Original paper

# The relationship between mutation carriage of *BRCA1/2* and clinicopathological characteristics in women with breast cancer – experience from a diagnostic centre in Turkey

Neslihan Duzkale[1], Onur Can Guler[2], Suat Kutun[2], Canan Emiroglu[3], Serdar Saridemir[2], Aysun Gokce[4], Olcay Kandemir[4], Tugba Taskin Turkmenoglu[4], Serap Yorubulut[5], Bahadir Kulah[6]

[1]Department of Medical Genetic, Diskapi Yildirim Beyazit Training and Research Hospital, Ankara, Turkey
[2]Department of Surgical Oncology, Ankara Dr Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital, Ankara, Turkey
[3]Department of Family Medicine, Diskapi Yildirim Beyazit Training and Research Hospital, Ankara, Turkey
[4]Department of Pathology, Ankara Etlik City Hospital, Ankara, Turkey
[5]Department of Statistics, Kırıkkale University, Faculty of Science and Letters, Ankara, Turkey
[6]Department of General Surgery, Ankara A Life Hospital, Ankara, Turkey

The 5–10% of breast cancers (BC) are hereditary, and *BRCA1/2* are causative in 25% of those inherited. It was aimed to examine the *BRCA1/2* genotype-BC phenotype relationship.

In 170 female patients with BC, *BRCA1/2* genes were investigated using Next Generation Sequencing. Demographic and clinicopathological characteristics of the patients and correlations of pedigree analysis with *BRCA1/2* mutation status were analysed.

*BRCA1/2* carriage was found to be 9.4%. When the patients were grouped as ≤ 40 and > 40 according to the age at diagnosis of BC, the tumour grade was higher in the ≤ 40 groups. In the study, *BRCA1/2* carriage and tumour grade were higher in patients with triple-negative breast cancers (TNBC). The risk of TNBC was 5.560 times higher in *BRCA1/2* carriers than in non-carriers.

There is a significant relationship between *BRCA1/2* carrier and BC hormone receptor negativity, tumour grade, and BC diagnosis age.

**Key words:** breast cancer, triple-negative, progesterone receptor, oestrogen receptor, *BRCA1, BRCA2*.

## Introduction

According to the Global Cancer Statistics report, female breast cancer (BC) is the most frequently diagnosed cancer type and ranks fifth in cancer-related deaths [1]. An estimated 90% of these cancers are sporadic and 5–10% of cases show hereditary transition. Germline causal variants of the *BRCA1/2* genes are responsible for about a quarter of inherited BCs [2]. *BRCA1* and *BRCA2* are tumour suppressor genes that play a role in maintaining genomic stability, cellular response to DNA damage, transcriptional regulation, cell cycle regulation, and cellular proliferation [3]. The frequency of causal variants of the *BRCA1/2*

genes is estimated to be 1/400–500 in the general population except for the Ashkenazi Jewish population [4]. It is believed that the risk of BC is 12% in the general female population, 46–87% in *BRCA1* carriers, and 38–84% in *BRCA2* carriers.These high penetrance genes cause *BRCA1*- and *BRCA2*- associated hereditary breast and ovarian cancer syndrome (HBOC). Individuals with HBOC syndrome have an increased risk of developing breast and ovarian cancer and many other types of cancer, including melanoma, pancreatic, and prostate cancer [4, 5]. Our study aimed to determine the relationship of 170 women diagnosed with BC with *BRCA1/2* genotypes, demographic/clinicopathological details, and family history.

## Material and methods

### Design

This study was designed as a single-centre, retrospective, observational cohort series.

### Study population

In our retrospective series, 170 female patients whose *BRCA1/2* genes were analysed between 2016–2019, at the Department of Medical Genetics in Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital were included. Patients participating in our study were selected in accordance with the National Comprehensive Cancer Network guidelines for *BRCA1/2* testing standards [6]. Breast cancer diagnoses of all patients in the study were histologically confirmed by a specialized pathologist. All cancers were staged according to the guidelines in the sixth edition of the American Joint Cancer Committee. If the proportion of cells stained positively by immunohistochemistry (IHC) was less than 1%, the oestrogen receptor (ER) and progesterone receptor (PR) status were classified as negative. Membranous staining for HER2 gene amplification in IHC staining was graded from 0 to +3. Patients with a staining pattern of +2 were evaluated using the fluorescent *in situ* hybridisation method, and < 2 copies of the HER2 gene was considered negative [7]. Demographic characteristics, clinical, histopathological, and immunohistochemical details of patients were obtained from the patients themselves, their past medical records, and the hospital's electronic database during genetic counselling. The family histories of the patients were reviewed by examining pedigree analyses including at least 3 generations. This study was conducted considering ethical responsibilities according to the Declaration of Helsinki and was approved by an independent ethics committee. In this study, all patients were informed about genetic tests and the use of their information, and their consent was obtained.

### Genomic DNA isolation

Genomic DNA was isolated from the patient's peripheral blood samples using an automated device (Qiagen®, USA) and tested with next-generation sequencing methods to detect germline variants of the *BRCA1/2* genes.

### Genetic analysis

The sequencing was implemented on the Ion S5™ System (Ion Torrent™) and the Illumina MiSeq System (Illumina Inc., San Diego, CA, USA). In the study, Oncomine™ BRCA Research Assay (Life Technologies, Carlsbad, CA, USA), *BRCA* MASTR™ Dx (Multiplicom, Niel, Belgium), and QIAseq multiplex amplicon panel (Qiagen, Hilden, Germany) kits targeting the *BRCA1/2* genes were used. Ion Reporter Software (Thermo Fisher Scientific), QIAGEN Clinical Insight (QIAGEN, Hilden, Germany), and Sophia DDM (Sophia Genetics, Saint-Sulp) software were used for data analysis. The sequencing results were compared with the human genome of hg19.

### Genome interpretation using in silico predictors

In this analysis, all exon regions and exon-intron boundaries up to 20 base pairs were examined. For the examination of gene variants, silicon programs such as SIFT, PolyPhen2, DANN, PROVEAN, GERP, MPC, Mutation Assessor, Fathmm, and Mutation Taster were used. For *BRCA1* and *BRCA2* genetic analysis, the accession numbers used were NM_007294.3 and NM_000059.3, respectively. The genomic changes detected in this study were classified based on the criteria of the American College of Medical Genetics and Genomics [8].

### Statistical analysis

Statistical analysis was performed using the SPSS (IBM SPSS Statistics 24) software. Frequency tables and descriptive statistics were used in the interpretation of the results. To compare 2 qualitative variables the Fisher's exact test and the $\chi^2$ test, and when the normal distribution criterion was not met, the Mann-Whitney *U* test was used. The binary logistic regression model was employed based on risk groups. When missing values were observed, this case was omitted from the analysis for this variable. A *p*-value less than 0.05 was considered statistically significant.

## Results

The mean age of the patients was 40.84 (range 26–77) years and the mean age of BC diagnosis was 40.4 (range 19–76) years. Causal variants in the *BRCA1/2* genes were detected in 16 of the patients. Of these, 8 were observed in the *BRCA1*

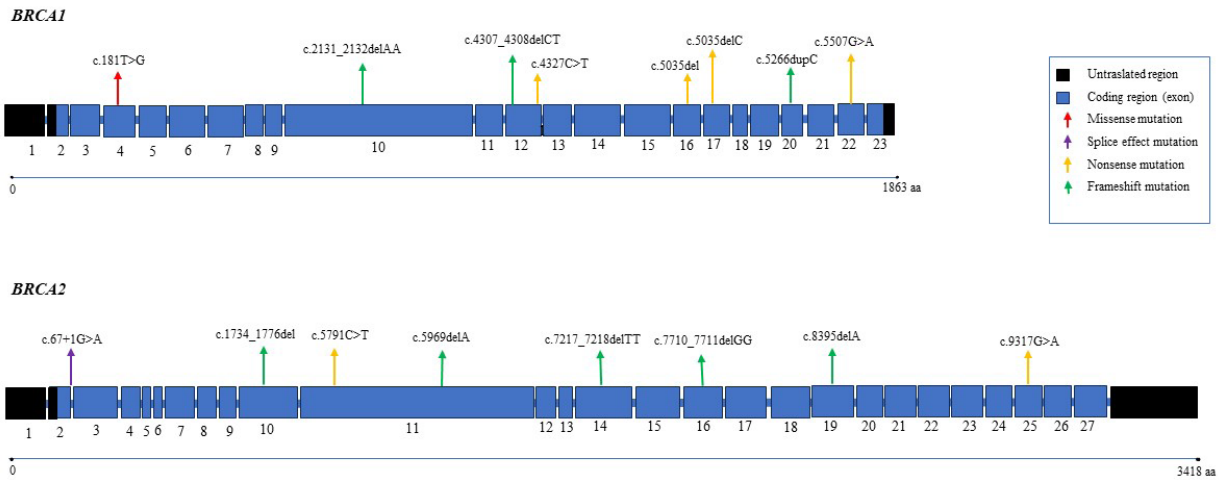


**Fig. 1.** Distribution of the identified pathogenic germline variants along the *BRCA1* and *BRCA2* genes

gene and 8 in the *BRCA2* gene (Fig. 1). In addition, 10 patients had variants of uncertain clinical significance (Table I). The mean age at diagnosis was 36.6 (21.0–62.0) years in 16 patients who were mutation carriers, and 40.8 (19.0–76.0) years in non-carriers. Two patients with *BRCA* carriers and 9 patients non-carriers had a history of multiple primary malignant neoplasms (MPMN). In 2 patients with a *BRCA* carrier, the other primary neoplasm was at the ovary, while in 9 non-carrier patients, the other primary tumour sites were the thyroid, ovary, colon, endometrium, and lung. Histopathological subtypes of BCs of all patients were reported as invasive ductal in 149 patients (87.6%), invasive lobular in 12 patients (7%), metaplastic in 3 patients (2%), medullary in 2 patients (1.2%), mucinous in 2 patients (1.2%), apocrine in one patient (0.5%) and mixed carcinoma in one patient (0.5%). All but one of the patients in the *BRCA* carrier group was diagnosed as invasive ductal carcinoma (15/16), while the other was metaplastic carcinoma (1/16). In the whole cohort, 16 patients (16/170, 9.4%) were diagnosed with bilateral BC and all were in the non-carrier group.

Patients were divided into 2 groups: *BRCA1/2* carriers and non-carriers. The two groups were compared statistically for the mean age at diagnosis, presence of MPMN, the histopathological subtype of BC, tumour grade, ER, PR, and HER2 expression status, Ki-67 proliferation index, and the number of relatives with BC/oviarian cancer (OC). Between both groups, no statistically significant difference was found in mean age at diagnosis, presence of MPMN, the histopathological subtype of BC, HER2 expression, Ki-67 proliferation index, and the number of relatives with BC/OC ($p > 0.05$).

Positive ER receptor expression was found in 5 patients (5/16, 38.5%) in the *BRCA1/2* mutation carrier group while it was positive in 82 patients (82/154, 75.2%) in the non-carrier group. The difference in ER expression status was statistically significant between the *BRCA* carrier and non-carrier groups ($p = 0.01$). Progesteron receptor expression positivity was observed in 3 patients (3/16, 25%) in the *BRCA* mutation carrier group and 73 patients (73/154, 68.2%) in the non-carrier group. Progesteron receptor expression status between the 2 groups was statistically significant ($p = 0.008$). When the group with and without *BRCA* mutation was evaluated in terms of tumour grade, it was seen that the tumour grade was higher in the group with mutation. This difference was statistically significant ($p = 0.036$) (Table II). As a result of the univariate analysis of the data, a multivariate model was created to investigate the relationship between ER and PR receptor status negativity and *BRCA1/2* carriage. According to the applied logistic regression model, no significant difference was observed for ER status, but it was found that those who were PR negative had a *BRCA1/2* carriage risk 6.621 times greater than those who were PR positive (Table III).

The patients were subdivided into 2 groups ≤ 40 years and > 40 years, according to the age of onset of BC. The analysis did not reveal any statistically significant difference between these 2 groups in terms of histopathological diagnosis, bilaterality, ER status, PR status, HER2 expression status, and Ki-67 proliferation index. Tumour grade was found to be higher in patients 40 years of age or younger, which was statistically significant ($p = 0.022$). In addition, it was observed that the frequency of MPMN was higher in the patient group over 40 years of age ($p = 0.003$) (Table IV).

There were 21 triple-negative breast cancers (TNBC) patients in the study, 6 of whom were mutation carriers and 15 were non-carriers. When all patients were grouped as TNBC type and others, the rate of *BRCA1/2* mutation carriage and tumour grade values were found to be higher in TNBCs compared to the non-TNBC group ($p = 0.006$, $p = 0.009$, respectively). There was no significant difference between the 2 groups in terms of age at diagnosis, bilaterality, and Ki-67 proliferation index (Table V).

**Table I.** *BRCA1/2* genes analysis results and details

| ID | Age | DX(s) | Ages at DX(s) | Gene | Nuc/AA change | Loc. | ACMG | Func | Cancer history on relatives | Ref |
|---|---|---|---|---|---|---|---|---|---|---|
| P2 | 39 | BC | 39 | *BRCA2* | c.2798C > A (p.Thr933Lys) | Ex11 | VUS | MS | 1 SC,1 GB,1BC | – |
| P3 | 50 | BC | 49 | *BRCA1* | c.4327C > T (p.Arg1443Ter) | Ex12 | PAT | NS | 2 BC, 1 SC, 1PC, 1EC | [38] |
| P11 | 48 | BC | 48 | *BRCA2* | c.67+1G > A | Int2 | PAT | SE | 3 BC | [39] |
| P18 | 42 | BC | 40 | *BRCA1* | c.2131_2132delAA (p.Lys711Valfs) | Ex10 | PAT | FS | 1 leukemia, 2 OC, 2 BC | [40] |
| P24 | 44 | BC | 44 | *BRCA2* | c.5386G > C (p.Asn1796His) | Ex11 | VUS | MS | 1 BC | – |
| P33 | 36 | BC | 35 | *BRCA1* | c.5035delC (p.Leu1679Ter) | Ex17 | PAT | NS | 1 BC, 1 LC, 1 PaC | [41] |
| P36 | 59 | BC | 35 | *BRCA2* | c.9364G > A (p.Ala3122Thr) | Ex25 | VUS | MS | – | [42] |
| P41 | 45 | BC | 36 | *BRCA1* | c.5266dupC (p.Gln1756Profs*74) | Ex20 | PAT | FS | 1 LC, 1 SkC, 1 BC | [43] |
| P42 | 49 | BC | 44 | *BRCA1* | c.794C > G (p.Ser265Cys) | Ex10 | VUS | MS | 1 SC, 1 BC | – |
| P47 | 31 | BC | 28 | *BRCA2* | c.3814A > G (p.Met1272Val) | Ex11 | VUS | MS | 1 LC, 1 BC | [44] |
| P54 | 65 | BC/OC | 65 (OC)/ 62 (BC) | *BRCA2* | c.7217_7218delTT (p.Phe2406CysfsTer5) | Ex14 | PAT | FS | 1 EC, 2 BC | – |
| P55 | 27 | BC | 25 | *BRCA2* | c.5969delA (p.Asp1990ValfsTer14) | Ex11 | PAT | FS | 1 LC, 1 PC | [40] |
| P56 | 45 | BC | 32 | *BRCA2* | c.8395delA (p.Arg2799AspfsTer22) | Ex19 | PAT | FS | 1 LC, 1 BC | – |
| P59 | 30 | BC | 29 | *BRCA1* | c.5507G > A (p.Trp1836Ter) | Ex22 | PAT | NS | 1 SC, 1 BC | [41] |
| P60 | 43 | BC | 42 | *BRCA2* | c.3562A > G (p.Ile1188Val) | Ex11 | VUS | MS | – | [40] |
| P68 | 57 | BC | 36 | *BRCA2* | c.9934A > G (p.Ile3312Val) | Ex27 | VUS | MS | 1 liver, 3 BC | [45] |
| P98 | 27 | BC | 27 | *BRCA1* | c.4307_4308delCT (p.Ser1436Phefs*4) | Ex12 | PAT | FS | 3 BC | [46] |
| P107 | 35 | BC | 34 | *BRCA1* | c.5035del (p.Asn1678Leu1679Ter) | Ex16 | PAT | NS | 1 LC, 1 BC | [41] |
| P111 | 60 | BC/OC | 47 (BC)/ 59 (OC) | *BRCA2* | c.5791C > T (p.Gln1931*) | Ex11 | PAT | NS | 1 BrC | [47] |
| P118 | 30 | BC | 21 | *BRCA1* | c.181T > G (p.Cys61Gly) | Ex4 | PAT | MS | 1 LC | [48] |
| P120 | 30 | BC | 30 | *BRCA2* | c.1734_1776del (p.Glu2571Lysfs*12) | Ex10 | PAT | FS | 1 SC, 1CC, 1PC, 1BrC, 3BC | – |
| P122 | 38 | BC | 37 | *BRCA2* | c.7710_7711delGG (p.Glu2571Lysfs*12) | Ex16 | PAT | FS | – | – |
| P131 | 46 | BC | 40 | *BRCA2* | c.9317G > A (p.Trp3106*) | Ex25 | PAT | NS | 1 BC, 1AC | [47] |
| P150 | 54 | BC | 49 | *BRCA2* | c.8360G > A (p.Arg2787His) | Ex19 | VUS | MS | 1 lymphoma, 1 BrC | [49] |
| P156 | 31 | BC | 30 | *BRCA2* | c.6080G > A (p.Arg2027Lys) | Ex11 | VUS | MS | 1 LC, 1SkC, 1 BC | – |
| P167 | 46 | BC | 40 | *BRCA2* | c.670G>A (p.Asp224Asn) | Ex8 | VUS | MS | 1 PC, 1 EC, 2 SC, 1 BC | [50] |

*AA – amino acid, ACMG – American College of Medical Genetics and Genomics scoring, Ages at DXs – ages at diagnoses, BC – breast cancer, BrC – brain cancer, CC – colon cancer, DXs – diagnoses, EC – endometrium cancer, Ex – exon, F – female, FS – frameshift, Func – function, GB – gall bladder, Int – intron, LC – lung cancer, Loc – location, M – male, MS – missense, NS – nonsense, Nuc – nucleotide, OC – ovarian cancer, PaC – pancreas cancer, PAT – pathogenic, PC – prostate cancer, Ref – reference number, SC – stomach cancer, SkC – skin cancer, SE – splice effect, VUS – variant of uncertain significance*

**Table II**. Clinical, demographic, histopathological and immunohistochemical features of breast cancer patients' regarding *BRCA1/2* mutation carrier status

| Parameters (n = 170) | Groups | | Statistical analysis* Probability |
|---|---|---|---|
| | *BRCA1/2* carrier (*N* = 16) | *BRCA1/2* non-carrier (*N* = 154) | |
| Age (year) | 44.7 (31.0–69.0) | 47.0 (30.0–81.0) | Z = –1.722<br>*p* = 0.085 |
| Age at diagnosis (year) | 36.6 (21.0–62.0) | 40.8 (19.0–76.0) | Z = –1.863<br>*p* = .063 |
| MPMN, *n* (%) | | | *p* = 0.277 |
| No | 14 (87.5) | 145 (94.2) | |
| Yes | 2 (12.5) | 9 (5.8) | |
| ER status, *n* (%) | | | ***p* = 0.010** |
| Negative | 8 (61.5) | 27 (24.8) | |
| Positive | 5 (38.5) | 82 (75.2) | |
| PR status, *n* (%) | | | ***p* = 0.008** |
| Negative | 9 (75.0) | 34 (31.8) | |
| Positive | 3 (25.0) | 73 (68.2) | |
| HER2, *n* (%) | | | *p* = 0.207 |
| Negative | 11 (100.0) | 77 (80.2) | |
| Positive | – | 19 (19.8) | |
| Ki-67, *n* (%) | | | *p* = 0.357 |
| < 15 | – | 12 (12.6) | |
| ≥ 15 | 11 (100.0) | 83 (7.4) | |
| Grade | 3.0 (2.0–3.0) | 2.0 (1.0–3.0) | ***p* = 0.036** |
| Metastases at the diagnosis, *n* (%) | | | *p* = 0.744 |
| No | 14 (87.5) | 130 (84.4) | |
| Yes (axillary) | 2 (12.5) | 24 (15.6) | |
| The presence of relatives with ATC, *n* (%) | | | *p* = 0.473 |
| No | 1 (6.3) | 26 (16.9) | |
| Yes | 15 (93.7) | 128 (83.1) | |
| The presence of relatives with BC, *n* (%) | | | |
| No | 5 (31.3) | 69 (44.8) | $\chi^2$ = 0.602<br>*p* = 0.438 |
| Yes | 11 (68.7) | 85 (55.2) | |
| The presence of relatives with OC, *n* (%) | | | *p* = 0.239 |
| No | 14 (87.5) | 146 (94.8) | |
| Yes | 2 (12.5) | 8 (5.2) | |
| The number of relatives with BC, *n* (%) | 1.0 (0–9.0) | 1.0 (0–9.0) | Z = –1.474<br>*p* = 0.140 |
| The number of relatives with OC, *n* (%) | 0 (0–2.0) | 0 (0–1.0) | Z = –1.231<br>*p* = 0.218 |
| The number of relatives with ATC, *n* (%) | 2.5 (0–7.0) | 2.0 (0–9.0) | Z = –1.157<br>*p* = 0.247 |

*ATC – all types of cancer, BC – breast cancer, OC – ovarian cancer*
** Mann-Whitney U test (Z – table value) test is used to compare the measurement values of two independent groups in data. Fisher-Exact and Pearson-$\chi^2$ cross tables were used to examine the relationships of two qualitative variables.*

**Table III**. Logistic regression model based on *BRCA1/2* carrier risk status

| Prameters | B | S.H. | Wald | sd | *p*-value | OR | 95% CI (OR)* | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Min | Max |
| PR status** | 1.863 | 0.698 | 7.117 | 1 | 0.008 | 6.441 | 1.639 | 25.311 |
| Constant | –3.192 | 0.589 | 29.357 | 1 | 0.000 | 0.041 | | |
| CCR = 89.9% | | | $\chi^2_{(3)} = 3.3–691, p = 0.297$ | | | | | |

*Backward – LR model was used*
** Binary logistic regression*
*** Reference category: positive group*

**Table IV**. Comparison of variables according to the ages of diagnosis of the patients

| Parameters (*N* = 170) | Age | | Statistical analysis* Probability |
|---|---|---|---|
| | ≤ 40 (*N* = 95) | > 40 (*N* = 75) | |
| MPMN, *n* (%) | | | |
| No | 94 (98.9) | 65 (86.7) | $p = 0.003$ |
| Yes | 1 (1.1) | 10 (13.3) | |
| Tumour subtype, *n* (%) | | | $p = 0.294$ |
| Invasive ductal carcinoma | 86 (90.5) | 63 (84.0) | |
| Others | 9 (9.5) | 12 (16.0) | |
| Bilaterality, *n* (%) | | | $p = 0.069$ |
| No | 90 (94.7) | 64 (85.3) | |
| Yes | 5 (5.3) | 11 (14.7) | |
| ER status, *n* (%) | | | $p = 0.193$ |
| Negative | 26 (33.3) | 9 (20.5) | |
| Positive | 52 (66.7) | 35 (79.5) | |
| PR status, *n* (%) | | | $p = 0.406$ |
| Negative | 29 (39.7) | 14 (30.4) | |
| Positive | 44 (60.3) | 32 (69.6) | |
| HER2, *n* (%) | | | $p = 0.551$ |
| Negative | 54 (81.8) | 34 (82.9) | |
| Positive | 12 (18.2) | 7 (17.1) | |
| Ki-67, *n* (%) | | | $p = 0.756$ |
| < 15% | 7 (10.4) | 5 (12.8) | |
| ≥ 15% | 60 (89.6) | 34 (87.2) | |
| Grade | 3.0 (1.0–3.0) | 2.0 (1.0–3.0) | ***p = 0.022*** |
| Metastases at the diagnosis, *n* (%) | | | $p = 0.99$ |
| No | 81 (85.3) | 63 (84.0) | |
| Yes (Axillary lymph node) | 14 (14.7) | 12 (16.0) | |

** Mann-Whitney U test (Z – table value) test is used to compare the measurement values of two independent groups in data. Fisher-Exact and Pearson-$\chi^2$ cross tables were used to examine the relationships of two qualitative variables.*

To identify the relationship between *BRCA1/2* mutation carriage and TNBC, a logistic regression analysis was performed. This test revealed a significant relationship between TNBC and *BRCA1/2* mutation carriage. Patients with *BRCA1/2* mutation were found be 5.560 times more likely to be TNBC type when compared to non-carriers (OR 95%; 1,772–17,450) (Table VI).

## Discussion

In our study, we investigated the effect of the mutation carrier status of *BRCA1/2* on demographic and clinicopathologic parameters in female BCs. In our patient group, the carrier rate of *BRCA1/2* was found to be 9.4% (16/170). Many studies investigating *BRCA1/2* genes have been reported in the Turk-

**Table V.** Comparison of variables in groups according to receptor (oestrogen and progesterone-receptor, HER2) status

| Parameters ($N = 170$) | TNBC status | | Statistical analysis* Probability |
|---|---|---|---|
| | TNBC | Others | |
| Age at diagnosis (years) | 37.0 (26.0–55.0) | 40.9 (19.0–76.0) | $p = 0.063$ |
| *BRCA1/2, n* (%) | | 10 (6.7) | $p = 0.006$ |
| Carrier | 6 (28.6) | 139 (93.3) | |
| Non-carrier | 15 (71.4) | | |
| **Bilaterality,** *n* (%) | | 134 (89.9) | $p = 0.696$ |
| No | 20 (95.2) | 15 (10.1) | |
| Yes | 1 (4.8) | | |
| Ki-67 index, *n* (%) | | 11 (12.6) | $p = 0.689$ |
| < 15 | 1 (5.3) | 76 (87.4) | |
| ≥ 15 | 18 (94.7) | 2.0 (1.0–3.0) | |
| Grade | 3.0 (2.0–3.0) | | $p = 0.009$ |
| Metastases at the diagnosis, *n* (%) | | 127 (85.2) | $p = 0.534$ |
| No | 17 (81.0) | 22 (14.8) | |
| Yes (Axillary lymph node) | 4 (19.0) | | |

** Mann-Whitney U test (Z – table value) test is used to compare the measurement values of two independent groups in data. Fisher's Exact Test was used to examine the relations of two qualitative variables.*

**Table VI**. Logistic regression model for *BRCA1/2* carrier risk status and triple-negative breast cancers

| Parameters | B | S.H. | Wald | sd | *p*-value | OR | 95% CI (OR)* | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Min | Max |
| *BRCA1/2* non-carriers | 1.761 | 0.584 | 8.643 | 1 | 0.003 | 5.560 | 1.772 | 17.450 |
| Constant | –2.226 | 0.272 | 67.112 | 1 | 0.000 | 0.108 | | |
| | CCR = 87.6% | | | $\chi^2 = 1.016$ $p = 0.602$ | | | | |

** Binary logistic regression. Backward: LR model was used.*

ish population (Table VII) [9–30]. In one of the first studies conducted in our population, 105 breast and/or ovarian patients were investigated and the mutation carrier rate was found to be 10.47% (11/105). Nine of these patients had mutations in the *BRCA1* gene and 2 had mutations in the *BRCA2* gene. Their study also predicted that the *BRCA1* 5382insC mutation could be a possible founder mutation for the Turkish population [11]. In a study in which they included 83 breast/ovarian cancer patients, Manguoğlu *et al*. examined the 11th exon of the *BRCA1* gene in all patients and the entire *BRCA2* gene in 53 patients and detected only 3 pathogenic variants [12]. In their study, they also investigated the presence of 4 dominant mutations reported in the Jewish population (185delAG in *BRCA1*, 5382insC Tyr978X and 6174delT in *BRCA2*) and were unable to detect any of these mutations in Turkish patients with BC [12, 31]. Their study showed that there was no dominant mutation in exon 11 of *BRCA1* and the *BRCA2* gene [12].

In another study conducted recently in our population, 181 cases diagnosed with BC were examined and 38 (21%) were found to be *BRCA1/2* carriers. The authors suggested that the c.5266dupC mutation, which is frequently observed in their studies and localised in the *BRCA1* gene, is a candidate founder mutation in the Turkish population [27]. In a study conducted by Ödemiş *et al*., in 2373 Turkish cases diagnosed with breast and/or ovarian cancer, the *BRCA1/2* gene mutation carrier rate was found to be 16.5%. *BRCA1* mutation was found in 57.5% of mutation carriers, *BRCA2* mutation was found in 41.9%, and both *BRCA1* and *BRCA2* mutations were detected in 0.6% of patients. In their study, the carrier rate was reported to be 28.5% in patients with a history of TNBC diagnosed before the age of 60 years [26]. In another study including 495 Turkish BC patients, the *BRCA1/2* gene mutation rate was found to be 9.89%. In this study, the *BRCA1* gene c.5266dupC mutation, which has been widely reported in Ashkenazi Jewish ancestry, was observed in 5 patients and was reported as the most frequently detected mutation [28]. *BRCA1* and *BRCA2* mutations were detected at different rates

**Table VII**. Summary of studies determined *BRCA1/2* mutations and founder mutations from Turkey

| Author, year | CS | S size | Mutation scanning | | Mutation frequency | | | Founder mutations (estimated) | | Ref |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | *BRCA1* | *BRCA2* | *BRCA1/2*, n (%) | *BRCA1*, n (%) | *BRCA2*, n (%) | *BRCA1* | *BRCA2* | |
| Balcı *et al.*, 1999 | BC, OC, HR | 15 | All coding exons | All coding exons | 3/15 (20) | 2/15 (13.3) | 1/15 (6.7) | Not reported | Not reported | [9] |
| Ozdag *et al.*, 2000 | BC, BC + OC | 50 | Exon 2, 5, 10, 11, 13, 20, 24 | Exon 11 | 4/50 (8) | 2/50 | 2/50 | Not reported | Not reported | [10] |
| Yazici *et al.*, 2000 | BC, OC, BC + OC | 105 | Exon 2, 11, 14, 20 (all exons in 24 patients) | Exon 10, 11 | 11/105 (10.5) | 9/105 (8.6) | 2/105 (1.9) | 5382insC | Not reported | [11] |
| Manguoglu *et al.*, 2003 | BC, OC, BC + OC | 83 | Three spesific mutations and exon 2, 11 | All coding exons | 3/83 (7.2) | 2/83 (2.4) | 1/53 (4.8) | Not reported | Not reported | [12] |
| Güran *et al.*, 2005 | BC, OC, HR | 22 | Nine spesific mutations | Four spesific mutations | 1/22 (4.5) | 1/22 (4.5) | 0/22 (0) | Not reported | Not reported | [13] |
| Egeli *et al.*, 2006 | BC, OC, BC + OC, HR | 38 | All coding exons | All coding exons | 4/38 (10.5) | 1/38 (2.6) | 3/38 (7.9) | c.5266dupC | Not reported | [14] |
| Manguoglu *et al.*, 2010 | BC, OC, BC + OC, PCa | 156 | All coding exons | All coding exons | 7/156 (4.5) | 3/156 (1.9) | 4/156 (2.6) | Not reported | Not reported | [15] |
| Manguoglu *et al.*, 2011 | BC, OC | 50 | Del and dup analysis in all exons | Del and dup analysis in all exons | 0/50 (0) | 0/50 (0) | 0/50 (0) | Not reported | Not reported | [16] |
| Aydın *et al.*, 2011 | BC | 211 | Del and dup analysis in all exons | Del and dup analysis in all exons | 4/208 (1.9) | 4/208 (1.9) | 0/185 (0) | Not reported | Not reported | [17] |
| Cecener *et al.*, 2014 | BC | 117 | All coding exons | All coding exons | 16/117 (13.7) | 16/117 (13.7) | 0/117 (0) | Not reported | Not reported | [18] |
| Yazıcı *et al.*, 2018 | BC, OC, BC + OC, BC/OC with OTC, HI | 1809 | All coding exons + del and dup analysis | All coding exons + del and dup analysis | 293/1785(17) | NA | NA | Not reported | Not reported | [19] |
| Bisgin *et al.*, 2019 | BC | 129 | All coding exons | All coding exons | 18/129 (13.9) | 7/129 (5.4) | 11/129 (8.5) | Not reported | Not reported | [20] |
| Geredeli *et al.*, 2019 | BC, BC + OC | 99 | All coding exons | All coding exons | 19/99 (19.2) | 11/99 (11.1) | 8/99 (8.1) | Not reported | Not reported | [21] |

**Table VII.** Cont.

| Author, year | CS | S size | Mutation scanning | | Mutation frequency | | | Founder mutations (estimated) | | Ref |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | *BRCA1* | *BRCA2* | *BRCA1/2*, *n* (%) | *BRCA1*, *n* (%) | *BRCA2*, *n* (%) | *BRCA1* | *BRCA2* | |
| Bahsi *et al.*, 2020 | NA | 1419 | All coding exons + del and dup analysis | All coding exons + del and dup analysis | 139/1419(10.8) [122/141 (9.61) and 17/1419 (1.2) pathogenic del and dup variants)] | 58/1419 (4.1) | 64/1419 (4.5) | c.5266dupC | c.1773_1776 delTTAT | [22] |
| Demir *et al.*, 2020 | BC, OC, BC + OC, OTC, HR | 493 | All coding exons + del and dup analysis | All coding exons + del and dup analysis | 88/493 (17.9) | 58/493 (11.8) | 30/493 (6.1) | c.5266dupC | Not reported | [23] |
| Eyupoglu *et al.*, 2020 | BC, BC + OC, HR | 113 | All coding exons | All coding exons | 48/113 (42.4) | 18/113 (15.9) | 30/113 (26.5) | Not reported | Not reported | [24] |
| Atcı *et al.*, 2021 | BC | 302 | All coding exons | All coding exons | 78/302 (25.9) | 41/302 (13.6) | 37/302 (12.3) | Not reported | Not reported | [25] |
| Ödemis *et al.*, 2022 | BC, OC | 2373 | All coding exons | All coding exons | 391/2373(16.5) | 225/2373 (9.5) | 164/2373 (7) | Not reported | Not reported | [26] |
| Gun-Bilgic *et al.*, 2022 | BC, OC | 181 | All coding exons | All coding exons | 38/181 (21) | 17/181 (9.4) | 21/181 (11.6) | c.5266dupC | Not reported | [27] |
| Bora *et al.*, 2022 | BC, BC + SM | 495 | All coding exons + del and dup analysis | All coding exons + del and dup analysis | 49/495 (9.9) | 25/495 (5.1) | 24/495 (4.8) | c.5266dupC | Not reported | [28] |
| Sunar *et al.*, 2022 | OC | 76 | All coding exons + del and dup analysis | All coding exons + del and dup analysis | 24/76 (31.6) | 17/76 (22.4) | 7/76 (9.2) | Not reported | Not reported | [29] |
| Boga *et al.*, 2023 | BC and HI with FH of BC | 2475 | All coding exons | All coding exons | 421/2475(17) for all samples/ 239/1444 (16.6) for BC | NA | NA | c.1444_1447delATTA | c.7689delC | [30] |
| This study (all data available upon request) | BC, BC + OC, BC + SM | 170 | All coding exons + del and dup analysis | All coding exons + del and dup analysis | 16/170 (9.4) | 8/170 (4.7) | 8/170 (4.7) | Not reported | Not reported | – |

*BC – breast cancer, CS – cancer status in tested individuals, FH – family history, HI – healthy individuals, HR – healthy relatives, NA – non-available, OC – ovarian cancer, OTC – other types of cancer, PCa – prostate cancer, Ref – reference, S size – sample size, SM – secondary malignancies*

**Table VIII**. Distribution of *BRCA1* and *BRCA2* mutations detected in high-risk breast and ovarian cancer families worldwide

| Continent of ascertainment | America (North) | Africa | Asia | America (South/Central) | Europe | Austria |
|---|---|---|---|---|---|---|
| *BRCA1* mutation frequency (%) | 14 | 43.5 | 17 | 27.8 | 10.9 | 29.8 |
| *BRCA2* mutation frequency (%) | 19.5 | 15.9 | 19.2 | 26.1 | 12.9 | 17.1 |

in studies conducted in our population (Table VII). Recently, a meta-analysis study involving approximately 30000 *BRCA1/2* mutation carriers from different geographies and various racial/ethnic populations was conducted and the distribution of carriers worldwide was reported (Table VIII). The heterogeneous distribution of *BRCA1/2* gene mutations was also remarkable in this meta-analysis study [32].

In studies, some *BRCA1/2* gene mutations are detected more frequently. The fact that these high-frequency mutations are common in certain ethnic groups and limited populations may be a result of the "founder effect". Recurrent mutations, unlike founder mutations, represent a relatively small portion of the *BRCA1/2* mutations reported in most populations. Identification of founder mutations with large-scale studies involving large numbers of patients may provide an advantage in choosing genetic testing for high-risk families. In this respect, it is important to determine whether the frequently observed mutations in studies are recurrent (observed independently more than once) or founder mutations (originating from a single origin). To determine the founder mutations, the haplotypes of individuals who are carriers of the same mutation must be compared by haplotype analysis and the minimum common ancestor haplotype between these carriers must be determined [33]. In the literature, many *BRCA1/2* variants that have been proven to be founder mutations have been reported in different populations from geographies such as Asia, Europe, the Middle East, North America and Latin America. The best-known populations with the founder mutation are the Ashkenazi Jewish population and the Icelandic population, respectively. Founder mutations identified in these populations represent approximately 60% of *BRCA1/2* carrier BC families [31, 34]. This rate was reported as 86% in Poland and 69% in Slovenia [35, 36]. In a recent study conducted in the Chilean population, founder mutations responsible for nearly 80% of mutation carriers of these genes have been identified [37].

In previous studies conducted on the Turkish population, the authors reported some variants that they claimed were founder mutations (Table VII). These are the mutations c.5266dupC, c.1444_1447delATTA in the *BRCA1* gene and c.1773_1776delTTAT, c.7689delC in the *BRCA2* gene [11, 14, 22, 23, 27–30]. Since none of the mutations thought to be founder mutations in the Turkish population have been confirmed by haplotype analysis studies, it remains unclear whether these are independently recurring mutations or whether they are founder mutations. In our study, c.5266dupC, one of the mutations previously claimed to be the "founder mutation" for our population, was detected in only one patient. In conclusion, a founder mutation was not detected in our study, as in many other studies in our population.

In our series, the mean age at diagnosis of 16 *BRCA1/2* carriers was 37 years (33.8 in *BRCA1* carriers and 40.13 in *BRCA2* carriers), and 40.8 years in non-carriers. While the BC risk of a woman with a *BRCA1* mutation is 20% after the age of 40 years, 51% after the age of 50 years, and 85% after the age of 70 years; this risk is known as 28% after the age of 50 years and 84% after the age of 70 years in women with *BRCA2* mutation. In women with BC who are carriers of *BRCA1/2*, the cumulative risk of developing bilateral BC is 2.2%, and the annual risk is 2.8% in carriers ≤ 40 years of age [38]. In this study, no significant difference in bilateral BC risk was detected between BRCA carriers and non-carriers. In previous studies in the literature, the risk of ovarian cancer by age 70 years was observed to be 39–63% in *BRCA1* mutation carriers and 16.5–27% in *BRCA2* mutation carriers [39]. In our study, 2 patients who were carriers of *BRCA2* were diagnosed with both breast and ovarian cancer.

Familial transition in BC is more common than in other organ cancers. In women with a history of BC in first-degree relatives, the risk of developing BC increases 2–3 times compared to the normal population [40]. In this study, the number and presence of breast, ovarian, or other cancers in the relatives of the patients were compared between the *BRCA1/2* carrier and non-carrier groups, but no significant difference was found. Although mutations of *BRCA1/2* genes are blamed in approximately 25% of families with a strong BC history, other high penetrance genes such as *TP53, CDH1, PTEN, STK11, RAD51C* and *RAD51D,* and low/medium penetrance genes such as *ATM, CHEK2, BRIP1* and *PALB2* may also be causative in these families. Most of these genes play a role in maintaining genomic integrity and DNA repair mechanisms [41]. Thus, other genes that increase BC risk were not investigated in our study, and it was not possible to elucidate the possible genetic aetiopathogenesis of our familial BC cases who were *BRCA1/2* non-carriers.

The Consortium of Investigators of Modifiers of *BRCA1/2* reported that BCs observed in those carrying mutations of these genes are primarily invasive ductal carcinoma. In the report, in BCs observed in *BRCA1* carriers, ER and PR negativity is about 80%, HER2 negativity is 90%, and the TNBC rate is about 70%, and in BCs observed in *BRCA2* carriers, ER negativity is 23%, PR negativity is 36%, HER2 negativity is 87%, and the rate of TNBC is reported to be 16% [42].

The immunohistochemical and histopathological properties of *BRCA1/2* carriers and non-carriers were first investigated in the literature by Palacios *et al*. In their study, conducted in a relatively small sample group, they found higher grades, more frequent ER/PR negativity, and higher proliferation rates in *BRCA1/2* carriers. In terms of *HER-2* amplification, they could not find any difference between the groups [43]. Lakhani *et al*. found that BC patients who are *BRCA1/2* carriers have a higher degree and mitotic index than non-carriers, and they also show more pleomorphism [44]. Another study showed that *BRCA1*-associated tumours were more frequently ER/PR negative, p53 positive, and higher grade than tumours of both *BRCA2*-related and non-*BRCA1/2* carriers. In the multivariate analysis performed in this study, *BRCA1*-related tumours were compared with tumours of non-*BRCA1/2* carriers, while independent factors were defined as age, grade, and PR negativity. In comparing these 2 groups, ER status could not be detected as an independent marker in multivariate analysis. In the same study, no significant differences were found between *BRCA2*-related tumours and tumours of non-*BRCA1/2* carriers, except grade [45]. In other studies in the literature, it has been reported that BCs seen in *BRCA1/2* carriers have high grades and a very aggressive prognosis [42, 46–48]. Our study has shown that *BRCA* carriers have a higher tumour grade than non-carriers and that the ER/PR status is mostly negative. The findings we obtained from our study were found to be compatible with the literature.

Multivariate analysis performed in this study proved that the PR-negative group had a 6.621-fold increased risk of *BRCA1/2* carriage compared to the PR positive group. There are several different cell types in the breast tissue that actively communicate with each other and with the extracellular matrix. Autocrine and paracrine actions of this tissue provide the activation of hormone receptors. A recent *in vitro* study proved that the *BRCA1* mutation carrier differentiates the hormone response of organoids and influences PR activity [49].

In our study, when the patients were grouped as TNBC and others, the *BRCA1/2* mutation carrying rate and tumour grade in TNBCs were found to be higher than the non-TNBC group ($p = 0.006$, $p = 0.009$, respectively). Triple-negative breast cancers, which is a highly heterogeneous type of BC in terms of clinical, genetic, and morphological features, constitutes 12–24% of all BCs. This type of cancer has a worse prognosis than other BCs [50]. *BRCA1/2* mutations are the most well-known genetic risk factors involved in the aetiopathogenesis of TNBCs, and the prevalence of germline mutations of these genes in TNBC patients has been reported to be 10–30% [51]. In this study, logistic regression analysis was performed to determine the relationship between *BRCA1/2* carriage and TNBC, and it was observed that carriers had 5.560 times greater TNBC than non-carriers (OR 95%; 1.772–17.450). One of the important limitations of our study was that it was conducted in a relatively small sample group and that other BC-related genes were not investigated in patients in whom a causal variant in the *BRCA1/2* genes was not detected. In addition, the effects of exogenous risk factors such as oral contraceptive use, hormone replacement therapy, alcohol consumption, overweight and physical inactivity could not be examined in the study. Information about the cancers of the relatives of the patients was obtained from pedigree analysis and segregation analysis could not be performed in many of these relatives.

## Conclusions

Our study showed that the *BRCA1/2* carriage rate was 9.4% in 170 Turkish female BC patients. In carriers, ER/PR was mostly negative and tumour grades were higher. The risk of *BRCA* carriage was found to be 6.621 times increased in PR negativity. The risk of *BRCA1/2* carriage was 5.560 times higher in the TNBC group and the tumour grade was found to be higher. Breast cancers caused by mutations in the *BRCA1/2* gene are quite different from sporadic BCs in terms of most clinical and histopathological features. Future large-scale studies will be useful in identifying the unique clinicopathological features of cancers for which these causative genes are responsible.

## Disclosures

1. The present study involved human participants, and it was conducted considering ethical responsibilities according to the World Medical Association and the Declaration of Helsinki. The independent Ethics Committee of the Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital approved this study (Document No: 2023-01/11).
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3. Financial support and sponsorship: None
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## Addresse for correspondence

**Neslihan Duzkale**, MD
Department of Medical Genetic
Diskapi Yildirim Beyazit Training and Research Hospital
Ankara, Turkey
e-mail: neslihanduzkale@gmail.com