

**Screening of Genetic Susceptibility Genes for Breast Cancer Patients and
Establishment of High-risk Populations in Chinese Communities (SIGHT)**

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Introduction

According to the estimation by Global cancer statistics in 2020, female breast cancer has become the leading cause of global cancer incidence ¹. Although China has a lower breast cancer burden compared with developed countries, the incidence and mortality of breast cancer in China has been increasing in the past decade ². It has been reported that 5% -10% of breast cancers have a hereditary background ³. Germline mutations in tumor-suppressor genes *BRCA1* and *BRCA2* are well known breast cancer predictors, which are involved in DNA repair and maintenance of genomic stability, and have been identified as genetic susceptibility genes of breast cancer ⁴.

BRCA1/2 mutation carriers have a significantly increased risk of developing breast cancer and ovarian cancer during their lifetime. A meta-analysis pooled 22 studies worldwide showed that, the average cumulative risks in *BRCA1*-mutation carriers by age 70 years were 65% for breast cancer and 39% for ovarian cancer, the corresponding estimates for *BRCA2* were 45% and 11%. Based on a prospective cohort of 9856 *BRCA1/2* mutation carriers, *BRCA1* and *BRCA2* Cohort Consortium found that, the cumulative risk 20 years for contralateral breast cancer after breast cancer diagnosis was 40% for *BRCA1* and 26% for *BRCA2* carriers ⁵. And the cumulative breast cancer risk to age 80 years was 72% for *BRCA1* and 69% for *BRCA2* carriers. The cumulative ovarian cancer risk to age 80 years was 44% for *BRCA1* and 17% for *BRCA2* carriers. Furthermore, *BRCA1/2* mutation could also increase the risk of male breast cancer ⁶, prostate cancer ⁷, pancreatic cancer, stomach cancer ⁸, and colorectal cancer ⁹.

During the past decades, several studies have been conducted to examine the distribution and prevalence of *BRCA1/2* mutations among different populations ¹⁰⁻¹⁴, including several studies in China ¹⁵⁻¹⁷. Notably, two studies in China have investigated the penetrance of breast cancer in *BRCA1/2* mutation carriers ^{18,19}, which provided evidence for the genetic counseling in China. Nevertheless, there are still many defects and many questions need to be answered. First, most studies conducted among Chinese were hospital-based research, and patients in some studies were recruited from one single city, limiting the application of results to larger populations. Hospital-based patient data, whose patients are opportunistic visits, may not reflect the real epidemiology in the population. Secondly, the *BRCA1/2* gene testing based on high genetic risk groups cannot reflect the true incidence of gene mutations in the general population of breast cancer. In addition, due to the incomplete collection of family history information in previous studies, it is not possible to establish a prediction model for the probability of *BRCA1/2* gene mutation in the Chinese population, nor to accurately calculate the breast cancer penetrance of mutation carriers. Moreover, current studies have shown that the commonly used clinical prediction models of *BRCA1/2* gene mutations derived from Western populations perform well in Caucasian patients, but the accuracy rate is not high in Asian populations.²⁰ Finally, the most important clinical significance of *BRCA1/2* gene mutation detection lies in the early prevention and diagnosis of breast cancer. The best place for the management and implementation of primary prevention of breast cancer is not in the hospital, but in the community, so it is very important to carry out screening of breast cancer genetic susceptibility genes in the community.

In order to fill the gaps in community research, we designed an observational, prospective

cohort study to screen genetic susceptibility genes of breast cancer patients in the Chinese community (from Eastern China, Jiangsu-Zhejiang-Shanghai Area) and establish a cohort of high-risk populations.

Objectives

This study plans to collect breast cancer patients from some communities in China for *BRCA1/2* mutation detection, understand the mutation rate of *BRCA1/2* and other genetic susceptibility genes in Chinese community breast cancer population, establish the *BRCA1/2* mutation carrier rate predictive model in the Chinese population, and accurately calculate the breast cancer penetrance (risk of incidence) of gene mutation carriers. It is expected to realize the unified management and follow-up of the high-risk population of breast cancer based on the community health service center, which will provide the basis for the research of early diagnosis, early prevention and intervention in the future.

Methods and analysis:

Study design

This is a multisite, prospective, cohort study conducted over 3 years beginning in July 2019 and ending in June 2022.

Participant recruitment

This study is carried out in Shanghai Municipality, Jiangsu Province and Zhejiang Province. These three provinces or municipality are adjacent to each other and located in the middle and lower reaches of the Yangtze River in eastern China. They are economically developed areas in China. Although China is a multi-ethnic country, the Han nationality accounts for 91.5%. In the area we study, the residents are basically Han nationality. Therefore, considering the regional convenience of patients' recruitment, the consistency of economic development level, and the unity of nationality and culture, we choose the breast cancer survivors of these three provinces or municipality as the research object. Our sample selection determines that our research can only represent data of breast cancer survivors in eastern China. At the same time, compared with the selection of all residents in the community as the research object, the choice of breast cancer survivors in the community is not perfect, but it should be the best choice to consider the cost and effectiveness at present.

All the recruitment of breast cancer survivors is carried out in the community. Shanghai Municipal Center for Disease Control and prevention (CDC) has been carrying out the registration of common malignant tumors in Shanghai since the 1960s. Therefore, the information of breast cancer survivors in Shanghai can be obtained directly from CDC. In Jiangsu Province and Zhejiang Province, since the local CDC have only started the registration of common malignant tumors in recent years, complete data cannot be obtained at the beginning of the recruitment, so it needs to be verified by data from other sources. The recruitment in Jiangsu Province is conducted in Jiangyin City. Jiangyin Cancer Club is an officially supported non-governmental group organized by cancer survivors themselves. With official support, the group can use the patient data registered by the local medical insurance

bureau and hospitals to develop new members. Its registered members include almost all cancer survivors in Jiangyin city. We can use the club's information to recruit breast cancer survivors, and the data of local CDC can be appropriately supplemented. The recruitment in Zhejiang Province is conducted in Zhoushan City. Zhoushan is an archipelago, relatively isolated from the mainland. Because of the traffic, most of the local breast cancer patients are diagnosed and treated in Zhoushan Hospital, the largest hospital in the city. Zhoushan Hospital also organize a club for breast cancer survivors. Combined with information from the local medical insurance bureau, it can get the most complete information about breast cancer survivors in the city. This information is also used to recruit breast cancer survivors in Zhoushan city, and the data of local CDC can also be appropriately supplemented.

Taking the community as the unit, sampling and information collection are carried out in batches. Each community collects blood samples and information at the same place in one day, with 50-200 patients each time. 2-4 weeks before the time of sampling, the staff of the community health service center or cancer club will notify all the breast cancer survivors in the community by letter and telephone. The letter contains the promotional materials to illustrate the clinical significance of the detection of breast cancer susceptibility genes and the time and place of sampling in the community. The phone also tells the same content.

We set the response rate of breast cancer survivors in the community to 70%. If we did not reach that response rate in the initial communication, we would recapture once through letters and telephone calls. If two or more patients belong to the same family and have blood relationship, only the patient with the youngest onset age can be taken as the proband, and the other patients will be removed from the database and not included in the statistics.

We estimated the sample size based on the pathogenic variant rate of *BRCA1/2* in Chinese population. A minimum sample size of 1927 produces a two-sided 95% confidence interval with a width equal to 2% when the pathogenic variant rate is 5%. We plan to recruit up to 5000 breast cancer patients from communities. As of 2021, blood samples and information of 2216 cases of breast cancer probands were collected from 13 communities in Jiangsu-Zhejiang-Shanghai Area. The data of 2216 cases of probands compared with the breast cancer survivors registered by local CDC are shown in Table 1. But due to the reason of COVID-19, it is impossible to complete the collection work of 5000 cases of breast cancer by June 2022. We have applied for an extension to the ethics committee.

Inclusion criteria

- Breast cancer survivors of any age;
- Both men and women;
- Subjects signed an informed consent form before being selected to express their consent to participate in the research and to comply with the research procedures.

Exclusion criteria

- Healthy people who have not suffered from breast cancer or patients with other malignant tumors;
- Unable to interview the investigator and sign the informed consent form due to any

reason;

- The inability to collect peripheral blood or oral mucosa samples for any reason.

Biological samples and clinical genetic information collection

In the letter and telephone notification before sampling, the patient will be informed of the documents to be carried on the day of sampling, including ID card, medical insurance card/medical record card, discharge summary from the hospital, postoperative pathological report and immunohistochemical results. On the day of the sampling, the investigator organized 6-10 doctors and research nurses to interview the patients on the spot, sign the informed consent form, fill in and review clinical and genetic information forms, collect family history information, and blood samples (4~5ml venous blood) or oral mucosal samples. After the event, If patients with recapture wish to receive genetic testing, they can go to the community hospital to have an interview with the designated doctor. Clinical genetic information and biological samples were managed by the researcher. 4~5ml of venous blood was divided into duplicates, one was kept in the tissue bank of Fudan University Affiliated Cancer Hospital, and the other was handed over to Shanghai AITA Biomedical Research Institute for *BRCA1*, *BRCA2*, *PTEN*, *CHEK2* and *PALB2* gene mutation detection. All gene mutation testing is free for patients.

Clinical genetic information form

All the participants will record the following information at baseline:

- 1) Personal information: including gender, age, birthplace, patrilineal and matrilineal origin.
- 2) Basic information: menstrual status, fertility history, oral contraceptive status, drinking and smoking status.
- 3) Important issues:
 - a) Whether a breast cancer patient with other malignancies;
 - b) Whether a breast cancer patient younger than 40 years old;
 - c) Whether a patient with bilateral breast cancer;
 - d) Whether a triple-negative breast cancer patient;
 - e) Whether there are any relatives in the family who are breast cancer patients?
 - f) Whether there are any relatives in the family who are patients with other malignant tumors

4) First-degree relatives' information: including the current age of parents, siblings and children, age of death (if any), the prevalence and survival of malignant tumors.

When the participants meet any of the prescribed conditions in important issues, they will receive more detailed tumor family history information collection and draw family trees.

All the above information will be collected in all patients. If any of the six questions in the "important issues" is answered "yes", the patient will be asked for a detailed family history and draw a pedigree. According to our current data, 62.7% of patients have drawn a pedigree.

Family trees

A family tree for the patient was constructed in accordance with guidelines of Pedigree Standardization Task Force²¹.

Quality Control

At the scene of collecting patients' clinical information and blood samples, each patient will be assigned a unique barcode. The barcode is four copies, one on the informed consent form, one on the clinical and family history information questionnaire, and two on the blood sample collection tube divided into two tubes. One tube of blood sample collected on site was sent to Shanghai AITA Biomedical Research Institute for genetic testing, and the other tube of blood sample, together with the informed consent and questionnaire, was kept by the Affiliated Cancer Hospital of Fudan University. When AITA received the samples, all of them with barcode were registered into AITA Sample management system. The barcode is the only Identifier for all the samples in the DNA extraction, library construction and sequencing process.

Mutation Analyses

Sample preparation and next generation sequencing were performed at the Shanghai AITA Biomedical Research Institute as previously described²². Some small-scale studies (results unpublished) were conducted before the start of this study. It was found that the detection rate of *TP53* and *ATM* was not high. Due to funding reasons, the research plan decided not to perform the mutation detection of these two genes. In previous studies, the *RECQL* gene was also included, but because Australian studies showed that *RECQL* gene mutation was not associated with breast cancer risk²³, the gene was not included in this study. Therefore, the genes selected in this study are *BRCA1*, *BRCA2*, *PTEN*, *CHEK2*, and *PALB2*.

Multiplex-PCR primer design and preparation: The target-specific primers for the coding sequences of *BRCA1* (NM_007300), *BRCA2* (NM_000059), *PALB2* (NM_024675), *CHEK2* (NM_007194) and *PTEN* (NM_000314) genes were designed using Primer3 (<http://bioinfo.ut.ee/primer3/>). Universal sequences (CS1: ACACTGACGACATGGTTCTACA and CS2: TACGGTAGCAGAGACTTGGTCT) were appended at 5'-ends of each forward and reverse primer, respectively. Preamplification for tagged amplicon deep sequencing (Tam-Seq) was conducted in 6 mL PCR mixture containing 3 mL of KAPA 2G Robust HotStart ReadyMix (2X) (Kapa Biosystems, Boston, Massachusetts, United States), 1 mL of primer mix (500 nM), and 2 mL of DNA template (10 ng/ml). Sequencing barcode primers (Fluidigm Corporation, South San Francisco, California) consisted of PE1 and PE2 sequences for Illumina cluster generation, a 10-bp barcode, and CS1 and CS2 adaptors, used in pairs (PE1-CS1/PE2-BC-CS2; PE1-CS2/PE2-BC-CS1). For the DNA library, PCR products were barcoded and analyzed using gel electrophoresis to ensure the expected insertion size. The library was quantified by Agilent BioAnalyzer and sequenced using the Illumina Xten platform with paired-end reads of 150 bp per the manufacturer's instructions. Custom sequencing primers for CS1 and CS2 targeted the paired reads, and 10-base indexing (barcode) reads per the recommendations of Fluidigm.

Sequencing reads were aligned to the hg19 reference genome using BWA1 (<http://bio-bwa.sourceforge.net>). Genome Analysis Toolkit (GATK) 2 (<https://software.broadinstitute.org/gatk/>) was used for base quality score recalibration,

indel realignment, and variant calling based on the following criteria: (i) QD < 2.0; (ii) MQ < 40.0; (iii) MQRankSum < -12.5; and (iv) ReadPosRankSum < -8.0. Variant functions were predicted using SnpEff (<http://snpeff.sourceforge.net>), PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>), PROVEAN (<http://provean.jcvi.org/index.php>), and SIFT (<https://omictools.com/sift-tool>). Variant population frequency was annotated with the ExAC database (<http://exac.broadinstitute.org>), the 1,000 Genomes database (<https://www.internationalgenome.org>), and an internal database.

Only novel BRCA1/2 variants or variants with < 1% population frequency in 1,000 Genomes or ExAC were collected. Clinical significance of each variant was annotated according to ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>), ACMG-AMP guidelines, and other supporting evidence from the public literature and curated databases. Variants were manually inspected with Integrative Genomics Viewer (<http://www.igv.org/>), to exclude false-positives. Classification of variants was collapsed from a 5-tier to 3-tier classification system comprised of pathogenic and benign variants, and VUS. All pathogenic variants were validated by Sanger sequencing.

Because even in patients with high risk of breast cancer, the detection rate of large genomic rearrangements of BRCA1/2 is not high²⁴. In view of the cost, the MLPA method is not used in this study.

Follow-up

Shanghai AITA Biomedical Research Institute delivers the results of the participants' genetic mutation test to the researcher. The researcher informs the patient's community health service center of the test result.

The participants with variants of benign, likely benign and uncertain significance are notified by the community health service center without special treatment, and only continue with routine treatment and follow-up.

The participants with variants of pathogenic and likely pathogenic are recommended to be notified by the investigator and discuss follow-up intervention plan in person. The notification content includes: the significance of gene variants, the necessity and possibility of family verification (once the patient agrees, the verification of the pathogenic or likely pathogenic variants of blood-relatives is also free), the risk of (contralateral) breast cancer, ovarian cancer and other malignant tumors for mutation carriers, the follow-up plans, the possibility of preventive surgery, and relevant information about targeted drug (PARP inhibitor drugs) intervention, etc.

Statistical analysis

The frequency of pathogenic or likely pathogenic variants and 95% confidence intervals of BRCA1/2 and other genetic susceptibility genes in Chinese community breast cancer population will be measured. After stratifying according to the different clinical characteristics of the patients, chi-square tests will be used to compare the mutation rates of BRCA1/2 gene in different subgroups.

Logistic regression will be performed to establish a prediction model for the probability of pathogenic or likely pathogenic variants of *BRCA1/2* and other genes. Candidate predictors included age, race or ethnic group, the mean number of tumors in a family etc. Missing data were imputed using multiple imputation by chained equations if needed. Model validation is based on k-fold cross validation approach. Model calibration and discrimination were evaluated using HL test and AUC, respectively. ORs and corresponding 95% CI will report.

Kin-Cohort method will be used to calculate the penetrance of breast cancer and other common malignancies in *BRCA1/2* and other genetic mutation carriers.

Ethics and dissemination

This study was approved by the Fudan University Shanghai Cancer Center Institutional Review Board (Registration number: 1905202-3). This study follows the Helsinki Declaration. Patients will be told the details of the study (purpose, risk and benefits), and patients have the right to quit any time. An informed consent form will be sent to each patient prior to enrolment to ensure that each patient understands the cohort study. The process of obtaining informed consent is in accordance with the Good Clinical Practice of Pharmaceutical Products (GCP) requirements. The results of this study will be presented at national and international meetings and published in a scientific peer-reviewed journal.

Discussion

An important aspect of the clinical significance of detecting genetic susceptibility genes for breast cancer is to be able to identify genetically susceptible populations from the general population, so as to carry out early diagnosis and prevention of breast cancer. The results of Olaparib clinical studies²⁵ in recent years made it possible to treat *BRCA1/2* mutation-positive breast cancer patients with targeted therapy, thus promoting the development of genetic testing. In order to clarify the value of *BRCA1/2* and other genetic susceptibility genes for early diagnosis and prevention of breast cancer, the information on the proportion of mutation carriers in the population, who are more likely to carry genetic mutations, and the cancer risk of mutation carriers are very important. That information directly determines the necessity of genetic testing and early prevention, including preventive surgery. There have been many studies on these in the Western population, but there is still a lack of large-scale reports among the Chinese population. As a result, the current diagnosis and treatment routines performed in the Chinese population are based on the guidelines obtained from the data of the Western population. However, a small number of studies have shown that the data of the Chinese population, especially the cancer risk of mutation carriers, are very different from those of the Western white population. This also raises questions: Is it necessary to promote expensive and traumatic preventive surgery among Chinese mutation carriers, if the risk of breast cancer in Chinese mutation carriers is much lower than that in Western populations?

An important method currently used to calculate the penetrance of mutation carriers is based on the family history of the proband. After identifying the proband, the researchers first collect information on close relatives at risk, then establish the probability that each relative is a carrier of a mutation in *BRCA1/2* by use of Mendelian inheritance, and finally

count cumulative breast cancer events and estimate age-specific risks, after adjusting for the probabilities that the contributing family members are carriers. A key assumption in both of these methods is that the risks are presumed to be same no matter for family members of a carrier who is a case patient or a control subject²⁶. Therefore, the sample selection is very critical, and we must strive to make the selected sample representative of the entire population. The ideal situation is to randomly select any individual from the population, regardless of whether they have cancer or not. However, the mutation rate of breast cancer genetic susceptibility genes in healthy people is very low. In order to obtain enough mutation carriers, more testing is needed, which makes the research more expensive. Therefore, most studies have selected breast cancer patients as research subjects. Because the mutation detection rate in patients is much higher than that of normal people, which improves the efficiency of detection and greatly saves research costs. In addition, there is another problem in selecting patients as samples. Because in the same case of carrying mutations, breast cancer patients and healthy people may have different risk factors other than mutations, which will cause bias. At present, the research reporting the penetrance rate of *BRCA1/2* mutation carriers in the Chinese population all come from patient samples in the hospital. One of the research samples came from a genetic high-risk clinic in a hospital¹⁸, and the other were samples of consecutive patients admitted in the hospital¹⁹. These studies are highly likely to overestimate the penetrance of the Chinese population due to sampling errors. Our research selects breast cancer patients in a specific geographic area from the communities, collects all patients as much as possible to enter the study, eliminates the factor of sample selection bias, so that the research sample can represent the entire population as much as possible. Although it cannot be compared with a sample randomly selected from the entire population, it should be the most economical and effective sample among the Chinese population so far.

Gail also raised some limitations of the kin-cohort design²⁷, we try to reduce the impact of these limitations through the study design. To reduce the selection bias, as described above, we recruit breast cancer patients in the community, regardless of their family history of breast cancer or other malignancies, rather than recruiting breast cancer patients with genetic predisposition in genetic high-risk clinics. For the information bias from inability of the proband to recall the disease histories of relatives accurately, the community we select is located in the economically developed eastern region of China, and the patient's education level is high, which can ensure more accurate family history information. We also leave contact information for further supplementary use. Finally, we strictly review each patient's family history to ensure that none of the patients were related to each other by blood, while maximizing the sample size to reduce bias.

Most of the existing models used to predict *BRCA1/2* gene mutations are derived from data among western populations, but it has great limitation to apply these models to Chinese populations. Kurian et al.²⁰ used BRCAPRO and Myriad II to predict the *BRCA1/2* gene mutations in Asian and Caucasian patients, and found that these two models performed well in whites, but only about 50% of mutation carriers can be predicted in Asian patients. Especially the *BRCA2* gene mutations can only be predicted 1/6 of the actual. Researchers

believe that it is due to differences in the incidence of *BRCA1/2* gene mutations, penetrance, and the incidence of breast cancer among different races or ethnicity groups. Therefore, it is also crucial to use the Chinese population's own data to establish a gene mutation prediction model suitable for the Chinese population. In recent years, with the development of next-generation sequencing technology, the cost of *BRCA1/2* gene mutation detection has dropped significantly. At the same time, due to the inability to find a perfect prediction model with an accuracy of 100%, some researchers suggested that all breast cancer patients should be tested for *BRCA1/2* gene mutations, and there is no need to apply predictive models for pre-detection evaluation²⁸. Studies have shown that the proportion of *BRCA1/2* gene mutations in Chinese breast cancer patients is similar to that of Western populations, only about 5%²². Even if the cost of testing is much lower than before, in a populous country like China, testing all breast cancer patients in order to find about 5% of mutation carriers will still consume a lot of medical resources. Therefore, the establishment of a mutation prediction model suitable for the Chinese population still has high socio-economic value.

Reference

1. Sung H, Ferlay J, Siegel RL, et al: Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 71:209-249, 2021
2. Zhang S, Sun K, Zheng R, et al: Cancer incidence and mortality in China, 2015. *Journal of the National Cancer Center* 1:2-11, 2021
3. Fackenthal JD, Olopade OI: Breast cancer risk associated with BRCA1 and BRCA2 in diverse populations. *Nat Rev Cancer* 7:937-48, 2007
4. Nathanson KL, Wooster R, Weber BL: Breast cancer genetics: what we know and what we need. *Nat Med* 7:552-6, 2001
5. Kuchenbaecker KB, Hopper JL, Barnes DR, et al: Risks of Breast, Ovarian, and Contralateral Breast Cancer for BRCA1 and BRCA2 Mutation Carriers. *Jama* 317:2402-2416, 2017
6. Tai YC, Domchek S, Parmigiani G, et al: Breast cancer risk among male BRCA1 and BRCA2 mutation carriers. *J Natl Cancer Inst* 99:1811-4, 2007
7. Oh M, Alkushaym N, Fallatah S, et al: The association of BRCA1 and BRCA2 mutations with prostate cancer risk, frequency, and mortality: A meta-analysis. *Prostate* 79:880-895, 2019
8. Cavanagh H, Rogers KM: The role of BRCA1 and BRCA2 mutations in prostate, pancreatic and stomach cancers. *Hered Cancer Clin Pract* 13:16, 2015
9. Oh M, McBride A, Yun S, et al: BRCA1 and BRCA2 Gene Mutations and Colorectal Cancer Risk: Systematic Review and Meta-analysis. *J Natl Cancer Inst* 110:1178-1189, 2018
10. Jara L, Morales S, de Mayo T, et al: Mutations in BRCA1, BRCA2 and other breast and ovarian cancer susceptibility genes in Central and South American populations. *Biol Res* 50:35, 2017
11. Abdulrashid K, AlHussaini N, Ahmed W, et al: Prevalence of BRCA mutations among hereditary breast and/or ovarian cancer patients in Arab countries: systematic review and meta-analysis. *BMC Cancer* 19:256, 2019
12. Sharma P, Klemp JR, Kimler BF, et al: Germline BRCA mutation evaluation in a prospective triple-negative breast cancer registry: implications for hereditary breast and/or ovarian cancer syndrome testing. *Breast Cancer Res Treat* 145:707-14, 2014
13. Bergman A, Flodin A, Engwall Y, et al: A high frequency of germline BRCA1/2 mutations in western Sweden detected with complementary screening techniques. *Fam Cancer* 4:89-96, 2005

14. Palomba G, Pisano M, Cossu A, et al: Spectrum and prevalence of BRCA1 and BRCA2 germline mutations in Sardinian patients with breast carcinoma through hospital-based screening. *Cancer* 104:1172-9, 2005
15. Suter NM, Ray RM, Hu YW, et al: BRCA1 and BRCA2 mutations in women from Shanghai China. *Cancer Epidemiol Biomarkers Prev* 13:181-9, 2004
16. Li WF, Hu Z, Rao NY, et al: The prevalence of BRCA1 and BRCA2 germline mutations in high-risk breast cancer patients of Chinese Han nationality: two recurrent mutations were identified. *Breast Cancer Res Treat* 110:99-109, 2008
17. Zhang J, Pei R, Pang Z, et al: Prevalence and characterization of BRCA1 and BRCA2 germline mutations in Chinese women with familial breast cancer. *Breast Cancer Res Treat* 132:421-8, 2012
18. Zhang L, Shin VY, Chai X, et al: Breast and ovarian cancer penetrance of BRCA1/2 mutations among Hong Kong women. *Oncotarget* 9:25025-25033, 2018
19. Yao L, Sun J, Zhang J, et al: Breast cancer risk in Chinese women with BRCA1 or BRCA2 mutations. *Breast Cancer Res Treat* 156:441-445, 2016
20. Kurian AW, Gong GD, Chun NM, et al: Performance of BRCA1/2 mutation prediction models in Asian Americans. *J Clin Oncol* 26:4752-8, 2008
21. Bennett RL, French KS, Resta RG, et al: Standardized human pedigree nomenclature: update and assessment of the recommendations of the National Society of Genetic Counselors. *J Genet Couns* 17:424-33, 2008
22. Liu Y, Wang H, Wang X, et al: Prevalence and reclassification of BRCA1 and BRCA2 variants in a large, unselected Chinese Han breast cancer cohort. *J Hematol Oncol* 14:18, 2021
23. Li N, Rowley SM, Goode DL, et al: Mutations in RECQL are not associated with breast cancer risk in an Australian population. *Nat Genet* 50:1346-1348, 2018
24. Park B, Sohn JY, Yoon KA, et al: Characteristics of BRCA1/2 mutations carriers including large genomic rearrangements in high risk breast cancer patients. *Breast Cancer Res Treat* 163:139-150, 2017
25. Tutt ANJ, Garber JE, Kaufman B, et al: Adjuvant Olaparib for Patients with BRCA1- or BRCA2-Mutated Breast Cancer. *N Engl J Med* 384:2394-2405, 2021
26. Begg CB: On the use of familial aggregation in population-based case probands for calculating penetrance. *J Natl Cancer Inst* 94:1221-6, 2002
27. Gail MH, Pee D, Carroll R: Kin-cohort designs for gene characterization. *J Natl Cancer Inst Monogr*:55-60, 1999
28. Beitsch PD, Whitworth PW, Hughes K, et al: Underdiagnosis of Hereditary Breast Cancer: Are Genetic Testing Guidelines a Tool or an Obstacle? *J Clin Oncol* 37:453-460, 2019

Table 1: The comparison between the data of breast cancer survivors recruited from this study and the data of all breast cancer survivors in the community provided by local CDC

Patient's area	Breast cancer survivors in this study*			Breast cancer survivors registered by local CDC**			Participation rate in this study
	No.	Proportion of patients by age of onset		No.	Proportion of patients by age of onset		
		<50 y	>=50 y		<50 y	>=50 y	
Shanghai	689	33.2%	66.8%	1085	38.8%	61.2%	63.5%
Jiangsu	470	52.6%	47.4%	846	37.1%	62.9%	55.6%
Zhejiang	1057	47.9%	52.1%	1589	36.2%	63.8%	66.5%
Total	2216	44.2%	55.8%	3520	37.1%	62.9%	63.0%

* The time of recruitment is from July 2019 to March 2021.

** The time cut-off point for breast cancer survivors data provided by local CDC is June 2021.

CDC: Center for Disease Control and prevention