ORIGINAL ARTICLE

Inherited mutations in *BRCA1* and *BRCA2* in an unselected multiethnic cohort of Asian patients with breast cancer and healthy controls from Malaysia

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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 germ line variants in an unselected cohort of Asian patients with breast cancer and healthy controls.

Methods Germ line DNA from a hospital-based study of 2575 unselected patients with breast cancer and 2809 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 patients with breast cancer and five (0.18%) *BRCA1* and six (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 guidelines for genetic testing.

Conclusion Five per cent of unselected Asian patients with breast cancer carry deleterious variants in *BRCA1* or *BRCA2*. While 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.

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 patients with breast cancer selected on the basis of age, family history and, some studies, tumour subtype, such as oestrogen receptor negative or triple negative breast cancer (TNBC).[2] These studies have reported the prevalence of deleterious germ line variants in *BRCA1* and *BRCA2* among Asian high-risk patients with breast cancer is similar to that in other populations, ranging between 10% and 20%.[2–6] However, it is estimated that less than 1% of the 560 000 patients with breast cancer 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 multiethnic cohort of unselected patients with breast cancer 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 germ line variants in *BRCA1* and *BRCA2* and lays the foundation for developing risk assessment tools for Asian populations.

METHODS

Study populations

We included patients with breast cancer and control subjects who participated in the Malaysian Breast Cancer Genetic Study between October 2002 and March 2015. Incident and prevalent cases and controls were recruited from two hospitals: University Malaya Medical Centre and Sime Darby

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Medical Centre.[10 11] Of the 2870 patients with breast cancer and 2999 control subjects recruited, 2575 and 2809 cases and controls, respectively, were included in this study (see tables 1 and 2 in the online supplementary file 1 for exclusion criteria). Of these 2575 cases, 887 (34%) women were considered to be a priori high or moderate risk and had been previously tested for germ line alterations in *BRCA1* and *BRCA2* by Sanger sequencing and Multiplex Ligation-dependent Probe Amplification (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, California, USA) 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 classified as Class 4 or Class 5 according to BRCA Mutation Database (http://arup.utah.edu/database/BRCA/) or Leiden Open Variation Database were considered deleterious, except for variants located at the C-terminal of *BRCA1* and *BRCA2* (amino acid position 1856–1863 and 3326–3385, 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 Next generation sequencing data only. Carriers of large genomic rearrangement (LGR), non-LGR deleterious variants and variants of unknown significance (VUS) previously identified but not detected in this sequencing study were considered as non-carriers. Categorical and continuous variables were compared using χ^2 test and t-test, respectively. Statistical tests were considered significant based on two-sided hypothesis tests with $p<0.05$.

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

The NCCN guidelines V.1.2017 and Malaysian Clinical Practice Guidelines (MyCPG)[9] for genetic testing of *BRCA1* and *BRCA2* for BRCA-related breast and ovarian cancer syndrome were used to identify patients with breast cancer 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 table 3 in the online supplementary file 1. Approximately two-thirds of cases and controls were of Chinese ancestry. Patients with breast cancer 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 2575 patients with breast cancer, 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% vs 1.2%) but less common

Table 1 Comparison of screening criteria between NCCN and MyCPG

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

MyCPG, Malaysian Clinical Practice Guidelines; NCCN, National Comprehensive Cancer Network; TNBC, triple negative breast cancer.

Table 2 Demographic characteristics and known breast cancer risk factors of study participants*

Category	Cases (n=2575)	Controls (n=2809)	p Value
Demographic factors			
Age (year±SD)	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)	1101 (39.3)	
50–59	804 (31.7)	1087 (38.8)	
≥60	490 (19.3)	607 (21.6)	
Ethnicity			
Chinese	1726 (67.0)	1686 (60.0)	<0.001
Malay	490 (19.0)	547 (19.5)	
Indian	359 (13.9)	576 (20.5)	
Family history			
Number of first-degree relatives with breast cancer			
0	2224 (86.4)	2454 (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)	
Number of second-degree relatives with breast cancer			
0	2322 (90.2)	2640 (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	

*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.

in Indian patients (2.8% vs 5.0%), while the frequencies were similar in Malay patients (3.3% vs 3.5%; p<0.01 for difference in *BRCA1:BRCA2* ratio).

Of 2809 control subjects, five (0.18%) had deleterious variants in *BRCA1* and six (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 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 with control subjects, with estimated ORs for breast cancer of 12.6 (95% CI 5.0 to 31.4) and 12.6 (95% CI 5.4 to 29.0) for *BRCA1* and *BRCA2*, respectively.

VUS in *BRCA1* were reported in 12 cases (0.47%) versus four 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); tables 4 and 5 in the online supplementary file 1).

One hundred and twenty-five of 887 a priori moderate-risk to high-risk patients previously screened had *BRCA1* germ line variants (nine LGR, 54 non-LGR deleterious variants and 63 missense, intronic, synonymous and inframe variants) and 242 had *BRCA2* germ line variants (four 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% to 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% to 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 (five *BRCA1* and 6 *BRCA2*) were identified in breast cancer cases and control subjects, respectively (online supplementary file 1). 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 patients with breast cancer and one in two (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 with non-carriers (table 4; mean ages at diagnosis 41, 46, and 50 years old, respectively). While 49% of patients with breast cancer were diagnosed before the age of 50, 72% of *BRCA1* and *BRCA2* carriers were diagnosed before the

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

Class	Cases (n=2575)	Controls (n=2809)	ExAC EA (n=4327)	OR (95% CI)†	OR (95% CI)‡
Non-carriers	2412 (93.7%)	2755 (98.1%)	4259 (98.4%)	1.00 (reference)	1.00 (reference)
BRCA1					
Deleterious	55 (2.1)	5 (0.2)	7 (0.2)	12.6 (5.0 to 31.4)	13.9 (6.3 to 30.5)
VUS	12 (0.5)	4 (0.1)	6 (0.1)	3.4 (1.1 to 10.6)	3.5 (1.3 to 9.4)
BRCA2					
Deleterious	66 (2.6)	6 (0.2)	9 (0.2)	12.6 (5.4 to 29.0)	12.9 (6.4 to 26.0)
VUS	30 (1.2)	39 (1.4)	46 (1.1)	0.9 (0.5 to 1.4)	1.2 (0.7 to 1.9)

*Unless otherwise specified, data are presented in no. (%)
†Cases versus controls.
‡Cases versus ExAC EA.
EA: East Asian; ExAC: Exome Aggregation Consortium; VUS, variants of unknown significance.

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=2454)	p Value†	p Value‡
Age (year±SD)	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)	2143 (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)	1952 (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)	2429 (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)	2321 (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)	2439 (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)	1241 (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)	1042 (51.1)		
III	10 (23.8)	13 (25.5)	291 (14.3)		
IV	1 (2.4)	8 (15.7)	93 (4.6)		
Oestrogen receptor					
Negative	38 (80.9)	11 (19.6)	759 (33.6)	<0.001	0.028
Positive	9 (19.1)	45 (80.4)	1497 (66.4)		
Progesterone receptor					
Negative	36 (83.7)	23 (44.2)	875 (43.0)	<0.001	0.857
Positive	7 (16.3)	29 (55.8)	1161 (57.0)		
Human epidermal growth factor 2					
Negative	43 (93.5)	44 (84.6)	1512 (70.5)	0.001	0.027
Positive	3 (6.5)	8 (15.4)	632 (29.5)		
Triple negative breast cancer					
No	9 (22.0)	43 (87.8)	1626 (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)		

*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.
†*BRCA1* carriers versus non-*BRCA1/2* carriers.
‡*BRCA2* carriers versus non-*BRCA1/2* carriers.

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 oestrogen receptors positive breast cancers, human epidermal growth factor receptor negative 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 (see table 10 in the online supplementary file 1).

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 (V.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 vs ≤35), age of onset of TNBC (≤60 vs ≤50) and the significance of family history of breast and other cancers. In the present study, 46% of patients with breast cancer, 91% of *BRCA1* carriers and 71% of *BRCA2* carriers fulfilled the NCCN criteria, whereas 24% of patients with breast cancer, 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 patients with breast cancer. We found *BRCA1* and *BRCA2* deleterious variants in 4.7% (95% CI 3.9% to 5.5%) of patients with breast cancer 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, indicates a sensitivity of 90%, suggesting that the true prevalence would be approximately 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 ORs 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 patients with breast cancer 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 patients with breast cancer 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 patients with breast cancer 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 threefold *BRCA2* carriers compared with *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 with Caucasians,[28–30] the higher *BRCA2:BRCA1* mutation ratio in Asian patients with breast cancer 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 with 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 patients with breast cancer 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 patients with breast cancer. Furthermore, some carriers may have been missed due to the sensitivity of our amplicon-based sequencing approach.

CONCLUSION

Five per cent of unselected Asian patients with breast cancer are carriers of germ line 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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