Inherited mutations in *BRCA1* and *BRCA2* in an unselected multi-ethnic cohort of Asian breast cancer patients and healthy controls from Malaysia

Wei Xiong Wen[1], Jamie Allen[2], Kah Nyin Lai[1], Shivaani Mariapun[1], Siti Norhidayu Hasan[1], Pei Sze Ng[1], Daphne Shin-Chi Lee[1], Sheau Yee Lee[1], Sook Yee Yoon[1], Joanna Lim[1], Shao Yan Lau[1], Brennan Decker[2,3], Karen Pooley[2], Leila Dorling[2], Craig Luccarini[4], Caroline Baynes[2], Don Conroy[2], Patricia Harrington[2], Jacques Simard[5], , Cheng Har Yip[1,6], Nur Aishah Mohd Taib[7,8], Weang Kee Ho[9], Antonis C Antoniou[2], Alison M Dunning[4], Douglas F Easton[2,4], Soo Hwang Teo[1,7,8]

[1] Cancer Research Malaysia, Subang Jaya, Selangor, Malaysia
[2] Department of Public Health and Primary Care, Centre for Cancer Genetic Epidemiology, University of Cambridge, Cambridge, United Kingdom
[3] Cancer Genetics and Comparative Genomics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland, United States of America
[4] Department of Oncology, Centre for Cancer Genetic Epidemiology, University of Cambridge, Cambridge, United Kingdom
[5] Genomics Center, Centre Hospitalier Universitaire de Québec - Université Laval Research Center, Quebec, Canada
[6] Sime Darby Medical Centre, Subang Jaya, Selangor, Malaysia
[7] Breast Cancer Research Unit, University Malaya Cancer Research Institute, Faculty of Medicine, University Malaya, Kuala Lumpur, Malaysia
[8] Department of Surgery, Faculty of Medicine, University Malaya Medical Centre, Kuala Lumpur, Malaysia
[9] Department of Applied Mathematics, Faculty of Engineering, The University of Nottingham Malaysia Campus, 43500, Semenyih, Selangor, Malaysia

Wei Xiong Wen: sean.wen@cancerresearch.my
Jamie Allen: jma73@medschl.cam.ac.uk
Kah Nyin Lai: kahnyin.lai@cancerresearch.my
Shivaani Mariapun: shivaani.mariapun@cancerresearch.my
Siti Norhidayu Hasan: norhidayu.hasan@cancerresearch.my
Pei Sze Ng: patsy.ng@cancerresearch.my
Daphne Shin-Chi Lee: daphne.lee@cancerresearch.my
Sheau Yee Lee: nanako1107@gmail.com
Sook Yee Yoon: sookyee.yoon@cancerresearch.my
Joanna Lim: joanna.lim@cancerresearch.my
Shao Yan Lau: shaoyan.lau@cancerresearch.my
Brennan Decker: bdecker@bwh.harvard.edu
Karen Pooley: kap28@medschl.cam.ac.uk
Leila Dorling: ld429@medschl.cam.ac.uk
Craig Luccarini: craig@srl.cam.ac.uk
Caroline Baynes: ceb63@medschl.cam.ac.uk
Don Conroy: dmc40@medschl.cam.ac.uk
Patricia Harrington: pah25@medschl.cam.ac.uk

Jacques Simard: jacques.simard@crchudequebec.ulaval.ca
Cheng Har Yip: chenghar.yip@gmail.com
Nur Aishah Mohd Taib: nuraish@gmail.com
Weang Kee Ho: weangkee.ho@nottingham.edu.my

Antonis C Antoniou: aca20@medschl.cam.ac.uk
Alison M Dunning: amd24@medschl.cam.ac.uk
Douglas F Easton: douglas@srl.cam.ac.uk
Soo Hwang Teo: soohwang.teo@cancerresearch.my

Corresponding Author: Professor Dr. Teo Soo Hwang,
Cancer Research Malaysia,
1 Jalan SS12/1A, 47500 Subang Jaya,
Selangor, MALAYSIA
Tel: +603 2712 3224
Fax: +603 2712 3225
Email: soohwang.teo@cancerresearch.my

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ABSTRACT

Background: Genetic testing for *BRCA1* and *BRCA2* is offered typically to selected women based on age of onset and family history of cancer. However, current internationally-accepted genetic testing referral guidelines are built mostly on data from cancer genetics clinics in women of European descent. To evaluate the appropriateness of such guidelines in Asians, we have determined the prevalence of germline variants in an unselected cohort of Asian breast cancer patients and healthy controls.

Methods: Germline DNA from a hospital-based study of 2,575 unselected breast cancer patients and 2,809 healthy controls were subjected to amplicon-based targeted sequencing of exonic and proximal splice site junction regions of *BRCA1* and *BRCA2* using the Fluidigm Access Array system, with sequencing conducted on a Illumina HiSeq2500 platform. Variant calling was performed with GATK UnifiedGenotyper and were validated by Sanger sequencing.

Results: Fifty-five (2.1%) *BRCA1* and 66 (2.6%) *BRCA2* deleterious mutations were identified among breast cancer patients and 5 (0.18%) *BRCA1* and 6 (0.21%) *BRCA2* mutations among controls. One-thousand one-hundred and eighty-six (46%) patients and 97 (80%) carriers fulfilled the National Comprehensive Cancer Network (NCCN) guidelines for genetic testing.

Conclusion: Five percent of unselected Asian breast cancer patients carry deleterious variants in *BRCA1* or *BRCA2*. Whilst current referral guidelines identified the majority of carriers, one in two patients would be referred for genetic services. Given that such services are largely unavailable in majority of low-resource settings in Asia, our study highlights the need for more efficient guidelines to identify at-risk individuals in Asia.

(247 words)

Keywords: Asian, breast cancer, *BRCA1*, *BRCA2*, National Comprehensive Cancer Network

INTRODUCTION

Genetic testing for mutations in *BRCA1* and *BRCA2* has led to the identification of individuals at higher risk of breast cancer, enabled risk stratified approaches for management of risk in relatives, and enabled the selection of individuals who may benefit from therapies targeting the DNA damage response.[1] The majority of studies have hitherto screened high-risk breast cancer patients selected on the basis of age, family history, and, some studies, tumour subtype, such as estrogen receptor negative or triple negative breast cancer.[2] These studies have reported the prevalence of deleterious germline variants in *BRCA1* and *BRCA2* among Asian high-risk breast cancer patients is similar to that in other populations, ranging between 10 to 20%.[2-6] However, it is estimated that less than 1% of the 560,000 breast cancer patients diagnosed in 14 Asian countries each year benefit from genetic testing services, because of high cost and limited accessibility.[7] In such resource-limited settings, it is critical to have appropriate guidelines for referral for genetic testing. While internationally accepted clinical criteria for referral can be obtained from the National Comprehensive Cancer Network (NCCN) guidelines on genetic/familial high-risk assessment,[8] such guidelines has been developed primarily from data from population of European ancestry. There are established differences in breast cancer epidemiology between Asian and Caucasian individuals,[9] but the appropriateness of such guidelines in identifying mutation carriers have hitherto not been assessed in Asian populations.

To evaluate current genetic testing referral guidelines, we have conducted an analysis of *BRCA1* and *BRCA2* in a multi-ethnic cohort of unselected breast cancer patients of Chinese, Malay, and Indian ethnicity from Malaysia. Our study provides data on the appropriateness of current guidelines for identifying individuals at higher

risk of carrying germline variants in *BRCA1* and *BRCA2*, and lays the foundation for developing risk assessment tools for Asian populations.

METHODS

Study populations

We included breast cancer patients and control subjects who participated in the Malaysian Breast Cancer Genetic Study (MyBrCa) between October 2002 and March 2015. Incident and prevalent cases, and controls were recruited from two hospitals: University Malaya Medical Centre and Sime Darby Medical Centre.[10 11] Of the 2,870 breast cancer patients and 2,999 control subjects recruited, 2,575 and 2,809 cases and controls, respectively, were included in this study (see Supplementary Table 1 and 2 for exclusion criteria). Of these 2,575 cases, 887 (34%) women were considered to be *a priori* high- or moderate-risk, and had been previously tested for germline alterations in *BRCA1* and *BRCA2* by Sanger sequencing and MLPA analysis as described.[12-15] All study participants provided written informed consent. The study was approved by the Medical Ethics Committee of University Malaya Medical Centre (application number: 842.9) and the Independent Ethics Committee of Sime Darby Medical Centre (application numbers: 201109.4 and 201208.1).

Sequencing library preparation and sequencing

Fluidigm D3 design software (Fluidigm, San Francisco, CA) was used to design a targeted sequencing panel that included the coding sequences and intron/exon boundaries of coding exons from 31 known or suspected breast cancer susceptibility genes, including *BRCA1* and *BRCA2*. Target sequence enrichment was performed

using 48.48 Fluidigm Access Arrays (Fluidigm, San Francisco, California, USA) then sequenced on Illumina Hi-Seq2500 instrument (Illumina, San Diego, California, USA) according to the manufacturer's protocol as previously described.[16] The median read depth across the 261 amplicons covering the *BRCA1* and *BRCA2* coding sequence was 673 (IQR: 534 – 909).

Bioinformatics analysis

Sequenced reads were demultiplexed and converted from the Illumina binary format into FASTQ format. Next, adaptor sequences were trimmed using Cutadapt (https://pypi.python.org/pypi/cutadapt). Sequenced reads were then aligned against the human genome reference sequence (hg19) with Burrows-Wheeler Aligner.[17] Subsequent local insertion/deletion (indel) realignment and base quality score recalibration were performed using the Genome Analysis Toolkit (GATK; https://www.broadinstitute.org/gatk). Genetic variants were called with GATK Unified Genotyper using the default parameters except –minIndelFrac (set to 0.05).[18] Variants were annotated using ANNOVAR (http://www.openbioinformatics.org/annovar)[19] and missense variants were further annotated using Align-GVGD (http://agvgd.iarc.fr).[20] Nonsense, frameshift, canonical splice site variants (positions -2 and -1 upstream of an exon start and +1 and +2 downstream of an exon end), and single nucleotide variants (SNVs) classified as Class 4 or Class 5 according to BRCA Mutation Database (http://arup.utah.edu/database/BRCA/) or Leiden Open Variation Database (LOVD) were considered deleterious, except for variants located at the C-terminal of *BRCA1* and *BRCA2* (amino acid position 1,856-1,863 and 3,326-3,385, respectively). All

deleterious and non-C0 variants as per Align-GVGD were validated by Sanger sequencing.

Statistical analysis

Analyses were based on the variants identified through the analysis of the NGS data only. Carriers of LGR, non-LGR deleterious variants, and VUS previously identified but not detected in this sequencing study were considered as non-carriers. Categorical and continuous variables were compared using Chi-squared test and *t* test, respectively. Statistical tests were considered significant based on 2-sided hypothesis tests with $P < .05$.

NCCN guidelines and MyCPG for *BRCA1* and *BRCA2* testing

The NCCN Guidelines Version 1.2017 and Malaysian Clinical Practice Guidelines (MyCPG) Version Nov.2010 for genetic testing of *BRCA1* and *BRCA2* for BRCA-related breast and ovarian cancer syndrome were used to identify breast cancer patients and *BRCA1* and *BRCA2* carriers whom met testing criteria for *BRCA1* and *BRCA2* screening. The NCCN Guidelines are a statement of evidence and consensus of the authors regarding their views of currently accepted approaches to treatment. The MyCPG are meant to be guides for clinical practice in Malaysia based on the best available evidence at the time of development. *BRCA1* and *BRCA2* testing criteria for both guidelines used in this study are described in Table 1.

RESULTS

Study population

Comparisons of the characteristics of breast cancer cases, and the healthy women attending opportunistic screening mammography are shown in Table 2 and Supplementary Table 3. Approximately two thirds of cases and controls were of Chinese ancestry. Breast cancer patients were, on average, younger than the controls and enriched for family history of breast cancers up to second degree.

BRCA1 and *BRCA2* mutations and VUS

Of the 2,575 breast cancer patients, 55 (2.1%) carried deleterious variants in *BRCA1* and 66 (2.6%) had deleterious variants in *BRCA2* (Table 3). The frequency of deleterious variants was similar in Indian (7.5%) and Malay patients (6.7%), but lower in Chinese patients (3.5%, $P<0.01$). *BRCA2* deleterious variants were more common than *BRCA1* deleterious variants among Chinese patients (2.3% versus 1.2%) but less common in Indian patients (2.8% versus 5.0%), while the frequencies were similar in Malay patients (3.3% versus 3.5%; $P<0.01$ for difference in *BRCA1*:*BRCA2* ratio).

Of 2,809 control subjects, 5 (0.18%) had deleterious variants in *BRCA1* and 6 (0.21%) had deleterious variants in *BRCA2* (Table 3). The deleterious variant frequencies of *BRCA1* and *BRCA2* in the controls were similar to those in the Exome Aggregation Consortium East Asian population [ExAC East Asian] with reported deleterious variant frequencies of 0.16% and 0.21% for *BRCA1* and *BRCA2*, respectively.

Deleterious variants in *BRCA1* and *BRCA2* were significantly more common in breast cancer cases compared to control subjects, with estimated odds ratios for breast cancer of 12.6 (95% CI: 5.0-31.4) and 12.6 (95% CI: 5.4-29.0) for *BRCA1* and *BRCA2*, respectively.

Variants of unknown significance (VUS) in *BRCA1* were reported in 12 cases (0.47%) versus 4 controls (0.14%) (*P*=0.03). In contrast, there was no difference in the frequency of VUS in *BRCA2* in cases versus controls (30 (1.2%) cases versus 39 (1.4%) controls (*P*=0.70); Supplementary Table 4 and 5).

One-hundred and twenty-five of 887 *a priori* moderate- to high-risk patients previously screened had *BRCA1* germline variants (9 large genomic rearrangement (LGR), 54 non-LGR deleterious variants and 63 missense, intronic, synonymous and inframe variants) and 242 had *BRCA2* germline variants (4 LGR, 49 non-LGR variants and 191 missense, intronic, synonymous and inframe variants). Of these, 98 *BRCA1* and 221 *BRCA2* variants were detected using this amplicon-based method, giving a sensitivity of 89% (95% CI: 86-92%, not inclusive of LGR). When examined, the variants missed by the amplicon sequencing method all showed preferential amplification of the wild-type allele (and hence were excluded due to high allelic imbalance) or had low amplicon coverage. Sensitivity for non-LGR deleterious variants was similar (90%; 95% CI: 85-96%) with 49 of 54 *BRCA1* and 44 of 49 *BRCA2* deleterious variants detected.

Types and spectrum of deleterious variants

Ninety-seven distinct deleterious variants (41 *BRCA1* and 56 *BRCA2*) and 11 distinct deleterious variants (5 *BRCA1* and 6 *BRCA2*) were identified in breast cancer cases and control subjects, respectively (Supplementary Tables 6-9). Notable recurrent variants were *BRCA1* c.68_69delAG, *BRCA1* c.2635G>T, and *BRCA2* c.262_263CT. *BRCA1* c.68_69delAG was observed exclusively in the Indians and constituted 4 of 17 (24%) of *BRCA1* deleterious mutations reported in Indian breast cancer cases. *BRCA1* c.2635G>T, a reported mutation among Southern Chinese

[21], was identified in two Chinese and one Malay breast cancer cases. Interestingly, principal component analysis derived from previous genome-wide genotyping data suggested that this Malay individual is of mixed Chinese and Malay descent (data not shown).[22] *BRCA2* c.262_263CT contributed 7 of 16 (44%) of *BRCA2* variants found in the Malay breast cancer patients and 1 in 2 (50%) *BRCA2* variants in Malay control subjects.

Clinicopathological characteristics of deleterious variant carriers

BRCA1 and *BRCA2* carriers were more likely to be diagnosed at a younger age compared to non-carriers (Table 4; mean ages at diagnosis 41, 46, and 50 years old respectively). While 49% of breast cancer patients were diagnosed before the age of 50, 72% of *BRCA1* and *BRCA2* carriers were diagnosed before the age of 50 (78% and 66% for *BRCA1* and *BRCA2*, respectively). *BRCA1* and *BRCA2* carriers were also significantly more likely to have family history of breast or ovarian cancer, and high grade tumours (grade III). In addition, *BRCA1* carriers were more likely to have bilateral breast cancer, personal history of ovarian cancer, and TNBC, whereas *BRCA2* carriers were more likely to have ER+ breast cancers, HER2- breast cancer and later stage of breast cancer presentation (stage IV). Further comparison of the clinical and pathological characteristics of *BRCA1* and *BRCA2* carriers showed no differences in these variables among the different ethnic groups (Supplementary Table 10).

Predictive value of testing guidelines

In order to determine the appropriateness of using age of onset, family history, and pathological features of breast cancer to identify women who may benefit most from

genetic testing, we determined the proportion of women and carriers who fulfilled the criteria for the NCCN Genetic/Familial High-Risk Assessment: Breast and Ovarian (Version 2.2017) and compared it with those who fulfilled the criteria for MyCPG for *BRCA1* and *BRCA2* testing. Both criteria included women with breast and ovarian cancer, bilateral breast cancer under the age of 50, male breast cancer and strong first degree relative with breast cancer. However, the criteria differ in age of primary breast cancer (≤45 versus ≤35), age of onset of triple negative breast cancer (≤60 versus ≤50) and the significance of family history of breast and other cancers. In the present study, 46% of breast cancer patients, 91% of *BRCA1* carriers and 71% of *BRCA2* carriers fulfilled the NCCN criteria, whereas 24% of breast cancer patients, 73% of *BRCA1* carriers and 50% of *BRCA2* carriers fulfilled the MyCPG criteria.

DISCUSSION

The prevalence of *BRCA1* and *BRCA2* deleterious variant carriers among Asian breast cancer cases has hitherto been largely investigated in *a priori* high-risk cohorts selected on the basis of age of diagnosis, family history of breast and ovarian cancer, and to a limited extent, pathological features of the cancers.[2] To the best of our knowledge, this is the largest study involving full exon screening of *BRCA1* and *BRCA2* in an unselected series of Asian breast cancer patients. We found *BRCA1* and *BRCA2* deleterious variants in 4.7% (95% CI: 3.9-5.5%) of breast cancer patients in this unselected hospital-based series, with the frequencies of *BRCA1* and *BRCA2* deleterious variants being similar. Comparison with previous clinical testing, including analysis of LGR, indicate a sensitivity of 90%, suggesting that the true prevalence would be ~5-6%.

The population frequencies of *BRCA1* and *BRCA2* deleterious variant carriers in the controls were similar to that observed in the Exome Aggregation Consortium East Asians, at approximately 0.2% for each gene. Our results were also consistent with previous estimates of 0.4% *BRCA1* and *BRCA2* mutation carrier frequency in Caucasian population.[23 24] The estimated breast cancer odds ratios associated with *BRCA1* and *BRCA2* deleterious variants (12.6 for both genes) were similar to those estimated in European populations.[25] These results suggest that *BRCA1* and *BRCA2* mutations are associated with similar relative risks in Asian and European populations, which would imply that the absolute risk of breast cancer in carriers would be lower in Asian women. However, the OR estimates have wide confidence limits, and larger studies will be needed to provide more precise estimates.

Consistent with previous studies,[5 6 26 27] we show that carriers of both *BRCA1* and *BRCA2* deleterious variants were more likely than non-carriers to be diagnosed at a younger age, have family history of breast or ovarian cancer and high tumour grade. In addition, bilateral breast cancer, personal history of ovarian cancer, and TNBC pathology were significantly associated with *BRCA1* deleterious variant carriers.

Full exon sequencing on an unselected series of breast cancer patients allowed us to evaluate how often *BRCA1* and *BRCA2* deleterious variant carriers might be missed in clinical practice in a typical resource-constrained Asian country such as Malaysia. Using the more stringent MyCPG genetic testing criteria, only 24% of breast cancer patients would be offered genetic counselling, but 40% of deleterious variant carriers would be missed. On the other hand, using the NCCN genetic testing criteria, 80% of deleterious variant carriers fulfilled the criteria and

would therefore be offered genetic counselling, but nearly half (46%) of all breast cancer patients would also need genetic counselling, making this a costly and potentially unaffordable risk-stratified management approach.

Notably, both NCCN genetic testing criteria and current risk prediction model underestimated *BRCA2* more significantly than *BRCA1* carriers. In this study, NCCN referral guidelines underestimated by 3-fold *BRCA2* carriers compared to *BRCA1* (29% vs. 9%). The underdetection of *BRCA1* and *BRCA2* carriers by current genetic testing guidelines and risk prediction models may be accounted by the lower absolute risks associated with *BRCA1* and *BRCA2* mutations in Asians compared to Caucasians,[28-30] the higher *BRCA2*:*BRCA1* mutation ratio in Asian breast cancer patients to that of Caucasian[2 5 6 26 31-33] compounded by under-reporting of family history of breast cancer cases in Asian settings,[7 34] and lower population incidence rates of breast cancer in Asian compared to Caucasian populations.[35] This highlights a need for additional biomarkers or methods to identify Asian women who would benefit from genetic counselling and genetic testing, particularly in families with insignificant family history from resource-constrained settings.

With the availability of Asian-specific estimates of *BRCA1* and *BRCA2* carrier prevalence in unselected breast cancer patients and unaffected population, and the risk estimates conferred by these genes, these may guide modifications to existing models and testing guidelines, or development of novel ones, to predict *BRCA1* and *BRCA2* carriers more accurately in Asian individuals.[36 37]

One limitation of our study is that LGRs were not included because MLPA was not performed in all breast cancer patients. Furthermore, some carriers may have been missed due to the sensitivity of our amplicon-based sequencing approach.

CONCLUSION

Five percent of unselected Asian breast cancer patients are carriers of germline deleterious variants in *BRCA1* or *BRCA2* and approximately 80% of carriers would have been offered genetic counselling based on current NCCN screening criteria. Our study provides the foundation for developing risk assessment tools for the Asian population, and highlights the need for cost-effective strategies to triage women for genetic counselling and testing in low resource settings.

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CONTRIBUTORS
Conception and design: Soo Hwang Teo, Douglas F Easton
Acquisition of data, and analysis and interpretation of data: Wei Xiong Wen, Jamie Allen, Kah Nyin Lai, Shivaani Mariapun, Siti Norhidayu Hasan, Pei Sze Ng, Daphne Shin-Chi Lee, Sheau Yee Lee, Sook Yee Yoon, Joanna Lim, Shao Yan Lau, Brennan Decker, Karen Pooley, Leila Dorling, Craig Luccarini, Caroline Baynes, Don Conroy, Patricia Harrington, Jacques Simard, Cheng Har Yip, Nur Aishah Mohd Taib, Weang Kee Ho, Antonis C Antoniou, Alison M Dunning,
Manuscript writing: Wei Xiong Wen, Soo Hwang Teo
Critical review and final approval of manuscript: All authors

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COMPETING INTEREST
None declared.

PATIENT CONSENT
Obtained.

ETHICS APPROVAL
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Table 1: Comparison of screening criteria between NCCN and MyCPG

Category	**NCCN and MyCPG**	
Personal history of cancer	Ovarian cancer	
	Bilateral breast cancer ≤50yo	
Family history of cancer	Male breast cancer	
	Ovarian cancer	
	Proband ≤50yo + ≥1 close blood relative with breast cancer	
Category	**NCCN**	**MyCPG**
Personal history of cancer	Primary breast cancer ≤45yo	Primary breast cancer ≤35yo
Family history of cancer	Proband any age + ≥1 close blood relative with breast cancer ≤50yo	Proband any age + ≥2 close blood relative with breast cancer ≤50yo
	Proband any age + ≥2 close blood relative with breast cancer	Proband any age + ≥3 close blood relative with breast cancer
	Proband ≤50yo + ≥1 close blood relative with pancreatic cancer	
	Proband any age+ ≥2 close blood relative with pancreatic cancer	
Pathology	TNBC ≤60yo	TNBC ≤50yo

NCCN: National Comprehensive Cancer Network
MyCPG: Malaysian Clinical Practice Guidelines

Table 2: Demographic characteristics and known breast cancer risk factors of study participants [a]

Category	Cases (N = 2,575)	Controls (N = 2,809)	*P* value
Demographic factors			
Age (year ± s.d.)	50.0 ± 10.8	52.6 ± 8.2	<0.001
Age distribution			
<30	67 (2.6)	0	<0.001
30-39	351 (13.9)	10 (0.4)	
40-49	821 (32.4)	1,101 (39.3)	
50-59	804 (31.7)	1,087 (38.8)	
≥60	490 (19.3)	607 (21.6)	
Ethnicity			
Chinese	1,726 (67.0)	1,686 (60.0)	<0.001
Malay	490 (19.0)	547 (19.5)	
Indian	359 (13.9)	576 (20.5)	
Family history			
No. of first degree relatives with breast cancer			
0	2,224 (86.4)	2,454 (87.5)	0.061
1	309 (12.0)	304 (10.8)	
2	35 (1.4)	45 (1.6)	
3	7 (0.3)	1 (0.04)	
No. of second degree relatives with breast cancer			
0	2,322 (90.2)	2,640 (94.2)	<0.001
1	219 (8.5)	148 (5.3)	
2	30 (1.2)	14 (0.5)	
3	3 (0.1)	2 (0.1)	
4	1 (0.04)	0	

s.d: Standard deviation

[a] Unless otherwise specified, data are presented in no. (%); For each data type, the total number of subjects may differ because of missing or incomplete data.

Table 3: Mutation frequencies of *BRCA1* and *BRCA2* in breast cancer cases compared with population [a]

Class	Cases (N = 2,575)	Controls (N = 2,809)	ExAC EA (N = 4,327)	OR (95% CI)[b]	OR (95% CI)[c]
Non-carriers	2,412 (93.7%)	2,755 (98.1%)	4,259 (98.4%)	1.00 (reference)	1.00 (reference)
BRCA1					
Deleterious	55 (2.1)	5 (0.2)	7 (0.2)	12.6 (5.0-31.4)	13.9 (6.3-30.5)
VUS	12(0.5)	4 (0.1)	6 (0.1)	3.4 (1.1-10.6)	3.5 (1.3-9.4)
BRCA2					
Deleterious	66 (2.6)	6 (0.2)	9 (0.2)	12.6 (5.4-29.0)	12.9 (6.4-26.0)
VUS	30 (1.2)	39 (1.4)	46 (1.1)	0.9 (0.5-1.4)	1.2 (0.7-1.9)

95% CI: 95% Confidence interval; EA: East Asian; ExAC: Exome Aggregation Consortium; OR: Odds ratio; rMS: rare missense

[a] Unless otherwise specified, data are presented in no. (%)

[b] Cases vs. controls

[c] Cases vs. ExAC EA

Table 4: Association between *BRCA1* and *BRCA2* mutation status and clinicopathological characteristics [a]

Clinical variables	*BRCA1* carriers (N = 55)	*BRCA2* carriers (N = 66)	Non-carriers (N = 2,454)	*P* value [b]	*P* value [c]
Age (year ± s.d.)	40.8 ± 10.6	45.7 ± 10.8	50.3 ± 10.7	<0.001	0.001
Age distribution					
<30	9 (16.4)	2 (3.1)	56 (2.3)	<0.001	0.001
30-39	19 (34.5)	20 (30.8)	312 (12.9)		
40-49	15 (27.3)	21 (32.3)	785 (32.5)		
50-59	10 (18.2)	12 (18.5)	782 (32.4)		
≥60	2 (3.6)	10 (15.4)	478 (19.8)		
Family history of breast cancer up to first-degree					
No	37 (67.3)	47 (71.2)	2,143 (87.3)	<0.001	<0.001
Yes	18 (32.7)	19 (28.8)	311 (12.7)		
Family history of breast cancer up to second-degree					
No	32 (58.2)	41 (62.1)	1,952 (79.5)	<0.001	0.001
Yes	23 (41.8)	25 (37.9)	502 (20.5)		
Family history of ovarian cancer up to first-degree					
No	50 (90.9)	63 (95.5)	2,429 (99.0)	<0.001	0.007
Yes	5 (9.1)	3 (4.5)	25 (1.0)		
Family history of ovarian cancer up to second-degree					
No	49 (89.1)	63 (95.5)	2413 (98.3)	<0.001	0.078
Yes	6 (10.9)	3 (4.5)	41 (1.7)		
Bilateral breast cancer					
No	45 (81.8)	60 (90.9)	2,321 (94.6)	<0.001	0.197
Yes	10 (18.2)	6 (9.1)	133 (5.4)		
Ovarian cancer					
No	53 (96.4)	65 (98.5)	2,439 (99.4)	0.007	0.362
Yes	2 (3.6)	1 (1.5)	15 (0.6)		
Grade (%)					
I	1 (2.8)	0	236 (12.2)	<0.001	0.030
II	8 (22.2)	25 (52.1)	957 (49.4)		
III	27 (75.0)	23 (47.9)	746 (38.5)		
Lymph node					
Negative	30 (63.8)	24 (44.4)	1,241 (56.6)	0.320	0.076
Positive	17 (36.2)	30 (55.6)	953 (43.4)		
Stage					
I	10 (23.8)	12 (23.5)	614 (30.1)	0.311	<0.001
II	21 (50.0)	18 (35.3)	1,042 (51.1)		
III	10 (23.8)	13 (25.5)	291 (14.3)		
IV	1 (2.4)	8 (15.7)	93 (4.6)		
ER					
Negative	38 (80.9)	11 (19.6)	759 (33.6)	<0.001	0.028
Positive	9 (19.1)	45 (80.4)	1,497 (66.4)		
PR					
Negative	36 (83.7)	23 (44.2)	875 (43.0)	<0.001	0.857
Positive	7 (16.3)	29 (55.8)	1,161 (57.0)		

HER2					
Negative	43 (93.5)	44 (84.6)	1,512 (70.5)	0.001	0.027
Positive	3 (6.5)	8 (15.4)	632 (29.5)		
TNBC					
No	9 (22.0)	43 (87.8)	1,626 (82.9)	<0.001	0.369
Yes	32 (78.0)	6 (12.2)	336 (17.1)		
Ki-67					
Low	2 (33.3)	4 (57.1)	277 (64.1)	0.119	0.703
High	4 (66.7)	3 (42.9)	155 (35.9)		

s.d: Standard deviation

[a] Unless otherwise specified, data are presented in no. (%); For each data type, the total number of subjects may differ because of missing or incomplete data.

[b] *BRCA1* carriers vs. non-*BRCA1/2* carriers

[c] *BRCA2* carriers vs. non-*BRCA1/2* carriers

Supplementary Table 1: Exclusion criteria for breast cancer patients for this study

Exclusion criteria	N
Male breast cancer patients	7
Diagnosed with non-invasive breast cancer	149
Insufficient or low-quality genomic DNA samples	66
Ethnicity other than Chinese, Malay, or Indian	65
Nationality other than Malaysian	10
Relatives of proband	3
Duplicate records	2

Supplementary Table 2: Exclusion criteria for control subjects for this study

Exclusion criteria	N
Personal history of breast cancer	6
Insufficient or low-quality genomic DNA samples	7
Ethnicity other than Chinese, Malay, or Indian	177

Supplementary Table 3: Demographic characteristics and known breast cancer risk factors of study participants stratified by ethnicity [a]

Category	Chinese			Malay			Indian		
	Cases (N = 1,726)	Controls (N = 1,686)	*P* value	Cases (N = 490)	Controls (N = 547)	*P* value	Cases (N = 359)	Controls (N = 576)	*P* value
Demographic factors									
Age (year ± s.d.)	52.7 ± 8.3	50.6 ± 10.8	<0.001	51.6 ± 7.5	46.8 ± 10.3	<0.001	53.6 ± 8.2	51.5 ± 10.7	0.001
Age distribution									
<30	34 (2.0)	0	<0.001	25 (5.1)	0	<0.001	8 (2.2)	0	<0.001
30-39	219 (13.0)	5 (0.3)		93 (19.1)	3 (0.5)		39 (10.9)	2 (0.3)	
40-49	551 (32.6)	669 (39.7)		166 (34.1)	238 (43.6)		104 (29.1)	194 (33.7)	
50-59	532 (31.5)	636 (37.8)		148 (30.4)	211 (38.6)		124 (34.6)	240 (41.7)	
≥60	352 (20.9)	374 (22.2)		55 (11.3)	94 (17.2)		83 (23.2)	139 (24.2)	
Family history									
No. of first degree relatives with breast cancer									
0	1,483 (85.9)	1,448 (86.0)	0.037	430 (87.8)	503 (92.0)	0.112	311 (86.6)	503 (87.8)	0939
1	217 (12.6)	202 (12.0)		49 (10.0)	38 (6.9)		43 (12.0)	64 (11.2)	
2	21 (1.2)	34 (2.0)		10 (2.0)	6 (1.1)		4 (1.1)	5 (0.9)	
3	5 (0.3)	0		1 (0.2)	0		1 (0.3)	1 (0.2)	
No. of second degree relatives with breast cancer									
0	1,542 (89.3)	1,571 (93.3)	0.001	450 (91.8)	520 (95.1)	0.066	330 (91.9)	549 (95.8)	0.018
1	156 (9.0)	102 (6.1)		36 (7.3)	22 (4.0)		27 (7.5)	24 (4.2)	
2	24 (1.4)	9 (0.5)		4 (0.8)	5 (0.9)		2 (0.6)	0	
3	3 (0.2)	2 (0.1)		0	0		0	0	
4	1 (0.1)	0		0	0		0	0	

Reproductive risk factors									
Age at menarche (year ± s.d.)	12.9 ± 1.4	13.0 ± 1.5	0.028	13.0 ± 1.4	12.8 ± 1.4	0.012	12.8 ± 1.4	12.9 ± 1.4	0.205
Postmenopausal									
No	688 (45.0)	745 (44.3)	0.665	245 (56.1)	266 (48.7)	0.022	133 (39.9)	206 (35.8)	0.210
Yes	840 (55.0)	938 (55.7)		192 (43.9)	280 (51.3)		200 (60.1)	370 (64.2)	
Age at menopause (year ± s.d.) [b]	49.5 ± 4.6	49.5 ± 4.8	0.971	49.5 ± 4.5	49.5 ± 4.3	0.934	48.6 ± 5.4	48.8 ± 4.9	0.669
No. of live birth (year ± s.d.) [c]	2.6 ± 1.0	2.8 ± 1.3	<0.001	3.5 ± 1.4	3.4 ± 1.5	0.247	2.7 ± 1.1	3.0 ± 1.4	0.003
Age at first live birth (year ± s.d.) [c]	27.9 ± 4.6	26.7 ± 4.9	<0.001	26.1 ± 4.2	25.4 ± 4.3	0.019	26.6 ± 5.2	25.9 ± 5.2	0.071
Other risk factors									
Oral contraceptive [d]									
No	1,156 (72.2)	1,202 (71.6)	0.716	294 (63.0)	334 (61.1)	0.536	289 (82.6)	480 (83.3)	0.764
Yes	445 (27.8)	476 (28.4)		173 (37.0)	213 (38.9)		61 (17.4)	96 (16.7)	
Hormone replacement therapy [d]									
No	1,439 (91.7)	1,461 (87.1)	<0.001	407 (92.7)	477 (87.4)	0.006	312 (91.8)	491 (85.4)	0.004
Yes	131 (8.3)	217 (12.9)		32 (7.3)	69 (12.6)		28 (8.2)	84 (14.6)	

s.d.: Standard deviation

[a] Unless otherwise specified, data are presented in no. (%); For each data type, the total number of subjects may differ because of missing or incomplete data.

[b] Among postmenopausal women

[c] Among parous women

[d] Ever user

Supplementary Table 4: The spectrum of *BRCA1* VUS identified in Malaysian cases and controls

No.	AGVGD class	Nucleotide change	Amino acid change	N (Cases)				N (Controls)			
				Chinese	Malay	Indian	Total	Chinese	Malay	Indian	Total
1	C15	c.3724A>G	p.T1242A	-	1	-	1	-	-	-	-
2	C15	c.4185G>T	p.Q1395H	-	1	-	1	-	-	-	-
3	C15	c.5489C>A	p.A1830E	-	1	-	1	-	-	-	-
4	C25	c.5057A>G	p.H1686R	-	2	-	2	-	-	-	-
5	C25	c.2597G>A	p.R866H	-	-	-	-	-	-	1	1
6	C45	c.533T>A	p.V178D	-	1	-	1	-	1	-	1
7	C65	c.190T>C	p.C64R	1	-	-	1	-	-	-	-
8	C65	c.216C>G	p.S72R	-	1	-	1	-	2	-	2
9	C65	c.3649T>C	p.S1217P	2	-	-	2	-	-	-	-
10	C65	c.5072C>A	p.T1691K	2	-	-	2	-	-	-	-

Supplementary Table 5: The spectrum of *BRCA2* VUS identified in Malaysian cases and controls

No.	AGVGD class	Nucleotide change	Amino acid change	N (Cases)				N (Controls)			
				Chinese	Malay	Indian	Total	Chinese	Malay	Indian	Total
1	C15	c.3391A>G	p.R1131G	1	-	-	1	-	-	-	-
2	C15	c.3569G>T	p.R1190L	-	1	-	1	-	1	-	1
3	C15	c.8201C>T	p.P2734L	1	-	-	1	-	-	-	-
4	C15	c.8227G>T	p.G2743C	-	-	1	1	-	-	1	1
5	C15	c.5048A>T	p.Q1683L	-	-	-	-	-	-	1	1
6	C15	c.7547C>T	p.S2516F	-	-	-	-	-	-	1	1
7	C15	c.7928C>T	p.A2643V	-	-	-	-	1	-	-	1
8	C25	c.8527A>T	p.N2843Y	-	1	-	1	-	-	-	-
9	C25	c.9097A>C	p.T3033P	2	-	-	2	-	-	-	-
10	C25	c.9857T>A	p.I3286N	-	1	-	1	-	-	-	-
11	C25	c.572A>T	p.D191V	-	-	-	-	1	-	-	1
12	C25	c.6231G>C	p.K2077N	-	-	-	-	-	1	-	1
13	C55	c.5986G>A	p.A1996T	-	-	5	5	-	-	9	9
14	C55	c.7522G>A	p.G2508S	5	-	-	5	10	-	-	10
15	C55	c.9104A>G	p.Y3035C	3	-	-	3	1	-	-	1
16	C55	c.6182C>G	p.A2061G	-	-	-	-	1	-	-	1
17	C65	c.7631G>A	p.G2544D	-	2	-	2	-	3	-	3
18	C65	c.7787G>T	p.G2596V	-	1	-	1	-	-	-	-
19	C65	c.7915C>T	p.P2639S	-	-	1	1	-	-	-	-
20	C65	c.8702G>A	p.G2901D	4	-	1	5	6	-	-	6
21	C65	c.7796A>C	p.E2599A	-	-	-	-	1	-	-	1
22	C65	c.8524C>T	p.R2842C	-	-	-	-	1	-	-	1

Supplementary Table 6: The spectrum of *BRCA1* deleterious variants identified in Malaysian breast cancer cases

No.	Nucleotide change	Amino acid change	N			
			Chinese	Malay	Indian	Total
1	c.61delA	p.I21fs	-	-	1	1
2	c.66dupA	p.E23fs	1	-	1	2
3	c.68_69delAG	p.E23fs	-	-	4	4
4	c.115T>C	p.C39R	-	2	-	2
5	c.134+1G>T	-	1	-	-	1
6	c.134+2delT	-	1	-	-	1
7	c.135-1G>C	-	-	-	1	1
8	c.150delA	p.K50fs	-	-	1	1
9	c.213-12A>G	-	1	-	-	1
10	c.470_471delCT	p.S157*	2	-	-	2
11	c.505C>T	p.Q169*	-	1	-	1
12	c.594-2A>G	-	1	-	-	1
13	c.686_687delCT	p.S229*	-	1	-	1
14	c.726delT	p.S242fs	-	1	1	2
15	c.850C>T	p.Q284*	-	-	1	1
16	c.981_982delAT	p.C328*	1	-	-	1
17	c.1054G>T	p.E352*	-	-	1	1
18	c.1104delA	p.D369fs	1	-	-	1
19	c.1204G>T	p.E402*	-	2	-	2
20	c.1504_1508delTTAAA	p.L502fs	1	-	-	1
21	c.2070_2071delAA	p.R691fs	-	1	-	1
22	c.2635G>T	p.E879*	2	1	-	3
23	c.3008_3009delTT	p.F1003*	-	1	-	1
24	c.3288_3289delAA	p.L1098fs	-	1	-	1
25	c.3323_3326delTAAA	p.I1108fs	-	1	-	1
26	c.3424delG	p.A1142fs	1	-	-	1
27	c.3607C>T	p.R1203*	-	-	1	1
28	c.3770_3771delAG	p.E1257fs	2	-	-	2
29	c.3856delA	p.S1286fs	-	1	-	1
30	c.3869_3870delAA	p.K1290fs	-	1	-	1
31	c.4065_4068delTCAA	p.N1355fs	1	-	-	1
32	c.4148C>G	p.S1383*	2	-	-	2
33	c.4258C>T	p.Q1420*	1	-	-	1
34	c.4327C>T	p.R1443*	1	-	1	2
35	c.4562_4563insAGGAG	p.N1521fs	-	-	1	1
36	c.4760C>A	p.S1587*	-	-	1	1
37	c.5211_5212delAG	p.G1738fs	-	-	1	1
38	c.5251C>T	p.R1751*	-	-	1	1
39	c.5328dupC	p.T1777fs	-	2	-	2
40	c.5332+1G>A	-	1	-	-	1
41	c.5503C>T	p.R1835*	-	1	-	1

Supplementary Table 7: The spectrum of *BRCA2* deleterious variants identified in Malaysian breast cancer cases

No.	Nucleotide change	Amino acid change	N			
			Chinese	Malay	Indian	Total
1	c.-39-1_-39delGA	-	1	-	-	1
2	c.262_263delCT	p.L88fs	-	7	-	7
3	c.631+1G>A	-	1	-	-	1
4	c.774_775delAA	p.E260fs	1	-	-	1
5	c.809C>G	p.S270*	2	-	-	2
6	c.956dupA	p.N319fs	1	-	-	1
7	c.1773_1776delTTAT	p.I591fs	1	-	-	1
8	c.2471_2476delTAAATG	p.L824*	-	1	-	1
9	c.2595delA	p.E866fs	1	-	-	1
10	c.2612C>A	p.S871*	1	-	-	1
11	c.2808_2811delACAA	p.A938fs	2	-	-	2
12	c.2830A>T	p.K944*	1	-	-	1
13	c.3109C>T	p.Q1037*	1	-	-	1
14	c.3680_3681delTG	p.L1227fs	-	1	-	1
15	c.3847_3848delGT	p.V1283fs	1	-	-	1
16	c.3865_3868delAAAT	p.K1289fs	-	-	1	1
17	c.3922G>T	p.E1308*	1	-	-	1
18	c.3957_3958delTG	p.N1319fs	1	-	-	1
19	c.4003G>T	p.E1335*	-	-	1	1
20	c.4037_4038delCT	p.T1346fs	-	2	-	2
21	c.4467_4474delinsTGTTTTT	p.K1489fs	1	-	-	1
22	c.4525C>T	p.Q1509*	1	-	-	1
23	c.4872_4873delTG	p.E1625fs	1	-	-	1
24	c.5047C>T	p.Q1683*	-	-	1	1
25	c.5073dupA	p.W1692fs	1	-	-	1
26	c.5213_5216delCTTA	p.T1738fs	-	-	1	1
27	c.5576_5579delTTAA	p.I1859fs	1	-	-	1
28	c.5645C>A	p.S1882*	1	-	-	1
29	c.5681dupA	p.Y1894 *	1	-	-	1
30	c.5727_5728insG	p.D1910fs	-	-	1	1
31	c.5967dupA	p.D1990fs	-	2	-	2
32	c.6082_6086delGAAGA	p.E2028fs	-	-	1	1
33	c.6325_6326delGT	p.V2109*	1	-	-	1
34	c.6405_6409delCTTAA	p.N2135fs	1	-	-	1
35	c.6468_6469dupTC	p.Q2157fs	-	-	1	1
36	c.6541G>T	p.G2181*	-	1	-	1
37	c.6591_6592delTG	p.E2198fs	1	-	-	1
38	c.6673delA	p.T2225fs	1	-	-	1
39	c.7007G>T	p.R2336L	1	-	-	1
40	c.7379_7382delACAA	p.N2460fs	1	-	-	1
41	c.7467dupT	p.I2490fs	-	1	-	1

42	c.7558C>T	p.R2520*	1	-	-	1
43	c.7629T>G	p.Y2543*	1	-	-	1
44	c.7673_7674delAG	p.E2558fs	-	1	-	1
45	c.7976+1G>A	-	1	-	-	1
46	c.8023A>G	p.I2675V	1	-	-	1
47	c.8234_8237delTGAC	p.L2745fs	1	-	-	1
48	c.8869C>T	p.Q2957*	-	-	1	1
49	c.9097delA	p.T3033fs	1	-	-	1
50	c.9097dupA	p.T3033fs	1	-	-	1
51	c.9098_9099insA	p.Q3034fs	1	-	-	1
52	c.9271_9274dupGTCT	p.Y3092fs	-	-	1	1
53	c.9276T>G	p.Y3092*	-	-	1	1
54	c.9294C>G	p.Y3098*	1	-	-	1
55	c.9294C>A	p.Y3098*	1	-	-	1
56	c.9330dupT	p.E3111*	1	-	-	1

Supplementary Table 8: The spectrum of *BRCA1* deleterious variants identified in Malaysian controls

No.	Nucleotide change	Amino acid change	N			
			Chinese	Malay	Indian	Total
1	c.594-1G>T	-	-	-	1	1
2	c.3097G>T	p.E1033*	-	1	-	1
3 [a]	c.4327C>T	p.R1443*	-	-	1	1
4	c.4356delA	p.A1453fs	1	-	-	1
5	c.5335delC	p.Q1779fs	1	-	-	1

[a] Also detected in breast cancer cases

Supplementary Table 9: The spectrum of *BRCA2* deleterious variants identified in Malaysian controls

No.	Nucleotide change	Amino acid change	N			
			Chinese	Malay	Indian	Total
1 [a]	c.262_263delCT	p.L88fs	-	1	-	1
2	c.2677C>T	p.Q893*	1	-	-	1
3	c.2957dupA	p.N986fs	1	-	-	1
4	c.4793_4794delTC	p.L1598fs	-	1	-	1
5	c.7558C>T	p.R2520*	1	-	-	1
6	c.9117G>A	p.P3039P	1	-	-	1

[a] Also detected in breast cancer cases

Supplementary Table 10: Clinicopathological characteristics of *BRCA1* and *BRCA2* mutation carriers across ethnicities [a]

Clinical variables	Chinese	Malay	Indian	*P* value
BRCA1	N = 21	N = 17	N = 17	
Age (year ± s.d.)	41.3 ± 9.8	39.4 ± 12.0	41.7 ± 10.6	0.804
Age distribution				
<30	2 (9.5)	5 (29.4)	2 (11.8)	0.688
30-39	9 (42.9)	5 (29.4)	5 (29.4)	
40-49	6 (28.6)	3 (17.6)	6 (35.3)	
50-59	3 (14.3)	3 (17.6)	4 (23.5)	
≥60	1 (4.8)	1 (5.9)	0	
Family history of breast cancer up to first-degree				
No	13 (61.9)	14 (82.4)	10 (58.8)	0.275
Yes	8 (38.1)	3 (17.6)	7 (41.2)	
Family history of breast cancer up to second-degree				
No	11 (52.4)	12 (70.6)	9 (52.9)	0.459
Yes	10 (47.6)	5 (29.4)	8 (47.1)	
Family history of ovarian cancer up to first-degree				
No	18 (85.7)	16 (94.1)	16 (94.1)	0.574
Yes	3 (14.3)	1 (5.9)	1 (5.9)	
Family history of ovarian cancer up to second-degree				
No	18 (85.7)	15 (88.2)	16 (94.1)	0.704
Yes	3 (14.3)	2 (11.8)	1 (5.9)	
Bilateral breast cancer				
No	16 (76.2)	17 (100)	12 (70.6)	0.059
Yes	5 (23.8)	0	5 (29.4)	
Ovarian cancer				
No	21 (100)	17 (100)	15 (88.2)	0.098
Yes	0	0	2 (11.8)	
Grade (%)				
I	1 (7.1)	0	0	0.083
II	3 (21.4)	0	5 (45.5)	
III	10 (71.4)	11 (100)	6 (54.5)	
Lymph node				
Negative	13 (68.4)	9 (60.0)	8 (61.5)	0.861
Positive	6 (31.6)	6 (40.0)	5 (38.5)	
Stage				
I	4 (25.0)	2 (14.3)	4 (33.3)	0.605
II	9 (56.3)	6 (42.9)	6 (50.0)	
III	3 (18.8)	5 (35.7)	2 (16.7)	
IV	0	1 (7.1)	0	
ER				
Negative	15 (78.9)	12 (80.0)	11 (84.6)	0.918
Positive	4 (21.1)	3 (20.0)	2 (15.4)	
PR				
Negative	13 (81.3)	12 (80.0)	11 (91.7)	0.677
Positive	3 (18.8)	3 (20.0)	1 (8.3)	
HER2				
Negative	15 (88.2)	14 (93.3)	14 (100)	0.418

Positive	2 (11.8)	1 (6.7)	0	
TNBC				
No	3 (21.4)	4 (26.7)	2 (16.7)	0.822
Yes	11 (78.6)	11 (73.3)	10 (83.3)	
Ki-67				
Low	2 (40.0)	0	0	0.439
High	3 (60.0)	1 (100)	0	
BRCA2	N = 40	N = 16	N = 10	
Age (year ± s.d.)	45.6 ± 11.2	43.6 ± 10.0	49.8 ± 10.5	0.369
Age distribution				
<30	0	2 (12.5)	0	0.053
30-39	14 (35.9)	3 (18.8)	3 (30.0)	
40-49	11 (28.2)	7 (43.8)	3 (30.0)	
50-59	9 (23.1)	3 (18.8)	0	
≥60	5 (12.8)	1 (6.3)	4 (40.0)	
Family history of breast cancer up to first-degree				
No	31 (77.5)	8 (50.0)	8 (80.0)	0.097
Yes	9 (22.5)	8 (50.0)	2 (20.0)	
Family history of breast cancer up to second-degree				
No	27 (67.5)	7 (43.8)	7 (70.0)	0.218
Yes	13 (32.5)	9 (56.3)	3 (30.0)	
Family history of ovarian cancer up to first-degree				
No	39 (97.5)	16 (100)	8 (80.0)	0.036
Yes	1 (2.5)	0	2 (20.0)	
Family history of ovarian cancer up to second-degree				
No	39 (97.5)	16 (100)	8 (80.0)	0.036
Yes	1 (2.5)	0	2 (20.0)	
Bilateral breast cancer				
No	36 (90.0)	15 (93.8)	9 (90.0)	0.902
Yes	4 (10.0)	1 (6.3)	1 (10.0)	
Ovarian cancer				
No	40 (100)	16 (100)	9 (90.0)	0.058
Yes	0	0	1 (10.0)	
Grade (%)				
I	0	0	0	0.574
II	17 (53.1)	6 (60.0)	2 (33.3)	
III	15 (46.9)	4 (40.0)	4 (66.7)	
Lymph node				
Negative	17 (47.2)	4 (33.3)	3 (50.0)	0.675
Positive	19 (52.8)	8 (66.7)	3 (50.0)	
Stage				
I	8 (24.2)	4 (28.6)	0	0.679
II	11 (33.3)	5 (35.7)	2 (50.0)	
III	9 (27.3)	2 (14.3)	2 (50.0)	
IV	5 (15.2)	3 (21.4)	0	
ER				
Negative	7 (18.9)	2 (16.7)	2 (28.6)	0.805
Positive	30 (81.1)	10 (83.3)	5 (71.4)	
PR				

Negative	17 (48.6)	4 (36.4)	2 (33.3)	0.660
Positive	18 (51.4)	7 (63.6)	4 (66.7)	
HER2				
Negative	29 (82.9)	9 (81.8)	6 (100)	0.538
Positive	6 (17.1)	2 (18.2)	0	
TNBC				
No	28 (84.8)	10 (100)	5 (83.3)	0.414
Yes	5 (15.2)	0	1 (16.7)	
Ki-67				
Low	4 (57.1)	0	0	NA
High	3 (42.9)	0	0	

s.d.: Standard deviation

[a] Unless otherwise specified, data are presented in no. (%); For each data type, the total number of subjects may differ because of missing or incomplete data.