

Germline mutation landscape of Chinese patients with familial breast/ovarian cancer in a panel of 22 susceptibility genes

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Abstract

Genetic testing for germline mutations in *BRCA1/2* of patients with breast cancer (BC) is part of routine patient care. However, *BRCA1/2* mutations account only for a fraction of familial BC. A custom panel of 22 gene sequencing was performed on each patient. Among the 481 female patients, 135 patients were detected to carry pathogenic (P)/likely pathogenic (LP) mutations (28.1%), which corresponded to 12 different cancer predisposition genes [14.6% (70/481) on *BRCA1* gene, 5.0% (24/481) on *BRCA2* gene, 8.5% (41/481) on non-*BRCA1/2* genes]. Moreover, 24.7% (119/481) of patients had mutation of unknown significance (VUS) in these genes. The most

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common (8/481) pathogenic mutation is *BRCA1* c.5470_5477del, while *BRIP1* 2392 C > T of patients was detected. All the mutations detected were mainly seen in the homologous recombinant repair pathway. Compared to *BRCA2* mutation, *BRCA1* mutation is higher in younger female patients ($P < 0.01$). Some pathogenic mutations were detected in the patients' family members without the past history of tumor and 92 novel mutations were detected (31 on *BRCA* including 2 P, 16 LP, 13 VUS; 61 on non-*BRCA1/2* including 9 LP, 52 VUS). The detection rate of *BRCA1/2* mutations was higher in patients with three or more cancer family members than those with one or two. However, the difference was not statistically different. The results suggest that multigene panel testing can increase mutation detection rate for high-risk BC patients. Detailed family history can help to categorize new mutations.

KEYWORDS

BRCA1, *BRCA2*, familial breast cancer, multigenes, novel mutation

1 | INTRODUCTION

BRCA1/2 mutations are characterized as an increased lifetime risk for hereditary breast and ovarian cancer syndrome.¹ Clinical genetic testing for familial breast cancer (BC) has been transformed by the advent of massively parallel sequencing, which allows simultaneous screening of a large number of genes at a fraction of the cost on one gene sequencing previously.² However, there is a large portion of familial BC not associated with *BRCA1/2* mutations. Familial BC often related to mutations of non-*BRCA1/2* genes in homologous recombination (HR) pathway (*ATM*, *CHEK2*, *BARD1*, *BRIP1*, *MRE11A*, *NBN* and etc), by DNA damage response pathway (*MSH2*, *MLH1*, *MSH6*, *PMS2* and etc)^{3,4} and mismatch recognition pathway (*MUTYH*, *EPCAM* and etc).^{5,6} Mutations of these genes have been reported to have medium-to-high penetrance of hereditary BC.⁷⁻⁹ The prevalence and spectrum of BC germline mutations in Chinese female patients have not been well investigated. Meanwhile, they are important for patient management.

In this study, we used a custom-designed 22-gene panel (Table 1) in order to evaluate the clinical value of multigene panel testing in Chinese patients with familial BC. Most of the genes are associated with hereditary BC.

2 | MATERIALS AND METHODS

2.1 | Study cohort

Total of 481 female BC patients were selected in 28 hospitals in China from 2016 to 2017. Patients whose first and secondary degree family members diagnosed of breast or ovarian cancer were included in this study. Primary BC patients with family history were selected by the attending doctors, or they

volunteer to participate. All the patients signed the informed consent.

2.2 | Multigene panel design

In this study, 22 cancer susceptibility genes (Table S1) were included in this panel for their possible role in the development of hereditary cancer based on published literatures. All exons, partial intronic and UTR regions of these genes were covered by this panel which consists of 120 kb pairs approximately. Probes of this panel were synthesized by iGeneTech (China).

2.3 | Next-generation sequencing and data processing

Genomic DNA (gDNA) was extracted from peripheral blood samples (2-5 ml) of each patient using QIAamp DNA Blood Midi Kit (Qiagen, Germany) according to manufacturer's instruction. The target gene library was generated using KAPA Hyper prep Kits (Roche NimbleGen, INC). The prepared libraries were sequenced by NextSeqCN500 (BerryGenomics, China). The sequencing depth was about 1000X. Qualified reads were aligned to human reference genome hg19 by Burrows-Wheeler Alignment (BWA 0.5.9). Germline mutations were detected using Genome Analysis Toolkit (GATK) and SAMtools. Annotations were defined using ANNOVAR (<http://www.openbioinformatics.org/annovar>). Population allele frequencies were extracted from ExAC (<http://exac.broadinstitute.org/>), GnomAD (<http://gnomad.broadinstitute.org/>) and 1000 Genomes Project (<http://www.1000genomes.org/>). Mutation databases including HGMD (<http://www.hgmd.cf.ac.uk/>), OMIM (<http://omim.org/>), ClinVar (<http://www.ncbi.nlm.nih.gov/clinvar/>), and BIC (

TABLE 1 Clinicopathological characteristics between mutation carriers and noncarriers in 481 patients

Characteristics	Noncarriers (N = 227)		BRCA1 carriers (N = 70)		BRCA2 carriers (N = 24)		Others (N = 41)		P1	P2	P3
	No.	%	No.	%	No.	%	No.	%			
Age at diagnosis, years											
Mean ± SD	47.5 ± 10.6	43.2 ± 10.2							<0.01	0.991	0.011
≤40 years	40	17.60%	31	44.30	5	20.80	15	36.60			
>40 years	168	74.00%	33	47.10	18	75.00	23	56.10			
Unknown	19	8.40%	6	8.60	1	4.20	3	7.30			
Family history of other cancer									<0.01	0.955	0.998
Yes	41	18.10%	29	41.40	5	20.80	8	19.50			
No	186	81.90%	41	58.60	19	79.20	33	80.50			
Unknown	0	0.00%	0	0.00	1	4.20	0	0.00			
Lateral of breast cancer									0.068	1	0.596
Bilateral	6	2.60%	6	8.60	1	4.20	0	0.00			
Unilateral	175	77.10%	51	72.90	20	83.30	35	85.40			
Unknown	46	20.30%	13	18.60	3	12.50	6	14.60			
Class									<0.01	<0.01	0.868
TNBC	12	5.30%	27	38.60	19	79.20	2	4.90			
Non-TNBC	132	58.10%	24	34.30	2	8.30	33	80.50			
Unknown	23	10.10%	19	27.10	3	12.50	6	14.60			
History									0.081	0.075	0.135
Ductal	86	37.90%	35	50.00	14	58.30	21	51.20			
Others	141	62.10%	35	50.00	10	41.70	20	48.80			

P1: BRCA1 carriers vs noncarriers; P2: BRCA2 carriers vs noncarriers; P3: Others non-BRCA1/2 genes carriers vs noncarriers

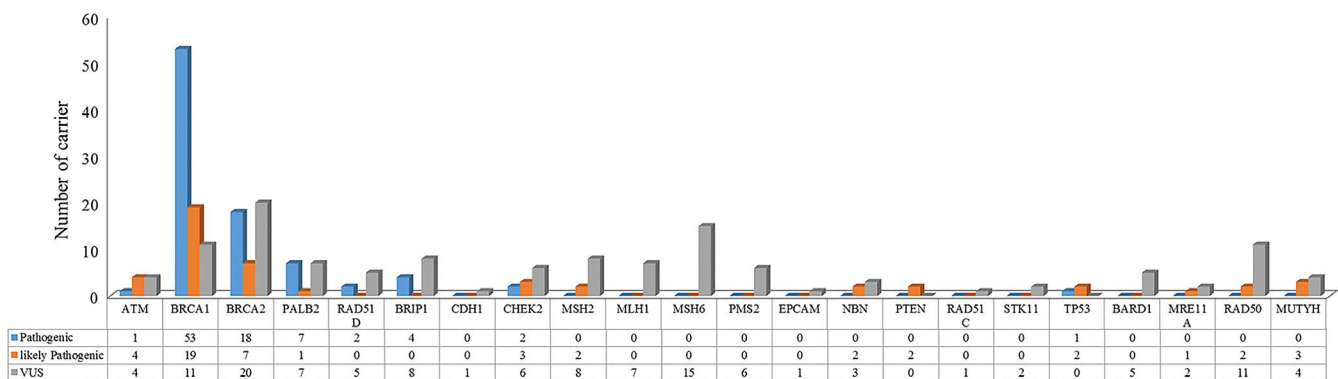
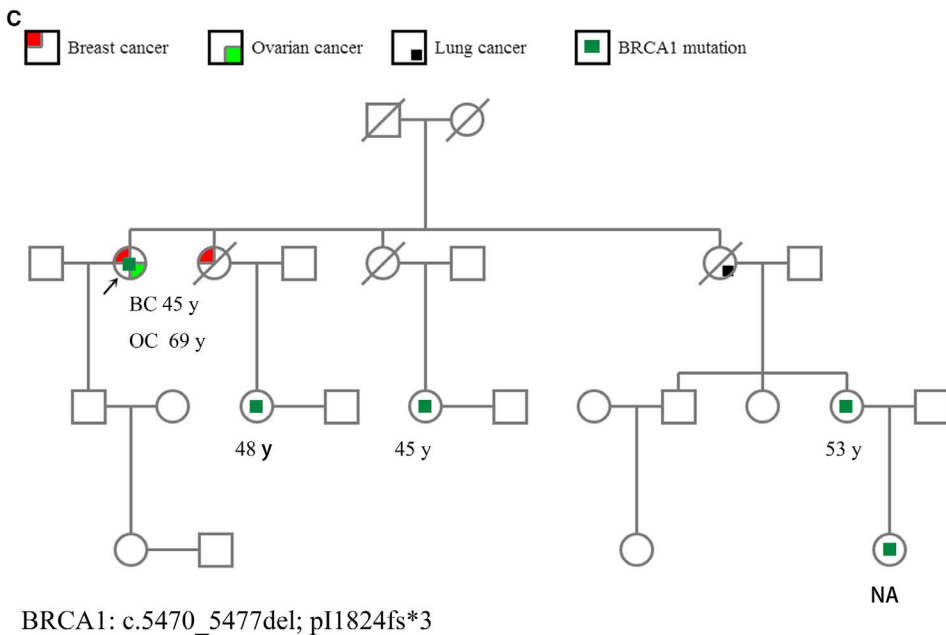
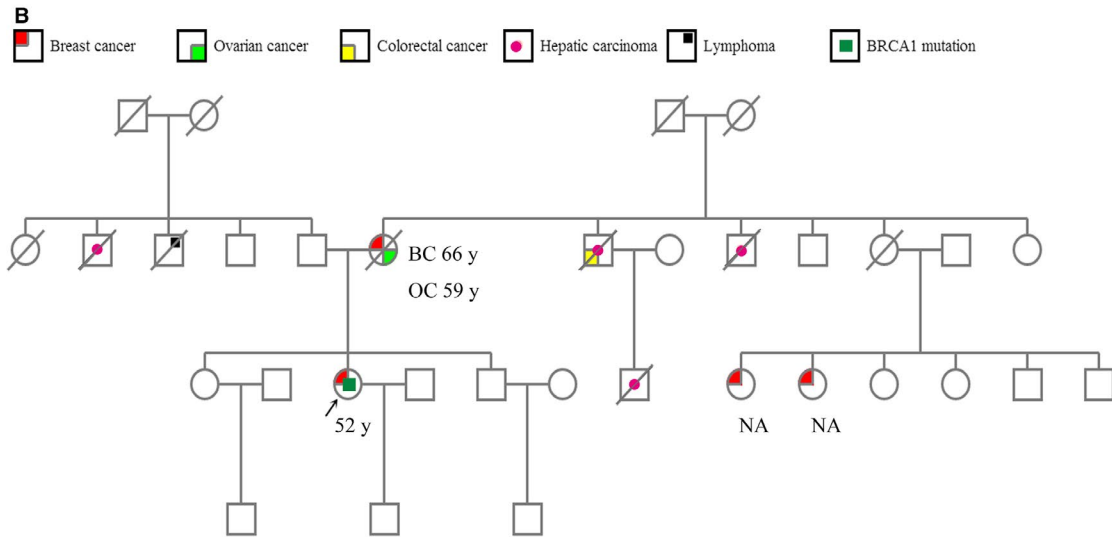
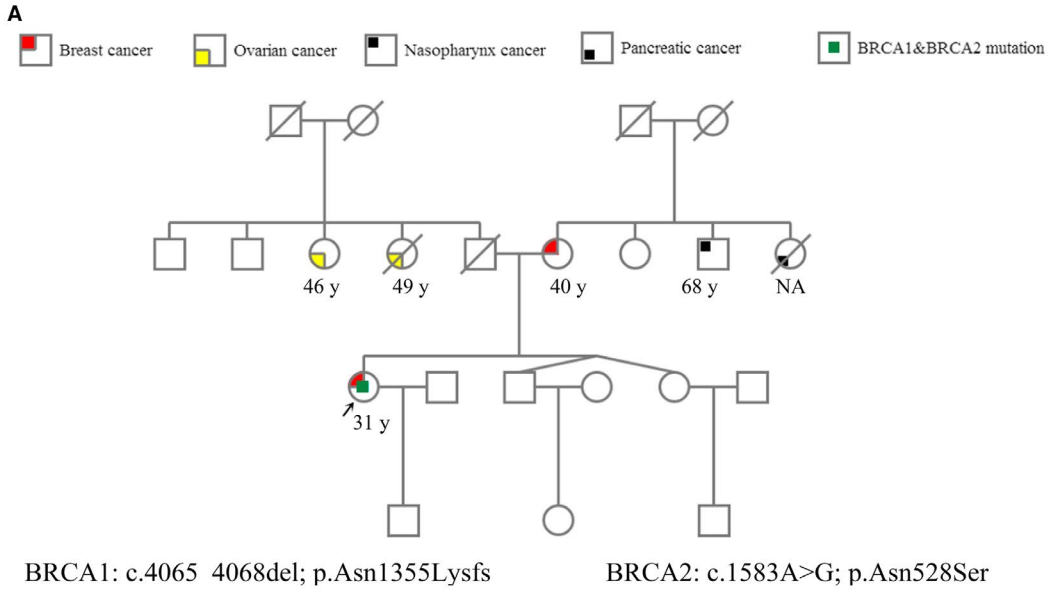
**FIGURE 1** Distribution of different mutations identified with multiple-gene panel

FIGURE 2 Pedigrees. (A) Pedigree of patient with c.4065_4068del; p.Asn1355LysfsTer mutation in BRCA1 gene. (B, C) Pedigree of patients with c.5470_5477del; p.I1824fs*3 mutation in BRCA1 gene. The probands are indicated by arrowheads. Cancer type and age at cancer diagnosis are indicated in the legend



nhgri.nih.gov/bic/) were also included in the analysis pipeline. In this study, we just analyzed point mutations, short insertion, and deletions. In addition, mutations (pathogenic, likely pathogenic, VUS) were confirmed by Sanger sequencing.

2.4 | Germline mutation classification

All mutations were classified according to the American College of Medical Genetics (ACMG) professional practice and guidelines [five-tier mutation: P (Pathogenic); LP (Likely Pathogenic); uncertain significance (VUS); LB (Likely Benign); and B (Benign)].¹⁰ Mutation classification was generated by genetic Counselor and verified by two curators.

2.5 | Statistical analysis

Statistical analysis of clinicopathological characteristics between mutation carriers and non-carriers were performed by the Chi-square test or the Fisher exact test. Two-sided *P* values less than 0.05 was considered to be statistically significant. All analyses were performed using R language (<https://www.r-project.org/>).

3 | RESULTS

3.1 | Mutation status and patient clinical characteristics

In this study, blood samples from 481 Chinese BC patients who have a family history were analyzed by using a custom panel of 22 genes. The median age at diagnosis was 47 years (range, 19–77 years). The younger group (<40 years) carried more *BRCA1* pathogenic mutations than the elder group (>40 years) (17.6% vs 44.3%, *P* < 0.01) (Table 1). Moreover, the rate of non-negative mutation in *BRCA1/2* detected (including P, LP, VUS) in the younger group (≤50 years) was higher than that of the elder group (>50 years) (25% vs 9.9%). The mutation rate in non-*BRCA1/2* mutation carriers and non-carriers are similar (Figure S1). Mutation rates of *BRCA1* (5.3% vs 38.6%, *P* < 0.01) and *BRCA2* (5.3% vs 79.2%, *P* < 0.01) were higher in the triple-negative breast cancer (TNBC) group compared to non-TNBC group (Table 1). A patient diagnosed with TNBC carried two pathogenic mutations c.2155A > T and c.2143_2147delCTGGT in *BRCA1* gene. We found only one study that reported a similar case.¹¹ Compared with noncarriers, the mutation rate of *BRCA1* was higher in patients without a BC family history while the mutation rate of *BRCA2* and other genes showed no

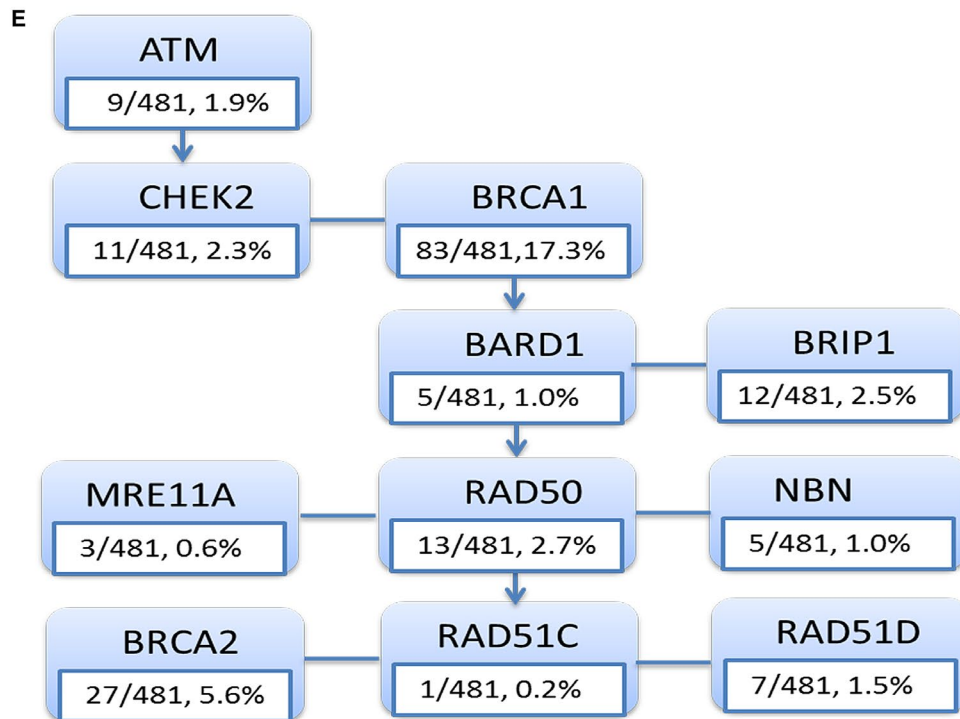
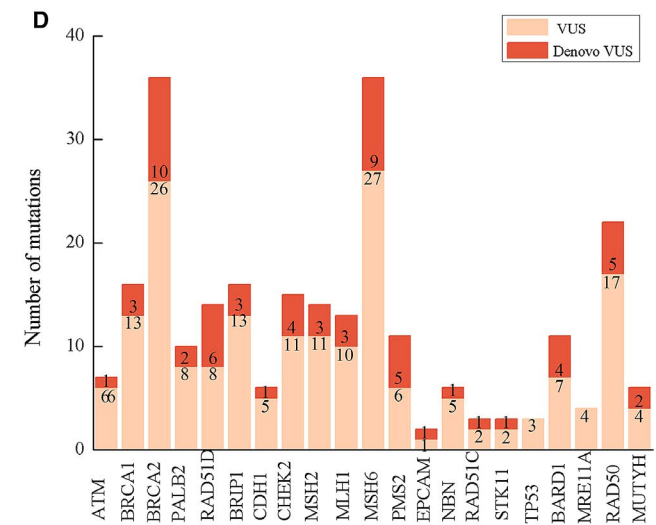
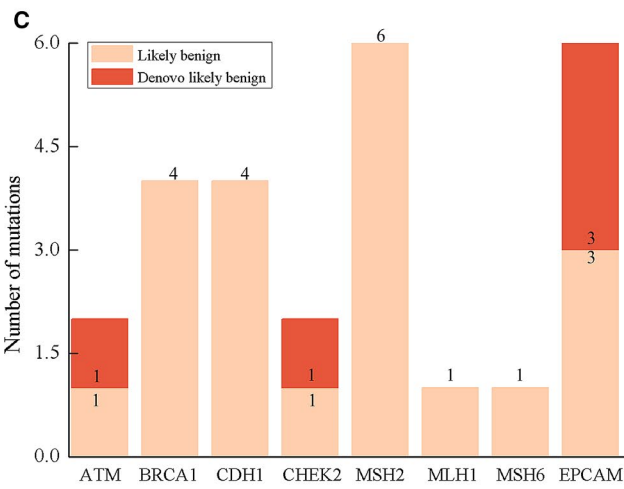
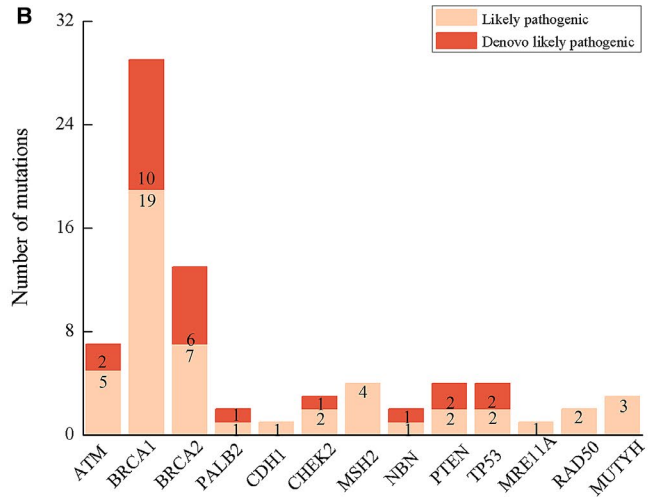
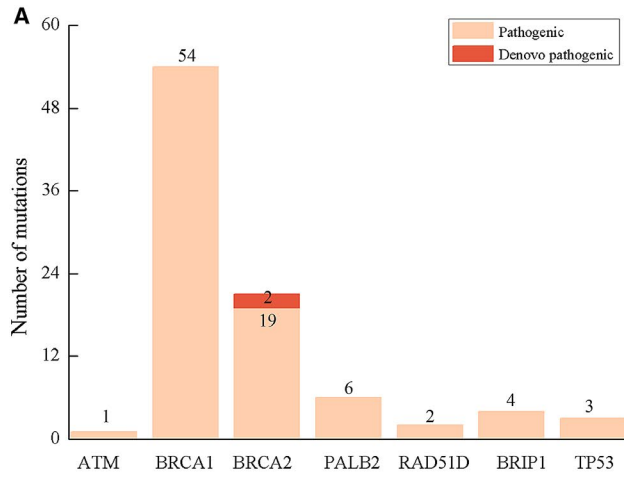
statistically significant difference in age of diagnosis, family history of other cancer, lateral of BC, and tumor type.

3.2 | Rate and spectrum of germline mutations in female Chinese patients with familial BC

Sequencing results of the custom 22 gene panel showed that 135 (28.1%) of the 481 patients carried at least one pathogenic (LP and P) mutation and 70 (14.6%) and 24 (5.0%) patients carried pathogenic mutation of *BRCA1* and *BRCA2*, respectively. The remaining 41 (8.5%) patients carried mutations in non-*BRCA1/2* genes (Figure 1 and Table S2). Furthermore, 39 (8.1%) patients carried more than one mutation. For example, one patient was identified to carry three mutations: *BRCA1* (c.5202del, P), *BRCA2* (c.4408_4412del, P), and *TP53* (c.884C > T, VUS) (Tables S3 and S4). Two *BRCA1* mutations were present in patients with a family history of breast, ovarian, pancreatic, and nasopharyngeal cancer (Figure 2A). Among the mutations detected in this cohort, 73 of 104 (70.2%) pathogenic mutations were in *BRCA1/2* genes (50 in *BRCA1*, 23 in *BRCA2*), while 41 (29.8%) were in non-*BRCA1/2* genes (Figure 1). The *BRIPI* c.2392C > T (n = 1) was detected in patients with a family history of nasopharyngeal carcinoma in addition to BC. The most frequent pathogenic mutation in this study is c.5470_5477del of *BRCA1* was identified in eight families (Table S5). As shown in Figure 2B, the family with this mutation has a apparent cancer family history. The mutation was found in one concurrent BC and ovarian cancer patient and her four healthy family members (Figure 2C). It is noteworthy that the patient's daughter was not a carrier, while her second, and third degree female relatives (sister's daughter and sister's granddaughter) were carriers. Moreover, thirteen mutations in BC-related genes were detected in more than one family (Table S5).

More VUSs were found in non-*BRCA1/2* than *BRCA1/2* genes. Here, 42 (27.5%) of 153 patients carried VUS in *BRCA1/2* genes while 127 in non-*BRCA1/2* genes (16 patients carried both *BRCA1/2* and non-*BRCA1/2* mutations) (Figure 1). It was found that at least one VUS was identified in *EPCAM*, *MLH1*, *MSH6*, *PMS2*, *RAD50*, *RAD51C*, *STK11*, and *BARD1* genes, respectively. The mutation type of VUSs is mainly SNVs, which were found in more than one patient with significant family history. For example *MSH6* c.3244C > T was found in 3 families (Table S2). In addition, 31 mutations detected in this cohort in *BRCA1/2* genes and 61 mutations in non-*BRCA1/2* genes were not found in the BIC database (Figure 3A–D). The discussion of novel mutations was based on the disease onset age,

FIGURE 3 The reported mutations were identified by the multiple-gene panel method. (A) (B) (C) (D) The number of novel and non-novel mutations in non-negative mutations (Pathogenic/likely Pathogenic/likely benign/unknown significance). (E) The percent of gene mutations in homologous recombination pathway



cancer type, unilateral/bilateral lesion, and family history.¹⁰ Meanwhile, the type of mutation is important supporting evidence for the classification of novel mutations. The *BRCA1* c.3919G > T is a nonsense mutation, which was detected in two families with a family history of BC from different regions in China.

The pathway enrichment study further confirms that a majority of mutations (39.7%; 191/481) were identified in the HR (Figure 3E). In the MMR-related genes, only one likely pathogenic mutation was detected in the *MSH2* gene (c.2197G > A, 4 family), which was found in patients diagnosed with pediatric medulloblastoma.¹²

In this study, we found that sites of the mutation were scattered in different regions of a gene (Figure 4A). Here, 257 patients were detected with gene mutations (104/257 P, 50/257 LP, 151/257 VUS) in this study, including SNVs (214), insertions (Ins) (9) and deletions (Del) (34) (Figure 4B). The distribution of mutations is close to that in the whole population (Figure 4C).

Family history studies demonstrated that BC is the most common type (230/481, 47.8%) followed by ovarian (37/481), colorectal (21/481), gastric (20/481), and cervical (12/481). Twenty-two patients (22/481) had a history of more than one cancer type (Table S6). The pathogenic *BRCA2* (c.3919G > T) mutation was detected in one family with a history of BC and prostatic cancer (Figure 5A). The patient with the *BRCA2* (c.9070_9073del) mutation had a family history of four BC relatives (Figure 5B). In addition, the family with lung and esophageal cancers, two VUS *BRCA2* and *MRE11A* mutations were detected in two BC patients with *BRCA1* likely pathogenic mutation (Figure 5C). In eight families, even though there were three relatives in each family had history of cancer, there were no mutations detected by the panel (Table S4). In general, there was no significant difference between gene mutation and family history. However, the data showed that there is a linear trend, which means that more relatives with cancer, the higher proportion of probands with *BRCA* mutations (Figure S2).

4 | DISCUSSION

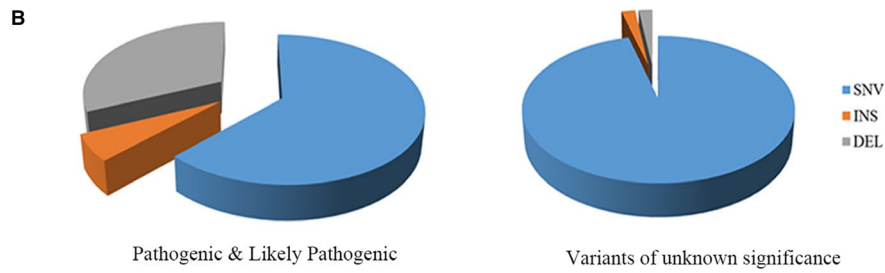
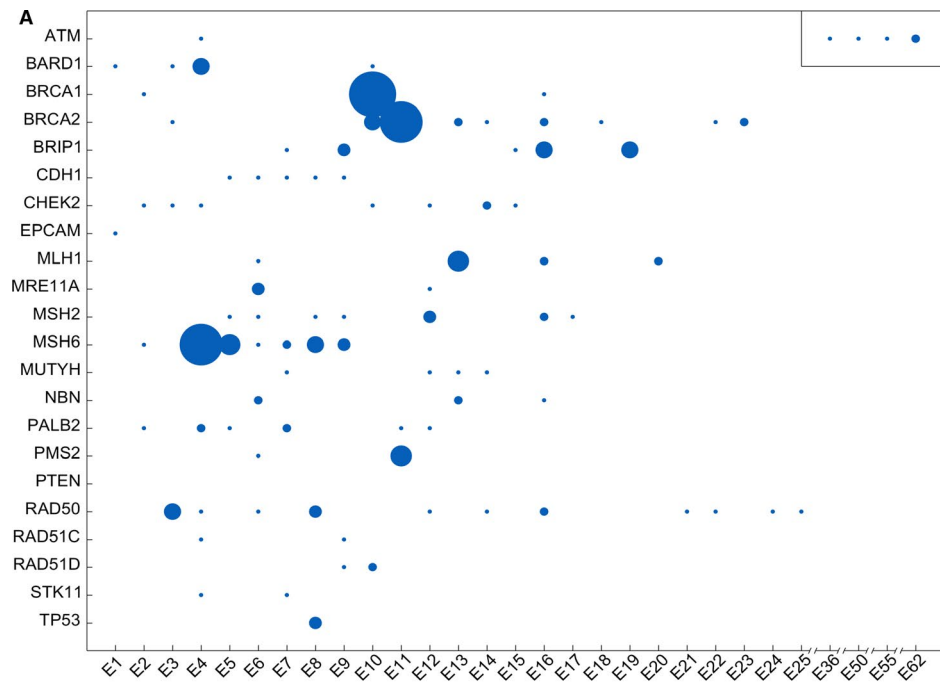
In our cohort study of 481 patients who underwent genetic testing, *BRCA1* mutations were significantly enriched in younger patients (<40 years). *BRCA1/2* mutations were observed more in the TNBC patients.¹³ All 22 genes had higher detection rates in patients less than 50. Thus, we concluded that genetic screening in this populations is essential.¹⁴

In this study, pathogenic mutations were identified in 135 patients most of them in the *BRCA1/2* gene. The mutation carrier rate was higher than the unselected BC patients (19.5% vs 5.3%).¹⁵ Mutations found in the *BRCA1* gene (14.6%) in this cohort were almost three times higher compared to *BRCA2* (5%). A higher mutation burden in *BRCA1* was also reported in

other studies in patients with a cancer history or unselected patients in Asian population.^{16,17} Besides, 8.5% of patients carried non-*BRCA1/2* pathogenic mutations, which were mainly found in *ATM*, *CHEK2*, *PALB2*, and *BRIP1* genes.⁷ Previous studies have reported that approximately 11.4% of BC patients carried mutations in non-*BRCA1/2* genes.⁸ It is worth noting that rare mutations of *BRIP1* c.2392C > T was detected in one patient in our cohort. However, this mutation is reported in three studies and found in 18 Fanconi anemia patients, and is associated with BC in the Irish.^{18,19} Multigene panel testing is likely to provide a more complete mutation capture than *BRCA1/2* alone.^{9,20} No pathogenic mutations were found in *STK11* and *RAD51C*. It was also reported that *STK11* mutations have been limited to individuals with clinical features indicative of the Peutz-Jegher syndrome and *RAD51C* mutations reported in OC.¹⁷ Of note, *BARD1* and *BRIP1* pathogenic mutations were not detected in this study. Other studies showed that *BARD1* mutation might be rare and responsible for a few familial BC patients.¹⁴ It has been reported that *BRIP1* had a higher penetrance for OC.²¹ On the other hand, about 47.2% (227/481) patients had no pathogenic mutations detection in our cohort. One possible explanation for this was that only 22 genes was included in the panel and there are other genes involved in BC patients.^{7,8} Furthermore, the limitation of the detection method used may affect mutation detection.²⁰

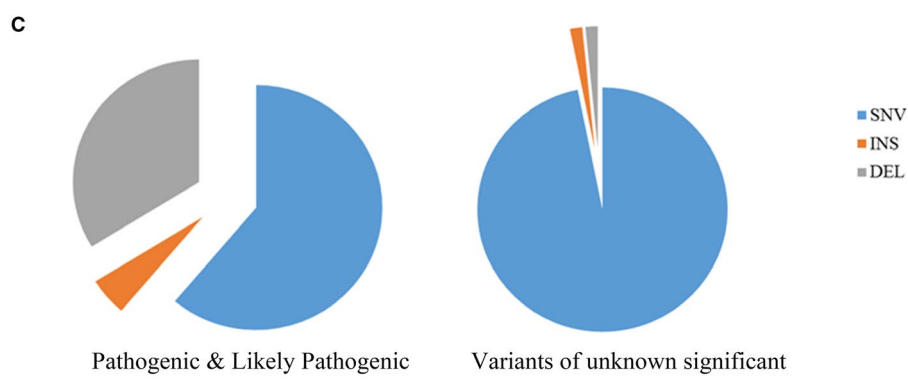
About 24.7% (119/481) of patients carried VUS mutations, mostly in non-*BRCA* genes.² Previous studies often focused on *BRCA* genes other than non-*BRCA* genes, classification of VUS was investigated in more detail and some of them were classified as non-VUS.¹⁰ The rate of VUS detection depends on the number of genes included and has been reported ranging from 6.7 (6-gene panel) to 41.7% (25-gene panel).^{8,22} In this study, the rate of VUS was 24.7%, partially for the study included patients with a family history of multiple tumors. Some of the VUS will eventually be classified as non-VUS with further investigations. However, segregation analysis in the same family is not conducive to the categorization of the VUS. The multifactorial approach should be considered in this process.²³ Throughout all the mutations, it is worth noting that the pathogenic mutations are more frequent in nonsense and frameshift mutations while VUS mutations are more missense. This also confirmed that the mutation type had a large difference according to the structure and function of genes, among which nonsense and frameshift mutations were likely to be pathogenic.¹⁰ This study showed that a majority of mutations identified were in the HR signaling pathway.²¹ Therefore, this pathway might play an important role in familial BC in China.

Some patients with a unique family history were discussed in this study. The common characteristics of these families were with multiple cancers diagnosed and multiple family members affected. Most of the tumors were associated with hereditary BC, such as colorectal, endometrial, ovarian cancer, and pancreatic cancer, and so on.



	Pathogenic & Likely Pathogenic(N)	VUS (N)
SNV	65	149
INS	6	3
DEL	33	1

N: Number of patients



	Pathogenic & Likely Pathogenic(N)	VUS (N)
SNV	85	183
INS	7	3
DEL	47	3

N: Number of mutation Total=349

FIGURE 4 The distribution of germline mutations in BC families. (A) The distribution of 22 gene mutations in exon regions. (B) Distribution of different mutations identified with multiple-gene panel

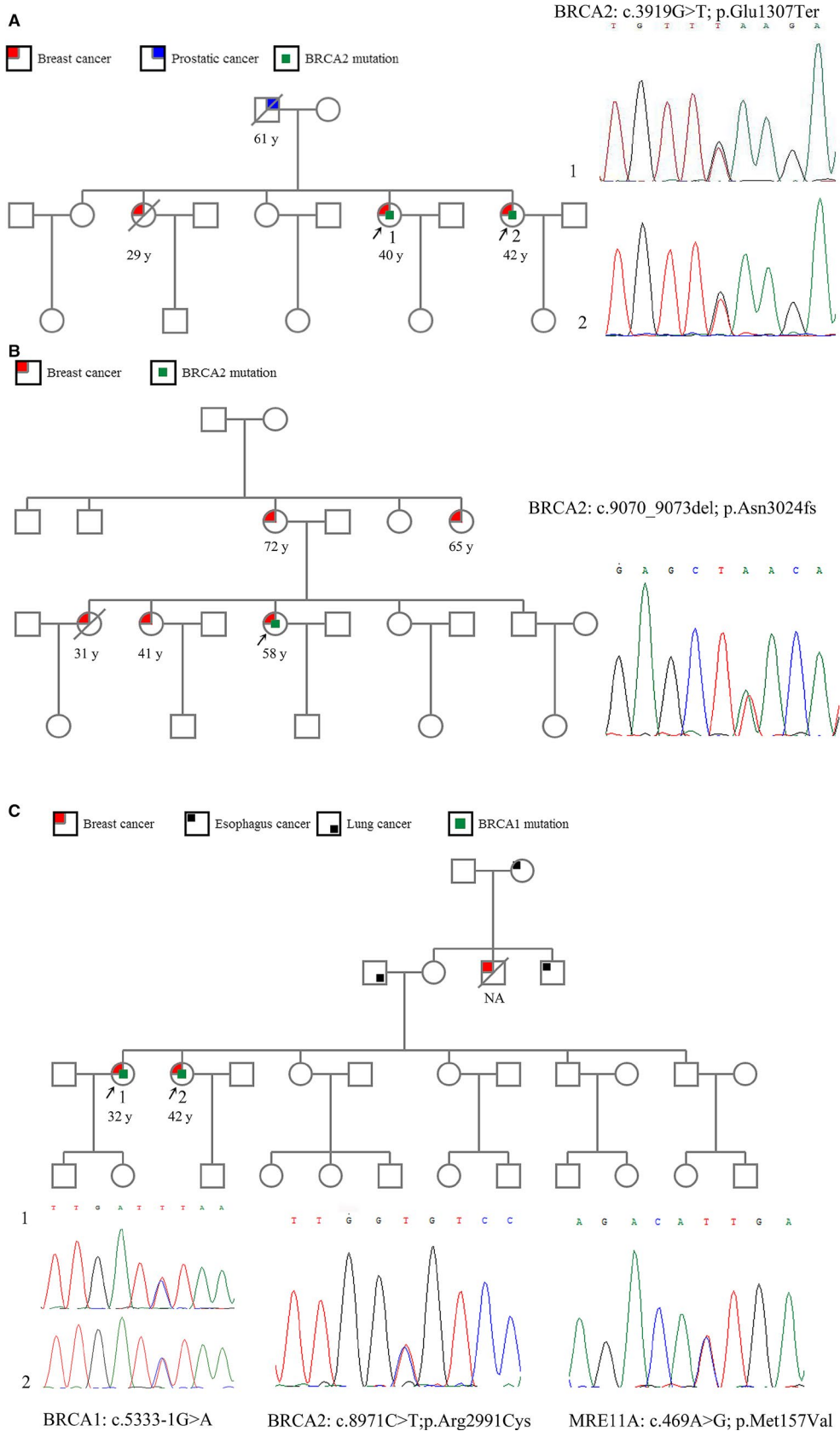


FIGURE 5 Pedigrees. (A) Pedigree of patient with c.3919G > T; p.Glu1370Ter mutation in *BRCA2* gene. (B) Pedigree of patient with c.9070_5073del; p.I1824fs*3 mutation in *BRCA2* gene. (C) Pedigree of patient with three different mutations in *BRCA1*, *BRCA2*, and *MRE11A* genes. The probands are indicated by arrowheads. Cancer type and age at cancer diagnosis are indicated in the legend

These results are consistent with previous findings that the increase in the incidence of pancreatic cancer was related with *BRCA* mutation.¹² Phelan et al, suggested that CRC screening should be done among women with *BRCA1* mutation.²⁴ The number of relatives with BC were positively correlated with *BRCA1/2* mutations. It is one of the characteristics of hereditary tumor. Family studies supported the selection of management choices, especially for carriers without cancer.¹⁵ Additionally, familial BC is not only related to colorectal and other cancers, but also to other rare cancer types that should be investigated further in some families. This study also indicated that selected population screening may help to reduce cancer-related mortality.²³

In conclusion, appropriately selected patients may benefit from multiple-gene sequencing, especially those with personal or family history of more than one possible genetic syndrome. The detailed family history and clinical characteristics are useful for mutation classification, specifically in cases of unreported mutations. Moreover, family separation is more valuable in the classification of pathogenic/likely pathogenic mutations rather than VUS. Our findings are important for the promotion of large panels in high-risk breast/Ovarian cancer populations and clinical genetic testing of patient management.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

- Cortesi L, Turchetti D, Marchi I, et al. Breast cancer screening in women at increased risk according to different family histories: an update of the Modena Study Group experience. *BMC Cancer*. 2006;6:210.
- Hermel DJ, McKinnon WC, Wood ME, Greenblatt MS. Multi-gene panel testing for hereditary cancer susceptibility in a rural Familial Cancer Program. *Fam. Cancer*. 2017;16:159-166.
- Kappil M, Terry MB, Delgado-Cruzata L, Liao Y, Santella RM. Mismatch repair polymorphisms as markers of breast cancer prevalence in the breast cancer family registry. *Anticancer Res*. 2016;36:4437-4441.
- Zhao Q, Yang J, Li L, et al. Germline and somatic mutations in homologous recombination genes among Chinese ovarian cancer patients detected using next-generation sequencing. *J Gynecol. Oncol*. 2017;28.
- Espenschied CR, LaDuca H, Li S, et al. Multigene panel testing provides a new perspective on Lynch syndrome. *J. Clin. Oncol*. 2017;35:2568-2575.
- Rashid MU, Naeemi H, Muhammad N, et al. A novel deleterious c. 2656G> T MSH2 germline mutation in a Pakistani family with a phenotypic overlap of hereditary breast and ovarian cancer and Lynch syndrome. *Hered. Cancer Clin. Pract.* 2016;14:14.
- Economopoulou P, Dimitriadis G, Psyrris A. Beyond BRCA: new hereditary breast cancer susceptibility genes. *Cancer Treat. Rev*. 2015;41:1-8.
- Tung N, Battelli C, Allen B, et al. Frequency of mutations in individuals with breast cancer referred for BRCA1 and BRCA2 testing using next-generation sequencing with a 25-gene panel. *Cancer*. 2015;121:25-33.
- Kapoor NS, Curcio LD, Blakemore CA, et al. Multigene panel testing detects equal rates of pathogenic BRCA1/2 mutations and has a higher diagnostic yield compared to limited BRCA1/2 analysis alone in patients at risk for hereditary breast cancer. *Ann Surg Oncol*. 2015;22:3282-3288.
- Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17:405.
- Sawyer SL, Tian L, Kahkonen M, et al. Biallelic mutations in BRCA1 cause a new Fanconi anemia subtype. *Cancer Discov*. 2015;5:135-142.
- Iqbal J, Ragone A, Lubinski J, et al. The incidence of pancreatic cancer in BRCA1 and BRCA2 mutation carriers. *Br J Cancer*. 2012;107:2005.
- Gomez-Flores-Ramos L, Alvarez-Gomez RM, Villarreal-Garza C, Wegman-Ostrosky T, Mohar A. Breast cancer genetics in young women: what do we know? *Mutat Res*. 2017;774:33-45.

14. Gupta S, Provenzale D, Regenbogen S, et al. Guidelines insights: genetic/familial high-risk assessment: colorectal, version 3.2017. *J. Natl. Compr. Canc. Netw.* 2017;15:1465-1475.
15. Walsh T, Casadei S, Coats KH, et al. Spectrum of mutations in BRCA1, BRCA2, CHEK2, and TP53 in families at high risk of breast cancer. *JAMA.* 2006;295:1379-1388.
16. Zhang J, Sun J, Chen J, et al. Comprehensive analysis of BRCA1 and BRCA2 germline mutations in a large cohort of 5931 Chinese women with breast cancer. *Breast Cancer Res. Treat.* 2016;158:455-462.
17. Sun J, Meng H, Yao L, et al. Germline mutations in cancer susceptibility genes in a large series of unselected breast cancer patients. *Clin Cancer Res.* 2017;23:6113-6119.
18. McInerney NM, Miller N, Rowan A, et al. Evaluation of variants in the CHEK2, BRIP1 and PALB2 genes in an Irish breast cancer cohort. *Breast Cancer Res. Treat.* 2010;121:203-210.
19. Kooshyar MM, Nassiri M, Karimiani EG, Doosti M, Rodbari K. Chromosomal analysis and BRCA2* 617delT/88delTG and BRIP1 (c. 2392C> T) mutations of Fanconi anemia in Iranian family, and its correlation to breast cancer susceptibility. *J. Chem. Pharm. Res.* 2015;7:147-153.
20. Schmidt AY, Hansen T, et al. next-generation sequencing-based detection of germline copy number variations in BRCA1/BRCA2: validation of a one-step diagnostic workflow. *J Mol Diagn.* 2017;19:809-816.
21. Minion L, Dolinsky J, Chao E, Monk B. Hereditary predisposition to ovarian cancer, looking beyond BRCA1/BRCA2. *Gynecol. Oncol.* 2014;135:383-384.
22. Doherty J, Bonadies DC, Matloff ET. Testing for hereditary breast cancer: panel or targeted testing? Experience from a clinical cancer genetics practice. *Journal of genetic counseling.* 2015;24:683-687.
23. Chenevix-Trench G, Healey S, Lakhani S, et al. Genetic and histopathologic evaluation of BRCA1 and BRCA2 DNA sequence variants of unknown clinical significance. *Can Res.* 2006;66:2019-2027.
24. Phelan C, Iqbal J, Lynch H, et al. Incidence of colorectal cancer in BRCA1 and BRCA2 mutation carriers: results from a follow-up study. *Br. J. Cancer.* 2014;110:530.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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