks, which causes replication forks to stop, and double-strand breaks to occur. In cells with normal homologous recombination, double-strand DNA breaks can be repaired by the homologous recombination repair pathway. However, in cells with homologous recombination deficiency, treatment with a PARP inhibitor results in cell cycle arrest, a concept called synthetic lethality, and apoptosis (42).

Tumors with changes that inactivate *BRCA1/2*, can respond to PARP inhibitors, such as olaparib, talazoparib, rucaparib or niraparib, and chemotherapeutics that cause DNA damage, such as cisplatin and carboplatin (43, 44). In the "triple negative trial", a randomized phase III trial comparing carboplatin with docetaxel in patients with locally advanced metastatic or recurrent TNBC, patients with germline *BRCA1/2* mutations showed significantly better response to carboplatin compared to docetaxel (41). Two randomized phase III trials reported PARP inhibitor efficacy compared to physician's choice of chemotherapy in patients with metastatic breast cancer and one germline *BRCA* pathogenic variant. In the OlympiAD study (ClinicalTrials.gov descriptor: NCT02000622), olaparib was shown to improve progression-free survival with a hazard ratio of 0.58 [95% confidence interval (CI), 0.43–0.80] compared to standard chemotherapy. In the EMBRACA study (ClinicalTrials.gov descriptor: NCT01945775), talazoparib showed a progression-free survival contribution with a hazard ratio of 0.54 (95% CI, 0.42–0.71) (45). That these inhibitors offer oral treatments, and perhaps even more importantly, improve the quality of life of patients, makes them more attractive. In the NCCN Guidelines version 3.2022, both treatments are indicated as category 1 recommendation in the presence of a *BRCA1/2* mutation. In addition to metastatic diseases, there are studies that also report the positive results of PARP inhibitors in neoadjuvant and adjuvant therapies (46).

*CDH1* may also play a potential strategic role in the clinical management of breast cancer patients as a predictor of prognosis and survival. To date, *CDH1* has not been defined as a molecular target for treatment, but *in vitro* studies have shown that the germ line function could be suitable for targeted therapy. Important new crosstalk mechanisms have been described. Growth factor signals are hyperactivated upon loss of *CDH1,* regardless of the somatic activating mutations in downstream effectors. In particular, the PI3K/Akt pathway is activated upon loss of *CDH1* in the absence of specific oncogens. *ROS1* gene rearrangements are a defined and approved therapeutic target in relation to different cancers. Bajrami et al. (47) have described a synthetic lethal interaction between *CDH1* and *ROS1*. The authors showed that ROS1 inhibition in *CDH1* defective breast tumor cell sequences and breast tumor xenografts derived from patients resulted in tumor cell death.

In contrast to the biomarker role of *TP53*, until recently very few studies were performed that target therapeutically mutant *TP53*. With the recent identification of several compounds capable of reactivating the mutant protein, however, PRIMA-1MET (p53 reactivation and induction of massive apoptosis, methylated derivative) also called APR-246 has been reported (48). In addition to inhibiting cell proliferation, APR-246 was seen to induce apoptosis and reduce migration in the investigated mutant p53 breast cancer cell sequences. Another anti-p53 compound, which has been investigated for anticancer activity in breast cancer cells is the 2-sulfo-pyrimidine molecule known as PK11007. Like APR-246, PK11007 stabilizes and reactivates mutant p53 (49). There are no FDA-approved therapeutic targets yet, either for *CDH1* or *T53*, but ongoing studies are promising. Studies are ongoing to identify the other mutations identified in the present study, both to determine suitability as treatment targets and to define their characteristics predicting prognoses.

This study identified a total of 58 mutations in a cohort of 195 women who had been operated for breast cancer, of which 35 were VUS and 14 were novel variants that have not been previously reported. We have highlighted the VUS in this report because the clinical significance of VUS is changing over time and variant classification is important for clinical molecular genetic testing and clinical guidance. In a previous study, the researchers reclassified specific VUS identified over a 13-year period, and the results showed that 5.6% were reclassified as pathogenic or likely pathogenic variants. Further investigation into the pathogenicity of VUS is required. With continued monitoring of patients with VUS mutations, reclassification must be suggested when sufficient evidence is collected (50). Besides clinical follow-up, genetic follow-up should occur. Knowledge and identification of mutations have important implications for predictive, preventive, personalized medicine, besides genetic counseling and development of specific treatment protocols.

This study has also provided risk assessment data, which may be useful for clinical management for *TP53, CDH1, PALB2* and *PTEN,* together with *BRCA1* and *BRCA2* genes in breast cancer patients and may also be important for surveillance of other family members. Further studies are needed to identify common variants in the Turkish population and to evaluate the pathogenity of VUS.

**Ethics Committee Approval:** The study was conducted in accordance with the Declaration of Helsinki and was approved by İzmir Katip Çelebi Non-Interventional Clinical Studies Institutional Review Board (0028/20.01.2022).

**Informed Consent:** Written informed consent forms were obtained from all patients.

**Peer-review:** Externally and internally peer-reviewed.

## Authorship Contributions

Surgical and Medical Practices: A.S., Z.G.G., E.Ö.G., M.K.A.; Concept: A.S., Z.G.G., E.Ö.G., M.K.A.; Design: A.S., Z.G.G., E.Ö.G., M.K.A.; Data Collection or Processing: A.S., Z.G.G., E.Ö.G., M.A.T.; Analysis or Interpretation: A.S., Z.G.G., E.Ö.G., M.A.T.; Literature Search: A.S., Z.G.G., E.Ö.G.; Writing: A.S., Z.G.G., E.Ö.G.

**Conflict of Interest:** No conflict of interest was declared by the authors.

**Financial Disclosure:** The authors declared that this study received no financial support.

## References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin 2020; 70: 7-30. (PMID: 31912902) [Crossref]
2. Petrucelli N, Daly MB, Pal T. BRCA1- and BRCA2-Associated Hereditary Breast and Ovarian Cancer. 1998 Sep 4 [updated 2022 May 26]. In: Adam MP, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, Gripp KW, Amemiya A, editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993–2022. (PMID: 20301425) [Crossref]
3. Kuchenbaecker KB, Hopper JL, Barnes DR, Phillips KA, Mooij TM, Roos-Blom MJ, et al. Risks of Breast, Ovarian, and Contralateral Breast Cancer for BRCA1 and BRCA2 Mutation Carriers. JAMA 2017; 317: 2402-2416. (PMID: 28632866) [Crossref]
4. Kwong A, Shin VY, Ho CYS, Au CH, Slavin TP, Weitzel JN, et al. Mutation screening of germline TP53 mutations in high-risk Chinese breast cancer patients. BMC Cancer 2020; 20: 1053. (PMID: 33138793) [Crossref]
5. Adib E, El Zarif T, Nassar AH, Akl EW, Abou Alaiwi S, Mouhieddine TH, et al. CDH1 germline variants are enriched in patients with colorectal cancer, gastric cancer, and breast cancer. Br J Cancer 2022; 126: 797-803. (PMID: 34949788) [Crossref]
6. van der Post RS, Vogelaar IP, Carneiro F, Guilford P, Huntsman D, Hoogerbrugge N, et al. Hereditary diffuse gastric cancer: updated clinical guidelines with an emphasis on germline CDH1 mutation carriers. J Med Genet 2015; 52: 361-374. (PMID: 25979631) [Crossref]
7. Zhou J, Wang H, Fu F, Li Z, Feng Q, Wu W, et al. Spectrum of PALB2 germline mutations and characteristics of PALB2-related breast cancer: Screening of 16,501 unselected patients with breast cancer and 5890 controls by next-generation sequencing. Cancer 2020; 126: 3202-3208. (PMID: 32339256) [Crossref]
8. Seo M, Cho N, Ahn HS, Moon HG. Cowden syndrome presenting as breast cancer: imaging and clinical features. Korean J Radiol 2014; 15: 586-590. (PMID: 25246819) [Crossref]
9. Daly MB, Pal T, Berry MP, Buys SS, Dickson P, Domchek SM, et al. Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic, Version 2.2021, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw 2021; 19: 77-102. (PMID: 33406487) [Crossref]
10. Woodward ER, van Veen EM, Evans DG. From BRCA1 to Polygenic Risk Scores: Mutation-Associated Risks in Breast Cancer-Related Genes. Breast Care (Basel) 2021; 16: 202-213. (PMID: 34248461) [Crossref]
11. Gorodetska I, Serga S, Levkovich N, Lahuta T, Ostapchenko L, Demydov S, et al. The frequency of BRCA1 founder mutation c.5266dupC (5382insC) in breast cancer patients from Ukraine. Hered Cancer Clin Pract 2015; 13: 19. (PMID: 26468334) [Crossref]
12. Hirotsu Y, Nakagomi H, Sakamoto I, Amemiya K, Mochizuki H, Omata M. Detection of BRCA1 and BRCA2 germline mutations in Japanese population using next-generation sequencing. Mol Genet Genomic Med 2015; 3: 121-129. (PMID: 25802882) [Crossref]
13. National Center for Biotechnology Information. ClinVar; [VCV000054380.10], https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV000054380.10 (accessed June 26, 2022). [Crossref]
14. Rebbeck TR, Friebel TM, Friedman E, Hamann U, Huo D, Kwong A, et al. Mutational spectrum in a worldwide study of 29,700 families with BRCA1 or BRCA2 mutations. Hum Mutat 2018; 39: 593-620. (PMID: 29446198) [Crossref]
15. Tsaousis GN, Papadopoulou E, Apessos A, Agiannitopoulos K, Pepe G, Kampouri S, et al. Analysis of hereditary cancer syndromes by using a panel of genes: novel and multiple pathogenic mutations. BMC Cancer 2019; 19: 535. (PMID: 31159747) [Crossref]
16. Mehta A, Vasudevan S, Sharma SK, Kumar D, Panigrahi M, Suryavanshi M, et al. Germline BRCA1 and BRCA2 deleterious mutations and variants of unknown clinical significance associated with breast/ovarian cancer: a report from North India. Cancer Manag Res 2018; 10: 6505-6516. (PMID: 30555256) [Crossref]
17. Mai PL, Chatterjee N, Hartge P, Tucker M, Brody L, Struewing JP, et al. Potential excess mortality in BRCA1/2 mutation carriers beyond breast, ovarian, prostate, and pancreatic cancers, and melanoma. PLoS One 2009; 4: e4812. (PMID: 19277124) [Crossref]
18. Machackova E, Claes K, Mikova M, Házová J, Ťahlová EH, Vasickova P, et al. Twenty Years of BRCA1 and BRCA2 Molecular Analysis at MMCI - Current Developments for the Classification of Variants. Klin Onkol 2019; 32: 51-71. (PMID: 31409081) [Crossref]
19. Loizidou MA, Hadjisavvas A, Pirpa P, Spanou E, Delikurt T, Tanteles GA, et al. BRCA1 and BRCA2 mutation testing in Cyprus; a population based study. Clin Genet 2017; 91: 611-615. (PMID: 27882536) [Crossref]
20. National Center for Biotechnology Information. ClinVar; [VCV000051652.16], https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV000051652.16 (accessed June 5, 2022). [Crossref]
21. Zehir A, Benayed R, Shah RH, Syed A, Middha S, Kim HR et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. Nat Med 2017; 23: 703-713. (PMID: 28481359) [Crossref]
22. National Center for Biotechnology Information. ClinVar; [VCV000096840.14], https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV000096840.14 (accessed July 5, 2022). [Crossref]
23. Dutil J, Teer JK, Golubeva V, Yoder S, Tong WL, Arroyo N, et al. Germline variants in cancer genes in high-risk non-BRCA patients from Puerto Rico. Sci Rep 2019; 9: 17769. (PMID: 31780696) [Crossref]
24. Bishop MR, Omeler-Fenaud SM, Huskey ALW, Merner ND. Gene panel screening for insight towards breast cancer susceptibility in different ethnicities. PLoS One 2020; 15: e0238295. (PMID: 32866190) [Crossref]

25. Briceño-Balcázar I, Gómez-Gutiérrez A, Díaz-Dussán NA, Noguera-Santamaría MC, Díaz-Rincón D, Casas-Gómez MC. Mutational spectrum in breast cancer associated BRCA1 and BRCA2 genes in Colombia. Colomb Med (Cali) 2017; 48: 58-63. (PMID: 29021639) [Crossref]
26. Wang L, Wang H, Wang T, Liu J, Chen W, Wang Y, et al. Analysis of polymorphisms in genes associated with the FA/BRCA pathway in three patients with multiple primary malignant neoplasms. Artif Cells Nanomed Biotechnol 2019; 47: 1101-1112. (PMID: 30942098) [Crossref]
27. National Center for Biotechnology Information. ClinVar; [VCV000254486.3], https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV000254486.3 (accessed June 6, 2022). [Crossref]
28. National Center for Biotechnology Information. ClinVar; [VCV000052341.13], https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV000052341.13 (accessed July 4, 2022). [Crossref]
29. Schon K, Tischkowitz M. Clinical implications of germline mutations in breast cancer: TP53. Breast Cancer Res Treat 2018; 167: 417-423. (PMID: 29039119) [Crossref]
30. Mai PL, Best AF, Peters JA, DeCastro RM, Khincha PP, Loud JT, et al. Risks of first and subsequent cancers among TP53 mutation carriers in the National Cancer Institute Li-Fraumeni syndrome cohort. Cancer 2016; 122: 3673-3681. (PMID: 27496084) [Crossref]
31. National Center for Biotechnology Information. ClinVar; [VCV000141920.11], https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV000141920.11 (accessed June 5, 2022). [Crossref]
32. Momozawa Y, Iwasaki Y, Parsons MT, Kamatani Y, Takahashi A, Tamura C, et al. Germline pathogenic variants of 11 breast cancer genes in 7,051 Japanese patients and 11,241 controls. Nat Commun 2018; 9: 4083. (PMID: 30287823) [Crossref]
33. Antoniou AC, Casadei S, Heikkinen T, Barrowdale D, Pylkäs K, Roberts J, et al. Breast-cancer risk in families with mutations in PALB2. N Engl J Med 2014; 371: 497-506. (PMID: 25099575) [Crossref]
34. Ece Solmaz A, Yeniay L, Gökmen E, Zekioğlu O, Haydaroğlu A, Bilgen I, et al. Clinical Contribution of Next-Generation Sequencing Multigene Panel Testing for BRCA Negative High-Risk Patients With Breast Cancer. Clin Breast Cancer 2021; 21: e647-e653. (PMID: 33980423) [Crossref]
35. National Center for Biotechnology Information. ClinVar; [VCV000233066.7], https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV000233066.7 (accessed June 6, 2022). [Crossref]
36. Manahan ER, Kuerer HM, Sebastian M, Hughes KS, Boughey JC, Euhus DM, et al. Consensus Guidelines on Genetic` Testing for Hereditary Breast Cancer from the American Society of Breast Surgeons. Ann Surg Oncol 2019; 26: 3025-3031. (PMID: 31342359) [Crossref]
37. Jakub JW, Peled AW, Gray RJ, Greenup RA, Kiluk JV, Sacchini V, et al. Oncologic Safety of Prophylactic Nipple-Sparing Mastectomy in a Population With BRCA Mutations: A Multi-institutional Study. JAMA Surg 2018; 153: 123-129. (PMID: 28903167) [Crossref]
38. Honold F, Camus M. Prophylactic mastectomy versus surveillance for the prevention of breast cancer in women's BRCA carriers. Medwave 2018; 18: e7161. (PMID: 30052622) [Crossref]
39. Domchek SM, Friebel TM, Singer CF, Evans DG, Lynch HT, Isaacs C, et al. Association of risk-reducing surgery in BRCA1 or BRCA2 mutation carriers with cancer risk and mortality. JAMA 2010; 304: 967-975. (PMID: 20810374) [Crossref]
40. Mavaddat N, Barrowdale D, Andrulis IL, Domchek SM, Eccles D, Nevanlinna H, et al. Pathology of breast and ovarian cancers among BRCA1 and BRCA2 mutation carriers: results from the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA). Cancer Epidemiol Biomarkers Prev 2012; 21: 134-147. (PMID: 22144499) [Crossref]
41. Tutt A, Tovey H, Cheang MCU, Kernaghan S, Kilburn L, Gazinska P, et al. Carboplatin in BRCA1/2-mutated and triple-negative breast cancer BRCAness subgroups: the TNT Trial. Nat Med 2018; 24: 628-637. (PMID: 29713086) [Crossref]
42. Faraoni I, Graziani G. Role of BRCA Mutations in Cancer Treatment with Poly (ADP-ribose) Polymerase (PARP) Inhibitors. Cancers (Basel) 2018; 10: 487. (PMID: 30518089) [Crossref]
43. Robson M, Im SA, Senkus E, Xu B, Domchek SM, Masuda N, et al. Olaparib for Metastatic Breast Cancer in Patients with a Germline BRCA Mutation. N Engl J Med 2017; 377: 523-533. (PMID: 28578601) [Crossref]
44. Drew Y, Ledermann J, Hall G, Rea D, Glasspool R, Highley M, et al. Phase 2 multicentre trial investigating intermittent and continuous dosing schedules of the poly (ADP-ribose) polymerase inhibitor rucaparib in germline BRCA mutation carriers with advanced ovarian and breast cancer. Br J Cancer 2016; 114: 723-730. Erratum in: Br J Cancer 2016; 114: e21. (PMID: 27002934) [Crossref]
45. Ettl J, Quek RGW, Lee KH, Rugo HS, Hurvitz S, Gonçalves A, et al. Quality of life with talazoparib versus physician's choice of chemotherapy in patients with advanced breast cancer and germline BRCA1/2 mutation: patient-reported outcomes from the EMBRACA phase III trial. Ann Oncol 2018; 29: 1939-1947. (PMID: 30124753) [Crossref]
46. Tung NM, Zakalik D, Somerfield MR; Hereditary Breast Cancer Guideline Expert Panel. Adjuvant PARP Inhibitors in Patients With High-Risk Early-Stage HER2-Negative Breast Cancer and Germline BRCA Mutations: ASCO Hereditary Breast Cancer Guideline Rapid Recommendation Update. J Clin Oncol 2021; 39: 2959-2961. (PMID: 34343058) [Crossref]
47. Bajrami I, Marlow R, van de Ven M, Brough R, Pemberton HN, Frankum J, et al. E-Cadherin/ROS1 Inhibitor Synthetic Lethality in Breast Cancer. Cancer Discov 2018; 8: 498-515. (PMID: 29610289) [Crossref]
48. Bykov VJ, Zache N, Stridh H, Westman J, Bergman J, Selivanova G, et al. PRIMA-1(MET) synergizes with cisplatin to induce tumor cell apoptosis. Oncogene 2005; 24: 3484-3491. (PMID: 15735745) [Crossref]
49. Bauer MR, Joerger AC, Fersht AR. 2-Sulfonylpyrimidines: Mild alkylating agents with anticancer activity toward p53-compromised cells. Proc Natl Acad Sci USA 2016; 113: E5271-E5280. (PMID: 27551077) [Crossref]
50. Ha HI, Ryu JS, Shim H, Kong SY, Lim MC. Reclassification of BRCA1 and BRCA2 variants found in ovarian epithelial, fallopian tube, and primary peritoneal cancers. J Gynecol Oncol 2020; 31: e83. (PMID: 33078592) [Crossref]