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Prevalence of hereditary breast and ovarian cancer (HBOC) predisposition gene mutations among 882 HBOC high-risk Chinese individuals

Di Shao[1,2] (iD) | Shaomin Cheng[1,2] | Fengming Guo[1,2] | Changbin Zhu[1] | Yuying Yuan[1] | Kunling Hu[1,2] | Zhe Wang[1] | Xuan Meng[1] | Xin Jin[1] | Yun Xiong[1] | Xianghua Chai[1] | Hong Li[1] | Yu Zhang[1] | Hongyun Zhang[1] | Jihong Liu[3] | Mingzhi Ye[1,2]

[1]BGI Genomics, BGI-Shenzhen, Shenzhen, China

[2]BGI-Guangzhou Medical Laboratory, BGI-Shenzhen, Guangzhou, China

[3]Department of Gynecology, Sun Yat-sen University Cancer Center, Guangzhou, China

Correspondence
Jihong Liu, Department of Gynecology, Sun Yat-sen University Cancer Center, Guangzhou, China.
Email: liujih@mail.sysu.edu.cn

Mingzhi Ye, BGI Genomics, Beishan Industrial Zone, Yantian District, Shenzhen, China.
Email: yemingzhi@genomics.cn

Abstract

Identification of deleterious variants in hereditary breast and ovarian cancer (HBOC) susceptibility genes allows for increased clinical surveillance and early detection, and could predict the response to poly (ADP-ribose) polymerase (PARP) inhibitor in patients with advanced ovarian carcinomas. To determine the prevalence and clinical prediction factors for HBOC syndrome, 882 selected individuals underwent multigene panel testing for HBOC risk assessment during the period from January 2015 to March 2018. Overall, 176 deleterious mutations were observed in 19.50% (n = 172) of individuals. Twenty-six of 176 mutations could not be retrieved in related public databases and were considered to be novel. Among patients with ovarian cancer, 115 deleterious mutations were identified in 429 patients (48.6%) with significant enrichment for a family history of breast or ovarian cancer syndrome (P < .05). In the breast cancer subgroup, 31 deleterious mutations were identified in 261 patients. Besides *BRCA1* (8; 25.8%) and *BRCA2* (11; 35.5%), the most frequently occurring genes, an additional 12 deleterious mutations (38.7%) were found in seven other susceptibility genes. Higher mutation incidence (57.9%) was observed in subjects with histories of breast and ovarian cancer. Our results highlighted the genetic heterogeneity of HBOC and the efficiency of a multigene panel in carrying out risk assessment.

KEYWORDS
BRCA1, *BRCA2*, HBOC, mutation, NGS

1 | INTRODUCTION

Breast and ovarian cancers fiercely impact health of Chinese women as well as their families.[1,2] As is known, these two diseases are heritable. Inherited mutations of *BRCA1* and *BRCA2* are pathogenetic in a majority of HBOC patients.[3,4] In addition to *BRCA1/2*, genetic alterations of other HR genes (*ATM*, *BRIP1*, *CHEK2*, *RAD50*, *RAD51C*) are also associated with HBOC.[5-8] Moreover, research

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has indicated that mutations in the susceptibility genes (*TP53*, *PTEN*, *STK11*, and *CDH1*) were also associated with hereditary cancer syndromes such as Li-Fraumeni syndrome, Cowden syndrome, Peutz-Jeghers syndrome, and hereditary diffuse gastric cancer syndrome.[9-13] Moreover, congenital aberrations in mismatch repair genes also contributed to the onset of epithelial ovarian cancer. For instance, germline MSH6 alterations were proven to increase the risk of ovarian cancer.[14]

In addition to genetic counseling and risk management, it has been widely shown that germline *BRCA1/2* as well as other HR gene mutations may serve as prognostic markers for several tumor types including breast and ovarian cancers. Therapeutically, *BRCA1/2* and HR mutations ought to be good predictors of platinum-based (neo) adjuvant regimens for patients with triple-negative breast cancer or advanced epithelial ovarian cancer. Recently, several phase 3 clinical trials indicated germline *BRCA1/2* pathogenic mutations well predicated the response to PARP inhibitors of patients with advanced ovarian carcinomas as well as patients with HER2-negative breast cancer.[15,16]

Traditional genetic testing methods, such as PCR-based assay and the DGGE mutation scanning system, have been applied to detect *BRCA1/2* widely in China.[8,17] However, *BRCA1/2* are high-risk tumor suppressor genes without significant mutation hotspots; as a result, some mutations would be missed by conventional approaches. Results from recent studies confirmed that next-generation sequencing (NGS) showed multiple advantages in cancer genetic testing in terms of time and cost-effectiveness.[18-20] However, there are insufficient related reports on HBOC patients in the Chinese population.

To investigate the mutation frequency among individuals with a suspected HBOC risk in the Chinese population, we used multigene testing to show the distribution and prevalence of deleterious germline mutations among 882 patients with suspected HBOC risk in 21 HBOC hereditary susceptibility genes. Our results evaluated the benefits and limitations of multigene panel testing and provided insights into choosing appropriate multigene tests to assess the risk of hereditary cancer.

2 | MATERIALS AND METHODS

2.1 | Participants

Subjects were selected from patients referred for genetic testing using a 21-gene panel Oseq-BRCA (BGI Genomics) between January 2015 and March 2018. Enrollment criteria of this study were based on the current NCCN for genetic risk evaluation for HBOC: (i) diagnosed at any age with ovarian cancer or pancreatic cancer; (ii) diagnosed with breast cancer with one or more of the following: diagnosed at age ≤50 years, diagnosed with triple-negative breast cancer at ≤60 years, two or more separate breast cancer primaries, breast cancer at any age with at least one close blood relative with: breast cancer age ≤50 years, male breast cancer, pancreatic cancer or ovarian cancer, and breast cancer at any age with at least two close blood relatives with breast cancer; (iii) had a first- or second-degree relative with one or more of the following: breast cancer diagnosed at ≤45 years, ovarian cancer, male breast cancer or pancreatic cancer; (iv) three or more close blood relatives on the same side of the family diagnosed with any cancer.[21] Demographic and clinical information, including gender, personal cancer history, and family cancer history, were collected from test requisition forms by ordering clinicians at the time of testing. All patients signed informed consents approved by the Institutional Review Board of BGI Genomics.

2.2 | Next-generation sequencing library construction and gene capture

Genomic DNA (gDNA) was extracted from participants' peripheral blood samples using the Qiagen Blood Midi Kit (Qiagen). DNA concentration and quality were assessed by Qubit (Life Technologies) and agarose gel electrophoresis. Genomic DNA (250 ng) was used for sequencing library construction. Briefly, gDNA was fragmented randomly by the Covaris LE220 sonicator (Covaris, Inc.) to generate gDNA fragments with a peak of 250 bp and then subjected to three enzymatic steps: end-repair, A-tailing, and adapter ligation. DNA libraries were purified with Agencourt Ampure XP beads (Beckman-Coulter), and PCR was carried out during which a unique 8 bp barcode was added to label each sample. Five to ten PCR products were pooled equally and hybridized to a custom hereditary cancer panel (Roche NimbleGen). Hybridization product was subsequently purified, amplified, and qualified. Finally, sequencing was carried out with paired end and barcode on the BGISEQ-500 sequencer or Hiseq 2000 (Illumina) following the manufacturer's protocols.

2.3 | Sequencing data analysis and mutation calling

Raw fastq data generated by the sequencer were first filtered by SOAPnuke to exclude low-quality reads. Clean reads were then aligned to the reference human genome (UCSC hg19) using the Burrows-Wheeler Aligner (BWA) ALN algorithm. Single nucleotide variants (SNV) were detected by the GATK Unified Genotyper. Small insertions and deletions (InDels) were called using GATK HaplotypeCaller. Copy number variants (CNV) were called using read-depth analysis. All the above variants were further filtered by quality depth, strand bias, mapping quality, and reads position. Finally, each variant was annotated with respect to gene location and predicted function in Human Genome Variation Society (HGVS) nomenclature and was prepared for interpretation.

2.4 | Data interpretation

Interpretation was focused on variants in 21 selected susceptibility genes in HBOC (Table 1). These 21 genes were selected through

TABLE 1 Twenty-one selected susceptibility genes in hereditary breast and ovarian cancer

Risk category	Genes	Gene names
BRCA1/2	2	*BRCA1, BRCA2*
BRCA pathway/moderate risk	9	*ATM, BARD1, BRIP1, CHEK2, MRE11A, NBN, PALB2, RAD50, RAD51C*
High penetrance	4	*CDH1, PTEN, STK11, TP53*
Lynch syndrome	5	*MLH1, MSH2, MSH6, PMS1, PMS2*
Other moderate-risk genes	1	*MUTYH*

NCCN guidelines and published research articles, and they include core genes in the Fanconi anemia (FA) pathway and HR genes. Variants were classified into the following five categories according to the American College of Medical Genetics (ACMG) recommendations: class 1, benign; class 2, likely benign; class 3, VUS; class 4, likely pathogenic (LP); and class 5, pathogenic (P).[22] Population allele frequencies were collected from NCBI dbSNP, HapMap, 1000 human genome dataset, and an internal database of 100 Chinese healthy adults. Individuals with likely pathogenic or pathogenic variants were defined as having deleterious variants. Every deleterious variant was validated by qPCR, Sanger sequencing, or time-of-flight mass spectrometry.
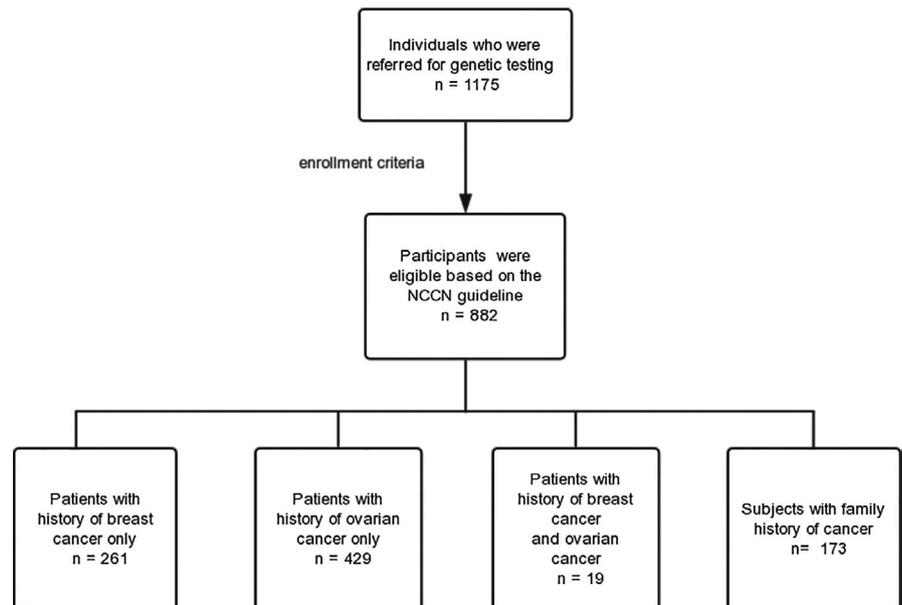
3 | RESULTS

3.1 | Participant characteristics

A total of 1175 individuals were referred to our clinical test center for Oseq-BRCA multigene testing, from which 882 participants were included in our study based on NCCN guidelines (Figure 1). Demographics for these 882 subjects are shown in Table 2. Among them, 709 samples had a diagnosis of either breast cancer or ovarian cancer, whereas 173 additional samples had a family history of

cancer. Of participants with a cancer diagnosis, 261 subjects had a personal history of breast cancer, 429 subjects had a personal history of ovarian cancer, and 19 had personal histories of both breast and ovarian cancer. Age at diagnosis ranged from 13 to 80 years, with an average age of 47 years. Regarding family cancer history information, 108 (62.4%) had at least one first- or second-degree relative with breast cancer only, and 96 (10.9%) had a relative with ovarian cancer only.

3.2 | Deleterious mutations identified in this cohort

Exons and splice sites of 21 HBOC susceptibility genes were examined for mutations by Oseq-BRCA in all 882 recruited participants. Overall, 176 deleterious (LP/P) mutations were observed in 19.50% (n = 172) individuals (Table 3, Figure 2). Of these mutations, 89 (50.6%) were found in *BRCA1*, 49 (27.8%) in *BRCA2*, and 38 (21.6%) mutations in 14 other susceptibility genes (Figure 3A). In addition, two individuals with ovarian cancer carried mutations in both *BRCA1* and another gene (*TP53* or *MRE11A*). Additionally, two individuals with breast cancer had mutations in both *CHEK2* and another gene (*BRCA2* or *TP53*). Deleterious mutations were identified in all individual genes, except *ATM, PTEN, CDH1, BARD1* and *PMS2*.

FIGURE 1 Flowchart of the study design. NCCN, National Comprehensive Cancer Network

TABLE 2 Demography and clinical characteristics of 882 participants included in the present study

	Total (n = 882)	BC (n = 261)	OC (n = 429)	BC & OC (n = 19)	FHx (n = 173)
Gender					
Male	29	0	0	0	29 (16.8%)
Female	853	261	429	19	144
Age at testing (y)					
[<35]	170	78	22	0	70
(35-50)	369	165	130	5	69
[>50]	340	17	276	14	33
NA	3	1	1	0	1
Mean (±SD)	47.0 (±12)	39.8 (±7)	53.5 (±10)	57.1 (±10)	40.4 (±12)
Median	47	40	54	57	38.5
Range	13-80	20-62	24-79	39-80	13-78
Family history					
BC	108	30	31	0	47
OC	96	3	25	2	66
BC & OC	21	2	3	0	16

BC, breast cancer; BC & OC, breast and ovarian cancer; FHx, subjects recruited based on family cancer history; NA, not available; OC, ovarian cancer.

In the breast cancer-only subgroup, 31 deleterious mutations were identified in 261 patients (Table 3). Most mutations occurred in *BRCA1* (8; 3.07%) and in *BRCA2* (11; 4.21%). An additional 12 mutations (13.9%) were found in seven other susceptibility genes (Figure 3B). Deleterious *BRCA1* mutations consisted of eight truncating (2 deletion, 2 frameshift, 2 nonsense, and 2 splice) mutations. Deleterious *BRCA2* mutations were 11 truncating mutations (9 frameshift, 2 nonsense). Among the other HR pathway genes, mutations were most commonly found in *CHEK2* (n = 5; 1.92%) and *BRIP1* (n = 2; 0.77%). In addition, mutations were also observed in *RAD51C*, *PALB2*, and *MRE11A* in one individual per gene. Only one Lynch syndrome gene mutation was identified in *PMS1* in the breast cancer subgroup. Among the other highly penetrant genes, mutations were found in *TP53* (n = 1; 0.38%) whereas no mutations were identified in *STK11*, *PTEN*, and *CDH1*.

In the ovarian cancer-only subgroup (n = 429), 115 deleterious mutations were identified in 113 individuals (Table 3). Of these, 66 (57%) occurred in *BRCA1*, 33 (29%) in *BRCA2*, and 16 (14%) in nine of 19 other susceptibility genes (Figure 3C). Deleterious *BRCA1* mutations consisted of 61 truncating (5 deletion, 35 frameshift, 16 nonsense and 5 splice) mutations and five known deleterious missense mutations. The 33 deleterious *BRCA2* mutations consisted of 30 truncating mutations (1 deletion, 19 frameshift, 7 nonsense, and 3 splice mutations) and three known deleterious missense mutations. Among the HR pathway genes, the most frequent mutations were found in *BRIP1* (n = 5; 1.17%), and *RAD51C* (n = 2; 0.47%). Mutations in *CHEK2*, *MRE11A* and *RAD50* were identified in three individuals, respectively. For Lynch syndrome-related genes (*MLH1*, *MSH2*, *MSH6*, *PMS1*, *PMS2*), deleterious mutations were identified in *MSH2* (n = 3) and *MSH6* (n = 1), accounting for 3.5% of all mutations in the ovarian

cancer subgroup. Among the other highly penetrant genes, mutations were found in *TP53* (n = 1; 0.23%) and *STK11* (n = 1; 0.23%).

In the subgroup of subjects with disease histories of both breast and ovarian cancer (n = 19) (Table 3), a higher frequency of mutation rate was observed. Eleven (57.9%) subjects had a mutation, of which 10 had a mutation in *BRCA1*, and one had a *MUTYH* mutation (Figure 3D).

Furthermore, 173 subjects did not have a cancer diagnosis but had a family history of cancer. In this subgroup, 19 mutations were identified in 21 cancer susceptibility genes with a prevalence of 10.98% (Table 3) in which 10 were mutations in *BRCA1/2* genes, two in *BRIP1*, two in *MLH1*, one in *CHEK2*, one in *MRE11A*, one in *NBN*, one in *RAD51C*, and one in *MUTYH* (Figure 3E). Interestingly, in this subgroup, the proportion of missense mutations was significantly higher than in other subgroups (Figure 3F). No mutations were found in *PALB2*, *RAD50*, *STK11*, *TP53*, *MSH2*, *MSH6*, and *PMS1* genes.

3.3 | Recurrent mutations, founder mutations, and novel mutations

In our cohort, recurrent mutations (n ≥ 3) were found in *BRCA1* p.Ile1824AspfsX3, *CHEK2* p.His371Tyr, *BRCA1* p.Glu1257GlyfsX9, and *BRCA2* p.Ser2670Leu (Table S1). *BRCA1* p.Ile1824AspfsX3 was also one of the Chinese founder mutations. The other Chinese founder mutations included *BRCA1* p.Cys328*, *BRCA2* p.Thr3033Asnfs*11, and *BRCA2* p.Gln1037Ter. No Ashkenazi Jewish or European founder mutations were observed. We confirmed 26 novel mutations that are not reported in public databases (ClinVar, UMD, LOVD, BIC) and in the literature. Of these, seven in *BRCA1* (p.Val14Glyfs*3, p.Asn298LysfsX2, p.Asn599Ilefs*13, p.Phe901Leufs*99, p.Glu1288Glnfs*18, p.Arg1753Ter, p.Glu1849Ter),

TABLE 3 Frequency of mutations by personal cancer history

Gene	No. of individuals (%)				
	Total (n = 882)	BC (n = 261)	OC (n = 429)	BC & OC (n = 19)	FHx (n = 173)
BRCA1/2					
BRCA1	89 (10.09)[a,b]	8 (3.07)	66 (15.38)[a,b]	10 (52.63)	5 (2.89)
BRCA2	49 (5.56)[c]	11 (4.21)[c]	33 (7.69)	0	5 (2.89)
BRCA pathway/moderate risk					
BRIP1	9 (1.02)	2 (0.77)	5 (1.17)	0	2 (1.16)
CHEK2	7 (0.79)[c,d]	5 (1.92)[c,d]	1 (0.23)	0	1 (0.58)
MRE11A	3 (0.34)[a]	1 (0.38)	1 (0.23)[a]	0	1 (0.58)
NBN	1 (0.11)	0	0	0	1 (0.58)
PALB2	1 (0.11)	1 (0.38)	0	0	0
RAD50	1 (0.11)	0	1 (0.23)	0	0
RAD51C	4 (0.45)	1 (0.38)	2 (0.47)	0	1 (0.58)
High penetrance					
STK11	1 (0.11)	0	1 (0.23)	0	0
TP53	2 (0.23)[b,d]	1 (0.38)[d]	1 (0.23)[b]	0	0
Lynch syndrome					
MLH1	2 (0.23)	0	0	0	2 (1.16)
MSH2	3 (0.34)	0	3 (0.70)	0	0
MSH6	1 (0.11)	0	1 (0.23)	0	0
PMS1	1 (0.11)	1 (0.38)	0	0	0
Other moderate-risk genes					
MUTYH	2 (0.23)	0	0	1 (5.26)	1 (0.58)
Total	176 (19.95)	31 (11.88)	115 (26.81)	11 (57.89)	19 (10.98)

Abbreviations: BC, breast cancer; BC & OC, breast and ovarian cancer; FHx, subjects recruited based on family cancer history; OC, ovarian cancer.

[a]One subject had both BRCA1 and MRE11A mutations.
[b]One subject had both BRCA1 and TP53 mutations.
[c]One subject had both BRCA2 and CHEK2 mutations.
[d]One subject had both CHEK2 and TP53 mutations.

nine in BRCA2 (p.Ser942Glnfs*18, p.Asn1066Lysfs*1, p.Asn1287LysfsX6, p.Lys1765Glnfs*13, p.Asp1868Valfs*5, p.Thr2125Asnfs*4, p.Pro2827 Leufs*36, p.Ser3080CysfsX30, p.Asn3124Glnfs*26) (Figure 4), three in BRIP1 (p.Ser206Ter, p.Ser230Ter, p.Lys998AsnfsX5), three in RAD51C (p.Ser231Ter, p.Gln62Ter, p.Val41Glyfs*18), and one in CHEK2 (Leu303_ E8splice), MSH2 (p.Asn412Metfs*22), NBN (p.Asn639Argfs*6), and PMS1 (p.Tyr90*), respectively.

3.4 | Variants of uncertain significance

The VUS rate in our cohort was 38.55% (n = 340), and 406 VUS mutations (339 missense, 58 splice, four in-frame, two frameshift, two duplication, one deletion) were detected in 882 individuals. VUS mutations were most prevalent in ATM (n = 53), followed by 29 VUS in BRCA1 and 42 VUS in BRCA2 (Figure 5). Apart from BRCA1 and BRCA2, other genes had insufficient interpretation

information of pathogenicity for a given mutation, resulting in a high proportion of VUS. Of these mutations, VUS were found most frequently in MRE11A p.Met157Val (n = 8), BRIP1 p.Gln944Glu (n = 8), and ATM p.His42Arg (n = 8). In addition, both PMS1 p.Arg919Cys and MSH6 p.Pro1082Ser occurred in six individuals. In addition, we also selected the VUS with a high level of evidence (Table S2), which can provide a basis for further analysis in subsequent studies.

3.5 | Mutation frequency in subgroups with different family histories and ages at diagnosis

The deleterious mutation rate for each subgroup according to age at diagnosis is detailed in Table 4. In the breast cancer-only subgroup, mean age at diagnosis was 39 among mutation-positive probands and 40 among mutation-negative probands (P = .66). In the ovarian
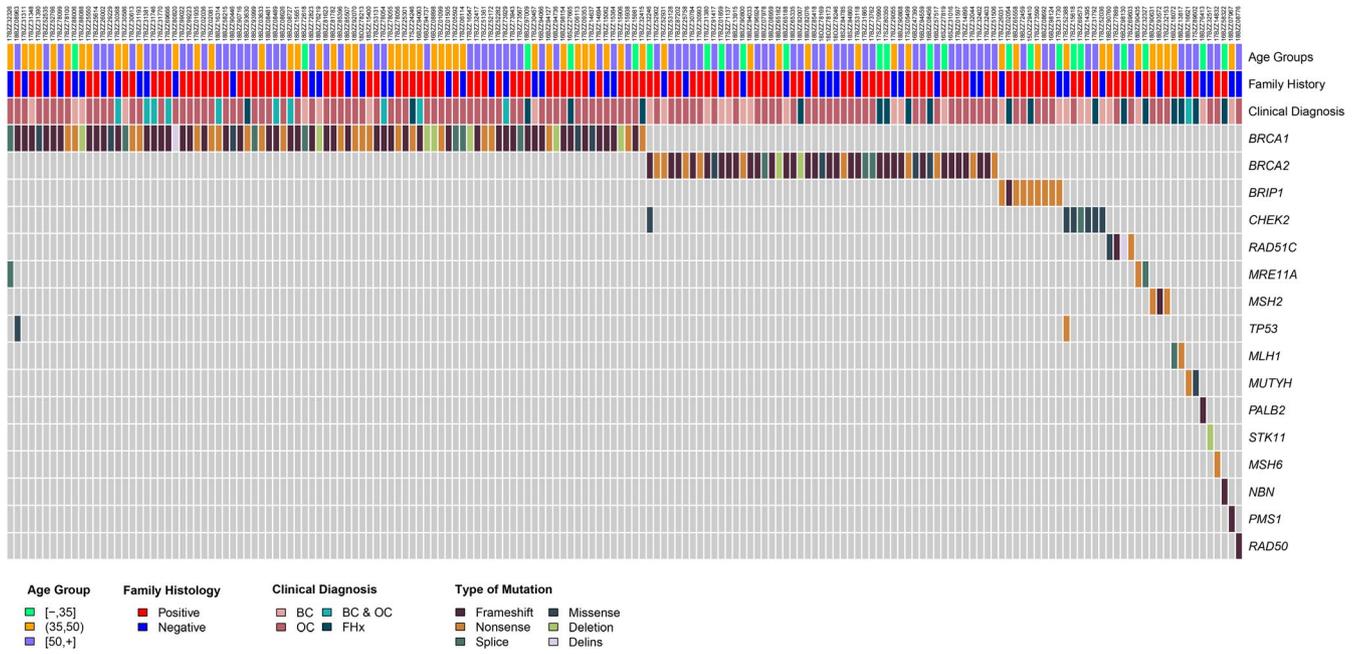
FIGURE 2 Deleterious mutations and clinicopathological features in individuals (n = 172) with class IV and class V mutations. Each column represents the cancer profile in one patient. Samples were sorted by age, family history, clinical diagnosis and type of mutation distinguished by different colors. BC, breast cancer; FHx, subjects with family history of cancer; OC, ovarian cancer
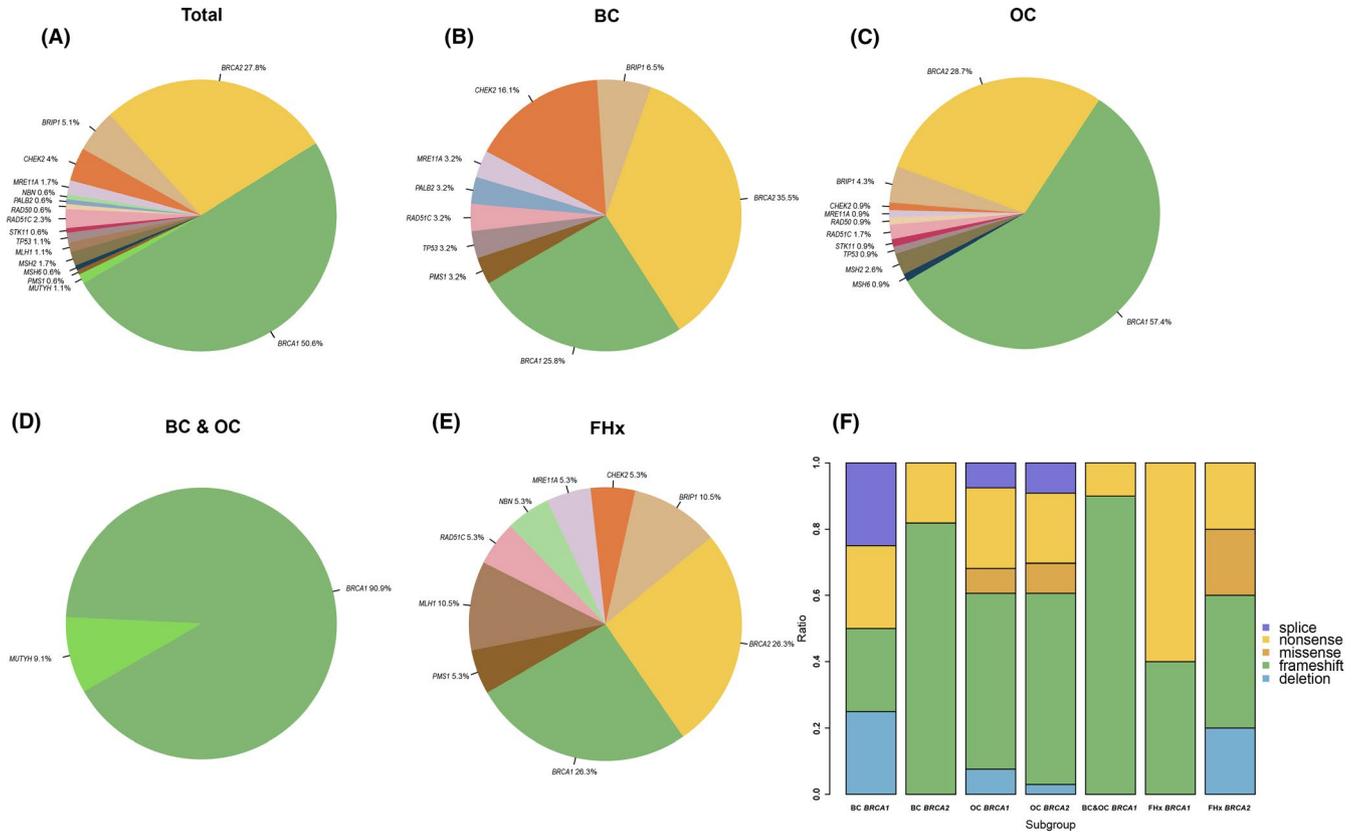

FIGURE 3 Deleterious mutations identified with a multigene panel. A, Total cohort n = 882. B, Breast cancer (BC) subgroup n = 261. C, Ovarian cancer (OC) subgroup n = 429. D, Patients with history of both breast and ovarian cancer (BC & OC) n = 19. E, Subjects with family history of cancer (FHx) n = 173. F, Types of BRCA deleterious mutations by subgroup. cancer

cancer-only cohort, average age at diagnosis was 53 among mutation-positive probands and 54 among mutation-negative probands (P = .90). In the breast and ovarian cancer cohorts, age at diagnosis was slightly older among mutation-negative individuals compared to those positive for a mutation; however, the difference was not significant (P = .41) (Table 4). We also evaluated whether patient subjects with deleterious
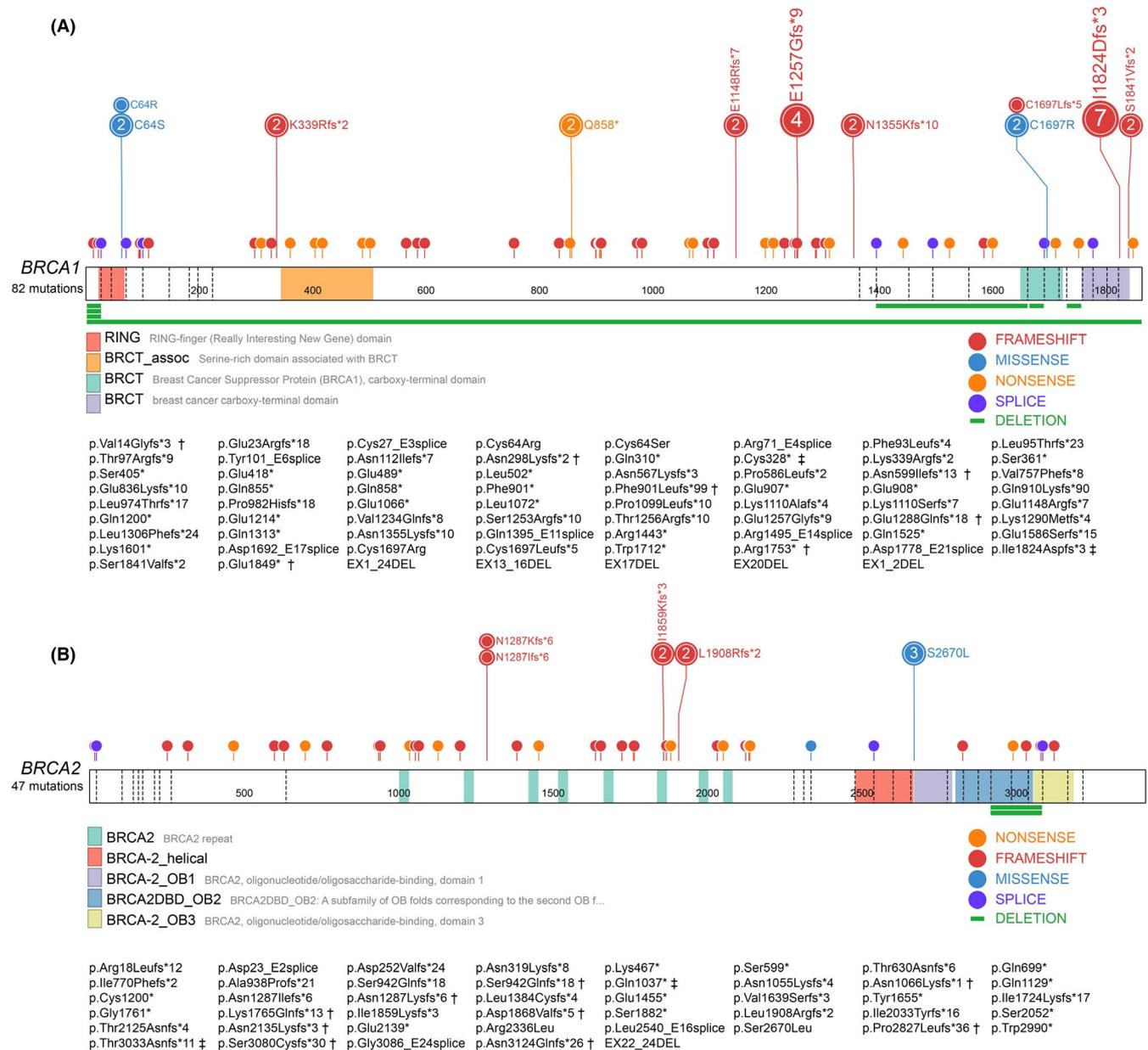
FIGURE 4 Distribution of deleterious mutations detected in (A) *BRCA1* and (B) *BRCA2* genes. Pins represent mutation position. Number represents the number of samples with the mutation (unmarked represents 1). Green bars represent deletion locations, and each segment represents a sample. The figure was created using ProteinPaint. *termination codon according the HGVS mutation nomenclature guidelines. https://varnomen.hgvs.org/recommendations/general/; †novel mutation; ‡Chinese founder mutation

mutations in the 21 susceptibility genes were associated with a greater family history of breast and/or ovarian cancers than nonmutated patient subjects (Table 5). Among breast cancer patients, no significant association was identified between mutations and family history of either breast cancer or ovarian cancer. However, among ovarian cancer patients, individuals with mutations were more likely to have a family history of either breast or ovarian cancer (*P* < .05) (Table 5).

4 | DISCUSSION

Using a HBOC multigene panel, we showed the prevalence of deleterious germline mutations among 882 subjects who were high-risk individuals and referred for Oseq-BRCA testing. This test uses liquid solution hybridization-based target enrichment and NGS to identify all types of variants in 21 HBOC genes. Our results support the views that panel testing could increase the diagnostic detection rate of deleterious germline mutations compared with testing for *BRCA1/2* mutations alone. In our cohort, 172 (19.50%) subjects had a deleterious mutation, and 21.6% of deleterious mutations were in genes other than *BRCA1* and *BRCA2*. The positive rate for the ovarian group was 26.81%, which was higher than for the breast cancer group (11.88%). Moreover, the mutation frequency of the *BRCA* gene in 173 subjects who were recruited based on family cancer history was significantly higher than that in the healthy population reported by a previous study (5.78% vs.

FIGURE 5 Overall proportion of variant of uncertain significance (VUS) in 21 cancer susceptibility genes. B/LB, benign or likely benign; P/LP, pathogenic or likely pathogenic

TABLE 4 Correlation between deleterious mutations and age in each subgroup

Age (y)	BC		OC		BC & OC		FHx	
	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
Mean	39.7	39.8	53.6	53.4	54.8	60.3	41.1	40.4
Median	39	40	53	54	52	59	35	39
Range	26-56	20-62	27-76	24-79	44-68	39-80	27-71	13-78
P-value	.6643		.9033		.4083		.9027	

Abbreviations: BC, breast cancer; BC & OC, breast and ovarian cancer; FHx, subjects recruited based on family cancer history; Neg, negative: no deleterious mutation; OC, ovarian cancer; Pos, positive: carried deleterious mutation.

TABLE 5 Correlation between deleterious mutations and HBOC family history in subgroups

	BC		OC	
	Pos	Neg	Pos	Neg
With HBOC family history	6 (2.3%)	29 (11.1%)	37 (8.6%)	22 (5.1%)
Without HBOC family history	23 (8.8%)	203 (77.8%)	76 (17.7%)	294 (68.5%)
P[a]	.246		<.05	

Abbreviations: BC, breast cancer; HBOC, hereditary breast and ovarian cancer; OC, ovarian cancer.
[a]Chi-squared test was used.

0.38%),[23] indicating the validity of the inclusion criteria. Our study is distinguished from other studies in the following ways. First, our large-size cohort is recruited from multiple general hospitals across China which is a representative population for the target population in China. The prevalence of mutations in this population was rarely reported in previous studies. In addition, our cohort was selected according to the NCCN guidelines, including breast cancer patients, ovarian cancer patients, and high-risk volunteers. Our results reflect the mutation frequency in individuals defined by the guidelines and have great clinical practical significance.

In the breast cancer-only subgroup, the prevalence of *BRCA1* and *BRCA2* deleterious mutations were 3.07% and 4.21%, respectively. In a previous Chinese population-based study, Sun et al[24] reported that *BRCA1/2* deleterious mutation frequencies were

4.24% and 6.60% in an early-onset breast cancer cohort and in a familial breast cancer cohort, respectively, which is similar to our subgroup and those observed in other studies in China.[25,26] However, the prevalence of *BRCA1/2* deleterious variants in breast cancer in other countries ranges from 9.3% to 18%.[27-29] Among African American women, Churpek et al[29] reported that the prevalence in deleterious mutations in *BRCA1 and BRCA2* genes was 10% and 8%, respectively. Differences in the definition of early-onset or familial breast cancer and genetic testing methods for hereditary breast cancer between studies may influence results. Previous studies showed that 4%-5% of breast cancer patients carried deleterious mutations beyond *BRCA1/2*,[27-29] which was consistent with our finding that 4.21% of this subgroup carried deleterious variants in neither *BRCA1* nor *BRCA2*. The third

commonly mutated gene in the present study was *CHEK2*, which encodes a checkpoint kinase 2 interacting with cell cycle regulators and DNA repair proteins. Also, the deleterious mutation of *CHEK2* would increase the risk of breast cancer.[14] Five patients carried *CHEK2* deleterious mutations, four in p.His371Tyr and one in c.908+2T>A. Although the recurrent mutation p.His371Tyr in *CHEK2* was marked as VUS in the ClinVar database, we interpreted it as a likely pathogenic variant. This mutation results in the change of a histidine to a tyrosine at position 371 of the CHEK2-encoded protein. Liu et al[30] found that the *CHEK2* p.H371Y mutation which occurred in a domain of protein kinase resulted in decreased kinase activity. This mutation is a suspected disease-causing mutation with one strong pathogenicity (PV3: functional studies supportive of a damaging effect) and one moderate pathogenicity (PM2 low frequency in 1000 Genomes Project). Noteworthily, our study found only one breast cancer patient carried the *PALB2* mutation but no patients carried the *ATM* mutation, whereas *ATM* and *PALB2* mutations were commonly identified in other studies.[27-29]

The frequency of *BRCA1/2* mutations was 23.07% in the ovarian cancer-only subgroup, 15.38% for *BRCA1* and 7.69% for *BRCA2*, respectively. The mutation rate of *BRCA1/2* in our ovarian cancer subgroup was slightly higher than that in other studies that found *BRCA1/2* deleterious mutations with rates of 13% to 15%.[31-33] Overall, *BRCA1/2* mutation accounted for 86% of total mutations in hereditary ovarian cancer, and the *BRCA1* mutation rate was more pronounced than the *BRCA2* in ovarian cancer patients, which is similar to the results from both Li et al[34] (2018, n = 1331, *BRCA1* for 17.1% and *BRCA2* for 5.3%) and Norquist et al[31] (2016, n = 1915, *BRCA1* for 9.5% and *BRCA2* for 5.1%). Apart from the B*RCA1/2* mutation, 0.9% of the subgroup carried *BRIP1* mutation (BRCA1-interacting protein C-terminal helicase 1), which is comparable to other studies in which the prevalence ranges from 0.8% to 1.5%.[31,35] BRIP1, a member of the BRCA-Fanconi anemia DNA repair pathway, is one of ovarian cancer moderate-risk genes and *BRIP1* mutations are associated with a 10%-15% increased risk of lifetime ovarian cancer.[36] Reviewing five patients with *BRIP1* deleterious mutations, all subjects had a family history of cancer (ovarian cancer, breast cancer, pancreatic cancer, colon cancer, gallbladder cancer). These data suggest that *BRIP1* mutation may be the pathogenic cause in ovarian cancer patients with a family history of cancer. In reviewing the mutations in mismatch repair genes (MMR; *MLH1*, *MSH2*, *MSH6*, *PMS2*), mainly causing Lynch syndrome, they were of low frequency in our subgroup (n = 4; 0.93%). However, in our cohort, MMR mutations only occurred on the *MSH2* and *MSH6* genes, and no mutations in *MLH1* were found, which is different from the spectrum of hereditary colorectal cancer. This phenomenon also occurred in the study of Norquist et al[31] (7 of 8 MMR mutations occurred in *PMS2* or *MSH6*). Although the values of these genes are unknown with respect to risk assessment, we cannot completely rule out the benefit of these genes when doing genetic testing in ovarian cancer.

In the subgroup of subjects with a diagnosis of both breast and ovarian cancer, high deleterious mutation rates (52.63% and 5.26%) were observed in *BRCA1* and *MUTYH*. Kwong et al[37] (2018, n = 20) reported that the prevalence of mutation Chinese patients with breast cancer complicated with ovarian cancer were 40% and 20% for *BRCA1* and *BRCA2*, respectively. Walsh et al[32] reported that the frequencies of *BRCA1* and *BRCA2* mutation were 38.71% and 22.58%, and three additional subjects carried *BRIP1*, *CHEK2*, and *MRE11A* mutations, respectively. It seems that the frequency of *BRCA1* mutations in this subgroup is higher than in the group carrying the *BRCA2* mutation; in particular, the *BRCA1* p.I1824Dfs*3 mutation was found in two patients. Given the limited sample size, more evidence is needed to support this assumption.

The rate of VUS in a similar multigene panel study (27 genes) was 32.7%, in which the authors used a panel with fewer genes but with *BRCA1/2* included.[38] Indeed, VUS rate in our cohort was 38.55%, which is slightly higher than the results of previous study. It is possible that the incidence of breast cancer and ovarian cancer in the Chinese population is lower than that in Caucasians, and the variants are relatively sporadic. *ATM* has the most frequent VUS detected due to the long transcript length. When we normlized the length of coding sequence (CDS) to make comparisons, it shows that *RAD51C* has the greatest number of VUS in per 1000 bases, up to 14.15 (Table S3). According to the NCCN guidelines, *RAD51C* specifically increases the risk of ovarian cancer. In our ovarian cancer patients, only one deleterious mutation of *RAD51C* was detected, which is relatively low compared with previous populations.[39] This may be due to the lack of reports on *RAD51C* mutation in the Chinese population.

Identification of deleterious variants in cancer susceptibility genes allows us to find eligible patients for surveillance screening, and it may also provide targeted therapy and prevention strategies for both patients and family members. Clinical interventions and recommendations of *BRCA1* and *BRCA2* mutation carriers have been well established and widely used in clinical practice. Most genes in our panels (*CDH1*, *MSH2*, *MLH1*, *MSH6*, *PMS2*, *PTEN*, *STK11*, and *TP53*) had corresponding current management suggestions in the NCCN guidelines. However, other moderate penetrance genes (*BARD1*, *RAD50*, *ATM*, *BRIP1*, *CHEK2*, *NBN*, *PALB2*, *RAD51C*) are not available in the management guidelines, whereas mutations in these genes were found in 2.60% of subjects. When encountering these mutations, it is a big challenge for clinicians. It is necessary to combine the family history and personal history to make a medical decision. Therefore, guidelines recommend that multigene testing is ideal in the context of professional genetic expertise for pre- and post-test counseling.

In conclusion, we reported the successful utility of multiple gene testing for identification of HBOC relevant risk gene mutations in a large-scale mutation screening. Results of the present study indicated that multigene panel testing can identify more individuals with relevant cancer risk gene mutations than *BRCA1/2* genetic testing alone. Although current NCCN guidelines recommend management of patients with mutations in the majority of risk genes, clinicians should be prepared to deal with VUS and mutations in moderate

penetrance genes. Our findings provide insights for the clinician to consider multigene tests to diagnose cancer predisposition in clinical practice.

CONFLICT OF INTEREST

Di Shao, Shaomin Cheng, Fengming Guo, Changbin Zhu, Kunling Hu, Yuying Yuan, Zhe Wang, Xuan Meng, Xin Jin, Yun Xiong, Xianghua Chai, Hong Li, Yu Zhang, Hongyun Zhang and Mingzhi Ye are employees of BGI Genomics that produces the 21-gene panel test used in this study. Yanling Fen and Jihong Liu have no conflict of interest.

ORCID

Di Shao 🆔 https://orcid.org/0000-0002-1485-2074

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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