







Spectrum of *PALB2* Germline Mutations and Characteristics of *PALB2*-Related Breast Cancer: Screening of 16,501 Unselected Patients With Breast Cancer and 5890 Controls by Next-Generation Sequencing

Jiaojiao Zhou, MD, PhD ^{1,2}; Honglian Wang, PhD ³; Fangmeng Fu, MD ⁴; Zhanwen Li, MD⁵; Qingjian Feng, MD⁶; Weizhu Wu, MD⁷; Yun Liu, PhD ^{8,9}; Chuan Wang, MD, PhD ⁴; and Yiding Chen, MD, PhD ^{1,2}

BACKGROUND: Partner and localizer BRCA2 (*PALB2*) is a breast cancer predisposition gene, but the clinical relevance of *PALB2* germline mutations in Chinese patients with breast cancer remains unknown. This study attempted to investigate the full prevalence and spectrum of *PALB2* germline mutations in China and the associations between *PALB2* germline mutations and breast cancer risk. **METHODS:** A total of 21,216 unselected patients with breast cancer were enrolled from 10 provinces in China, and 5890 Chinese women without cancer were enrolled as healthy controls. *PALB2* screening was based on next-generation sequencing. **RESULTS:** A total of 16,501 *BRCA1/2*-negative patients with breast cancer were analyzed. Deleterious *PALB2* mutation carriers accounted for 0.97% ($n = 160$) in the breast cancer cohort and for 0.19% ($n = 11$) in the healthy control cohort. Forty-one novel *PALB2* germline mutations were identified. A high frequency of *PALB2* c.751C>T was detected, and it accounted for 10.63% of the *PALB2* germline mutations detected (17 of 160). *PALB2* mutations were significantly associated with increased breast cancer risk (odds ratio [OR], 5.23; 95% confidence interval [CI], 2.84-9.65; $P < .0001$), especially among women 30 years old or younger (OR, 10.09; 95% CI, 3.95-25.79; $P < .0001$). Clinical characteristics, including a family history, bigger tumor size, triple-negative breast cancer, positive lymph nodes, and bilateral breast cancer, were closely related to *PALB2* mutations. **CONCLUSIONS:** This study revealed a comprehensive spectrum of *PALB2* germline mutations and characteristics of *PALB2*-related breast cancer in China. *PALB2* germline mutations confer a moderately increased risk for breast cancer but profoundly increase breast cancer risk for those 30 years old or younger in the Chinese population. **Cancer** 2020;126:3202-3208. © 2020 Zhejiang University School of Medicine Second Affiliated Hospital. *Cancer* published by Wiley Periodicals, Inc. on behalf of American Cancer Society This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

KEYWORDS: breast cancer risk, clinical characteristics, germline mutation, next-generation sequencing, partner and localizer BRCA2 (*PALB2*).

INTRODUCTION

Partner and localizer BRCA2 (*PALB2*) is an important DNA repair gene that is essential for its function in homologous recombination. In the DNA damage response, *PALB2* links BRCA1 and BRCA2¹ and implements the recombinational repair and checkpoint functions of BRCA2 in maintaining genome integrity.^{2,3}

Germline mutations in the *PALB2* gene confer a predisposition to breast cancer.^{4,5} Several studies in familial breast cancer have found a substantially increased risk of breast cancer in *PALB2* mutation carriers.⁵⁻⁹ However, because of the low frequency of *PALB2* mutations in women, we still lack data for the prevalence and spectrum of *PALB2* in

Corresponding Author: Yiding Chen, MD, PhD, Department of Breast Surgery, Second Affiliated Hospital, Zhejiang University School of Medicine, 88 Jie-Fang Rd, Hangzhou, Zhejiang, China 310009 (ydchen@zju.edu.cn).

Co-corresponding authors: Chuan Wang, MD, PhD, Department of Breast Surgery, Affiliated Union Hospital, Fujian Medical University, 29 Xinquan Rd, Fuzhou, Fujian, China 350001 (chuanwang1968@yahoo.com); Yun Liu, PhD, Key Laboratory of Molecular Medicine, Ministry of Education, Department of Biochemistry and Molecular Biology, Shanghai Medical College, Fudan University, Shanghai, China 200032 (superliuyun@fudan.edu.cn).

¹Department of Breast Surgery, Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China; ²Key Laboratory of Cancer Prevention and Intervention, China National Ministry of Education, Zhejiang University School of Medicine, Hangzhou, China; ³AITA Biomedical Research Institute, Shanghai, China; ⁴Department of Breast Surgery, Affiliated Union Hospital, Fujian Medical University, Fuzhou, China; ⁵Department of Breast Surgery, Women and Children's Hospital of Ningbo, Ningbo, China; ⁶Department of Breast Surgery, Yiwu Maternity and Child Care Hospital, Yiwu, China; ⁷Department of Thyroid and Breast Surgery, Ningbo Medical Center, Li Huili Eastern Hospital, Ningbo, China; ⁸Key Laboratory of Molecular Medicine, Ministry of Education, Department of Biochemistry and Molecular Biology, Shanghai Medical College, Fudan University, Shanghai, China; ⁹Institutes of Biomedical Sciences, Fudan University, Shanghai, China

The first 3 authors contributed equally to this article.

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unselected patients with breast cancer. A study in Poland investigated 2 recurrent *PALB2* mutations in a large cohort of unselected patients with breast cancer, and it confirmed an increased risk of breast cancer for carriers of these 2 recurrent *PALB2* mutations.¹⁰ Limited studies of *PALB2* germline mutations have been conducted in Chinese women. Because of the possibility of differences in *PALB2* mutations among different ethnic groups, it is of interest to establish the full prevalence and spectrum of *PALB2* germline mutations in Chinese women.

In this study, we sequenced *PALB2* genes of 21,216 unselected patients with primary breast cancer and 5890 healthy women in the Chinese population. We attempted to investigate the frequencies of *PALB2* germline mutations in Chinese women with and without breast cancer to depict the full spectrum of *PALB2* mutations, to assess the odds ratio (OR) for breast cancer associated with a *PALB2* germline mutation, and to explore the effects of *PALB2* mutations on the clinical outcomes of patients with breast cancer.

MATERIALS AND METHODS

Study Population

We recruited a total of 21,216 unselected patients with primary breast cancer between 2015 and 2018 from 10 provinces in China (Supporting Table 1). The recruited patients were diagnosed from 2000 to 2017. Among these patients, 3767 were excluded because of uncertain clinical or pathological information or poor DNA quality and quantity. Nine hundred forty-eight patients who carried deleterious *BRCA1/2* germline mutations were also excluded. The remaining 16,501 *BRCA1/2*-negative patients with breast cancer were analyzed. A total of 5890 healthy people (from the health examination crowd in medical centers) were further enrolled as the control group. The cohort of patients with breast cancer and healthy people in this study was a prospective cohort, and blood was collected prospectively. The ethics committee of the Second Affiliated Hospital of the Zhejiang University School of Medicine in Hangzhou (ethics approval 2015073) approved the study. All patients and healthy controls provided written informed consent. All the patients and controls will be (or have been) informed about the sequencing results.

Clinical Information Collection

Information about the age at diagnosis, tumor histology, tumor size, lymph node status, estrogen receptor status, progesterone receptor status, HER2 status, and bilaterality was abstracted from medical records. National Comprehensive Cancer Network and American Society of Clinical Oncology/College of American Pathologists

guidelines were used to define the tumor characteristics. A family history of breast, ovarian, or any other cancer was collected by questionnaire. The questionnaires were handed out to patients during clinic visits. For patients with available medical records, the questionnaires about family histories were further checked against their medical records. Genetic test results from this study were considered only for research and were not used for clinical decision making. A family history of breast and/or ovarian cancer was defined as 1 or more patients with breast and/or ovarian cancer among the patient's first-, second-, or third-degree relatives; a family history of any other cancer was defined as 1 or more patients with cancer (any kind of cancer except for breast and/or ovarian cancer) among the first- or second-degree relatives of the patient with breast cancer.

PALB2 and *BRCA1/2* Sequencing Assay

Blood samples were obtained from all patients with breast cancer and from healthy controls for DNA extraction and genotyping. All protein-coding exons and proximal intron-exon junctions (adjacent splice site, 10 bp) of *PALB2*, *BRCA1*, and *BRCA2* were screened. The sequencing methods and analysis of the sequencing data are described in detail in our previous study.^{11,12} Variant pathogenicity was explored on the basis of the ClinVar database and American College of Medical Genetics and Genomics recommendations. The null variants (due to nonsense, frameshift, canonical ± 1 or 2 splice sites, initiation codons, or single-exon or multi-exon deletions) that resulted in a loss of function were classified as deleterious variants. All the pathogenic mutations were validated by Sanger sequencing. National Center for Biotechnology Information transcript NM_024675.3 was used as a reference sequence for *PALB2*. All *PALB2* variants were named according to Human Genome Variation Society nomenclature.

Statistical Analysis

The distributions of *PALB2* mutations among patients with breast cancer and healthy controls were assessed with a Pearson chi-square test or Fisher exact test. ORs and 95% confidence intervals (CIs) were calculated with healthy controls as the reference group. Distributions of clinicopathological characteristics of patients with breast cancer and *PALB2* mutations and noncarriers were compared with a Pearson chi-square test or Fisher exact test for dichotomous variables and with a Student *t* test for continuous variables. Two-sided *P* values less than .05 were considered statistically significant. We performed all

statistical analyses with R software (version 3.5.3) unless otherwise noted.

RESULTS

Prevalence and Spectrum of *PALB2* Germline Mutations in Breast Cancer

PALB2 germline mutations were determined in our large cohort of 16,501 unselected *BRCA1/2*-negative patients with breast cancer. Our study revealed that a total of 79 deleterious *PALB2* germline mutations were identified in 160 of the 16,501 unselected patients with breast cancer (Fig. 1 and Supporting Table 2). The prevalence of *PALB2* germline mutations was 0.97% (160 of 16,501).

The 79 deleterious *PALB2* germline mutations included 25 nonsense mutations, 47 frameshift mutations, and 7 splicing mutations (Fig. 1 and Supporting Tables 2 and 3). The hotspot regions for *PALB2* mutations were exon 4 (74 of 160 [46.25%]) and exon 5 (36 of 160 [22.5%]; Fig. 2). There were 25 recurrent deleterious *PALB2* germline mutations, of which the 3 most common were c.751C>T (17 of 160 [10.63%]), c.2167_2168delAT (12 of 160 [7.5%]), and c.3114-1G>A (10 of 160 [6.25%]; Supporting Tables 2 and 3). A total of 41 novel mutations were identified in *PALB2*; they included 8 nonsense mutations, 31 frameshift mutations, and 2 splicing mutations (Supporting Tables 2 and 3). Most of these novel mutations were located in exon 4 (16 of 41 [39.02%]) or exon 5 (12 of 41 [29.27%]; Supporting Table 2).

***PALB2* Mutations and Breast Cancer Risk**

PALB2 mutations were found in 160 of 16,501 unselected *BRCA1/2*-negative patients with breast cancer (0.97%) and in 11 of 5890 healthy controls (0.19%). The OR for breast cancer risk in women with a *PALB2* mutation was 5.23 (95% CI, 2.84-9.65; $P < .0001$; Table 1). With subdivision by age groups, the OR for breast cancer risk in women with a *PALB2* mutation was 10.09 (95% CI, 3.95-25.79; $P < .0001$) for those 30 years old or younger and 5.06 (95% CI, 2.74-9.34; $P < .0001$) for those older than 30 years (Table 1).

***PALB2* Mutations and Tumor Characteristics**

There were several significant clinicopathological differences between *PALB2* mutation carriers and noncarriers (Table 2). Compared with noncarriers, significantly more *PALB2* mutation carriers were 30 years old or younger at diagnosis (6.88% vs 3.56%; $P = .04$). *PALB2* mutation carriers were significantly more likely

to have triple-negative breast cancers than noncarriers (22.83% vs 13.56%; $P = .004$). *PALB2* mutation carriers were more likely than noncarriers to have bigger tumors (tumor size ≥ 2 cm, 55.93% vs 45.93%; $P = .04$), positive axillary lymph nodes (lymph nodes positive, 49.60% vs 38.80%; $P = .018$), bilateral breast cancers (6.29% vs 2.01%; $P = .003$), a family history of breast and/or ovarian cancer (20.63% vs 7.96%; $P < .0001$), and a family history of any other cancer (23.75% vs 9.42%; $P < .0001$).

DISCUSSION

Because loss-of-function mutations in the *PALB2* gene confer a predisposition to breast cancer, clinical genetic testing for breast cancer risk increasingly includes *PALB2* in addition to *BRCA1* and *BRCA2*.⁶ Many breast cancer-related studies on *PALB2* germline mutations have been conducted in European and American populations^{6,10,13}; it is important and necessary to have a detailed spectrum of *PALB2* germline mutations and robust risk estimates for women who carry *PALB2* germline mutations in the Chinese population.

In our study, we found that 0.97% of patients carried *PALB2* germline mutations among 16,501 unselected *BRCA1/2*-negative patients with breast cancer. On the basis of a rate of *PALB2* mutation-positive cases of 0.19% among 5890 healthy controls, our study presents an estimated OR of 5.23 (95% CI, 2.84-9.65; $P < .0001$) for breast cancer associated with a *PALB2* mutation. *PALB2* germline mutations are rare in breast cancer and in the general population. Thus, only a few previous studies have investigated *PALB2* germline mutations in unselected breast cancer cases. Two deleterious mutations in *PALB2* (509_510delGA and 172_175delTTGT) were examined in a Polish population, and that study's findings showed that the frequency of these 2 *PALB2* germline mutations was 0.93% and that the estimated OR for breast cancer in *PALB2* carriers was 4.39.¹⁰ In an American population, Tung et al¹⁴ found that the proportion with *PALB2* germline mutations was 0.2% among sequential patients with breast cancer at the Dana-Farber Cancer Institute, and the breast cancer relative risk for *PALB2* carriers was 5.3. However, only 1 patient with breast cancer and a *PALB2* germline mutation was identified in Tung et al's study cohort, and this indicated the limited power to estimate the penetrance and breast cancer risk of *PALB2* mutations. Another study by Couch et al,¹⁵ based on a nationwide sample population of the United States, presented a *PALB2* germline mutation frequency of

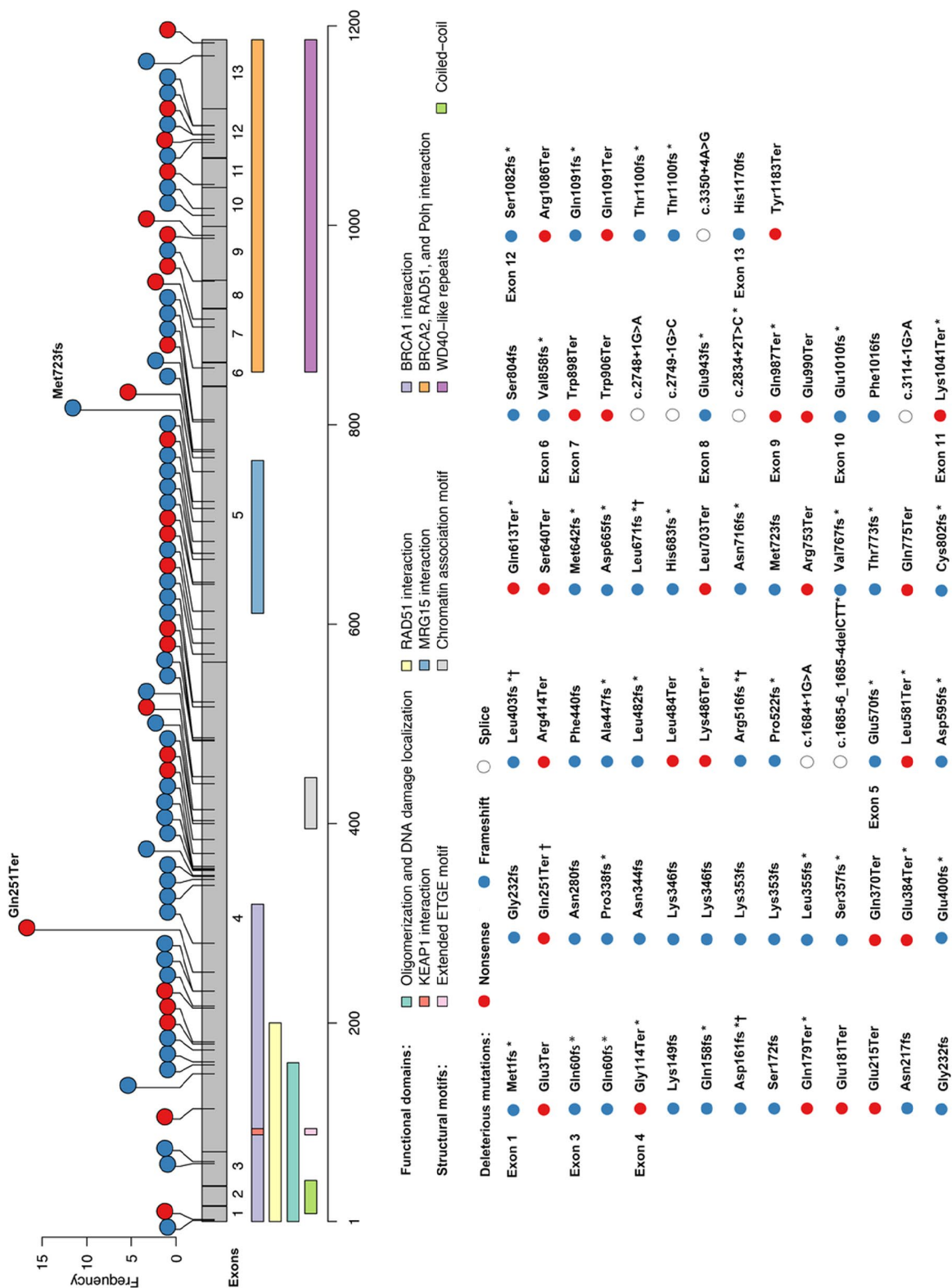


FIGURE 1. Spectrum of deleterious PALB2 mutations identified among 16,501 unselected BRCA1/2-negative patients with breast cancer. The schematic representation of the PALB2 gene was superimposed onto the exonic structure of the gene, with structural motifs and functional domains related. The x-axis represents the amino acid residues of the PALB2 protein. The y-axis represents the mutation frequency. *Novel PALB2 mutations; †PALB2 mutations identified in healthy controls. PALB2 indicates partner and localizer BRCA2.

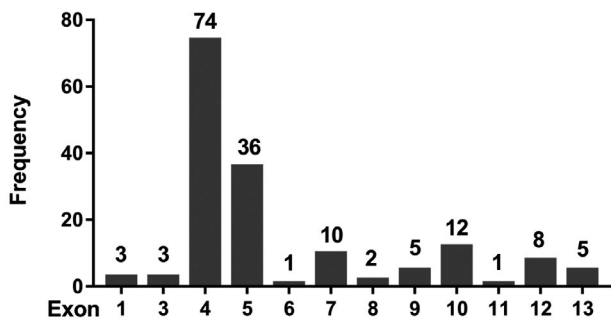


FIGURE 2. Distribution of deleterious *PALB2* mutations among its exons. *PALB2* indicates partner and localizer *BRCA2*.

0.8% among white women and an OR of 7.46 for breast cancer risk in *PALB2* carriers. Data from the Chinese population are very limited. Sun et al¹⁶ recently reported that *PALB2* germline mutations accounted for 0.69% of 8085 unselected Chinese patients with breast cancer who were treated at their breast center. It was a single-center study, and the breast cancer risk for *PALB2* germline mutation carriers is still unknown. To date, the current study is the largest *PALB2* mutations analysis in Chinese patients and healthy controls. The results of this study indicate that *PALB2* mutations confer a moderately increased risk for breast cancer. Notably, subdivided by age group, the breast cancer risk in our study was especially high for *PALB2* mutation carriers aged 30 years or younger (OR, 10.09). This underscores the necessity of regular surveillance (breast ultrasound or screening magnetic resonance imaging) for *PALB2* mutation carriers, especially those aged 30 years or younger.

In agreement with previous studies of *PALB2* mutations in breast cancers,¹³ the hotspot region for *PALB2* mutations in our study was exon 4. The most common mutation in our study, *PALB2* c.751C>T (n = 17), has been reported only in Asian populations (Chinese¹⁷ and Malaysian populations¹⁸) before, and this indicates that this mutation may be enriched in Asian populations. The frequency of the *PALB2* c.751C>T mutation in our unselected breast cancer cohort (17/16,501 = 0.1%) was lower than the frequency in the Chinese familial breast cancer cohort (2/360 = 0.56%)¹⁷ or the Malaysian unselected breast cancer cohort (1/467 = 0.21%).¹⁸ Another common mutation in our study, *PALB2* c.2167_2168delAT (n = 12), has been found in 3 European hereditary breast cancer cohorts before.^{6,19,20} The frequency of the *PALB2* c.2167_2168delAT mutation in our unselected breast cancer cohort (12/16,501 = 0.07%) was much lower than

the frequencies in the European hereditary breast cancer cohorts (UK cohort, 2/362 = 0.55%⁶; Italian cohort 1, 1/575 = 0.17%¹⁹; Italian cohort 2, 2/255 = 0.78%²⁰). Although some common *PALB2* mutations previously reported in European, US, and Australian breast cancers¹³ such as *PALB2* c.172_175delTTGT, c.2323C>T, and c.3549C>G were detected in our study, other known and common *PALB2* mutations, such as *PALB2* c.196C>T, c.509_510delGA, c.1592delT, and c.3113G>A, did not occur in our study. This further underlines the variety of *PALB2* mutations across different spatial distributions and ethnicities of the world.

In our study, 22.83% of the patients with breast cancer and *PALB2* germline mutations (29 of 127) had a triple-negative phenotype, whereas the frequency was 13.56% among mutation-negative cases ($P = .004$). Obviously, in our study, *PALB2* mutation breast cancers were significantly more likely to be the triple-negative phenotype in comparison with noncarriers. Nevertheless, the proportion of triple-negative breast cancers with *PALB2* germline mutations in our study (22.83%) was lower than those found in previous studies (Finnish familial and sporadic breast cancers, 54%²¹; UK hereditary breast cancer cohort, 30%⁶; Polish unselected breast cancer cohort, 34%¹⁰). In addition, *PALB2* mutation carriers were more likely to have bigger, axillary lymph node-positive, and bilateral tumors, and this indicates that *PALB2* mutation carriers present an aggressive phenotype. *PALB2* is a predisposition gene for breast and ovarian cancer, and it is reasonable that in this study, more *PALB2* mutation carriers had family histories of breast and/or ovarian cancer than noncarriers. Intriguingly, compared with noncarriers, *PALB2* mutation carriers here also more frequently had a family history of cancers other than breast and ovarian cancer, and the underlying mechanism needs to be further investigated.

Treatment regimens and effects were not assessed for *PALB2* mutation carriers in this study. A poly(adenosine diphosphate ribose) polymerase (PARP) inhibitor has been successfully used in patients with breast or ovarian cancer carrying *BRCA1* or *BRCA2* germline mutations.^{22,23} Although there is still a lack of solid clinical evidence for the use of PARP inhibitors in *PALB2*-associated cancer treatment, independent studies have provided evidence that *PALB2*-deficient cells are sensitive to PARP inhibitors.^{24,25} This suggests a potential treatment option for patients with breast cancer and *PALB2* germline mutations.

The preeminent strength of this study is that it was a large, population-based study. All the recruited patients

TABLE 1. Association of Deleterious *PALB2* Mutations With Breast Cancer Risk

	Total, No.	Deleterious <i>PALB2</i> Mutation Frequency, No.	Prevalence, %	OR (95% CI)	<i>P</i>
Healthy controls	5890	11	0.19	1.00 (reference)	
Patients with breast cancer	16,501	160	0.97	5.23 (2.84-9.65)	<.0001
Age group					
≤30 y	593	11	1.85	10.09 (3.95-25.79) ^a	<.0001
>30 y	15,883	149	0.94	5.06 (2.74-9.34)	<.0001

Abbreviations: CI, confidence interval; OR, odds ratio; *PALB2*, partner and localizer BRCA2.

^aFisher exact test.

TABLE 2. Clinicopathological Characteristics of *PALB2* Mutation Carriers and Noncarriers in the 16,501 *BRCA1/2*-Negative Patients With Breast Cancer

Characteristic	Patients With Breast Cancer	<i>PALB2</i> Mutation Carriers (n = 160)	<i>PALB2</i> Mutation Noncarriers (n = 16,341)	<i>P</i>
Age at diagnosis				
Mean ± SD, y	48.0 ± 10.2	47.4 ± 10.8	48.0 ± 10.2	.52
Range, n/N (%)				
≤30 y	593	11/160 (6.88)	582/16,341 (3.56)	.04
>30 y	15,908	149/160 (93.12)	15,759/16,341 (96.44)	
Tumor size, n/N (%)				
<2 cm	4652	52/118 (44.07)	4600/8507 (54.07)	.04
≥2 cm	3973	66/118 (55.93)	3907/8507 (45.93)	
Histology, n/N (%)				
Carcinoma in situ	1016	6/160 (3.75)	1010/16,341 (6.18)	.27
Invasive ductal carcinoma	14,179	140/160 (87.50)	14,039/16,341 (85.91)	
Other invasive carcinoma	1306	14/160 (8.75)	1292/16,341 (7.91)	
Estrogen receptor–positive, n/N (%)	10,111	92/133 (69.17)	10,019/14,300 (70.06)	.90
Progesterone receptor–positive, n/N (%)	9135	81/133 (60.90)	9054/14,310 (63.27)	.64
HER2–positive, n/N (%)	3347	26/123 (21.14)	3321/11,888 (27.94)	.12
Triple-negative breast cancer, n/N (%)	1519	29/127 (22.83)	1490/10,989 (13.56)	.004
Lymph nodes positive, n/N (%)	3415	62/125 (49.60)	3353/8642 (38.80)	.018
Bilateral, n/N (%)	231	9/143 (6.29)	222/11,032 (2.01)	.003^a
Distant metastasis, n/N (%)	697	5/160 (3.13)	692/16,341 (4.23)	.49
Family history of breast and/or ovarian cancer, n/N (%)	1331	33/160 (20.63)	1298/16,311 (7.96)	<.0001
Family history of any other cancer, n/N (%)	1574	38/160 (23.75)	1536/16,311 (9.42)	<.0001

Abbreviation: *PALB2*, partner and localizer BRCA2.

^aFisher exact test. The significance of the bold values were less than .05, which were considered statistically significant.

with breast cancer were unselected, and the number of *PALB2* mutation carriers was sufficient for analysis. This study comprehensively pictured the spectrum and penetrance of *PALB2* germline mutations in the Chinese breast cancer population. This study calculated the breast cancer risk for *PALB2* germline mutation carriers and illustrated characteristics of *PALB2*-related breast cancer in China. However, there are still several limitations to our study. There was quite a bit of missing data for the tumor size and lymph node status of *PALB2* mutation noncarriers, although the proportions of bigger tumors (tumor size ≥ 2 cm, 46%) and positive lymph nodes (39%) were similar to those reported before (results for *PALB2* mutation noncarriers in Poland [n = 12,413]: tumor size ≥ 2 cm, 48%; positive lymph nodes, 44%¹⁰; our single-center results published previously [n = 2203]: tumor size > 2 cm, 43%; positive lymph nodes, 38%¹²). Treatment

regimens and effects were not evaluated for *PALB2* mutation carriers because the mortality rate of those carriers was too low to support the analysis of treatment effects. In addition, we did not investigate the survival of *PALB2* mutation carriers and noncarriers because at the time of this writing the follow-up for those patients had not been fully finished, and this analysis will be presented in the future.

In conclusion, this study has revealed a comprehensive spectrum of *PALB2* germline mutations and characteristics of *PALB2*-related breast cancer in China. We showed that 0.97% of unselected *BRCA1/2*-negative patients with breast cancer carried deleterious *PALB2* germline mutations in this large Chinese cohort. *PALB2* germline mutations conferred a moderately increased risk for breast cancer but profoundly increased breast cancer risk for those aged 30 years or younger. Patients with breast cancer and *PALB2* germline mutations had

distinctive clinical characteristics. An analysis of survival and different treatment effects for *PALB2* mutation carriers needs to be further investigated.

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CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

AUTHOR CONTRIBUTIONS

Jiaojiao Zhou: Data analysis, writing of the manuscript, recruitment of the patients, collection of the blood samples and clinical information, and approval of the final manuscript. **Honglian Wang:** Data analysis, writing of the manuscript, gene sequencing, and approval of the final manuscript. **Fangmeng Fu:** Data analysis and approval of the final manuscript. **Zhanwen Li:** Recruitment of the patients, collection of the blood samples and clinical information, and approval of the final manuscript. **Qingjian Feng:** Recruitment of the patients, collection of the blood samples and clinical information, and approval of the final manuscript. **Weizhu Wu:** Recruitment of the patients, collection of the blood samples and clinical information, and approval of the final manuscript. **Yun Liu:** Planning and design of the study, recruitment of the patients, collection of the blood samples and clinical information, gene sequencing, and approval of the final manuscript. **Chuan Wang:** Data analysis, recruitment of the patients, collection of the blood samples and clinical information, and approval of the final manuscript. **Yiding Chen:** Planning and design of the study, data analysis, recruitment of the patients, collection of the blood samples and clinical information, and approval of the final manuscript.

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