Prevalence of *BRCA1* and *BRCA2* mutations in Japanese patients with triple-negative breast cancer: A single institute retrospective study

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Abstract. Numerous databases for risk assessment of *BRCA1/2* gene mutations contain insufficient data about Asians. Furthermore, few studies have reported the prevalence of germline *BRCA1/2* mutations in Japanese patients, particularly those with triple-negative breast cancer (TNBC). The present study was a retrospective analysis of data from patients with TNBC who underwent *BRCA1/2* mutation testing at Osaka International Cancer Institute (Osaka, Japan) between October 2014 and March 2020. A total of 65 patients with TNBC underwent a test for *BRCA1/2* mutations, and 13 (20.0%) had deleterious mutations in the *BRCA1* or *BRCA2* genes. Furthermore, 12 out of 29 patients with a family history of breast or ovarian cancer had deleterious *BRCA1/2* mutations, and only 1 of 34 without a family history had a mutation (41.4 vs. 2.9%; P=0.014). No patients aged >60 years had *BRCA1/2* mutations; however, the age of diagnosis was not a significant risk factor for *BRCA1/2* mutations (P=0.60). The prevalence of *BRCA1/2* mutations in the present cohort of Japanese patients with TNBC was slightly higher than those reported in other larger studies from Europe and North America. Further data from large prospective studies are required to more precisely define the prevalence of *BRCA1/2* mutations.

Introduction

Approximately 9% of all breast cancers are caused by pathological germline mutation of cancer susceptibility genes, and ~48 to 56% of these cancers have *BRCA1* or *BRCA2* mutations (1,2). Approximately 70% of breast cancers caused by germline BRCA1 mutation are the triple negative subtype, which is defined as estrogen receptor (ER) negative, progesterone receptor (PgR) negative, and human epidermal receptor 2 (HER2) negative (3,4). Therefore, triple-negative breast cancer (TNBC) is listed as one of the criteria for obtaining an evaluation for genetic risk of hereditary breast and ovarian cancer syndrome (5).

TNBC, which accounts for 12-15.5% of breast cancers in Japan (6,7), is characterized by rapid growth and worse prognosis compared with other subtypes of breast cancer (8). Recently, several poly (ADP-ribose) polymerase (PARP) inhibitors were shown to be effective for breast and ovarian cancers with germline *BRCA1/2* mutations, and the PARP inhibitor olaparib has been approved for clinical use in Japan (9). A *BRCA1/2* genetic testing for breast cancer patients with high risk of hereditary breast and ovarian cancer syndrome has been covered by the Japanese national insurance system since April 2020. Therefore, breast cancer patients who would like to have the genetic testing for *BRCA1/2* mutations, especially those with TNBC, will now have better access to the test in Japan.

The prevalence of germline *BRCA1/2* mutations in TNBC varied from 9.3 to 15.4% in large (N>100) studies from mainly Europe and North America (10,11) and was higher in several small studies from the USA and China (12,13). However, few studies have reported the prevalence of germline *BRCA1/2* mutations in Japanese patients, especially those with TNBC (4,14). Here, we report a retrospective analysis for the prevalence of *BRCA1/2* mutations among Japanese TNBC patients who had genetic testing in a single institute. Additionally, we assessed the risk factors for *BRCA1/2* mutation positivity in the same cohort.

Patients and methods

Target patients. Patients who were diagnosed with TNBC and underwent genetic testing for germline *BRCA1/2* mutations from October 2014 to March 2020 at Osaka International Cancer Institute (formerly Osaka Medical Center for Cancer and Cardiovascular Diseases) were included in our study.

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Determination of breast cancer subtypes and BRCA genetic testing. TNBC was determined as both ER and PR negativity (<1%) and HER2 negativity and was evaluated according to the American Society of Clinical Oncology/College of American Pathologists guidelines. Most patients underwent genetic testing because of a wish to participate in clinical trials of a PARP inhibitor or an immune checkpoint inhibitor. Many patients had no family history concerns. Genetic counseling was performed for all patients undergoing genetic testing. Written informed consent was obtained from all patients prior to genetic testing. Mutation analysis and interpretation was performed by Myriad Genetics, Inc. or FALCO Biosystems Ltd..

Retrospective analysis. Medical records and genetic counseling reports were examined retrospectively. In patients who had bilateral TNBCs, the age at first diagnosis was adopted. Family history was defined as having at least one relative with breast or ovarian cancer within the patient's third-degree relatives.

Statistical analysis. All statistical analyses were performed using EZR ver.1.4.0 (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R ver. 3.6.0 (The R Foundation for Statistical Computing, Vienna, Austria) (15). We analyzed by using Logistic regression analysis for univariate analysis and multivariate analysis. We performed a Mann-Whitney U test to examine if there was any difference in age between the *BRCA*-positive and *BRCA*-negative groups, and created a box-and-whisker plot to visualize the bias. In addition, an F-test was performed to examine whether there was a difference in age variability between the two groups.

Results

Patient characteristics. The patients' characteristics are summarized in Table I. Sixty-five TNBC patients were evaluated in this study; all were female. Fifty-five patients (84.6%) were 60 years old or younger, and 50 (76.9%) underwent genetic testing for clinical trials. Thirty patients (46.2%) had a family history of at least one relative with breast or ovarian cancer within their third-degree relatives. One patient received genetic counseling at another hospital before visiting our institute for a genetic testing; therefore, her family history was not obtained.

Seven deleterious mutations of *BRCA1*, six deleterious mutations of *BRCA2*, and one *BRCA2* variant of uncertain significance were found in a total of 14 patients. One patient had mutations in both genes, which were a deleterious *BRCA1* mutation and benign *BRCA2* mutation (not shown). The prevalence of germline *BRCA1/2* mutations in this cohort was 20.0% (13/65; Table I).

Bias in age distribution of the BRCA-positive group. The median age was 46 and 49 years for the *BRCA*-positive and -negative subjects respectively, and the logistic regression analysis showed no statistically significant difference (Table II). No deleterious *BRCA1/2* mutations were observed among patients older than 60 years old; the prevalence of mutations among patients 60 years old or younger was 24.1% (13/54; Table II). No deleterious mutations were observed in patients from 30-39 years old (0/12). The Mann-Whitney U test showed no statistically significant difference in age between the *BRCA*-positive and *BRCA*-negative groups, P=0.527, but the box-and-whisker plot appeared to be biased, and when the F test was performed to verify homoscedasticity, the variance was statistically significantly smaller (P=0.00984) and was concentrated around 46 years of age (Fig. 1).

Table I. Characteristics of the patients with triple-negative breast cancer.

Variable	Number of patients (n=65)
Age, years	
<30	3
30-39	12
40-49	23
50-59	16
≥60	11
Motives for genetic counseling	
Clinical trial	50
Others	15
Family history[a]	
Yes	30
No	34
Unknown	1
Genetic mutation	
BRCA1 deleterious	7
BRCA2 deleterious	6
VUS	1

[a]At least one relative with breast cancer or ovarian cancer within third degree relatives. VUS, variant of uncertain significance.

Correlation with family history. Only one patient among those without family history had a deleterious *BRCA2* mutation. The prevalence of germline *BRCA1/2* mutations among patients with family history was 41.4% (12/29; Table II).

Table II shows the result of the univariate and multivariate analysis. Univariate analysis and multivariate analysis using logistic regression analysis showed a significant relationship between *BRCA1/2* mutations and family history (P=0.00425, P=0.0136), but did not show a significant relationship between germline *BRCA1/2* mutations and age (P=0.462, P=0.605). Tumor size, lymph node metastasis, and histological grade were not related to *BRCA1/2* mutation.

Discussion

In 2012, the Japanese Hereditary Breast and Ovarian Cancer Consortium was established, and a nationwide registration system began in 2013 (4). However, the *BRCA1/2* genetic testing was not initially covered by the national insurance system and few clients underwent the test. Therefore, few reports show the prevalence of *BRCA1/2* mutation carriers in Japan, especially those with TNBC. Arai *et al* (4) reported the analysis of germline *BRCA1/2* mutations among 963 Japanese individuals who received a *BRCA1/2*

Table II. Risk factors for the presence of *BRCA1/2* deleterious mutations in patients with triple-negative breast cancer.

	BRCA1/2 deleterious mutation		P-value[a]	
Variable	Positive, n	Negative, n	Univariate	Multivariate
Age, years			0.462	0.605
Median (range)	46 (29-52)	49 (28-78)		
<30	1	2		
30-39	0	12		
40-49	9	14[b]		
50-59	3	13		
≥60	0	11		
Family history[c,d]			0.004[e]	0.014[e]
Yes	12	18		
No	1	33		
Tumor size			0.613	0.960
Tis/T1/T2	10	45		
T3/T4	3	7		
Lymph node metastasis			0.077	0.853
N0/N1	10	47		
N2/N3	3	5		
Histological grade			0.994	0.995
G1/G2	0	9		
G3	8	37		
Not assessed	5	6		

[a]Univariate analysis and multivariate analysis using logistic regression analysis. [b]Including 1 patient with variant of uncertain significance. [c]At least one relative with breast cancer or ovarian cancer within third degree relatives. [d]1 patient with an unknown family history was excluded. [e]P<0.05.

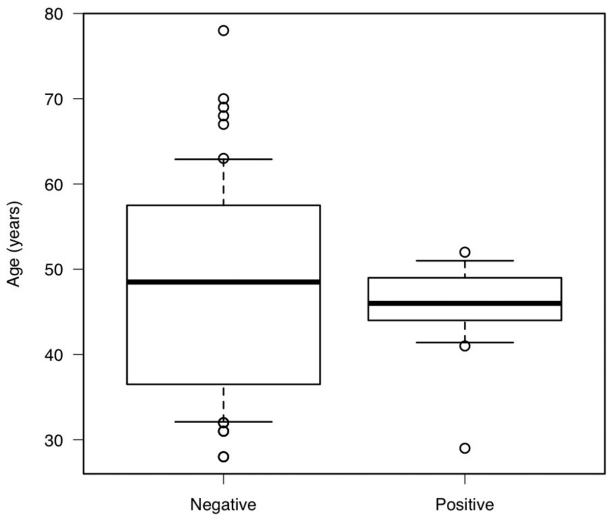

Figure 1. Box-and-whisker-plot of age in the BRCA mutation-positive and -negative groups. The median age in the BRCA mutation-positive and -negative groups was 49 and 46 years, respectively. Mann-Whitney U test revealed no significant difference; however, F-test showed that the variance of ages in the *BRCA*-positive group was smaller than that in the *BRCA*-negative group.

genetic testing and were registered in the database mentioned above from 2012 to 2014. The ratios of TNBC in patients with *BRCA1* and *BRCA2* mutations were 75.8 and 18.6%, respectively. However, the prevalence of germline *BRCA1/2* mutations among TNBC patients was not shown in their report. In 2015, Nakamura *et al* (14) reported an analysis of *BRCA1/2* mutations in 320 Japanese individuals with a strong family history of breast cancer and/or ovarian cancer. The analysis included 41 TNBC patients, and 22 of these TNBC patients had deleterious germline *BRCA1/2* mutations (53.7%). However, all TNBC patients in the study had a high-risk condition, which was a cancer diagnosis at an age younger than 40 years old or having more than one family member with breast and/or ovarian cancer. Therefore, the prevalence of *BRCA1/2* mutations in the general TNBC cohort in Japan was unclear in the study. In our study, 76.9% of patients received a genetic testing as part of clinical trials targeting TNBC patients and 53.1% (34/65) of patients subjected to a test had no family history. Although the patients were not consecutive patients and comprised only a small proportion of all TNBC patients treated in our institute during the study period, our report is the first to show the prevalence of germline *BRCA1/2* mutations in the near general cohort of Japanese TNBC patients. The prevalence value of 20% in our study is higher than in larger studies (11,16). The higher prevalence of *BRCA1/2* mutations in our study may be because of the small number of patients and the fact that patients with a strong family history of ovarian cancer were consciously enrolled as candidates for clinical trials, which are limitations of this study.

As for risk factors for *BRCA1/2* mutations in TNBC, the age of diagnosis is important. Emborgo *et al* (11) reported that 49 out of 294 patients (16.7%) with TNBC diagnosed at age 60 years or younger were positive for *BRCA1/2* deleterious mutations. Conversely, only 2 out of 86 patients (2.3%) with TNBC diagnosed at >60 years had *BRCA1/2* mutations. In line with other reports, these results indicate that being diagnosed with TNBC at >60 years of age was not significantly correlated with a positive *BRCA1/2* mutation. In our study, 10 patients were aged >60 years, and none had a *BRCA1/2* mutation. In addition, no patients from 30-39 years old were positive for *BRCA1/2* mutation. The results of the F test also showed a bias in the age of the positive subjects, with a statistically significantly smaller variance than the negative group and a concentration near the median age of 46 years. This may be related to the age at which the *BRCA* mutation-positive patients develop breast cancer. Regardless of the underlying reason, for younger breast cancer patients, testing with a gene panel for detecting mutations associated with hereditary cancer other than *BRCA* might be considered.

Another risk factor is family history, which is one of the criteria for a genetic testing for *BRCA1/2* mutations. However, whether family history is a risk factor for *BRCA1/2* mutation in TNBC patients is unclear. Sharma *et al* (17) examined 207 TNBC patients who prospectively underwent genetic testing for *BRCA1/2* mutations and reported that the *BRCA1/2* mutation prevalence rates in patients with and without family history were 21.1 and 6.3% (P=0.00425), respectively. Our study also showed a significant difference in *BRCA1/2* mutation positivity between patients with or without family history. The prevalence of *BRCA1/2* mutations was 41.4% among patients with family history, while only one patient had *BRCA2* mutation in the group without family history. This patient's mother died of a carcinoma of unknown origin in her abdominal cavity, which might have been ovarian or peritoneal cancer. Therefore, there may have been no patients with *BRCA1/2* mutations in the group without family history.

In conclusion, the prevalence of *BRCA1/2* mutations among Japanese TNBC patients in our cohort was 20.0%, which is similar to or slightly higher than that in reports from Europe or North America with large cohorts. Family history is a significant risk factor for *BRCA1/2* mutation positivity in TNBC patients. However, more prospective studies with greater numbers of consecutive TNBC patients are needed to clarify the accurate prevalence of *BRCA1/2* mutations. Furthermore, because young women under 30 years of age who may harbor germline mutations in other genes such as TP53 are included in the *BRCA1/2*-negative TNBC cohort, studies using multi-gene panel tests for cancer susceptibility genes should be planned in the future.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YT conceived the design of the study and wrote the outline of the manuscript. FF analyzed the data and wrote the final manuscript. TI was responsible for genetic counseling as the subjects underwent BRCA1/2 genetic testing. TN, TYa, NK, TYo, MN, SM, HK and SK were involved in the treatment of their patients as attending physicians and provided important advice for decision making. TN, TYa, NK, TYo, MN, SM, HK and SK also performed data curation to conduct this study and contributed to the writing of the final manuscript. YT and TI confirm the authenticity of all the raw data. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present retrospective study was approved by the OICI Institutional Review Board and conducted in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The approval number is 20044. Written informed consent was obtained from all patients prior to genetic testing of BRCA1/2. All confirmations of consent for research participation were described with the option to opt-out on the institution's website.

Patient consent for publication

All confirmations of consent for publication were described with the option to opt-out on the institution's website.

Competing interests

The authors declare that they have no competing interests.

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