Breast Cancer Res Treat (2017) 165:687–697
DOI 10.1007/s10549-017-4356-8

EPIDEMIOLOGY

Prevalence and spectrum of germline rare variants in *BRCA1/2* and *PALB2* among breast cancer cases in Sarawak, Malaysia

Xiaohong R. Yang[1] · Beena C. R. Devi[2] · Hyuna Sung[1] · Jennifer Guida[1] · Eliseos J. Mucaki[3] · Yanzi Xiao[1] · Ana Best[1] · Lisa Garland[4] · Yi Xie[1] · Nan Hu[1] · Maria Rodriguez-Herrera[1] · Chaoyu Wang[1] · Kristine Jones[4] · Wen Luo[4] · Belynda Hicks[4] · Tieng Swee Tang[2] · Karobi Moitra[1,5] · Peter K. Rogan[3] · Michael Dean[1]

Received: 17 June 2017 / Accepted: 23 June 2017 / Published online: 29 June 2017

Abstract
Purpose To characterize the spectrum of germline mutations in *BRCA1*, *BRCA2*, and *PALB2* in population-based unselected breast cancer cases in an Asian population.
Methods Germline DNA from 467 breast cancer patients in Sarawak General Hospital, Malaysia, where 93% of the breast cancer patients in Sarawak are treated, was sequenced for the entire coding region of *BRCA1*; *BRCA2*; *PALB2*; Exons 6, 7, and 8 of *TP53*; and Exons 7 and 8 of *PTEN*. Pathogenic variants included known pathogenic variants in ClinVar, loss of function variants, and variants that disrupt splice site.
Results We found 27 pathogenic variants (11 *BRCA1*, 10 *BRCA2*, 4 *PALB2*, and 2 *TP53*) in 34 patients, which gave a prevalence of germline mutations of 2.8, 3.23, and 0.86% for *BRCA1*, *BRCA2*, and *PALB2*, respectively. Compared to mutation non-carriers, *BRCA1* mutation carriers were more likely to have an earlier age at onset, triple-negative subtype, and lower body mass index, whereas *BRCA2* mutation carriers were more likely to have a positive family history. Mutation carrier cases had worse survival compared to non-carriers; however, the association was mostly driven by stage and tumor subtype. We also identified 19 variants of unknown significance, and some of them were predicted to alter splicing or transcription factor binding sites.
Conclusion Our data provide insight into the genetics of breast cancer in this understudied group and suggest the need for modifying genetic testing guidelines for this population with a much younger age at diagnosis and more limited resources compared with Caucasian populations.

Keywords Breast cancer · Germline mutation · *BRCA1/2* · *PALB2* · Malaysia

Electronic supplementary material The online version of this article (doi:10.1007/s10549-017-4356-8) contains supplementary material, which is available to authorized users.

✉ Xiaohong R. Yang
royang@mail.nih.gov

[1] Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, NCI/NIH, Bethesda, Rockville, MD, USA

[2] Department of Radiotherapy, Oncology and Palliative Care, Sarawak General Hospital, Kuching, Sarawak, Malaysia

[3] Department of Biochemistry, Schulich School of Medicine and Dentistry, University of Western Ontario, London, ON, Canada

[4] Cancer Genomics Research Laboratory, Leidos Biomedical Research, Frederick National Laboratory for Cancer Research, Frederick, MD, USA

[5] Department of Biology, Trinity Washington University, Washington, DC, USA

Introduction

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death among females worldwide [1]. Incidence rates of breast cancer vary across the globe and are likely due to differences in the reproductive patterns, lifestyle/dietary factors, genetic susceptibility, and differences in detection methods [2]. The incidence of breast cancer may also vary within geographic locations and ethnic groups [3]. In general, the highest risks/rates occur in the more economically developed regions of the world and among non-Hispanic Caucasian women. The incidence for invasive female breast cancer in Asia is much lower than in North America and Europe; however, rates

have been increasing rapidly in many Asian populations [4]. Although the adoption of a Westernized lifestyle has been proposed to account for much of the rate increase, Asian women have a distinct profile of breast cancer that differs from that seen in Western populations, such as an earlier age at onset and a higher frequency of more aggressive tumors [5]. In addition, the prevalence of some known breast cancer susceptibility loci also varies in Western and Asian populations [6].

Germline mutations in the *BRCA1* and *BRCA2* genes result in predisposition to breast and ovarian cancer; carriers of germline mutations in either one of these genes have a 47–55% probability of developing breast cancer and a 17–39% risk of ovarian cancer by the age 70 years [7]. Breast cancer patients carrying inherited mutations in these genes, especially *BRCA1*, are more likely to be diagnosed at young age, have a family history of breast cancer, and develop triple-negative (estrogen receptor [ER]-negative, progesterone receptor [PR]-negative, and human epidermal growth factor receptor 2 [HER2]-negative) tumors [8, 9]. In addition, the spectrum of *BRCA* mutations varies depending on geographic origin, population, and ethnic group [10, 11]. Knowledge of *BRCA* mutation status for women with newly diagnosed breast cancer may influence the clinical management and immediate treatment recommendations, such as performing prophylactic mastectomy to reduce the risk of contralateral breast cancer and using poly ADP ribose polymerase (PARP) inhibitors, which are particularly effective in *BRCA* mutation carriers [12]. However, the prevalence of *BRCA* mutations and clinical characteristics associated with these mutations in non-Caucasian populations have been poorly characterized, especially in population-based settings. Further, studies on germline variants in other breast cancer genes are more limited.

In this study, we intend to determine the frequency of germline mutations in *BRCA1*, *BRCA2*, and *PALB2* in a nearly population-based unselected series of breast cancer patients and to assess their relationship with clinical features and prognosis in Sarawak, Malaysia. Sarawak is the largest state in Malaysia, which maintains a certain level of autonomy in administration, immigration, and judiciary, differentiating it from the Malaysian Peninsula states. Sarawak comprises multiple ethnic groups including natives of Borneo (51%, comprising 27 ethnic subgroups), Malays (23%), and Chinese (29%) [13]. The breast cancer incidence rate in Sarawak (age-standardized rate [ASR], 22 per 100,000 women) is much lower compared to Western Europe (ASR, 96) and the incidence varies by ethnicity, with the highest rate observed in Chinese (ASR: 36.7) followed by Malays (ASR, 26.5) and natives (ASR, 10.6) [14, 15].

Materials and methods

Study population

The study population includes invasive breast cancer cases diagnosed and treated from 2010 to 2015 in the Department of Radiotherapy, Oncology and Palliative Care, Sarawak General Hospital, Malaysia, where ~93% of all breast cancer cases in Sarawak are treated [15]. Tumor characteristics including histology, grade, size, nodal status, and receptor status were extracted from the pathology report. Data on breast cancer risk factors were obtained through a detailed questionnaire. Active clinical follow-up was conducted for all cases with the annotation of treatment (yes/no for hormone, radiation, and chemo therapies), relapse, and survival status. Among 1700 patients who were treated during this period of time, saliva samples were collected from 480 patients. The project was approved by the Ethics Committee of the National Institute of Health, Malaysia, and exempted from review by the National Institutes of Health (NIH) Office of Human Subject Research Protections since it did not involve the use of personal identifying information (OHSRP number: 5410).

Breast cancer subtypes

Estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2) expression were assessed with immunohistochemical (IHC) staining of formalin-fixed, paraffin-embedded tissue sections, while HER2 expression was further determined by fluorescence in situ hybridization (FISH), as previously described [16]. Breast cancer subtypes were defined as ER-positive or PR-positive/HER2-negative (ER+ or PR+/HER2−, luminal A), ER+ or PR+/HER2+ (luminal B), ER−/PR−/HER2+ (HER2-enriched), and ER−/PR−/HER2− (triple-negative, TN).

Sequencing

Saliva samples were collected using Oragene™ DNA Self-Collection Kit (DNA Genotek), and genomic DNA was purified according to manufacturer's instructions and quantitated by Nanodrop. Patients with insufficient or low-quality DNA ($n = 13$) and missing clinical information ($n = 3$) were excluded from the further investigation. To determine the nature and frequency of mutations in the *BRCA1*, *BRCA2*, and *PALB2* genes, we performed targeted sequencing to an average depth of 250 reads on a total of 464 subjects using a custom-designed AmpliSeq panel including the entire coding region of *BRCA1*; *BRCA2*; *PALB2*; Exons 6, 7, and 8 of *TP53*; and Exons 7 and 8 of

PTEN. The sequencing and quality control measures were described previously in detail [17].

Variant annotation

Variants were identified and quality filtered with the Genome analysis toolkit (GATK) [18] and the Torrent Variant Caller (version 5.0-7) and annotated by Annovar. Common variants with frequency >1% in any public database (1000 Genome, Exome Sequencing Project [ESP], The Exome Aggregation Consortium [ExAC]) were excluded. Putative functional variants were annotated by reference to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/).

All rare variants were further analyzed using an information-theory-based method for in silico prioritization of variants to assess effects on DNA–protein and RNA–protein binding, including transcription factors, splicing factors, and other RNA binding proteins [19]. The Shannon Human Splicing Mutation Pipeline (http://www.mutationforecaster.com) was used to analyze variants for their potential effects on both natural and cryptic splicing [20]. The predicted information content is known as its R_i, which is a measure of binding site strength (in bits) [21]. Changes to splicing regulatory factor binding were also examined using information models described in Mucaki et al. [22]. To assess potential effects on transcription factor binding, a modified version of the Shannon pipeline was used which utilized transcription factor information models built from ENCODE ChIP-seq data sets [23]. RNA protein binding sites were analyzed using models obtained from the RNA-Binding Protein Database (BPDB; http://rbpdb.ccbr.utoronto.ca/) and the Catalog of Inferred Sequence Binding Preferences of RNA binding protein (CISBP-RNA; http://cisbp-rna.ccbr.utoronto.ca/). Furthermore, potential changes to mRNA stability due to variants found in untranslated regions were evaluated using SNPfold [24] and mFOLD [25].

Pathogenic variants included known pathogenic alleles in ClinVar and unreported loss of function variants (frameshift and stop-gain) or variants that alter the first or second base of the splice site. Variants of unknown significance (VUS) included variants that were designated as VUS by ClinVar, non-synonymous that have never been reported, and rare (absent in 1000 Genome) synonymous or intronic variants that were predicted to be deleterious. Other synonymous or intronic variants and variants classified as benign or likely benign by ClinVar were grouped into a single "benign" category.

Multiplex ligation-dependent probe amplification (MLPA)

A sample of 80 ng of genomic DNA was processed per the manufacturer's protocol (MRC-Holland, MLPA P002 *BRCA1* probemix) for MLPA and run on an ABI-3730X. Data files were processed by Coffalyser software and reactions included an artificial positive duplication controls for *BRCA* (MRC-Holland, Artificial Duplication DNA).

Statistical analyses

Chi-square, Fisher's exact test, or Mantel–Haenszel Chi-square test for categorical variables and ANOVA test for continuous variables were used to test the variation of patient characteristics by mutation status. Patients with pathogenic variants and VUS variants were separately compared to the reference group which consisted of carriers of benign variants and mutation non-carriers. Multivariable unconditional logistic regression was used to calculate the odds ratios (ORs) and 95% confidence intervals (95% CIs) for the associations between mutation status and patient characteristics including age at diagnosis (continuous), ethnicity (Chinese, Malay, Native; reference = Chinese), family history of breast cancer (yes vs. no), grade (poorly differentiated vs. well or moderately differentiated), tumor subtype (luminal A, ER+ or PR+/HER2+, HER2-enriched, and TN; reference = luminal A), body mass index (BMI ≥25 vs. <25 kg/m^2), parity (parity vs. nulliparity).

Overall survival was defined as the time between the date of diagnosis and either the date of death or the date at last follow-up (if death did not occur during the follow-up period). Cases with unknown vital status or follow-up duration ($n = 37$) were excluded from the analysis. The Kaplan–Meier method was used to assess overall survival among patients, stratified by mutation status. The log-rank test was used to compare survival curves between groups. A multivariable Cox-proportional hazard regression model was also used to test the differences in survival between mutation carriers and non-carriers with the adjustment of age, stage, tumor subtype, and treatments (endocrine, radiation, and chemo therapies; yes vs. no). Sensitivity analyses excluding patients who were followed up within 3 or 6 months and remained alive from the analysis did not lead to significant changes in results, and we therefore included all patients in the survival analyses. All statistical analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC).

Results

A total of 464 breast cancer patients with sequencing data were included in this analysis. The distribution of clinical characteristics and major breast cancer risk factors for women included in this analysis was similar to that for all other women treated in SGH during the same period of time ($n = 1700$; Supplementary Table 1).

Table 1 Pathogenic variants in *BRCA1, BRCA2, PALB2*, and *TP53* identified among unselected breast cancer patients in Sarawak, Malaysia

Chr	Position	dbSNP[a]	Ref	Var	Gene	Variant	Age	FHBC[b]	Subtype
chr17	41197802		C		*BRCA1*	c.5548delG	41	N	TN
chr17	41201209	rs80357590	G		*BRCA1*	c.5335delC	38	Y	TN
chr17	41201209	rs80357590	G		*BRCA1*	c.5335delC	34	Y	TN
chr17	41243941	rs62625308	G	A	*BRCA1*	R1203X	41	Y	TN
chr17	41244320	rs80357635	CT		*BRCA1*	c.3228_3229delAG	65	Y	Luminal A
chr17	41244334	rs80357923	G		*BRCA1*	c.3214delC	42	N	TN
chr17	41244334	rs80357923	G		*BRCA1*	c.3214delC	23	Y	TN
chr17	41245481		CTTTTA		*BRCA1*	S689X	37	N	Luminal A
chr17	41246407	rs876659327		C	*BRCA1*	c.1140dupG	44	N	TN
chr17	41247940		C	T	*BRCA1*	c.594 +1G > A	61	N	Luminal A
chr17	41258474	rs80357382	T	C	*BRCA1*	R71G	36	N	TN
chr17	41258503	rs80357093	C	T	*BRCA1*	C61Y	45	N	HER2-enriched
chr17	41267767	rs80356880	G	T	*BRCA1*	T37K	31	N	TN
chr13	32900701	rs80358810	G	A	*BRCA2*	W194X	54	N	Luminal A
chr13	32903605		T		*BRCA2*	c.657delT	43	N	Luminal A
chr13	32905127	rs80359659	GACA		*BRCA2*	c.755_758delACAG	42	UNK	Luminal A
chr13	32907379	rs80359303	AAAT		*BRCA2*	c.1763_1766delATAA	39	Y	TN
chr13	32907502	rs80359314		A	*BRCA2*	c.1888dupA	53	N	TN
chr13	32910932	rs397507627	C		*BRCA2*	c.2442delC	48	Y	TN
chr13	32911601	rs80358557	C	T	*BRCA2*	Q1037X	60	Y	TN
chr13	32913844	rs397507780	AC		*BRCA2*	c.5353_5354delAC	49	UNK	Luminal A
chr13	32913844	rs397507780	AC		*BRCA2*	c.5353_5354delAC	50	Y	Luminal A
chr13	32913844	rs397507780	AC		*BRCA2*	c.5353_5354delAC	45	N	Luminal A
chr13	32914069	rs80359520	AATT		*BRCA2*	c.5575_5578delATTA	47	N	TN
chr13	32953896	rs80359734	TGAG		*BRCA2*	c.8961_8964delGAGT	58	Y	Luminal A
chr13	32953896	rs80359734	TGAG		*BRCA2*	c.8961_8964delGAGT	48	N	Luminal A
chr13	32953896	rs80359734	TGAG		*BRCA2*	c.8961_8964delGAGT	47	N	Luminal A
chr13	32953896	rs80359734	TGAG		*BRCA2*	c.8961_8964delGAGT	38	Y	Luminal A
chr16	23635336		T		*PALB2*	c.2828delA	38	N	Luminal A
chr16	23641792		T	G	*PALB2*	c.1691-2A > G	49	N	TN
chr16	23646182		C	T	*PALB2*	c.1559 + 1G >A	24	N	HER2-enriched
chr16	23647116	rs180177091	G	A	*PALB2*	N251X	64	Y	TN
chr17	7577539	rs121912651	G	A	*TP53*	R254 W	34	Y	TN
chr17	7578475		G		*TP53*	c.455delC	45	N	Luminal A

[a] Those without dbSNP ID are novel variants

[b] FHBC: family history of breast cancer

Pathogenic variants

We identified 11 *BRCA1* pathogenic variants in 13 patients, 10 *BRCA2* pathogenic variants in 15 patients, 4 *PALB2* pathogenic variants in four patients, and 2 *TP53* pathogenic variants in two patients (Table 1; Fig. 1), which gave a prevalence of germline mutations of 2.8, 3.2, and 0.86% for *BRCA1*, *BRCA2*, and *PALB2*, respectively, among unselected breast cancer cases in Sarawak, Malaysia. All these variants were extremely rare in the general population (<0.05% in 1000 Genome, ESP, and ExAC). Only 4 of them were recurrent variants (c.3214delC and c.5335delC in *BRCA1*; c.5353_5354delAC and c.8961_8964delGAGT in *BRCA2*) and all four are known variants that were reported previously in other populations. The pathogenic mutations included 9 previously undescribed, four in *BRCA1*, 1 in *BRCA2*, 3 in *PALB2* and one in *TP53* (Table 1). Large deletions in *BRCA1* were not detected in any of the 150 patients we analyzed using MLPA (data not shown).

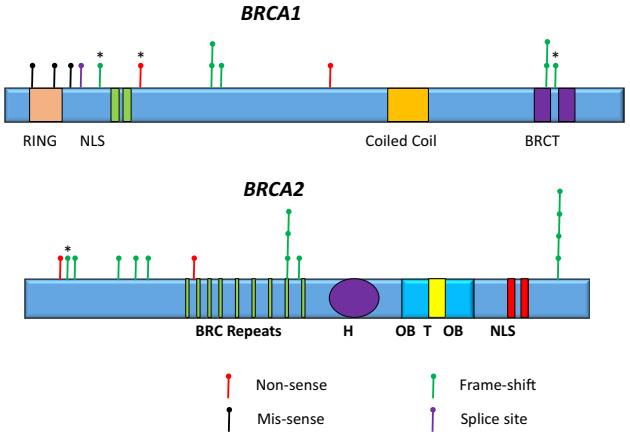

Fig. 1 Locations of *BRCA1/2* pathogenic variants identified among breast cancer cases in Sarawak, Malaysia. Novel variants are indicated by *asterisk*

Characteristics of pathogenic variant carriers

The average age of diagnosis among carrier cases of pathogenic variants was 44 years (range 23–65 years), eight years younger compared with non-carrier cases ($p = 0.0002$) (Table 2). Furthermore, cases with any pathogenic mutation were more likely to have a positive family history of breast cancer (41 vs. 21%), develop triple-negative (50 vs. 16%) or poorly differentiated (47 vs. 31%) tumors, and be nulliparous (32 vs. 19%). In contrast, these mutation carriers were less likely to have HER2-positive tumors (luminal B or HER2-enriched, 6 vs. 29%) and be overweight or obese (33 vs. 52%) (Table 2). There was no significant difference in mutation frequency across different ethnic groups. In the multivariable logistic regression model including age, ethnicity, tumor subtype, grade, family history of breast cancer, BMI, and parity, the associations remained significant for age (OR 0.94, 95% CI 0.90, 0.98; $p = 0.0051$) and tumor subtype ($OR_{TNvs.LuminalA} = 2.66$, 95% CI 1.08, 6.57; $p = 0.033$) and borderline significant for family history of breast cancer (OR 2.18, 95% CI 0.92, 5.15; $p = 0.076$) (Table 3).

When comparing carriers for *BRCA1* and *BRCA2* mutations separately to non-carriers, we found that the associations with age and triple-negative subtype were stronger for *BRCA1* mutations carriers, whereas the association with family history was stronger for *BRCA2* mutation carriers (Table 3; Supplementary Table 2). Interestingly, the association for BMI was only seen among *BRCA1* mutation carriers but not among *BRCA2* mutation carriers (Table 3).

Patients carrying pathogenic variants appeared to have worse survival compared with non-carriers ($P_{log\text{-}rank} = 0.11$) (Fig. 2); however, the association was mostly driven by tumor stage and subtype and became less significant after the adjustment of these variables and treatments (Hazard Ratio [HR] 1.88, 95% CI 0.52, 6.72; $p = 0.33$).

Variants of unknown significance (VUS)

In addition to pathogenic mutations, we also identified 20 variants whose pathogenic status could not be determined and were designated as VUS, 6 in *BRCA1*, 5 in *BRCA2*, 6 in *PALB2*, and 3 in *TP53* in 30 patients (Table 4). These variants were either designated VUS by clinical laboratories (ClinVar), or non-synonymous and have never been reported, or rare intronic variants with predicted deleterious effect. In aggregate, carriers of VUS were not significantly different compared with non-carriers in age of onset (OR 1.00, 95% CI 0.96, 1.04; p 0.92) or TN status (OR 0.81, 95% CI 0.27, 2.40; p 0.70); however, like pathogenic variant carriers, they were also less likely to have HER2-positive tumors (OR 0.25, 95% CI 0.068, 0.92; p 0.037) and more likely to have poorly differentiated tumors (OR 2.90, 95% CI 1.17, 7.19; $p = 0.021$) (Table 2). In addition, a subset of these VUS carriers were diagnosed with breast cancer before age 45 years; most of them had TN tumors, suggesting that some of these VUS variants might be pathogenic. Interestingly, the VUS carriers tended to have lower frequency of positive family history (OR 0.17, 95% CI 0.023, 1.32; $p = 0.091$) (Table 2).

In silico variant analysis using information theory

Three novel pathogenic variants abolish natural splice sites (*PALB2*:c.1691-2A > G, *PALB2*:c.1559 + 1G > A, and *BRCA1*:c.594 + 1G > A) (Supplementary Table 3). A known missense variant (rs80357382 [R71G]) simultaneously weakens *BRCA1* exon 3 natural donor (5.9 to 3.4 bits). ASSEDA predicts the activation of a cryptic site within this donor splice site which would result in a 22nt deletion (cryptic site shown to be used in *BRCA1* isoform NR_027676.1). This predicted splice form has been observed in Spanish familial breast cancer patients with the R71G mutation [26]. In addition, a rare intronic variant in *BRCA1* (g.41243354delAACTA) was flagged as a significant cryptic site, which increased the strength of a cryptic site from 1.2 to 4.2 bits (natural site is 2.9 bits, Supplementary Table 3). Other predicted changes involving cryptic splice sites were generally not deemed significant.

The impact of variants on splicing factor recognition sequences (SFRS) was also assessed with information theory-based models. *TP53* missense mutation (R254W) is deemed clinically significant in ClinVar. It is predicted to create a 4.7 bit hnRNP A1 site 42nt from the natural donor site of exon 6, which has been shown to induce exon

Table 2 Selected clinical characteristics and breast cancer risk factors by mutation class

	All ($n = 464$)		Pathogenic ($n = 34$)		VUS ($n = 29$)		Non-carriers ($n = 401$)		p^a	p^b
	n	%	*n*	%	*n*	%	*n*	%		
Age										
Mean (SD)	51.4	11.2	44.5	10.11	50.3	8.96	52.1	11.3	0.0002[c]	0.41[c]
20–40	64	13.8	11	32.4	3	10.3	50	12.5	0.0012	0.069
40–50	152	32.8	15	44.1	10	34.5	127	31.7		
50–60	141	30.4	4	11.8	14	48.3	123	30.7		
60+	107	23.1	4	11.8	2	6.9	101	25.2		
Vital status										
Allive	419	90.5	28	82.4	27	93.1	364	91.0		
Death	44	9.5	6	17.7	2	6.9	36	9.0		
Unknown	1						1			
Ethnicity										
Chinese	215	46.3	15	44.1	11	37.9	189	47.1	0.22	0.60
Malay	112	24.1	13	38.2	7	24.1	92	22.9		
Natives	126	27.2	6	17.7	10	34.5	110	27.4		
Others	11	2.4			1	3.5	10	2.5		
Family history										
No	360	78.4	19	59.4	27	96.4	314	78.7	0.012	0.025
Yes	99	21.6	13	40.6	1	3.6	85	21.3		
Missing	5		2		1		2			
ER										
Positive	298	65.2	15	44.1	18	64.3	265	67.1	0.0069	0.76
Negative	159	34.8	19	55.9	10	35.7	130	32.9		
Missing	7				1		6			
PR										
Positive	265	58.0	13	38.2	18	64.3	234	59.2	0.017	0.60
Negative	192	42.0	21	61.8	10	35.7	161	40.8		
Missing	7				1		6			
HER2										
Positive	118	26.2	2	5.9	3	11.1	113	29.1	0.0022	0.047
Negative	332	73.8	32	94.1	24	88.9	276	71.0		
Missing	14				2		12			
Subtype										
Luminal A	243	54.1	15	44.1	16	59.3	212	54.6	0.00002	0.12
Luminal B	64	14.3			1	3.7	63	16.2		
HER2-enriched	54	12.0	2	5.9	2	7.4	50	12.9		
TN	88	19.6	17	50.0	8	29.6	63	16.2		
Missing	15				2		13			
Stage										
0	11	2.4			1	3.6	10	2.5	0.79	0.68
1	67	14.7	6	17.7	6	21.4	55	13.9		
2	185	40.5	14	41.2	11	39.3	160	40.5		
3	157	34.4	10	29.4	9	32.1	138	34.9		
4	37	8.1	4	11.8	1	3.6	32	8.1		
Missing	7				1		6			
Grade										
Well differentiated	57	12.4			4	14.3	53	13.3	0.017	0.03
Moderately differentiated	249	54.0	18	52.9	9	32.1	222	55.6		

Table 2 continued

	All ($n = 464$)		Pathogenic ($n = 34$)		VUS ($n = 29$)		Non-carriers ($n = 401$)		p^a	p^b
	n	%	*n*	%	*n*	%	*n*	%		
Poorly differentiated	155	33.6	16	47.1	15	53.6	124	31.1		
Missing	3				1		2			
Chemotherapy										
No	102	22.5	4	11.8	8	27.6	90	23.0	0.19	0.57
Yes	352	77.5	30	88.2	21	72.4	301	77.0		
Missing	10						10			
Surgery										
No	36	7.9	2	5.9	4	13.8	30	7.7	0.99	0.28
Yes	418	92.1	32	94.1	25	86.2	361	92.3		
Missing	10						10			
Ratiotherapy										
No	107	25.0	5	15.6	5	19.2	97	26.2	0.19	0.50
Yes	321	75.0	27	84.4	21	80.8	273	73.8		
Missing	36		2		3		31			
BMI										
Mean (SD)	25.4	4.84	23.1	4.5	24.6	4.27	25.6	4.9	0.005[c]	0.28[c]
<23	148	32.3	16	48.5	11	39.3	121	30.5	0.13	0.66
23–25	77	16.8	6	18.2	3	10.7	68	17.1		
25–30	162	35.4	9	27.3	11	39.3	142	35.8		
>30	71	15.5	2	6.1	3	10.7	66	16.6		
Missing	6		1		1		4			
Age at menarche										
<13	136	29.4	11	32.4	9	31.0	116	29.1	0.69	0.82
13	160	34.6	13	38.2	11	37.9	136	34.1		
13+	166	35.9	10	29.4	9	31.0	147	36.8		
Missing	2						2			
Parity										
Nulliparous	94	20.3	11	32.4	6	20.7	77	19.2	0.067	0.84
1 or more children	370	79.7	23	67.7	23	79.3	324	80.8		
Age at pregnancy[d]										
<21	71	19.5	3	13.0	4	17.4	64	20.1	0.61	0.43
21–25	156	42.7	9	39.1	13	56.5	134	42.0		
26+	138	37.8	11	47.8	6	26.1	121	37.9		
Missing	5						5			
Breastfeeding[d]										
No	131	28.2	12	35.3	10	34.5	109	27.2	0.31	0.40
Yes	333	71.8	22	64.7	19	65.5	292	72.8		

[a] Comparison between pathogenic and non-carriers (including carriers of benign variants); Chi-Square test or Fisher's Exact test whichever appropriate

[b] Comparison between VUS and non-carriers (including carriers of benign variants); Chi-Square test or Fisher's Exact test whichever appropriate

[c] Student's *t* test

[d] Among parous women

skipping in other instances of inherited breast cancer [27]. Other pathogenic mutations were found to simultaneously alter SF binding sites; however their impact on phenotype cannot be discerned without additional functional evidence. There were 9 VUS which were predicted to alter the strengths of splicing factors (Supplementary Table 3).

Table 3 Associations between *BRCA1/2* mutations and patient characteristics and risk factors

	Overall			*BRCA1*			*BRCA2*		
	OR	95% CI	*p*	OR	95% CI	*p*	OR	95% CI	*p*
Age									
Per 5-year increase	**0.94**	**0.90–0.98**	**0.0051**	**0.93**	**0.87–0.99**	**0.028**	0.97	0.92–1.03	0.36
Ethnicity									
Chinese	Ref			Ref			Ref		
Malay	1.49	0.56–3.91	0.42	1.07	0.22–5.32	0.94	2.25	0.62–8.18	0.22
Natives	0.79	0.26–2.40	0.67	1.7	0.35–8.23	0.51	0.28	0.03–2.48	0.25
Tumor subtype									
Luminal A	Ref			Ref			Ref		
TN	**2.66**	**1.08–6.57**	**0.033**	**6.45**	**1.39–29.9**	**0.017**	2.13	0.61–7.45	0.24
Grade									
Well/moderately differentiated	Ref			Ref			Ref		
Poorly differentiated	1.48	0.62–3.54	0.38	1.23	0.31–4.92	0.77	1.03	0.27–3.85	0.97
Family history of breast cancer									
No	Ref								
Yes	2.18	0.92–5.15	0.076	2.29	0.59–8.83	0.23	**3.45**	**1.04–11.43**	**0.043**
Parity									
Nulliparous	Ref			Ref			Ref		
Parous	0.50	0.20–1.25	0.14	0.51	0.12–2.13	0.36	0.42	0.11–1.57	0.2
BMI									
≤25	Ref			Ref			Ref		
>25	0.53	0.23–1.27	0.15	**0.17**	**0.034–0.88**	**0.035**	1.43	0.43–4.72	0.56

Bold values indicate $p < 0.05$

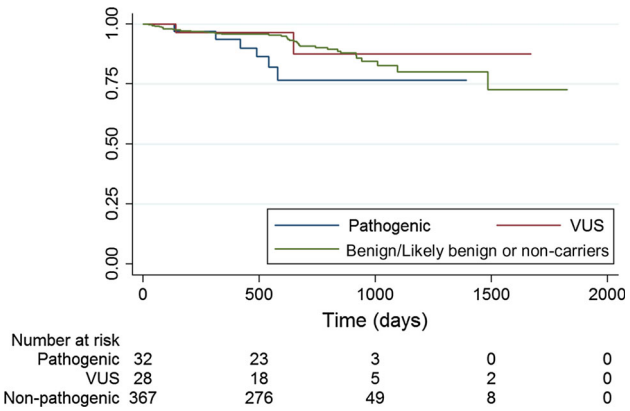

Fig. 2 Kaplan–Meier curve for overall survival among breast cancer cases in Sarawak, Malaysia, stratified by carrier status of carrying rare variants in *BRCA1*, *BRCA2*, *PALB2*, and *TP53*

Variants found either in the first exon or first intron of each gene were analyzed for their potential impact on transcription factor binding, resulting in three flagged VUS (Supplementary Table 3). A novel synonymous *TP53* mutation (T170T) found in two patients is predicted to abolish a pre-existing ETV1 while simultaneously creating a TCF7L2 site (the latter has been shown to bind to *TP53* promoter based on ENCODE data [28]). A novel variant identified in IVS1 of *BRCA1* (g.41275988G > A) was also predicted to create a 4.7 bit ETV6 site, a transcriptional repressor.

Discussion

While breast cancer is the most common cause of cancer incidence and mortality for women worldwide, the knowledge of breast cancer epidemiology and genetics in populations outside North America and Europe is limited. In this study, we conducted a comprehensive analysis of *BRCA1*, *BRCA2*, and *PALB2* mutations in a population-based study of breast cancer in the Sarawak region of Malaysia. We showed that the prevalence of pathogenic mutations was 2.8, 3.2, and 0.86%, respectively, for *BRCA1*, *BRCA2*, and *PALB2*, which is similar to those reported among unselected patients in other populations [29]. We also documented the spectrum of mutations and demonstrated that, as in other populations, pathogenic mutations were associated with earlier age of diagnosis, triple-negative tumors, and positive family history.

Table 4 Variants of unknown significance (VUS) identified among unselected breast cancer patients in Sarawak, Malaysia

Chr	Position	dbSNP	Ref	Var	Gene	Variant	Clinvar	Age	FHBC[a]	Subtype
chr17	41203101		G	A	*BRCA1*	P1771S	VUS	58	N	TN
chr17	41234479		T	C	*BRCA1*	I1433M	VUS	50	N	HER2-enriched
Chr17	41243354		AACTA		*BRCA1*	Intron, rare				
chr17	41244100	rs80357272	G	A	*BRCA1*	P1150S	VUS	48	N	HER2-enriched
chr17	41245262	rs273898682	T	A	*BRCA1*	A762S	VUS	63	N	Luminal A
chr17	41275988		G	A	*BRCA1*	Intron, Rare		53	N	Luminal A
chr17	41275988		G	A	*BRCA1*	Intron, Rare		47	N	Luminal A
chr17	41275988		G	A	*BRCA1*	Intron, Rare		53	N	Luminal B
chr17	41275988		G	A	*BRCA1*	Intron, Rare		38	N	TN
chr13	32899216		G	C	*BRCA2*	R107T	VUS	56	N	Luminal A
chr13	32900718	rs587781402	C	T	*BRCA2*	T200I	VUS	41	N	Luminal A
chr13	32907093	rs786202916	C	T	*BRCA2*	P493L	VUS	59	N	TN
chr13	32907093	rs786202916	C	T	*BRCA2*	P493L	VUS	53	N	Luminal A
chr13	32931892	rs397507926	G	A	*BRCA2*	G2544D	VUS	41	N	TN
chr13	32944554		A	C	*BRCA2*	T2783P	VUS	58		TN
chr16	23632742	rs183489969	C	G	*PALB2*	E1018D	VUS	74	N	Luminal A
chr16	23632742	rs183489969	C	G	*PALB2*	E1018D	VUS	44	N	
chr16	23632742	rs183489969	C	G	*PALB2*	E1018D	VUS	41	N	
chr16	23632742	rs183489969	C	G	*PALB2*	E1018D	VUS	59	N	Luminal A
chr16	23641379	rs780397699	G	C	*PALB2*	S699C	VUS	40	N	Luminal A
chr16	23641520	rs587781818	C	T	*PALB2*	S652N	VUS	59	N	Luminal A
chr16	23646141	rs568935449		TCA	*PALB2*	Rare Inton		50	Y	Luminal A
chr16	23646436		G	A	*PALB2*	T477T	VUS	55	N	Luminal A
chr16	23646436		G	A	*PALB2*	T477T	VUS	36	N	Luminal A
chr16	23646436		G	A	*PALB2*	T477T	VUS	47	N	Luminal A
chr16	23647143		A	C	*PALB2*	F242V	VUS	59	N	Luminal A
chr17	7578129		C	A	*TP53*	UTR3		48	N	Luminal A
chr17	7578420		C	T	*TP53*	T170T		41	N	TN
chr17	7578420		C	T	*TP53*	T170T		50	N	TN
chr17	7578470	rs137852789	C	T	*TP53*	G154S	VUS	38	N	TN

[a] FHBC: family history of breast cancer

Consistent with what was reported in other populations, *BRCA1* mutation carriers were more likely to have younger ages at diagnosis and develop TN tumors compared with *BRCA2* mutation carriers among Malaysian breast cancer cases in Sarawak. Interestingly, we found that *BRCA2* mutation carriers seemed to have a stronger association with family history of breast cancer compared with *BRCA1* mutation carriers, which is consistent with data from a recent study based on a larger number of sequenced Chinese breast cancer patients [30]. Although genetic testing guidelines such as the National Comprehensive Cancer Network (NCCN) are well established in Western countries, the criteria may need to be modified in Malaysia where resources are more limited and the average age at breast cancer onset is much younger. For example, if using $\leq$45 years as age threshold, one-third of the cases in Sarawak would qualify for genetic testing. On the other hand, four *BRCA2* carriers would not have been detected using NCCN criteria (no family history, diagnosed at age $>$45 years, with luminal A tumors), indicating the need for gene-specific testing guidelines.

PALB2 pathogenic mutations were identified in four subjects (2 Chinese and 2 Malay), less than 1% of our study group. While the two Malay *PALB2* mutation carriers both had a very early age of onset (24 and 38), the Chinese carriers did not (49 and 64 years of age) but they both had TN tumors. Sequencing exons 6, 7, and 8 of the *TP53* gene yielded two pathogenic mutation carriers, one Chinese diagnosed at age 34 with family history and one Native patient diagnosed at age 45 without family history. These

results suggest that using multiple-gene panel sequencing might be beneficial to identify additional mutation carriers in Malaysia.

Our survival analysis was limited by short follow-up time (median = 1.8 years) and few events. Nevertheless, we did observe a worse outcome among pathogenic mutation carriers, mostly among *BRCA1* mutation carriers (only one death among *BRCA2* mutation carriers). However, stage and tumor subtype were much stronger predictors for survival in multivariable analysis than mutation status. After the adjustment of stage and subtype as well as treatment variables, the association between mutation status and survival became insignificant.

When evaluating key breast cancer risk factors among mutation carriers, we found that *BRCA1* mutation carriers were less likely to be parous and overweight/obese compared with non-carrier cases. Recent studies suggest that *BRCA1* mutations accelerate ovary aging and women with *BRCA1* mutations had decreased ovarian reserve [31], which is consistent with the reduced parity observed among *BRCA1* mutation carriers in our study. It is unclear why *BRCA1* mutation carriers were thinner and the association did not appear to be driven by age, ethnicity, tumor subtype, or other examined variables. Although based on the small number of cases, the association remained significant in the multivariable model and showed clear distinction between *BRCA1* and *BRCA2* mutation status. Given that the questionnaire was taken at the diagnosis, it is unlikely that the lower BMI was treatment related.

As expected from the diversity of this population, there were a number of previously uncharacterized pathogenic mutations and other variants. Our variant analysis results suggest that several of these variants, including both pathogenic and VUS, may disrupt splicing and transcription binding sites. For example, three previously unreported pathogenic variants (two in *PALB2* and one in *BRCA1*) abolish natural splicing sites. A rare intronic *BRCA1* variant strengthens a cryptic splicing site. However, the cryptic site is associated with *BRCA1* exon 10, which is $\sim$4000 nt in length. The likelihood that this cryptic site will be activated is uncertain, as splicing of large exons has been demonstrated to be dependent on strong splicing regulatory elements. In addition, 9 VUS were predicted to alter the strengths of splicing factors and their possible impact on natural exon splicing implores further study. Furthermore, 3 VUS were predicted to have the potential of impacting transcription binding. While these variants remain classified as VUS, these predictions could provide insight into the potential effects of the nucleotide changes on overall transcriptional regulation of these genes.

Our study is limited by the small number of mutation carriers, particularly for analyses of each gene separately. Nevertheless, ours is the first study to demonstrate the frequency and spectrum of mutations in *BRCA1*, *BRCA2*, and *PALB2* among unselected breast cancer patients in a nearly population-based setting in Sarawak, Malaysia. We identified a number of novel pathogenic variants and VUS that may impact splicing or transcription binding. Our data provide insight into the genetics of breast cancer in this understudied group and highlight the need for modifying guidelines for genetic testing that better serve the need for this population.

Acknowledgements This research was supported by the Intramural Research Program of the NIH, National Cancer Institute (NCI), Division of Cancer Epidemiology and Genetics (DCEG), and Natural Sciences and Engineering Research Council of Canada-Discovery Grant [371758-2009], Canadian Foundation for Innovation, Canada Research Chairs and Cytognomix Inc.

Compliance with ethical standards

Conflicts of interest PKR is the inventor of US Patent 5867402 and other patents pending, which underlie the prediction and validation of mutations. He cofounded Cytognomix Inc., which is developing software based on this technology for complete genome or exome mutation analysis.

References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A (2015) Global cancer statistics, 2012. CA Cancer J Clin 65(2):87–108. doi:10.3322/caac.21262
2. Torre LA, Siegel RL, Ward EM, Jemal A (2016) Global cancer incidence and mortality rates and trends–an update. Cancer Epidemiol Biomark Prev 25(1):16–27. doi:10.1158/1055-9965.EPI-15-0578
3. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM (2010) Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 127(12):2893–2917. doi:10.1002/ijc.25516
4. Sung H, Rosenberg PS, Chen WQ, Hartman M, Lim WY, Chia KS, Wai-Kong Mang O, Chiang CJ, Kang D, Ngan RK, Tse LA, Anderson WF, Yang XR (2015) Female breast cancer incidence among Asian and Western populations: more similar than expected. J Natl Cancer Inst. doi:10.1093/jnci/djv107
5. Lim SE, Back M, Quek E, Iau P, Putti T, Wong JE (2007) Clinical observations from a breast cancer registry in Asian women. World J Surg 31(7):1387–1392. doi:10.1007/s00268-007-9086-3
6. Wen W, Shu XO, Guo X, Cai Q, Long J, Bolla MK, Michailidou K, Dennis J, Wang Q, Gao YT, Zheng Y, Dunning AM, Garcia-Closas M, Brennan P, Chen ST, Choi JY, Hartman M, Ito H, Lophatananon A, Matsuo K, Miao H, Muir K, Sangrajrang S, Shen CY, Teo SH, Tseng CC, Wu AH, Yip CH, Simard J, Pharoah PD, Hall P, Kang D, Xiang Y, Easton DF, Zheng W (2016) Prediction of breast cancer risk based on common genetic variants in women of East Asian ancestry. Breast Cancer Res 18(1):124. doi:10.1186/s13058-016-0786-1
7. Chen S, Parmigiani G (2007) Meta-analysis of BRCA1 and BRCA2 penetrance. J Clin Oncol 25(11):1329–1333. doi:10.1200/JCO.2006.09.1066

8. Atchley DP, Albarracin CT, Lopez A, Valero V, Amos CI, Gonzalez-Angulo AM, Hortobagyi GN, Arun BK (2008) Clinical and pathologic characteristics of patients with BRCA-positive and BRCA-negative breast cancer. J Clin Oncol 26(26): 4282–4288. doi:10.1200/JCO.2008.16.6231
9. Foulkes WD, Smith IE, Reis-Filho JS (2010) Triple-negative breast cancer. N Engl J Med 363(20):1938–1948. doi:10.1056/NEJMra1001389
10. Ferla R, Calo V, Cascio S, Rinaldi G, Badalamenti G, Carreca I, Surmacz E, Colucci G, Bazan V, Russo A (2007) Founder mutations in BRCA1 and BRCA2 genes. Ann Oncol 18(Suppl 6):vi93–vi98. doi:10.1093/annonc/mdm234
11. Liede A, Narod SA (2002) Hereditary breast and ovarian cancer in Asia: genetic epidemiology of BRCA1 and BRCA2. Hum Mutat 20(6):413–424. doi:10.1002/humu.10154
12. Rottenberg S, Jaspers JE, Kersbergen A, van der Burg E, Nygren AO, Zander SA, Derksen PW, de Bruin M, Zevenhoven J, Lau A, Boulter R, Cranston A, O'Connor MJ, Martin NM, Borst P, Jonkers J (2008) High sensitivity of BRCA1-deficient mammary tumors to the PARP inhibitor AZD2281 alone and in combination with platinum drugs. Proc Natl Acad Sci USA 105(44): 17079–17084. doi:10.1073/pnas.0806092105
13. Central Statistical Office of Malaysia (2010) Population Census of Malaysia. https://web.archive.org/web/20150301154300/, http://www.statistics.gov.my/portal/download_Population/files/census2010/
14. Huck OC, Wahab M (2009) Epidemiology of cancer in sarawak 2001-2005. Sarawak Health Department, Malaysia
15. Devi CR, Tang TS, Corbex M (2012) Incidence and risk factors for breast cancer subtypes in three distinct South-East Asian ethnic groups: Chinese, Malay and natives of Sarawak,Malaysia. Int J Cancer 131(12):2869–2877. doi:10.1002/ijc.27527
16. Horne HN, Beena Devi CR, Sung H, Tang TS, Rosenberg PS, Hewitt SM, Sherman ME, Anderson WF, Yang XR (2015) Greater absolute risk for all subtypes of breast cancer in the US than Malaysia. Breast Cancer Res Treat 149(1):285–291. doi:10.1007/s10549-014-3243-9
17. Dean M, Boland J, Yeager M, Im KM, Garland L, Rodriguez-Herrera M, Perez M, Mitchell J, Roberson D, Jones K, Lee HJ, Eggebeen R, Sawitzke J, Bass S, Zhang X, Robles V, Hollis C, Barajas C, Rath E, Arentz C, Figueroa JA, Nguyen DD, Nahleh Z (2015) Addressing health disparities in Hispanic breast cancer: accurate and inexpensive sequencing of BRCA1 and BRCA2. Gigascience 4:50. doi:10.1186/s13742-015-0088-z
18. DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, Philippakis AA, del Angel G, Rivas MA, Hanna M, McKenna A, Fennell TJ, Kernytsky AM, Sivachenko AY, Cibulskis K, Gabriel SB, Altshuler D, Daly MJ (2011) A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat Genet 43(5):491–498. doi:10.1038/ng.806
19. Mucaki EJ, Caminsky NG, Perri AM, Lu R, Laederach A, Halvorsen M, Knoll JH, Rogan PK (2016) A unified analytic framework for prioritization of non-coding variants of uncertain significance in heritable breast and ovarian cancer. BMC Med Genom 9:19. doi:10.1186/s12920-016-0178-5
20. Shirley BC, Mucaki EJ, Whitehead T, Costea PI, Akan P, Rogan PK (2013) Interpretation, stratification and evidence for sequence variants affecting mRNA splicing in complete human genome sequences. Genom Proteom Bioinform 11(2):77–85. doi:10.1016/j.gpb.2013.01.008
21. Rogan PK, Faux BM, Schneider TD (1998) Information analysis of human splice site mutations. Hum Mutat 12(3):153–171. doi:10.1002/(SICI)1098-1004(1998)12:3<153:AID-HUMU3>3.0.CO;2-I
22. Mucaki EJ, Shirley BC, Rogan PK (2013) Prediction of mutant mRNA splice isoforms by information theory-based exon definition. Hum Mutat 34(4):557–565. doi:10.1002/humu.22277
23. Lu R, Mucaki EJ, Rogan PK (2017) Discovery and validation of information theory-based transcription factor and cofactor binding site motifs. Nucleic Acids Res 45(5):e27. doi:10.1093/nar/gkw1036
24. Halvorsen M, Martin JS, Broadaway S, Laederach A (2010) Disease-associated mutations that alter the RNA structural ensemble. PLoS Genet 6(8):e1001074. doi:10.1371/journal.pgen.1001074
25. Zuker M (2003) Mfold web server for nucleic acid folding and hybridization prediction. Nucleic Acids Res 31(13):3406–3415
26. Vega A, Campos B, Bressac-De-Paillerets B, Bond PM, Janin N, Douglas FS, Domenech M, Baena M, Pericay C, Alonso C, Carracedo A, Baiget M, Diez O (2001) The R71G BRCA1 is a founder Spanish mutation and leads to aberrant splicing of the transcript. Hum Mutat 17(6):520–521. doi:10.1002/humu.1136
27. Peterlongo P, Catucci I, Colombo M, Caleca L, Mucaki E, Bogliolo M, Marin M, Damiola F, Bernard L, Pensotti V, Volorio S, Dall'Olio V, Meindl A, Bartram C, Sutter C, Surowy H, Sornin V, Dondon MG, Eon-Marchais S, Stoppa-Lyonnet D, Andrieu N, Sinilnikova OM, Genesis Mitchell G, James PA, Thompson E, kConFab, Swe B, Marchetti M, Verzeroli C, Tartari C, Capone GL, Putignano AL, Genuardi M, Medici V, Marchi I, Federico M, Tognazzo S, Matricardi L, Agata S, Dolcetti R, Della Puppa L, Cini G, Gismondi V, Viassolo V, Perfumo C, Mencarelli MA, Baldassarri M, Peissel B, Roversi G, Silvestri V, Rizzolo P, Spina F, Vivanet C, Tibiletti MG, Caligo MA, Gambino G, Tommasi S, Pilato B, Tondini C, Corna C, Bonanni B, Barile M, Osorio A, Benitez J, Balestrino L, Ottini L, Manoukian S, Pierotti MA, Renieri A, Varesco L, Couch FJ, Wang X, Devilee P, Hilbers FS, van Asperen CJ, Viel A, Montagna M, Cortesi L, Diez O, Balmana J, Hauke J, Schmutzler RK, Papi L, Pujana MA, Lazaro C, Falanga A, Offit K, Vijai J, Campbell I, Burwinkel B, Kvist A, Ehrencrona H, Mazoyer S, Pizzamiglio S, Verderio P, Surralles J, Rogan PK, Radice P (2015) FANCM c.5791C>T nonsense mutation (rs144567652) induces exon skipping, affects DNA repair activity and is a familial breast cancer risk factor. Hum Mol Genet 24(18):5345–5355. doi:10.1093/hmg/ddv251
28. Consortium EP (2012) An integrated encyclopedia of DNA elements in the human genome. Nature 489(7414):57–74. doi:10.1038/nature11247
29. Tung N, Battelli C, Allen B, Kaldate R, Bhatnagar S, Bowles K, Timms K, Garber JE, Herold C, Ellisen L, Krejdovsky J, DeLeonardis K, Sedgwick K, Soltis K, Roa B, Wenstrup RJ, Hartman AR (2015) Frequency of mutations in individuals with breast cancer referred for BRCA1 and BRCA2 testing using next-generation sequencing with a 25-gene panel. Cancer 121(1):25–33. doi:10.1002/cncr.29010
30. Lang GT, Shi JX, Hu X, Zhang CH, Shan L, Song CG, Zhuang ZG, Cao AY, Ling H, Yu KD, Li S, Sun MH, Zhou XY, Huang W, Shao ZM (2017) The spectrum of BRCA mutations and characteristics of BRCA-associated breast cancers in China: screening of 2,991 patients and 1,043 controls by next-generation sequencing. Int J Cancer 141(1):129–142. doi:10.1002/ijc.30692
31. Giordano S, Garrett-Mayer E, Mittal N, Smith K, Shulman L, Passaglia C, Gradishar W, Pavone ME (2016) Association of BRCA1 mutations with impaired ovarian reserve: connection between infertility and breast/ovarian cancer risk. J Adolesc Young Adult Oncol 5(4):337–343. doi:10.1089/jayao.2016.0009