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Research

Genetic insights into *BRCA1/2* associated breast cancer in Türkiye: focus on early-onset and aggressive subtypes

Betul Celik Demirbas[1] · Seda Kilic Erciyas[1] · Ozge Sukruoglu Erdogan[1] · Ozge Pasin[2] · Zubeyde Yalniz Kayim[1] · Merve Çiğdem Özgel[1] · Seref Bugra Tuncer[1]

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Abstract

Aim The prevalence of *BRCA1/2* pathogenic variants among Turkish breast cancer (BC) patients is not well-characterized. We specifically examine the age at onset and cancer sub-types concerning *BRCA1/2* mutation status, focusing on patients with no family history of breast or ovarian cancer.

Methods Peripheral blood samples were collected from 3184 BC patients applied to the Istanbul University Oncology Institute. Genetic testing for *BRCA1/2* mutations was conducted using the Illumina MiSeq® platform, with variant classification performed according to ACMG criteria.

Results Among the 3184 patients, 2764 (86.8%) were *BRCA1/2-*, while 247 (7.8%) were *BRCA1* + and 173 (5.4%) were *BRCA2* + . The mean age at BC onset was significantly lower in *BRCA1* + (39.73 years) and *BRCA2* + (41.07 years) patients compared to *BRCA1/2-* patients (43.17 years, $p < 0.001$). Among patients with no family history, HER2 positive cases had a significantly higher mean age at onset than Triple-Negative Breast Cancer(TNBC) cases (41.78 years vs. 40.44 years, $p = 0.017$).

Conclusions This study highlights the strong association between *BRCA1/2* + mutations and earlier BC onset, particularly in patients with no family history of breast or ovarian cancer in Türkiye.

Keywords *BRCA1/2* mutations · Breast cancer · HER2 positive · Triple-negative breast cancer (TNBC) · Turkish population

Abbreviations

BC	Breast cancer
NGS	Next-generation sequencing
HC	Hereditary cancer
HBOC	Hereditary breast and ovarian cancer syndrome
HER2	Human epidermal growth factor receptor 2
ER	Estrogen receptor
PR	Progesterone receptor
TNBC	Triple-negative breast cancer
ACMG	American college of medical genetics and genomics

✉ Seref Bugra Tuncer, seref.tuncer@istanbul.edu.tr; Betul Celik Demirbas, betul.celik@istanbul.edu.tr; Seda Kilic Erciyas, seda.kilic@istanbul.edu.tr; Ozge Sukruoglu Erdogan, ozge.erdogan@istanbul.edu.tr; Ozge Pasin, ozgepasin90@yahoo.com.tr; Zubeyde Yalniz Kayim, zyalniz@gmail.com; Merve Çiğdem Özgel, mervecigdemozgel@ogr.iu.edu.tr | [1]Department of Cancer Genetics, Oncology Institute, Istanbul University, Istanbul, Türkiye. [2]Department of Biostatistics and Medical Informatics, Hamidiye Medical Faculty, University of Health Sciences, Istanbul, Türkiye.

AMP	Association for molecular pathology
SNVs	Single nucleotide variants
MLPA	Multiplex ligation-dependent probe amplification
IHC	Immunohistochemistry
IDC	Invasive ductal carcinoma
ILC	Invasive lobular carcinoma
NCCN	National comprehensive cancer network
PBMc	Peripheral blood mononuclear cells
dbSNP	Single nucleotide polymorphism database
ExAC	The exome aggregation consortium
GnomAD	The genome aggregation database
SIFT	Sorting ıntolerant from tolerant
PolyPhen-2	Polymorphism phenotyping v2
HGMD	The human gene mutation database

1 Introduction

Breast cancer (BC) is one of the most frequently diagnosed malignancies worldwide, affecting both developed and developing nations. While established risk factors such as age, reproductive history, and lifestyle contribute to BC incidence, the underlying genetic and environmental influences remain incompletely understood [1].

Approximately 10% of BC cases are hereditary, with *BRCA1* and *BRCA2* germline pathogenic variants accounting for 25% of hereditary cases [2, 3]. Hereditary breast and ovarian cancer syndrome (HBOC) is an autosomal dominant disorder caused by pathogenic *BRCA1/2* mutations. The lifetime risk of developing invasive BC is approximately 12.8% in the United States and 10.2% in Türkiye [4]. Retrospective studies indicate that *BRCA1* mutation carriers have a cumulative BC risk of 40–87% by age 70, while *BRCA2* mutation carriers face a risk of 27–84% [3, 5]. Despite extensive research in various populations, comprehensive data on the prevalence and clinical impact of *BRCA1/2* mutations in the Turkish population remain limited.

Among individuals with familial early-onset BC, 10–40% carry *BRCA1/2* mutations, whereas these mutations are less frequent (1–10%) in sporadic cases [6]. In addition to *BRCA1/2*, other genes such as *TP53, ATM, PALB2*, and *CHEK2* contribute to familial BC, albeit at lower frequencies [7]. Tumors in *BRCA1/2* mutation carriers exhibit distinct morphological and genetic characteristics, differentiating them from sporadic tumors and hereditary BC cases unrelated to *BRCA1/2* [8]. This highlights the complexity of early-onset BC and the need for population-specific studies. Also, to elucidate the genetic foundation of hereditary cancer (HC), population-based studies targeting specific ethnic groups are crucial for identifying additional genetic variants that may contribute to HBOC within different populations.

Early age of onset is a hallmark of hereditary BC and a key diagnostic criterion for Lynch syndrome [9, 10]. Women with germline *BRCA1/2* mutations are more likely to develop BC at younger ages and with more aggressive subtypes, such as triple-negative breast cancer (TNBC) and HER2 positive BC, compared to those without such mutations [11, 12]. However, most data on both breast and ovarian cancer patients *BRCA1/2* related BC derive from Western populations, with few studies investigating Turkish patients. In a previous study, 57.5% (225/391) of Turkish HBOC patients were found to carry a BRCA1 mutation, 41.9% (164/391) had a *BRCA2* mutation, and 0.6% (2/391) harbored pathogenic variants in both *BRCA1* and *BRCA2* [13]. Additionally, a study conducted by Tuncer et al. reported a higher prevalence of *BRCA1* mutations (13.33%) compared to *BRCA2* mutations (6.19%) among Turkish patients with ovarian cancer [14]. Since most studies have analyzed HBOC cases without stratifying them into subgroups or age at diagnosis, there is a need to investigate these specifically within the Turkish population. To address this gap, we analyzed *BRCA1/2* germline pathogenic variants in Turkish BC patients using Next-Generation Sequencing (NGS), emphasizing subgroups and age at diagnosis.

Family history is also a major risk factor for early-onset BC. Studies have shown that individuals with multiple relatives affected by BC or ovarian cancer face a significantly higher risk of developing the disease [15]. Liu et al. [16] demonstrated a strong correlation between familial clustering and BC risk [16]. Similarly, Huang et al. [17]) found that *BRCA1/2* mutations are detected in up to 22.4% of early-onset BC cases with a family history, particularly in patients diagnosed before age 35, reinforcing the genetic basis of familial BC [17]. A systematic review by Liu et al. [18] further confirmed that *BRCA1/2* mutations are central to the pathogenesis of hereditary BC [18]. Lin et al. [19] emphasized that families with a history of

both BC and ovarian cancer are strongly associated with *BRCA1/2* mutations [19]. These findings highlight the importance of genetic screening and early detection in high-risk populations such as the Turkish population.

This study exclusively utilized peripheral blood samples to analyze *BRCA1/2* mutations in Turkish BC patients. While solid tumor tissues can identify both germline and somatic mutations, blood-based testing offers distinct advantages. Peripheral blood collection is non-invasive and readily accessible, making genetic testing feasible even when tumor biopsy is unavailable or degraded [20]. Additionally, blood samples facilitate risk assessment for patients and their families, aiding in preventive strategies [21]. Circulating tumor DNA (ctDNA) analysis may also provide insights into tumor dynamics and metastatic potential, though its primary role remains germline variant detection [22]. A limitation of blood-based testing is its inability to capture somatic mutations, which may influence tumor behavior and therapeutic response [23].

2 Materials and methods

2.1 Patients collection

Ethical approval for this study was granted by the Ethical Review Board of Istanbul University (Approval No: 2023/500), ensuring compliance with ethical standards for research involving human participants, as stated in the Declaration of Helsinki [24]. Peripheral blood samples were obtained from 3184 BC patients who presented to the Cancer Genetics Department of Istanbul University Oncology Institute between January 2021 and December 2024**.** Patients were enrolled during routine follow-ups, all with a confirmed prior BC diagnosis. Written informed consent was obtained from all participants before inclusion in the study. Comprehensive family histories spanning three generations(family history was defined as the occurrence of cancer among the patient's first-, second-, and third-degree relatives), along with detailed demographic, clinical staging, histological, and pathological data were recorded in patients' files. Blood samples were collected, and genomic DNA sequencing for *BRCA1/2* was conducted using NGS. Hormone receptor (HR) status, including estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), was retrieved from pathology reports. Receptor status was categorized as positive or negative based on established classification criteria from previous research by professional oncologists based on established clinical guidelines and supported by relevant literature [25, 26].

2.2 Mutational screening of *BRCA1/2*

BRCA1/2 genetic testing was conducted at Istanbul University Cancer Genetics Laboratory using the Illumina MiSeq® platform in conjunction with Sophia Genetics DDM analysis software (Illumina, CA, USA). These analyses were performed following a well-established NGS pipeline to ensure the accuracy and reliability of variant detection [27]. The identified variants were evaluated based on the American College of Medical Genetics and Genomics (ACMG) Criteria [28]. Sanger sequencing of *BRCA1/2* genes was performed using previously validated protocols with the CEQ 8000 System (Beckman Coulter, Inc, CA, USA) to confirm the presence of pathogenic variants [29].

2.2.1 Detection of *BRCA1/2* Germline Mutations by Next-Generation Sequencing

Sample Collection: Peripheral blood samples were obtained for genetic analysis from 3184 BC patients. Patients were included in the study based on their high risk for HBOC, as determined by the National Comprehensive Cancer Network (NCCN) guidelines for HBOC risk assessment [30]. Peripheral blood samples of the BC patients were isolated using the Ficoll gradient separation method (Sigma-Aldrich, Darmstadt, Germany). A total of 20 mL of peripheral blood was collected from BC patients and layered onto 3 mL of Ficoll for density gradient centrifugation. Lymphocytes were subsequently extracted and subjected to DNA isolation using the QIAamp DNA Micro Kit (Qiagen, Hilden, Germany) and the GeneAll® Exgene™ Kit (GeneAll Biotechnology, Seoul, Korea), following the manufacturer's protocols. DNA purity and concentration were assessed using a NanoDrop 2000c Spectrophotometer (Thermo Fisher Scientific, USA) to ensure high-quality genomic DNA for downstream analyses.

Library preparation: For library preparation, an amplicon-based gene panel protocol was employed using the Illumina MiSeq platform (Illumina, San Diego, CA, USA) with the BRCA MASTR Plus Dx kit (Sophia Genetics, Boston, USA), following the manufacturer's guidelines. Initially, five multiplex PCR reactions were conducted per sample using 50 ng

of genomic DNA, ensuring amplification of the entire target region. Post-amplification, the PCR products from each sample were pooled, and residual small DNA fragments were removed. The pooled products then served as templates for a universal PCR reaction, incorporating hybrid primers to assign unique multiplex identifiers (MID) and platform-specific primers to each amplicon. Finally, Agencourt AMPure XP beads (Beckman Coulter, CA, USA) were utilized for the purification of the tagged amplicon libraries. The concentration of each library was assessed using a NanoDrop 2000c Spectrophotometer (Thermo Fisher Scientific, USA) before sequencing. The DNA library was denatured and diluted by adding 5 µl of 0.2 N NaOH at a final concentration of 2 nM. Subsequently, the library was diluted to 10 pM using a pre-chilled HT1 buffer and spiked with 6% PhiX Control v3 (Illumina, CA, USA) to ensure sequencing quality and accuracy.

2.2.2 Variant analysis and classification

Variant analysis was conducted using bioinformatics pipelines to detect single nucleotide variants (SNVs), small insertions/deletions (indels), and large genomic rearrangements. To confirm NGS findings, Sanger sequencing was performed for point mutations, while Multiplex Ligation-dependent Probe Amplification (MLPA) was used for the detection of large deletions and duplications.

Sequence alignment and variant detection were conducted using SOPHiA DDM software (v5.10.6) (Saint-Sulpice, France), concerning the GRCh37/hg19 human genome assembly. Variant annotation and filtering were performed through multiple population and functional databases, including dbSNP[31], ExAC [32], GnomAD [33], SIFT [34], PolyPhen-2 [34], ClinVar [35], and HGMD [36].

2.3 Clinical subtypes of BRCA-related breast cancer

Cancer subtypes in *BRCA1/2* mutation carriers were analyzed using detailed clinical data. Subtype classification was based on the immunohistochemistry (IHC) status of ER, PR, and HER2 positive, as reported in the patient's pathology results. For this study, HER2 positive cases and TNBC cases were compared between patients with and without a family history of breast or ovarian cancer.

2.4 Statistical analysis

Descriptive statistics for qualitative variables were presented as frequencies and percentages, while quantitative variables were described using mean, standard deviation, median, and interquartile ranges (first and third quartiles). The Kolmogorov–Smirnov test was employed to assess the normality of the quantitative variables. The Mann–Whitney U test was used to compare the means of two independent groups, while the Kruskall-Wallis test was applied to compare the means across more than two groups, followed by the Dunn test for post hoc comparisons. Pearson's chi-square test was utilized to evaluate the relationships between qualitative variables. A significance level of $p < 0.05$ was adopted for all statistical tests. All calculations were performed using the Statistical Package for the Social Sciences (SPSS, version 28.0, Armonk, NY: IBM Corp.).

3 Results

3.1 Clinicopathological and demographic characteristics of breast cancer patients

This study included 3184 BC patients, with a median age at diagnosis of 38 years (IQR: 33–46) for BRCA1/2-positive cases and 40 years (IQR: 35–45) for BRCA1/2-negative cases shown in Table 1. *BRCA1/2* mutations were detected in 7.8% and 5.4% of patients, respectively, while 86.8% were *BRCA1/2*-. The majority (45.4%) were diagnosed at ≤ 40 years, and 98.2% were female. Most cases were diagnosed at Stage 2 (45.3%) and Stage 3 (37.7%), with 49.2% classified as Grade 3 tumors. Invasive ductal Carcinoma(IDC) was the predominant histological subtype (90.7%), and 25.4% of patients had metastatic disease. A family history of breast/ovarian cancer was reported in 54.2% of cases. These findings highlight the genetic and clinicopathological characteristics of BC patients, underscoring the need for early detection and genetic screening.

The detection rate of pathogenic *BRCA1/2* variants was assessed among 3184 patients who underwent genetic testing. Of these, 2764 patients (86.8%) were *BRCA1/2*-, while 247 patients (7.8%) were *BRCA1*+, and 173 patients (5.4%) were *BRCA2*+ (Fig. 1). The study cohort comprised 2702 women diagnosed with BC, 46 women diagnosed with both breast

Table 1 Clinicopathological and Demographic Characteristics of Breast Cancer Patients

Characteristics	Number	Percentage(%)
Age at diagnosis	Median	
BRCA1/2+	38(33–46)	
BRCA1/2-	40(35–45)	
Age Groups(years)		
< = 40	1446	
41–49	1001	45.4
> = 50	703	31.4
Missing	34	22.1
Ages		
< = 45	2059	
> 45	1091	64.7
Missing	34	34.3
BRCA1/2 Mutaion Status		
BRCA1 Positive	247	7.8
BRCA2 Positive	173	5.4
BRCA1/2 Negative	2764	86.8
Gender		
Female	3126	98.2
Male	58	1.8
Pathological Stage		
Stage 1	316	
Stage 2	1259	11.4
Stage 3	1047	45.3
Stage 4	157	37.7
Missing	405	5.6
Histological Grade		
Grade 1	169	
Grade 2	1244	6.1
Grade 3	1368	44.7
Missing	402	49.2
Menapausal Status		
Pre-menopausal	2307	
Post-menopausal	659	76.4
Missing	164	21.8
Histological Subtype		
IDC	2592	90.7
ILC	342	12
IDC + ILC	207	7.3
Metastasis Status		
Yes	806	
No	2058	25.4
Missing	320	64.6
Breast and ovarian cancer in 1st, 2nd, and 3rd degree family members		
Yes	1722	
No	1458	54.2
Missing	4	45.8

and ovarian cancer, 380 women diagnosed with BC alongside other malignancies, and 56 men diagnosed with BC (the

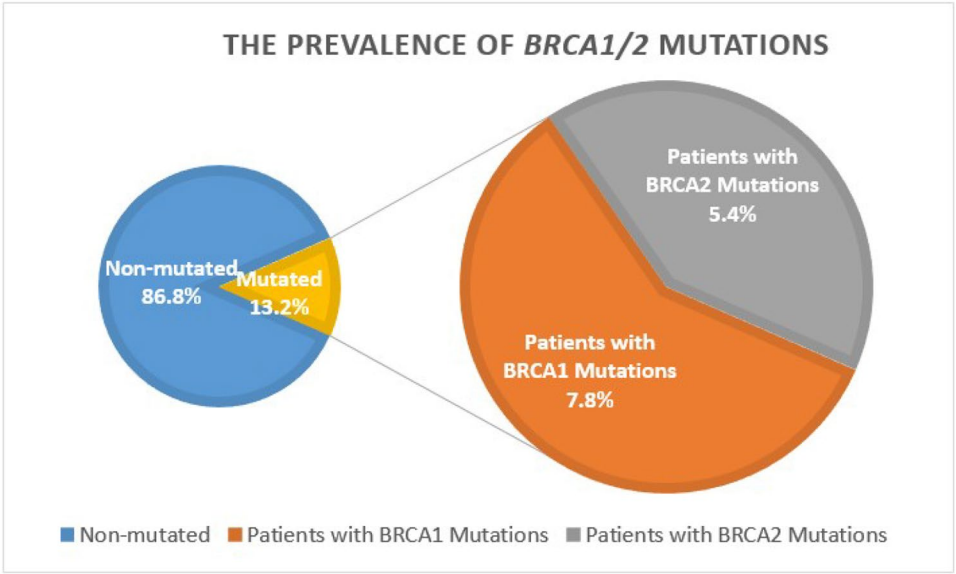

Fig. 1 Prevalence of *BRCA1* and *BRCA2* Mutations Among Patients Included in the Study

Table 2 The comparison with previous reports

Country	Study ID	Selection	Hormone Receptor Status	Total	*BRCA1* + Cases (%)	*BRCA2* + Cases (%)	*BRCA1/2* + Cases (%)
Japan	Momozawa [37]	Japanese	Mixed	7051	102 (1.45)	191 (2.71)	293 (4.15)
United States	Vidula [38]	NR/unclear	Mixed	202	NR	NR	15.2
United States	Susswein [39]	Mixed	Mixed	10.000	63 (1.2)	73 (1.4)	136 (2.6)
Germany	Fasching [40]	Mixed	NR/unclear	1462	1.4	2.9	4.3
France	Meynard [41]	Unselected	Mixed	407	0.7	2.0	2.7
Japan	Okano [5]	Japanese	Mixed	2,366	262 (11.1)	211 (8.9)	476 (20.1)
Israel	Dagan [42]	Ashkenazi	Mixed	149	33(22.1%)	15(10.1%	48 (32.2)
Canada	Ghadirian [43]	French-Canadia	Mixed	1093	13 (1.2)	43 (3.9)	56 (5.1)
Spain	Gonzalez-Rivera [44]	Spanish-Peruvian	TNBC	105	12.4	1.9	14.3
Australia	Wong-Brown [45]	Australian	TNBC	439	5.9	3.4	9.3
Italy	Palomba [46]	Italian	Mixed	726	1.0	1.9	2.9
South Korea	Kim [47]	Korean	NR/unclear	471	1.5	1.5	3.0

NR: Not Reported

male patients and those with unknown receptor status were omitted from further analysis to ensure consistency in the study population). The detection rates in each subgroup were compared with findings from previous studies (Table 2), providing a contextual understanding of the variant distribution.

3.2 The age at the onset of BC is lower in patients with *BRCA1/2* + pathogenic variants

To explore its association with the presence or absence of pathogenic *BRCA1/2* variants, genetic testing was conducted on 3184 patients, all of whom had documented ages of cancer onset before the study. The distribution of BC onset across different age groups is shown in Fig. 2, highlighting distinct patterns associated with *BRCA1/2* mutation status.

We divided the dataset into four quartiles to facilitate structured analysis, and age was categorized accordingly based on these quartile data. Table 3 provides a detailed comparison of the mean age of onset across *BRCA1/2* mutation groups, emphasizing the significantly younger onset observed in *BRCA1* + patients compared to *BRCA1/2*- ($p < 0.001$). A significant difference in the mean age of onset was observed between *BRCA1/2*- patients and those with *BRCA1* + ($p < 0.001$) or *BRCA2* + ($p = 0.010$) mutations. *BRCA1/2*- patients exhibited a significantly higher mean age at onset (43.17 ± SS years)

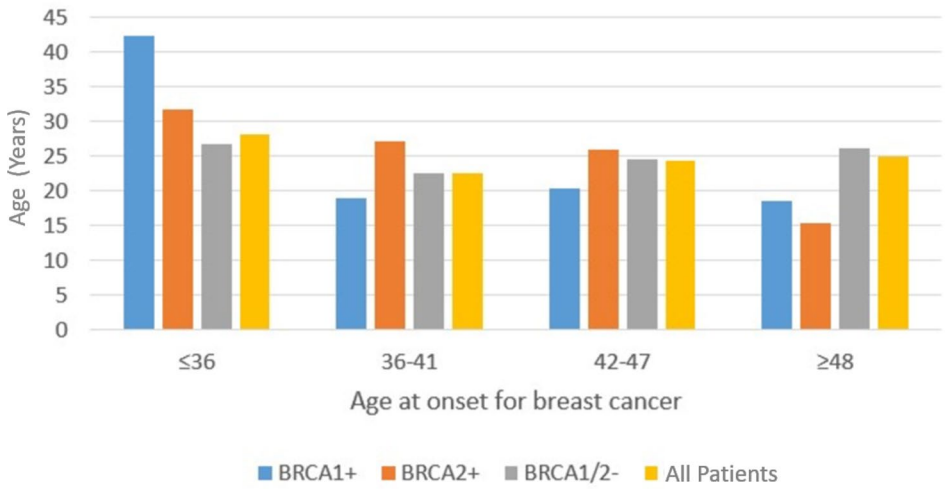

Fig. 2 Age at onset distribution in *BRCA1+*, *BRCA2+*, and, *BRCA1/2-* patients. The dataset was divided into four equal parts, and age was categorized based on the resulting quartile data

Table 3 The mean of age at the onset of breast cancer in *BRCA1/2-*, *BRCA1+*, and *BRCA2+* patients

BRCA1/2 Status	Age at onset(year) Mean ± Std. Deviation		Q2(Q1-Q3)			p	Pairwise comparison of p values		
			Q1	Q2(Median)	Q3		*BRCA1/2*-p	*BRCA1*+p	*BRCA2*+p
BRCA1/2-(n = 2764)	43.17	10.238	36.00	42.00	49.00	< 0.001	-		
BRCA1+(n = 247)	39.73	9.023	33.00	38.00	46.00		< 0.001	-	
BRCA2+(n = 173)	41.07	9.271	35.00	40.00	45.00		0.010	0.144	-

SD: standard deviation *Kruskal–Wallis and Dunn test was used. (n = number)

compared to *BRCA1+* (39.73 ± SS years) and *BRCA2+* (41.07 ± SS years). No statistically significant difference was found between the mean ages of *BRCA1+* and *BRCA2+* patients (p = 0.144). These findings indicate that *BRCA1+* patients are associated with a notably earlier onset of BC compared to *BRCA1/2-* while the age of onset for *BRCA2+* falls between *BRCA1+* and *BRCA1/2-*, without a significant distinction from either group.

The age distribution among *BRCA1/2+* and *BRCA1/2-* groups is shown in Fig. 3, revealing distinct patterns of onset across mutation statuses. Patients in the *BRCA1/2-*exhibit a wider age distribution, with a median age slightly above 40 years and several outliers, particularly among individuals aged over 60 years. In contrast, the *BRCA1+* patients show a lower median age, with a more concentrated age distribution and fewer outliers, indicating a stronger association with earlier onset. The *BRCA2+* group has a median age comparable to the *BRCA1+* group, but the overall age distribution is broader, particularly among patients aged over 40 years. These findings underscore the distinct age distribution patterns among Turkish BC patients with different *BRCA1/2+*, highlighting the earlier onset associated with *BRCA1+* carriers and the more variable onset in *BRCA2+* carriers. This variability may affect personalized screening and prevention strategies in mutation-positive individuals.

3.3 In both *BRCA1/2+* patients, age at onset of the disease showed no statistical difference between the HER2 positive and TNBC groups

3.3.1 Comparison of the Mean age at onset across breast cancer sub-types and *BRCA1/2* status

The age at onset of BC showed no statistically significant difference between HER2 positive and TNBC groups in patients with *BRCA1+* or *BRCA2+* mutations. However, distinct patterns were observed across clinical subtypes and *BRCA1/2* mutation statuses, as detailed in Table 4. Among *BRCA1/2-* patients, the mean age at onset was slightly higher in HER2 positive cases (43.85 years) compared to TNBC cases (42.06 years), with a statistically significant difference (p < 0.001). For *BRCA1+* patients, the mean age at onset was lower in the TNBC group (39.04 years) than in the HER2 positive group (40.67 years), although this difference was not statistically significant (p = 0.252). Similarly, in *BRCA2+* patients, no

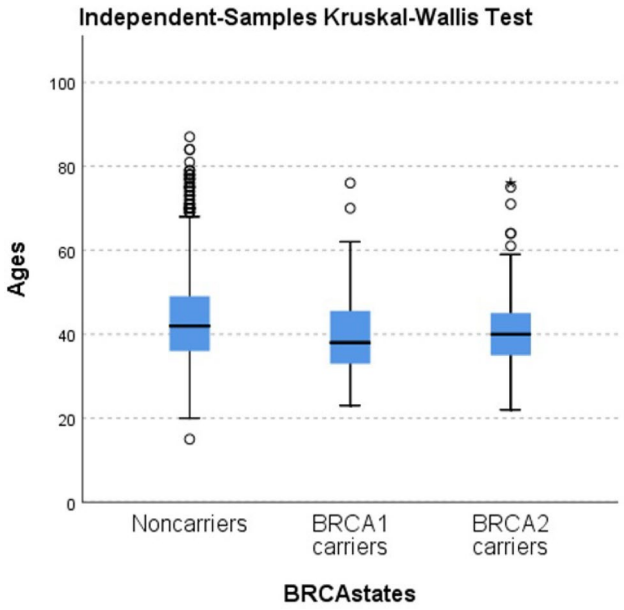

Fig. 3 Age Distribution in BRCA1/2 ± groups: Box Plot Analysis. The median age in the BRCA mutation-positive and negative groups was 38 (33–46) and 40 (35–45) years, respectively

Table 4 Comparison of the mean Age Onset in Breast Cancer Subtypes According to *BRCA1/2* Status

BRCA1/2 Status	Groups	Age at onset Mean ± Std. Deviation		Q2(Q1-Q3)			P values
				Q1	Q2(Median)	Q3	
BRCA1/2-(n = 2764)	HER2 + (n = 1343)	43.85	10.089	37.00	42.00	49.00	< 0.001
	TNBC (n = 1069)	42.06	10.029	35.00	41.00	48.00	
BRCA1 + (n = 247)	HER2 + (n = 61)	40.67	9.943	33.50	40.00	46.00	0.252
	TNBC (n = 146)	39.04	8.296	33.00	37.00	44.00	
BRCA2 + (n = 173)	HER2 + (n = 94)	40.73	8.941	35.00	40.50	45.25	0.810
	TNBC (n = 57)	41.67	9.777	36.00	39.00	46.50	

(n = number)

significant difference was observed between the HER2 positive (40.73 years) and TNBC (41.67 years) subtypes (p = 0.810). These findings highlight that while HER2 positive and TNBC subtypes exhibit a significant age difference in *BRCA1/2*- patients, such differences are not evident among patients with *BRCA1* + or *BRCA2* + mutations. This suggests that genetic factors may influence the relationship between BC subtypes and age at onset differently in mutation-positive and mutation-negative populations. The sample sizes for the *BRCA1* + and *BRCA2* + groups are smaller compared to the non-carriers group (*BRCA1* + *:* n = 247, *BRCA2* + : n = 173, *BRCA1/2-:* n = 2764). To account for this difference, we used the Kruskal–Wallis test, a non-parametric method that is robust to unequal sample sizes and does not assume normality.

3.3.2 The prevalence of pathogenic variants is more frequent among younger patients in the HER2 positive and TNBC group with no family history of breast and ovarian cancer

The relationship between BC subtypes and age in patients with no family history is particularly noteworthy. Therefore, we examined the distribution of pathogenic *BRCA1/2* + variants across different age groups in BC patients with no family history of breast or ovarian cancer, providing valuable insights into how genetic mutations influence cancer onset in this specific population. As shown in Table 5, among patients without a family history of breast or ovarian cancer, the mean age at onset was significantly higher for *BRCA1/2*- individuals in the HER2 positive group (41.78 years) compared to those in the TNBC group (40.44 years; p = 0.017). However, in *BRCA1* + patients, no significant difference was observed in the mean age at onset between HER2 positive (38.57 years) and TNBC cases (38.29 years; p = 0.465). Similarly, in *BRCA2* + patients, the mean age at onset did not differ significantly between HER2 positive (36.29 years) and TNBC groups (40.74 years; p = 0.142).

Table 5 Comparison of the mean age at Onset in Breast Cancer Subtypes According to *BRCA1/2* Status With no Family History

BRCA1/2 Status	Groups	Age at onset Mean ± Std. Deviation		Q2(Q1-Q3)			p
				Q1	Q2(Medyan)	Q3	
BRCA1/2-	HER2 + (n = 605)	41.78	9.862	35.00	41.00	47.00	0.017
	TNBC (n = 563)	40.44	9.438	34.00	39.00	46.00	
BRCA1 +	HER2 + (n = 14)	38.57	13.949	27.75	35.00	45.25	0.465
	TNBC (n = 35)	38.29	7.691	32.00	37.00	44.00	
BRCA2 +	HER2 + (n = 34)	36.29	8.653	31.00	35.00	40.75	0.142
	TNBC (n = 19)	40.74	11.125	35.00	38.00	42.00	

(n = number)

Figure 4 illustrates the age at onset of BC among *BRCA1* + and *BRCA2* + patients stratified by HER2 positive and TNBC subtypes in individuals with no family history of breast or ovarian cancer. Across both subtypes, the *BRCA1* + or *BRCA2* + mutations were consistently associated with an earlier age of cancer onset, particularly in patients diagnosed at age ≤ 36 years. Notably, *BRCA2* + mutations in the TNBC group exhibited a slightly different age distribution compared to *BRCA1* + mutations. While *BRCA1* + mutations were predominantly seen in patients aged ≤ 36 years, *BRCA2* + mutations were more frequently observed in patients diagnosed between 36–41 years. In the HER2 positive group, although the number of *BRCA1/2* + patients was limited, the majority were diagnosed at a younger age, reinforcing the association between these mutations and early-onset BC.

4 Discussion

This study aimed to investigate BC patients based on *BRCA1/2* mutation status within a Turkish cohort, emphasizing age-related factors and family history of breast and ovarian cancer. To date, limited research has focused on the prevalence and characteristics of *BRCA1/2* variants in Turkish populations, particularly regarding the age of onset.

The analysis of *BRCA1/2* mutations in the Turkish population has drawn attention due to its implications for cancer risk assessment. Previous studies, such as Bisgin et al., demonstrated the significant presence of *BRCA1/2* mutations in unselected Turkish BC patients, highlighting the necessity of genetic screening in this demographic [48]. Similarly, research by Tepebası et al. corroborated these findings, revealing comparable mutation prevalence to other populations, with notable variability in specific mutation profiles [49]. Additionally, reports of double heterozygosity in Turkish patients, as documented by Gökce and Eyerci, underscore the complexity of genetic predisposition, emphasizing the need for comprehensive genetic counseling and localized databases [50]. Such findings underscore the diversity of the mutation spectrum across different ethnic groups and the necessity for localized genetic databases to inform clinical practices.

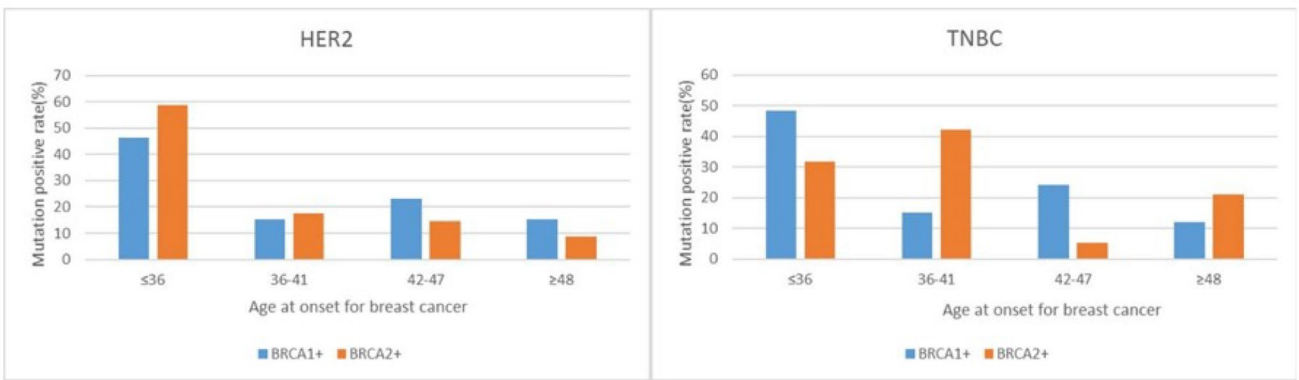

Fig. 4 The Prevalence of Age at Onset Among *BRCA1* + And *BRCA2* + Patients in HER2-Positive and Triple-Negative Breast Cancer(TNBC) Sub-Types in Patients with No Family History of Breast or Ovarian Cancer. Blue Bars indicate *BRCA1* + Patients and Orange Bars indicate *BRCA2* + patients

Age at diagnosis remains a critical factor in assessing hereditary BC risk, as highlighted in the NCCN guidelines [51]. While the association between familial BC and younger age of onset is well-established, studies have demonstrated that this relationship can vary significantly across different racial and ethnic groups [5]. Although numerous reviews have explored this topic, many fail to consistently account for family history, and the cohorts analyzed in these studies often differ substantially in their design and inclusion criteria [52]. To gain a more comprehensive understanding of these patterns, we need to examine this relationship in larger, well-defined cohorts, particularly within the Turkish population. Our analysis of a Turkish cohort revealed that BC associated with *BRCA1/2* + mutations were diagnosed at a relatively younger age compared to global averages. Notably, individuals with *BRCA1* + mutations exhibited an average age at diagnosis of 39.7 years, representing a significant decrease compared to earlier studies, which reported a mean age of approximately 42 years for *BRCA1/2* + mutation carriers [53, 54]. Existing literature predominantly focuses on Caucasian or white populations, leaving a critical gap in our understanding of how racial and ethnic differences may influence the impact of *BRCA1/2* + variants on the age of onset and other clinical characteristics of BC. This highlights the necessity of further research on populations that are not predominantly Caucasian to better elucidate these variations and refine genetic risk assessment strategies.

Numerous studies have investigated the relationship between *BRCA1/2* mutations and various BC subtypes. Consistent evidence indicates that tumors associated with *BRCA1* mutations are predominantly TNBC. In contrast, both *BRCA1* + and *BRCA2* + mutations are observed in HER2 positive BC cases, although *BRCA1* + mutations are less frequently associated with this subtype compared to *BRCA2* + mutations [55]. A comparable trend has been observed in cases of metastatic and recurrent BC [56]. However, only a limited number of studies have addressed age-related factors in this context. Data from Türkiye demonstrate that patients with TNBC *BRCA1* + mutations are diagnosed at a younger age compared to those with non-TNBC subtypes who harbor the same genetic mutations. Specifically, the average age at diagnosis for TNBC patients with *BRCA1* + mutations is reported as 38 years, while non-TNBC patients with *BRCA1* + mutations are diagnosed at an average age of 46 years, with a statistically significant difference ($p = 0.028$) [57]. In our current study, we found no significant difference in the age of onset between HER2 positive BC and TNBC among patients with *BRCA1/2* + mutations.

Conversely, among patients without *BRCA1/2* + mutations, the mean age at diagnosis for HER2 positive cases was significantly higher than that for TNBC cases ($p < 0.001$). These findings suggest that while the age of onset is comparable between HER2-positive and TNBC subtypes in patients with *BRCA1/2* + mutations, HER2 positive patients without these mutations tend to be diagnosed at a significantly older age compared to their TNBC counterparts. Furthermore, our data revealed that the majority of BC patients in the Turkish cohort were diagnosed in the third decade of their lives (age 36 and below). Notably, within the HER2 positive group, nearly 70% of patients carrying *BRCA2* + mutations were diagnosed before the age of 36. This highlights a significant trend in the age of diagnosis among BC subtypes in Türkiye.

Globally, the age of onset for BC varies significantly across populations. Western studies often report HER2 positive cases diagnosed in the late 40 s to early 50 s, while TNBC is more frequently diagnosed at younger ages, particularly in patients with *BRCA1/2* + mutations [58]. However, in this study, we detected that most patients were diagnosed at age 36 years old and younger. In contrast, TNBC is frequently diagnosed at a younger age, with various studies indicating that a significant percentage of patients are diagnosed in their 30 s [59]. For example, a study from China reported that a considerable number of TNBC patients were diagnosed before the age of 40 [60], similar to the findings in Türkiye. In the United States, the average age of diagnosis for TNBC is approximately 58 years; however, younger patients, particularly those with *BRCA1/2* + mutations, are often diagnosed in their 30 s [61].In our cohort, 55% of TNBC patients with *BRCA1* + mutations were diagnosed before the age of 36. These findings suggest that while Türkiye has a higher proportion of patients diagnosed before the age of 36 for both HER2 positive and TNBC subtypes, global trends indicate a broader age distribution. This underscores the importance of regional differences in BC epidemiology and highlights the need for tailored screening and prevention strategies.

While this study provides valuable insights, several limitations must be acknowledged. Although the cohort size (3184 patients) is substantial, the subtype distribution (e.g., HER2 positive, TNBC) may not be fully representative of the broader population. The difference in sample sizes could be a potential limitation and emphasize the findings. Additionally, the focus on patients without a family history of breast and ovarian cancer may overlook familial patterns influencing *BRCA1/2* mutation prevalence. Future research should include more diverse cohorts and consider family history to better understand mutation prevalence and its clinical implications across populations.

5 Conclusions

In conclusion, this study highlights the significant prevalence of pathogenic *BRCA1/2* variants among BC. This study underscores the significant prevalence of pathogenic *BRCA1/2* + variants among BC patients in Türkiye, with detection rates of 7.8% for *BRCA* + *1* and 5.4% for *BRCA2* + mutations. Patients with *BRCA1* + mutations were diagnosed at a younger mean age (39.73 years) compared to those with *BRCA2* + mutations (41.07 years) and *BRCA1/2*- patients (43.17 years). These findings highlight the critical role of genetic testing, particularly in patients without a family history of breast and ovarian cancer, where *BRCA1/2* + mutations were strongly associated with earlier cancer onset. Given the relatively younger age of diagnosis in the Turkish cohort compared to global populations, region-specific strategies for early detection and intervention are warranted. Establishing comprehensive genetic databases for the Turkish population would further enhance our understanding of mutation spectra and facilitate more personalized approaches to cancer prevention and treatment. Moreover, integrating *BRCA1/2* genetic testing into routine clinical workflows, particularly for high-risk groups such as young patients and those without a documented family history, could significantly improve outcomes through earlier diagnosis and targeted interventions. Future research should focus on the molecular and pathological characteristics of *BRCA1/2* related BC to uncover novel biomarkers for early diagnosis and treatment. Additionally, longitudinal studies tracking BC incidence and outcomes across diverse populations are necessary to validate these findings and assess their long-term clinical implications.

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Data availability Data availability: The datasets produced and/or analyzed in this study can be obtained from the corresponding author upon reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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