Research Article

Cancer Prevention Research

The NCCN Criterion "Young Age at Onset" Alone is Not an Indicator of Hereditary Breast Cancer in Iranian Population

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Abstract

Because the contribution of genetic factors to the burden of breast cancer is not well investigated in Iran, we aimed to examine the prevalence of mutations in breast cancer susceptibility genes, *BRCA1/2* and *PALB2*, and to investigate the predictive potential of hereditary breast cancer risk criteria for genetic testing in Iranian population. Next-generation sequencing was conducted on a population consisting of 299 and 125 patients with breast cancer, with and without hereditary cancer risk criteria for genetic testing, respectively. The pathogenic mutation frequency rate was 10.7% in patients with hereditary cancer criteria versus 1.6% in no criteria group ($P = 0.0017$). None of the 107 tested patients with only young age at onset (<40) criterion had a pathogenic mutation. Patients who had only a single heritable risk criterion [OR, 6.15; 95% confidence interval (CI), 1.26–58.59; $P = 0.009$] and patients with multiple heritable risk criteria (OR, 22.5; 95% CI, 5.19–201.31; $P < 0.0001$) had higher probabilities of carrying a mutation compared with no criteria group. Our results showed that young age at onset alone is not an indicator of hereditary breast cancer at least in the Iranian population. This is while women with multiple hereditary breast cancer risk criteria were enriched for *BRCA1/2* mutations. Given such high risk of identification of a disease-causing mutation, multiple hereditary criteria should be regarded as a strong predictor for a hereditary breast cancer syndrome. These findings are important concerning the optimization of genetic counseling and furthermore establishing criteria for *BRCA1/2* testing of the Iranian population.

Introduction

Iran with a multi-ethnic population, constitute of Persians, Azerbaijanis, Kurds, Lurs, Turkmens, Arabs, and Baluch (1), has a modest breast cancer incidence rate of 28.1 per 100,000 per year (2). Although Iran is among the Asian countries with the lowest breast cancer incidence, but that has been continuously rising in the recent years. A large proportion of this growth has been attributed to the increasing trend in the literacy, urbanization, and life expectancy and a decreasing trend in the family size and the total fertility rate (3).

Breast cancer is widely known as a multifactorial disease. Hereditary predisposition, as one of these factors, causes up to 10% of all breast cancer cases. Mutations in a number of genes have been associated with susceptibility to breast cancer. *BRCA1* and *BRCA2* are the best-known genes that account for a majority of hereditary breast cancer cases. Germline mutations in *BRCA1/2* are highly penetrant and predispose individuals to up to 65% lifetime risk of developing breast cancer and up to 40% lifetime risk of ovarian cancer (4, 5). Furthermore, the estimated risk of contralateral breast cancer after the first cancer diagnosis is up to 3% per year; persisted for 30 years (6). A higher likelihood of developing pancreatic, melanoma, and prostate cancers for *BRCA1* or *BRCA2* carriers have also been reported by several studies (7–9).

Apart from *BRCA1/2*, there are other known breast cancer susceptibility genes, including *ATM*, *CHEK2*, *NBN*, *PALB2*, *PTEN*, and *P53* (4). However, *PALB2* is the most clinically important one among these genes considering its mutation frequency and penetrance. *PALB2* belongs to the same

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DNA repair pathway as *BRCA1* and *BRCA2*. It has been estimated that the average risk of breast cancer associated with *PALB2* is ranged from 33% to up to 58%, which is comparable with the risk associated with *BRCA2* (10).

Because of the fact that *BRCA1/2* genes are not mutated in a large number of unselected breast cancers, this study screened the genes in patients with breast cancer who may be at an elevated risk of carrying a genetic mutation based on their cancer-related personal and family history. Identification of individuals who have a pathogenic mutation in breast cancer susceptibility genes is an important step to take advantage of genetic counseling, screening, and potentially life-saving prevention strategies. The National Comprehensive Cancer Network (NCCN), provide recommendations for the management of patients with high risk syndromes associated with an increased risk of breast cancer. The NCCN Clinical Practice Guideline in oncology for genetic/familial high risk evaluation suggests a set of clinical criteria as the first step for offering *BRCA1/2* genetic testing to patients with breast cancer. Referral indications for cancer predisposition assessment are young age at onset, positive family history of cancers, male breast cancer, or diagnosis with a multi-focal or triple-negative breast cancer (TNBC) (11). It is not known what proportion of breast cancers in Iran is hereditary and related to mutations in *BRCA1/2* and *PALB2* genes. All the prior Iranian investigations used limited approaches or restricted studies for specific gene mutations and had small sample sizes. According to the latest systematic review done in 2015, at least 15 valid investigations on *BRCA1/2* gene mutations in Iranian population exist. Of those more than half of them only examined the frequency of three Ashkenazi Jewish founder mutations, 185delAG, 5382insC, and 6174delT, and the rest only focused on other specific gene mutation or exons in *BRCA1/2* (12–16). Only a handful of studies have examined all the coding sequences of *BRCA1/2*. The first study identified two *BRCA2* pathogenic mutations in 10 high risk families (17). Another study found five pathogenic *BRCA1* mutations (5.8%) and one pathogenic *BRCA2* (1.17%) mutation in 85 selected breast cancers (18). Tabarestani and colleagues did identify three mutations in *BRCA1* (15%) and two in *BRCA2* (10%) among 20 indexed patients with high-risk breast cancer (19). Here, we screened *BRCA1/2* and *PALB2* genes using next-generation sequencing (NGS) technology in multi-ethnic Iranian population to determine the spectrum of the breast cancer susceptibility gene mutations and to further assess the predictive value of the hereditary breast cancer risk criteria for genetic testing.

Materials and Methods

Study population

In this project, a hospital-based case study was conducted on 958 patients with breast cancer. All cases were individuals who were referred to Cancer Institute, Imam Khomeini Hospital Complex due to their diagnosis of breast cancer between 2012 and 2016. Diagnosis of breast cancer was only confirmed by their surgical pathology report. On the basis of self-report, patients were categorized into Fars, Turk/Azari, Kurd, Lur, Gilaki/Mazandarani, and other (referred to individuals with mixed backgrounds or unknown ethnicity). All patients with breast cancer were categorized into two groups of "with criteria for genetic testing" and "no criteria for genetic testing" according to the hereditary breast cancer risks criteria. These criteria include age at onset of under 40 years, positive family history of breast/ovarian/related cancers, that is, pancreatic cancer and prostate cancer, male breast cancer (1st–3rd degree relatives in either side of the family), TNBC diagnosed $\leq$60 years, bilateral breast cancers of which one diagnosed $\leq$50 years, or multiple primary tumors in the same individuals.

Of the total 958 affected individuals, 410 patients were determined as a high-risk group with one or more of hereditary criteria. The rest of 548 affected individuals without the hereditary criteria constituted the low-risk group. All the eligible participants were actively cooperated in disclosing detailed information on the tumor hormone receptor status, demographics, ethnicity, and personal/family history of cancer and were asked for 10 mL blood samples. Of 410 individuals with hereditary criteria and 548 individuals with no hereditary criteria, 299 and 392 patients volunteered for providing blood samples, respectively.

Herein, as part of this investigation, we focused on all of the 299 patients with inclusion criteria for genetic testing. Besides, we randomly selected 125 individuals of 392 patients who did not fulfill the hereditary breast cancer selection criteria (Fig. 1). This study was conducted according to the Iranian National Codes for Research Ethics, which has been developed according to the Helsinki Declaration and International Ethical Guidelines for Biomedical Research Involving Human Subjects (CIOMS). All the participants signed a written informed consent. This study has the approval from the National Research Ethics Committee (code: IRAN.REC.1392.71).

Mutation analysis and variant classification

Total DNA from blood samples was extracted according to the manufacturer's instruction using Gentra Puregene Blood Kit (Qiagen). All coding sequences and intron–exon boundaries of *BRCA1* (NM_007294.3), *BRCA2* (NM_000059.3), and *PALB2* (NM_024675.3) were amplified using WaferGen SmartChip Technology (WaferGen Inc). Sequencing of the DNA libraries was conducted at 2×250 cycles using an Illumina MiSeq sequencer.

Generated sequence reads for each sample were aligned to the reference human genome using Burrows-Wheeler Aligner. Identification of genetic variations including

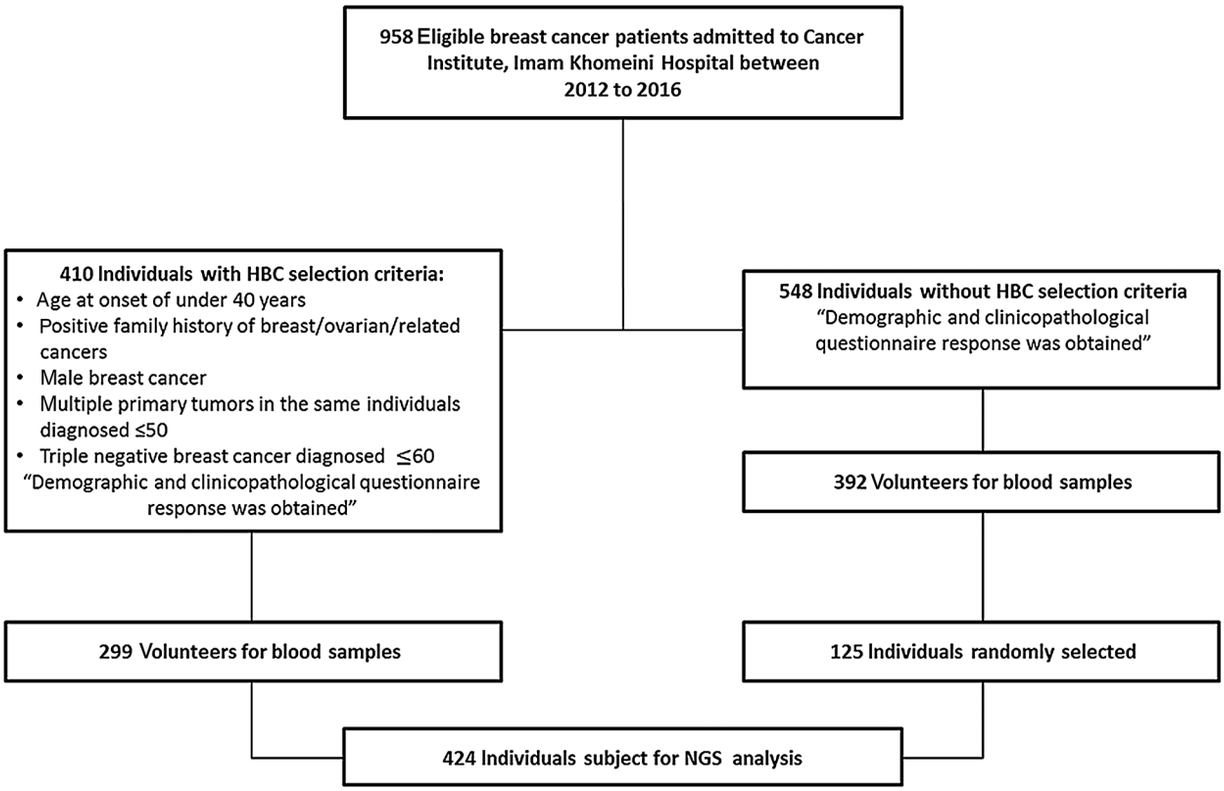

Figure 1.
Flowchart of patient selection.

SNP or insertion-deletion (indel) was done using the UnifiedGenotyper module of the GATK package. Regions with at least 20-fold depth of coverage were used for calling variants. A nucleotide that differed from the reference sequence in at least 25% of the reads aligned to a given position was called as a variant. In addition to the internal highly curated mutation database of Hereditary Cancer Center, Women's College Hospital, University of Toronto (Toronto, Canada), other databases such as ClinVar, HGMD, and BRCA Exchange were used for determining the pathogenicity of the identified mutations. Later, deleterious mutations were confirmed by Sanger sequencing.

Statistical analysis

To determine the frequencies of the categorical measures and the mean values for age at diagnosis, descriptive analysis was performed. The logistic regression was used to calculate the OR and 95% confidence interval (CI) to assess the odds of finding a mutation in patients with inclusion criteria for genetic testing compared with the no criteria group. Cochran–Armitage test (P_{trend}) was used to evaluate the dose–response association of being a mutation carrier and the number of criteria (categorized to: no criteria, single criterion, and multiple criteria). To estimate the number needed to screen, the reciprocal of the proportion of the mutation carrier in the "no criteria" group minus the proportion of the mutation carrier in each of the subgroups with hereditary risk criteria was calculated. This number was represented as the average number of patients who needs to be tested genetically to identify one additional carrier in the "single criterion" or "multiple criteria" group compared with the "no criteria" group. *P* values less than 0.05 were considered as statistically significant. The STATA 12 (Stata Corporation) was used for all the analysis.

Results

In this study, *BRCA1/2* and *PALB2* genetic testing was performed for 424 patients with breast cancer consisting of 299 and 125 patients with and without hereditary cancer criteria, respectively. Demographic characteristics of the two studied subgroups are summarized in Table 1.

In our entire studied population, 34 pathogenic mutations were identified; this was 13 *BRCA1* (4.34%), 17 *BRCA2* (5.68%), and two *PALB2* (0.66%) in 299 individuals with hereditary criteria, while 2 *BRCA2* carriers (1.6%) were found among 125 patients with no criteria ($OR_{high\ risk/low\ risk}$, 7.37; 95% CI, 1.82–64.27; $P = 0.0017$).

All the mutations were singleton and no recurrent mutation was found. Characteristics of the 34 patients

Table 1. Demographic characteristics of the two studied subgroups

Variable	With criteria ($n = 299$; %)	Without criteria ($n = 125$; %)	All cases ($N = 958$; %)
Sex			
Male	3 (1)	0	5 (0.52)
Female	296 (99)	125	953 (99.48)
Histology			
Infiltrative ductal carcinoma	236 (78.92)	95 (76)	741 (77.35)
Lobular carcinoma	10 (3.34)	6 (4.8)	42 (4.38)
Intraductal carcinoma	10 (3.34)	2 (1.6)	27 (2.82)
Medullar carcinoma	5 (1.67)	0	7 (0.73)
Other	12 (4.01)	3 (2.4)	59 (6.16)
Unknown	26 (8.69)	19 (15.2)	82 (8.56)
Laterality			
Unilateral	284 (94.98)	125	916 (95.62)
Bilateral	15 (5.01)	0	36 (3.76)
NA	0	0	6 (0.63)
ER+	166 (55.5)	91 (72.8)	589 (61.48)
ER−	91 (30.43)	20 (16)	233 (24.32)
NA	42 (14.04)	14 (11.2)	136 (14.2)
PR+	160 (53.5)	82 (65.6)	545 (56.9)
PR−	94 (31.43)	30 (24)	274 (28.6)
NA	45 (15.05)	13 (10.4)	139 (14.5)
HER2/neu+	69 (23.07)	34 (27.2)	261 (27.24)
HER2/neu−	175 (58.52)	71 (56.8)	546 (56.99)
NA	55 (18.39)	20 (16)	151 (15.76)
Molecular subtypes			
Luminal A or B	149 (49.83)	76 (60.8)	491 (51.25)
Triple negative	52 (17.39)	0	108 (11.27)
HER2-enriched	29 (9.69)	17 (13.6)	91 (9.5)
NA	63 (21.07)	23 (18.4)	223 (23.28)
Other	6 (2)	9 (7.2)	45 (4.70)
Age at diagnosis	41.14 ± 10.63	51.99 ± 8.74	47.18 ± 11
20–29	29 (9.70)	0	42 (4.38)
30–39	150 (50.17)	0	236 (24.63)
40–49	57 (19.06)	59 (47.20)	342 (35.7)
50–59	43 (14.38)	41 (32.80)	224 (23.38)
≥60	20 (6.69)	25 (20)	114 (10.9)
Ethnicity			
Fars	101 (33.78)	37 (29.60)	359 (37.59)
Turk	90 (30.10)	48 (38.40)	322 (33.72)
Kurd	23 (7.69)	8 (6.40)	74 (7.75)
Lur	17 (5.69)	12 (9.60)	57 (5.97)
Gilaki/Mazani	38 (12.71)	13 (10.40)	119 (12.46)
Other	30 (10.03)	7 (5.60)	24 (2.41)
Family history of cancer (any cancer in first–third-degree relatives)	184 (61.53)	49 (39.2)	446 (46.55)
Family history of related cancers with indication for genetic testing (in first–third-degree relatives)	155 (51.83)	0	251 (26.2)

with breast cancer who carried a pathogenic mutation in *BRCA1*, *BRCA2*, and *PALB2* genes have been shown in Table 2.

Categorizing patients with hereditary breast cancer criteria, there were 107 individuals designated as young age at onset (<40) who did not meet any other testing criteria. The remaining 192 patients were comprised of 110 patients with a single inclusion criterion (including male breast cancer, bilateral breast cancer, TNBC, or patients with a family history of breast, ovarian, or three or more related cancers) and 82 patients with multiple inclusion criteria for genetic testing. None of the 107 patients with an age at onset of <40 and no other hereditary criteria was a mutation carrier. Meanwhile, a direct association was observed between an increase in the number of other criteria and the probability of finding a mutation (χ^2, 33.387; $P_{trend} < 0.0001$). The likelihood of finding a mutation in patients with a "single criterion" (other than young age at onset <40) was 6.15 (95% CI, 1.26–58.59) times more compared with the no criteria group, whereas this was 22.5 (95% CI, 5.19–201.31) for patients with "multiple criteria" (Table 3).

In the "single criterion" and "multiple criteria" groups the number of women who needed to undergo a genetic testing to identify one additional mutation carrier compared with no criteria group was estimated 13 and 4, respectively.

A pathogenic mutation was found in 12 of 52 (23%) triple-negative cases, and in 14 of 166 estrogen receptor (ER)-positive breast cancers (8.43%). Among the 52 triple-negative cases, 7, 4, and 1 were *BRCA1*, *BRCA2*, and *PALB2* mutation carriers, respectively. Of 14 ER-positive patients who carry a pathogenic mutation, 3, 11, and 0 were *BRCA1*, *BRCA2*, and *PALB2* mutation carriers, respectively. Also, of all carriers, 3 were HER2-positive of them 2, 1, and 0 were *BRCA1*, *BRCA2*, and *PALB2* mutation carriers, respectively.

Fifty-one of 299 patients with hereditary criteria (17%) had a first-degree relative with breast cancer, and 89 of those patients (29.7%) had a first- or second-degree relative with breast cancer. Among 299 patients with hereditary criteria, 81.25% (26/32) of carriers had a family history of breast or ovarian cancer among their first- or second-degree relatives compared with 29.96% (80/267) of the noncarriers (OR, 10.12; 95% CI, 3.85–31.00; $P < 0.00001$).

Mutation frequencies per ethnic group have been summarized in Table 4. The noteworthy observation is that while Kurds constitute 7.7% of the tested patients with hereditary breast cancer criteria, 21.7% of them had mutations in *BRCA* genes.

Other than pathogenic mutations, 19 variants of little or unknown significance were also found; including two *BRCA1* (0.66%), nine *BRCA2* (3.01%), and four *PALB2* (1.33%) in a group with hereditary criteria for genetic testing, and four *BRCA2* (3.2%) in no criteria group (Table 5).

Discussion

Herein, we employed targeted NGS for the first time in Iran to screen Iranian patients with breast cancer for *BRCA1*, *BRCA2*, and *PALB2* gene mutations. Pathogenic mutation rate was 10.7% in patients with hereditary criteria for breast cancer versus 1.6% in no criteria group ($P = 0.0017$). All the patients who only met the young age at onset (<40) criterion tested negative for a gene mutation.

Table 2. Characteristics of the 34 patients with breast cancer who carry a pathogenic mutation in *BRCA1, BRCA2*, and *PALB2* genes

No.	Age	Classification	Gene	Exon	Mutation	Family history of BC/OC/related cancers[a] in the first- to third-degree relatives	BBC	TNBC
1	52	Stopgain	BRCA1	10	c.3544C>T	Yes (BC)	No	Yes
2	36	Stopgain	BRCA1	10	c.3607C>T	Yes (3 cancers)	No	No
3	34	Frameshift ins	BRCA1	10	c.2686_2687insA	Yes (BC)	No	No
4	38	Frameshift del	BRCA1	10	c.3359_3363delTTAAT	Yes (BC)	No	No
5	44	Frameshift del	BRCA1	10	c.1292del	Yes (OC)	No	Yes
6	26	Stopgain	BRCA1	10	c.3049_3050insGGAAATG	Yes (BC)	No	No
7	37	Nonsyn SNV	BRCA1	17	c.5095C>T	No	No	Yes
8	45	Frameshift del	BRCA1	10	c.2255_2259het_delTAAGT	Yes (OC)	No	No
9	35	Stopgain	BRCA1	10	c.3544C>T	Yes (BC) + (OC)	No	Yes
10	43	Frameshift del	BRCA1	10	c.4065_4068delTCAA	Yes (BC)	Yes	Yes
11	36	Frameshift ins	BRCA1	10	c.1442_1443insT	Yes (BC)	No	No
12	41	Frameshift del	BRCA1	2	c.68_69delAG	No	Yes	Yes
13	46	Splicing mutation	BRCA1	2	c.81-1G>C	Yes (BC)	No	Yes
14	28	Init codon	BRCA2	2	c.1A>G	Yes (BC)	No	No
15	43	Stopgain	BRCA2	20	c.8611G>T	Yes (BC)	No	No
16	29	Frameshift ins	BRCA2	10	c.1593_1594insA	Yes (BC) + (3 cancers)	No	Yes
17	59	Frameshift del	BRCA2	2	c.18_21delAGAG	Yes (BC) + (3 cancers)	No	No
18	33	Stopgain	BRCA2	7	c.523C>T	Yes (BC)	No	No
19	50	Stopgain	BRCA2	13	c.6952C>T	Yes (BC)	No	No
20	55	Frameshift del	BRCA2	22	c.8869delC	Yes (BC)	No	No
21	35	Frameshift del	BRCA2	11	c.2808_2811het_delCAA	Yes (BC) + (OC)	No	No
22	43	Frameshift ins	BRCA2	20	c.8585_8586insT	Yes (BC) + (OC)	No	No
23	39	Frameshift del	BRCA2	11	c.3189_3192het_delGTCA	Yes[b] (BC)	Yes	No
24	46	Frameshift del	BRCA2	11	c.3834_3835het_delTA	Yes[b] (BC)	Yes	No
25	37	Frameshift ins	BRCA2	10	c.1813_1814insA	Yes[b] (BC) + (3 cancers)	No	No
26	33	Frameshift del	BRCA2	25	c.9449delC	Yes (BC)	No	Yes
27	31	Frameshift del	BRCA2	10	c.1813delA	Yes (BC) + (3 cancers)	No	No
28	47	Frameshift ins	BRCA2	11	c.3751_3752insA	Yes[b] (BC)	No	Yes
29	49	Frameshift ins	BRCA2	11	c.3860_3861insA	No	No	No
30	56	Frameshift del	BRCA2	10	c.1041delA	No	No	Yes
31	55	Stop codon	BRCA2	11	c.3785C>G	No	No	No
32	81	Frameshift ins	BRCA2	24	c.9201_9202insTC	No	No	No
33	32	Frameshift ins	PALB2	4	c.1674_1675insTATT	No	No	Yes
34	44	Frameshift del	PALB2	4	c.1085_1086delTT	Yes[b] (BC)	No	No

Abbreviations: BC, breast cancer; BBC, bilateral breast cancer; Del, deletion; Ins, insertion; OC, ovarian cancer.

[a]Family history of three or more of the followings [pancreatic cancer, prostate cancer (Gleason score $\geq$ 7), melanoma, sarcoma, adrenocortical carcinoma, brain tumors, leukemia, diffuse gastric cancer, colon cancer, endometrial cancer, thyroid cancer, kidney cancer, dermatologic manifestations and/or macrocephaly, hamartomatous polyps of gastrointestinal tract; NCCN guideline version 2–2017].

[b]Age at diagnosis >50.

This is while patients who had only one hereditary criterion other than young age at onset and patients with multiple hereditary criteria including young age at onset had a significantly higher probability of finding a mutation compared with no risk criteria group. We only found 2 mutation carriers among 125 patients with no hereditary criteria and both of them were *BRCA2* mutation carriers.

The prevalence of the breast cancer susceptibility gene mutations has shown a great variability in different populations. The *BRCA* mutation frequencies among patients with high risk breast cancer were reported as 14%, 14.9%, 7.8%, and 13.5% in Turkey, Korea, China, and Malaysia, respectively (20, 21). In 2009, Hall and colleagues identified *BRCA* mutations in 12.5% of the 46,276 high-risk population consisted of patients with Western/Central European, Latin American, African, Asian, Native American, and Middle Eastern ancestries; of whom Middle Eastern had the lowest prevalence of 9.4% followed by patients with Western European (12.1%), Asian (12.7%), Central European (13.5%), American (Latin American,

Table 3. Identified mutations on the basis of the possible heritable breast cancer risks criteria studied in this project

Variable	Mutation carrier	None carrier	Total	OR (95% CI)	Significance level (*P*)	Positive predictive value (%; 95% CI)
No criteria	2	123	125	Reference	—	1.06 (0.19–5.66)
With criteria	32	267	299	7.37 (1.82–64.27)	0.0017	10.70 (7.43–14.77)
Only early aged BC (<40)	0	107	107	—	—	—
Other criteria except only early aged BC (<40)	32	160	192	12.30 (3.02–107.35)	<0.0001	16.66 (11.68–22.70)
Single criterion	10	100	110	6.15 (1.26–58.59)	0.009	9.09 (4.44–16.080)
Multiple (2$\leq$) criteria	22	60	82	22.5 (5.19–201.31)	<0.0001	26.82 (17.63–37.75)

Abbreviation: BC, breast cancer.

Table 4. Ethnicity-specific frequency of pathogenic mutations in patients with hereditary breast cancer criteria

Ethnicity	Number of tested patients	Number of mutation carriers	Number of BRCA1 carriers	Number of BRCA2 carriers	Number of PALB2 carriers
Fars	101	12 (12%)	6	5	1
Turk	90	6 (7%)	3	3	0
Kurd	23	5 (22%)	1	4	0
Lour	17	1 (6%)	1	0	0
Gilaki/ Mazani	38	3 (8%)	1	2	0
Other	30	5 (17%)	1	3	1
Total number	299	32 (11%)	13	17	2

14.7% and Native American, 13.2%), and African (15.6%) background. It is noteworthy to mention that the higher prevalence of *BRCA1* compared with *BRCA2* was observed in all studied ethnic groups except patients from Asia, in which the frequency of both were equal (6.3% each; ref. 22). In our study, the frequency of 10.7% was consistent with the average rate reported from Asia, while the frequency of the *BRCA2* mutations was higher than *BRCA1* ones (17 vs. 13).

Regarding *PALB2* mutation frequency, two carriers were identified in total, both among patients with hereditary breast cancer criteria; accounting for 0.66% of them. To the best of our knowledge, this is for the first time that heterogeneous Iranian population has been screened for *PALB2* gene mutations. Our finding added to the cumulative evidence that *PALB2* is involved in the hereditary breast cancer. This is consistent with previous reports from Asia where the *PALB2* mutation frequencies were less than 1%, which is comparable with the *PALB2* mutation frequency of 0.6%–2.7% reported from the western European families with multiple cases of breast cancer (23–25).

Genetic testing of 107 females with only young age at onset (<40) criterion, did not revealed any mutation carrier among them, while we found two mutation carriers among 125 patients (1.6%) with no hereditary criteria. Despite the fact that early age at onset breast cancer is considered as a generally accepted criterion for genetic testing, however, our results indicated that this criterion by itself is not associated with higher chance of carrying a germline mutation and other factors are probably more important. Because, about 20% of Iranian patients who diagnosed with breast cancer each year are under 40 years (26), this finding becomes even more important regarding hereditary breast cancer screening and cost effective strategies for genetic testing and suggests that young age at onset of <40 by its own may not be a good criterion for genetic testing and this criterion in combination with other hereditary cancer criteria should be considered for offering genetic testing.

The probability of finding a mutation carrier was directly associated with the number of heritable risk criteria in this study where the odds of finding a mutation was dramatically increased from patients with no criteria to patients with multiple criteria for genetic testing. Our result showed that 1 of 4 (26.8%) women with multiple hereditary breast cancer risk criteria will be detected as a *BRCA1/2* mutation carrier. Given such high risk of identification of a disease-causing mutation, multiple hereditary criteria should be regarded as a strong predictor for a hereditary breast cancer syndrome. These findings are important concerning

Table 5. Characteristics of the 19 patients with breast cancer who carry a nonpathogenic mutation in *BRCA1*, *BRCA2*, and *PALB2* genes

No.	Age	Classification	Gene	Exon	Mutation	Family history of BC/OC/related cancers[a] in the first–third-degree relatives	BBC	TNBC
1	37	Missense	BRCA2	14	c.7165A>G	No	No	No
2	32	Inframe del	BRCA1	5	c.248_250delTTG	Yes	No	Yes
3	55	Missense	PALB2	7	c.2590C>T	Yes	No	No
4	57	Missense	BRCA2	10	c.1234C>T	Yes	No	No
5	43	Missense	BRCA2	10	c.811G>A	Yes	No	No
6	63	Missense	PALB2	4	c.733G>A	Yes	No	No
7	36	Missense	BRCA2	27	c.9838C>T	No	No	No
8	48	Missense	BRCA2	3	c.70T>A	No	Yes	No
9	34	Missense	PALB2	13	c.3428T>A	Yes	No	No
10	29	Missense	BRCA1	17	c.5096G>A	No	No	No
11	38	Missense	BRCA2	26	c.9584C>G	No	No	No
12	59	Missense	PALB2	5	c.2344C>T	Yes	No	No
13	29	Missense	BRCA2	12	c.6935A>T	No	No	No
14	45	Nonsense	BRCA2	27	c.9976A>T	No	No	No
15	55	Nonsense	BRCA2	27	c.9976A>T	No	No	No
16	54	Nonsense	BRCA2	27	c.9976A>T	No	No	No
17	48	Nonsense	BRCA2	27	c.9976A>T	No	No	No
18	33	Nonsense	BRCA2	27	c.9976A>T	Yes	No	Yes
19	38	Nonsense	BRCA2	27	c.9976A>T	Yes	No	Yes

Abbreviations: BBC, bilateral breast cancer; Del, deletion; Ins, insertion.

[a]Family history of three or more of the followings [pancreatic cancer, prostate cancer (Gleason score $\geq$ 7), melanoma, sarcoma, adrenocortical carcinoma, brain tumors, leukemia, diffuse gastric cancer, colon cancer, endometrial cancer, thyroid cancer, kidney cancer, dermatologic manifestations and/or macrocephaly, and hamartomatous polyps of gastrointestinal tract; NCCN guideline version 2-2017].

optimization of genetic counseling and furthermore establishing criteria for *BRCA1/2* genetic testing of the Iranian population. In 2017, Cropper and colleagues did an investigation on the predictive values of NCCN guideline for genetic testing of patients with breast cancer who are at increased risk. Consistent with our findings (Table 3), they did indicate that patients who meet $\geq$2 NCCN criteria are enriched for a gene mutation and had a significant high predictive value of over 10% (27).

The highest ethnicity-specific mutation prevalence was reported among patients with Kurd ancestry. Among the predictive factors, ethnicity has a considerable role in the breast cancer heritability. According to the previous report, Iran is the second country in the Middle East and North Africa and the 28 of 160 nations in the globe with diverse ethnics and cultures. Persians (Fars) constitute the majority of the ethnic groups followed by Turks and Kurds (1). Stratification of patients according to their ethnicities, the highest proportion of the mutation carriers were found in the Kurd population. It encompassed 21.73% of the high-risk Kurd population, much of it were attributed to *BRCA2* gene mutations. However, further investigation with a larger study population is needed to confirm this observation.

With the knowledge of *BRCA* mutations spectrum, it is important to mention that in this study only 10% of patients with inclusion criteria for genetic testing were positive for a gene mutation. This result indicates that other known or unknown breast cancer susceptibility genes might account for additional cases. Another reason for this is that we did not examine large chromosomal rearrangement (i.e., insertion or deletion in three tested genes), which might make up the additional carriers of breast cancer susceptibility gene predisposition. Knowing this, however, the uncertainty regarding the interpretation of moderate to low penetrance genes in multiple-gene panel testing still exist as a concern and needed to be resolved.

The largest screening of Iranian breast cancer population added to the cumulative evidence that *BRCA1/2* mutations are seen commonly among Iranian patients with breast cancer especially those with hereditary breast cancer criteria and indicated that *PALB2* should be concerned in hereditary breast cancer screening alongside *BRCA1/2*. Investigating the predictive potential of hereditary breast cancer risk criteria, our results suggest that offering genetic testing to women with early age at onset of <40 with no other hereditary criteria may be not efficient. Therefore, until the time more evidence from larger investigations arises, these findings should be concerned for optimization of genetic counseling and genetic testing of the Iranian population for hereditary breast cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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