Germline and tumor *BRCA1/2* mutations in Chinese high grade serous ovarian cancer patients

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Background: Studies on the prevalence of *BRCA1/2* mutations in ovarian cancer mainly focused on germline single-nucleotide variant (SNV)/insertion/deletion (indel). The status of large genomic rearrangement (LRG) and somatic mutation were poorly investigated.

Methods: Paired blood and tumor DNA from an unselected cohort of 115 Chinese high grade serous ovarian cancer (HGSOC) patients were collected and analyzed for *BRCA1/2* SNV and indel by NGS. *BRCA1/2* LRG was detected by MLPA. Clinicopathological characteristics including age at diagnosis, FIGO stage, family history and follow-up data were collected for further analysis.

Results: A total of 115 HGSOC patients were screened. Among them, 30 (26.1%) had germline *BRCA1/2* mutations, including 19 (16.5%) SNV/indels, 5 (4.3%) LGRs in *BRCA1*, and 6 (5.2%) SNV/indels in *BRCA2*. Ten (8.7%) had somatic *BRCA1/2* mutations, including 5 (4.3%) in *BRCA1* and 5 (4.3%) in *BRCA2*. The entire tumor *BRCA1/2* mutation frequency was 34.8%. No patients were found with two or more deleterious *BRCA1/2* mutations. The proportion of germline (66.7%) and tumor (75%) mutation carriers was significantly increased for patients with family history when compared with those without (P<0.05). Patients with germline *BRCA1/2* mutation appeared to be younger than non-carriers (mean age, 50.9 *vs.* 54.4 years, P=0.004) and somatic mutation carriers (mean age, 50.9 *vs.* 58.7 years, P=0.009). No significant association was found between *BRCA1/2* status and clinicopathological characteristics including stage and family history of other cancer than breast and ovarian cancer. In univariate and Cox regression analysis, patients with tumor *BRCA1/2* mutations had significant improvements than non-carriers in overall survival in the first two years after surgery (P<0.05). No significant impacts were found between various mutation status in PFS.

Conclusions: There is a high germline and tumor *BRCA1/2* mutation incidences in Chinese HGSOC patients. Germline mutations were associated with family history and age at diagnosis, whereas somatic mutations were not. In our study, tumor *BRCA1/2* mutations showed a time-depended improved survival outcome. A larger cohort should be examined to clarify the relation between *BRCA1/2* mutation and survival outcomes.

Keywords: High grade serous ovarian cancer (HGSOC); *BRCA1/2*, somatic mutation; large genomic rearrangement (LRG)

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Introduction

Ovarian cancer is the third malignancy of the female genital tract, following cervical and uterine cancer. In 2020, there were 21,750 estimated new diagnoses of ovarian cancer and 13,940 deaths from the disease in the United States; deaths were higher than from cancer of the uterine corpus and cervix (1). High grade serous ovarian cancer (HGSOC) is the most common histological subtype of ovarian cancer, which is characterized by a high risk of recurrence and frequent genetic and epigenetic alterations of homologous recombination (HR) pathway genes, accounting for 70-80% of deaths from ovarian cancers (2-4). HR deficiency can be assessed by the presence of germline and somatic mutations in HR genes, including the breast cancer susceptibility genes 1 and 2 (*BRCA1* and *BRCA2*), Fanconi anemia genes (*BRIP1*, *PALB2*), the core *RAD* genes (*RAD51C*, *RAD51D*), and genes involved in HR pathways either directly (*CHEK2*, *BARD1*, *NBN*, *ATM*) or indirectly (*CDK12*) (5). The lifetime risk of epithelial ovarian cancer of well-established moderate- and high-penetrance susceptibility genes varies approximately between 5% and 60%, among which *BRCA1/2* genes had the highest risk coefficient (5). Mutations in *BRCA1/2* genes may lead to chromosomal instability, promote cell proliferation and prevent differentiation of normal cells, thus resulting in the occurrence and development of tumor (6). It is also an important biomarker for therapeutic intervention, as ovarian cancer patients with germline or somatic *BRCA1/2* mutation benefit more from chemotherapy and poly (ADP-Ribose) polymerase (PARP) inhibitors than non-carriers (7-10). Therefore, fully understanding *BRCA1/2* mutations is of great significance for clinical genetic counseling and guiding medical management strategies of ovarian cancer.

Common mutation types include single-nucleotide variants (SNV), insertion/deletion (indel) and large genomic rearrangement (LRG) and can occur in both germ cells and somatic cells. To our knowledge, due to the insufficient precision of bioinformatics algorithm on copy number variation in next-generation sequencing (NGS) method, previous studies on the prevalence of *BRCA1/2* mutation in ovarian cancer mainly evaluated germline SNV and indel, but usually lacked LGR detection (11-17). Besides that, studies on tumor *BRCA1/2* mutation are still limited for the relatively lower prevalence and more complex detection workflow (4,18,19). Although some studies investigated *BRCA1/2* mutations in ovarian cancer patients in our country, none of them has simultaneously clarified the overview of germline and tumor *BRCA1/2* mutation including SNV, indel and LGR, also none specifically in HGSOC patients. In this study, we took advantage of NGS and multiplex ligation-dependent probe amplification (MLPA) technologies to investigate deleterious germline and somatic *BRCA1/2* mutations in Chinese HGSOC patients, and analyze their association with clinicopathological factors and survival outcomes. We present the following article in accordance with the MDAR reporting checklist (available at http://dx.doi.org/10.21037/atm-20-6827).

Methods

Study patients and samples

An unselected cohort of 115 Chinese HGSOC patients undergone surgery in Fudan University Shanghai Cancer Center from 2014 to 2015 were sequentially selected for our study population. Patients enrolled were supposed to meet the following inclusion criteria: (I) pathologically confirmed HGSOC at the Department of Pathology of Fudan University Shanghai Cancer Center; (II) sufficient formalin-fixed paraffin-embedded (FFPE) sections for somatic testing; (III) DNA extracted was qualified for NGS and MLPA testing; and (IV) willingness to provide signed consent in advance of the trial. Patients not meeting all of these inclusion criteria were excluded. Blood and paired FFPE tissue samples were collected and retrieved from our biobank and the Department of Pathology for *BRCA1/2* testing. Genomic DNA extracted from tumor tissue sections and peripheral blood were performed using QIAamp DNA MiniKit and QIAamp DNA MidiKit (QIAgen, Valencia, CA), respectively. Clinicopathological parameters were electronically retrieved from the Hospital Information System (HIS) of Fudan University Shanghai Cancer Center. Median age at diagnosis in this cohort was 51 years (range, 38–79 years). All patients were followed up until July, 2020, or death. The median follow-up time was 44.8 (0.2–73.2) months. Disease relapse or progression was determined by medical imaging, serology, or histology. Progression-free survival (PFS) was measured from diagnosis to local or systemic recurrence or the last follow-up, while OS was measured from diagnosis to death or the last follow-up. Patients with one or more family members within two generations with breast or ovarian cancer were considered as having an HBOC family history. No kinship was found among them according to information of genetic

counseling. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Medical Ethics Committee of Fudan University Shanghai Cancer Center (NO.: FWA00030121) and informed consent was taken from all individual participants.

BRCA1/2 SNV/indel detection by NGS

For targeted NGS analysis, total 265 primer pairs in two pools (133 pairs in pool 1, and 132 pairs in pool 2) were used in Oncomine™ *BRCA* Research Assay (Thermo Fisher Scientific, Schaumburg, IL, USA), which can amplify the entire coding regions and 20 bp upstream or downstream of exon–intron boundaries of *BRCA1/2* genes. Multiplex PCR was performed using 20 ng genomic DNA with the following cycling conditions: 99 ℃ × 2 minutes, 20 cycles of 99 ℃ × 15 seconds, and 60 ℃ × 4 minutes. The amplicons were treated with 2 μL FuPa reagent to partially digest primers and phosphorylate the amplicons with the following conditions: 50 ℃ × 10 minutes, 55 ℃ × 10 minutes, and 60 °C × 20 minutes. The diluted barcodes (Thermo Fisher Scientific) were ligated with the following conditions: 22 ℃ × 30 minutes, and 68 ℃ × 5 minutes, and 72 ℃ × 10 minutes. Libraries were purified using Agencourt AMPure XP reagents (Beckman Coulter, Brea, CA, USA). Concentration was measured using an Ion Library Quantitation Kit (Thermo Fisher Scientific), then the same amount of 100 pmol/L libraries was pooled in one sequencing reaction. Emulsion PCR was implemented with the Ion OneTouch™ 2.0 System and Hi-Q™ View OT2 reagents (Thermo Fisher Scientific) following the manufacturer's instructions. The template-positive particles were purified using Ion OneTouch™ ES system and MyOne™ Streptavidin C1 Beads (Thermo Fisher Scientific). Parallel sequencing was performed on a Personal Genome Machine (PGM) sequencer using the Ion PGM™ Hi-Q™ Sequencing Kit according to the manufacturer's instructions. Sequencing was performed using 500 flow runs that generated ~200 bp reads.

The sequence data were processed using standard pipeline on Torrent Suite™ version 5.4 (Thermo Fisher Scientific) as previously described (20). Annotations including SNV, indel, and splice site alteration were performed using Ion Reporter™ version 5.4 (Thermo Fisher Scientific). Binary alignment map (BAM) files were visually confirmed with the Integrative Genomics Viewer (IGV) 2.4.4. Error artifacts of sequence, alignment, or variant call were discarded. Minor allele frequency (MAF) of variant less than 0.01 was considered for further pathogenicity evaluation. Variants were classified as pathogenic, likely pathogenic, uncertain significance, likely benign and benign according to ACMG guideline (21). Pathogenic/likely pathogenic (P/LP) variants were regarded as deleterious mutations with clinical significance. MAF was identified from population database including 1000 Genomes Project database (http://phase3browser.1000genomes.org/) and dbSNP (https://www.ncbi.nlm.nih.gov/snp/) and Exome Aggregation Consortium and Exome Sequencing Project. The variants pathogenic determination referred to databases such as the *BRCA* Exchange database (https://brcaexchange.org/favicon.ico), LOVD database (https://databases.lovd.nl/shared/genes) and ClinVar (http:// www.ncbi.nlm.nih.gov/clinvar/) and published papers. Bioinformatic tools including SIFT (http://sift.jcvi.org), Align GVGD (http://agvgd.iarc.fr/agvgd_input.php) and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2) were used as supplementary evidence to prove that a variant may affect normal function.

LGRs detection by MLPA

Genomic DNA extracted from the patient's peripheral blood was used for *BRCA* LGRs detection following the MLPA instructions (MRC-Holland, Amsterdam, The Netherlands). Probe mix P002 and P087 were used for the detection or confirmation of *BRCA1* LGRs. Similarly, probe mix P045 and P090 were used for *BRCA2* LGRs. Briefly, 100 ng genomic DNA dissolved in 5 μL TE buffer was used for denaturation with 98 ℃ × 5 minutes, then MLPA probe mix was added for hybridization with the following conditions: 95 ℃ × 1 minute, and 60 ℃ × 16–20 hours. After hybridization, a Ligase-65 master mix was added for ligation with the following conditions: 54 ℃ pause, 54 ℃ × 15 minutes, and 98 ℃ × 5 minutes. Multiplex PCR was performed with the following cycling conditions: 35 cycles of 95 ℃ × 30 seconds, 60 ℃ × 30 seconds, 72 ℃ × 60 seconds and an additional 72 ℃ × 20 minutes. Finally, electrophoresis and data analysis were performed on ABI 3500 machine (Thermo Fisher Scientific) and Coffalyser.NET software (MRC-Holland).

Statistical analysis

Pathologic characteristics tabulated by their types or ranges were compared between groups by chi-square test or Fisher's exact test, as appropriate. Survival analysis was

performed using log-rank test, Kaplan-Meier analysis and multivariate Cox proportional hazards regression models. All analyses were performed using GraphPad Prism 8.0 (GraphPad Software Inc., San Diego, USA) and SPSS version 19 (SPSS version 19; SPSS, Chicago, IL). P values <0.05 were considered as statistically significant.

Results

Germline and somatic BRCA1/2 mutations in HGSOC

The complete list of deleterious mutations identified in this cohort and their frequency were summarized in *Table 1* and *Figure 1*. There were 25 (21.7%) germline *BRCA1/2* mutations found by NGS testing, including 19 (16.5%) *BRCA1* and 6 (5.2%) *BRCA2* mutations (*Figure 1A*). In addition, 5 (4.3%) *BRCA1* LGRs were detected in blood DNA by MPLA, whereas no *BRCA2* LGRs were found in these patients (*Figure 1A*). The entire germline *BRCA1/2* mutation frequency was 26.1%. Among them, the proportion of frameshift, LGRs, nonsense, splice site alteration and nonsense were 50.0%, 16.7%, 16.7%, 10.0% and 6.6%, respectively (*Figure 1B*). Moreover, c.4228delG, c.1786delC, c.3059delC, c.2341G>T found in *BRCA1* and c.6382_6386delAAAGA, c.5851dupA found in *BRCA2* were novel that hasn't been reported in database (*Table 1*).

All germline mutations detected by NGS were also found in paired tumor tissues. In addition, 10 (8.6%) mutations were found only in tumor tissues, including 5 (4.3%) in *BRCA1* and 5 (4.3%) in *BRCA2* (*Figure 1A*). Nine (90%) of them were frameshift and the other one was nonsense (*Figure 1D*). c.1933del, c.3401_3405delAACAG found in *BRCA1* and c.4012_4024delGGCAGTGATTCAA found in *BRCA2* were not reported before. The mutational allele frequency ranged from 7.4% to 45.5%. Since LGRs detected in blood DNA theoretically exists in tumor tissues, the entire tumor *BRCA1/2* mutation frequency was 34.8%. Among these tumor *BRCA1/2* mutations, the proportion of frameshift, LGRs, nonsense, splice site alteration and nonsense were 60.0%, 12.5%, 15.0%, 7.5% and 5.0%, respectively (*Figure 1C*).

All *BRCA1/2* mutations including SNV, indel and LGRs were visualized by IGV software or Coffalyser software. Notably, no patient in this cohort was found with two or more deleterious mutations showing a mutual exclusion of *BRCA1/2* mutation. In terms of distribution, all mutations are scattered in various functional domains and protein binding regions of *BRCA1/2* (*Figure 2*).

The association between BRCA mutations and clinicopathological factors

Age at diagnosis, FIGO stage, family history of HBOC and family history of other cancer than breast and ovarian cancer, including esophageal cancer, gastric cancer, liver cancer, lung cancer, pancreatic cancer, intestinal cancer and malignant teratoma, were included in pathologic characteristics. The median age in this cohort was 51 years (range, 38–79 years). 10.4% of these patients had potentially significant family history of HBOC. The proportion of germline (66.7%) and tumor (75%) mutation carriers was significantly increased for patients with family history when compared with those without (P<0.05), while somatic *BRCA1/2* mutation carriers showed no significant association with inheritance patterns (8.3%, P=0.4, *Table 2*). In addition, patients with deleterious germline *BRCA1/2* mutation appeared to be younger than non-carriers (mean age, 50.9 years *vs.* 54.4 years, P=0.004), while somatic or tumor *BRCA1/2* mutation carriers showed no significant association with ages (mean age, 58.7 *vs.* 54.4 years for somatic, P=0.314, 52.9 years *vs.* 54.4 years for tumor, P=0.076, *Table 2*). Notably, the onset age of patients with germline mutations was significantly younger than that of patients with somatic mutations (mean age, 50.9 *vs.* 58.7 years, P=0.009). No significant association was found between *BRCA1/2* status and clinicopathological factors including stage and family history of other cancers. The relationships between *BRCA1* and *BRCA2* mutation and clinicopathological factors are listed in *Table 3*. No statistical significance was found in both ages (mean age, 50.5 *vs.* 52.5 years, respectively, P=1, *Table 3*) and family history of HBOC (P=0.155) between the two groups.

Survival analysis

At a median follow-up of 44.8 months (range, 0.2–73.2 months), there were 62 (53.9%) recurrences and 52 (45.2%) deaths in this cohort. The Kaplan Meier and Cox regression analysis for PFS and OS by mutation status are shown in *Figure 3* and Figures S1,S2. In univariate analysis, patients with tumor *BRCA1/2* mutations had significant improvements than non-carriers in OS in the first two years after surgery, with HR 22.322 (95% CI, 21.52–23.123, P=0.018). The results were confirmed by multivariate analysis with HR 0.35 (95% CI, 0.07–1.64, P=0.022) for germline mutations and HR 0.2 (95% CI, 0.04–0.9, P=0.008) for tumor mutations (Figure S2). No significant

Table 1 The list of patients with *BRCA1/2* mutation and related information

No	Age	FH	Stage	Gene	Exon/intron	Variation	AA change	Variant effect	Type
1	40	No	IIIc	*BRCA1*	Exon 13	c.4327C>T	p.(Arg1443*)	Nonsense	Gemline
2	42	No	IVb	*BRCA1*	Exon 13	c.4228delG	p.(Glu1410Lysfs*5)	Frameshift	Gemline#
3	44	Yes	IIIc	*BRCA1*	Exon 11b	c.1786delC	p.(Leu596Serfs*3)	Frameshift	Gemline#
4	44	No	IIIc	*BRCA1*	Exon 5	c.179_180insT	p.(Gln60Hisfs*6)	Frameshift	Gemline#
5	45	No	IIIc	*BRCA1*	Exon 11b	c.3916_3917delTT	p.(Leu1306Aspfs*23)	Frameshift	Gemline
6	45	No	IIIc	*BRCA1*	Exon 11b	c.3059delC	p.(Pro1020Glnfs*4)	Frameshift	Gemline#
7	47	No	IV	*BRCA1*	Exon 24	c.5470_5477delATTGGGCA	p.(Ile1824Aspfs*3)	Frameshift	Gemline
8	47	No	IIIc	*BRCA1*	Exon 24	c.5470_5477delATTGGGCA	p.(Ile1824Aspfs*3)	Frameshift	Gemline
9	47	No	IIIc	*BRCA1*	Exon 11b	c.3859delG	p.(Glu1287Argfs*20)	Frameshift	Gemline
10	47	Yes	IIIc	*BRCA1*	Exon 16	c.4801A>T	p.(Lys1601*)	nonsense	Gemline
11	48	No	IIIc	*BRCA1*	Exon 11b	c.3296delC	p.(Pro1099Leufs*10)	Frameshift	Gemline
12	48	Yes	IIIc	*BRCA1*	Exon 3	c.110C>A	p.(Thr37Lys)	Missense	Gemline
13	48	No	IIIa	*BRCA1*	Intron 3	c.135-2A>G	–	Splice	Gemline
14	49	No	IIb	*BRCA1*	Exon 8	c.493delC	p.(Leu165*)	Frameshift	Somatic
15	49	No	IIIc	*BRCA1*	Exon 11b	c.2217dupA	p.(Val740Serfs*3)	Frameshift	Gemline
16	50	No	IIIc	*BRCA1*	Intron 16	c.4987-2A>G	–	Splice	Gemline
17	51	No	IIIc	*BRCA1*	Exon 11b	c.3916_3917delTT	p.(Leu1306Aspfs*23)	Frameshift	Somatic
18	52	No	IV	*BRCA1*	Exon 20	c.5251C>T	p.(Arg1751*)	nonsense	Gemline
19	54	Yes	IIIc	*BRCA1*	Exon 11b	c.2341G>T	p.(Glu781*)	nonsense	Gemline#
20	57	Yes	IIIc	*BRCA1*	Exon 11b	c.4065_4068delTCAA	p.(Asn1355Lysfs*10)	Frameshift	Somatic
21	61	No	IIIc	BRCA1	Intron 2	c.81-2A>G	–	Splice	Gemline
22	61	No	IIIc	BRCA1	Exon 11b	c.1933del	p.(Ser645Leufs*6)	Frameshift	Somatic#
23	69	No	IIIc	*BRCA1*	Exon 11b	c.3401_3405delAACAG	p.(Glu1134Alafs*5)	Frameshift	Somatic#
24	79	No	IIIc	*BRCA1*	Exon 2	c.66dupA	p.(Glu23Argfs*18)	Frameshift	Gemline
25	44	Yes	IV	*BRCA1*	–	Exon 3 deletion	–	LGR	Gemline
26	51	Yes	IIIc	*BRCA1*	–	Exons 21-24 deletion	–	LGR	Gemline
27	68	Yes	IIIc	*BRCA1*	–	Exons 5-7 deletion	–	LGR	Gemline
28	70	No	IIIc	*BRCA1*	–	Exon 1 deletion	–	LGR	Gemline
29	43	No	IIIb	*BRCA1*	–	Whole gene deletion of exons 1-24	–	LGR	Gemline
30	43	No	IIIc	*BRCA2*	Exon 11	c.6382_6386delAAAGA	p.(Lys2128Ilefs*2)	Frameshift	Gemline#
31	46	No	IIIc	*BRCA2*	Exon 11	c.4012_4024delGGCAGTGATTCAA	p.(Gly1338Valfs*32)	Frameshift	Somatic#
32	47	No	IIIc	*BRCA2*	Exon 11	c.5495del	p.(Ser1832Leufs*8)	Frameshift	Gemline
33	47	No	IIIc	*BRCA2*	Exon 18	c.8009C>T	p.(Ser2670Leu)	Missense	Gemline
34	47	No	IIIc	*BRCA2*	Exon 11	c.4408_4412delATAAG	p.(Ile1470Lysfs*10)	Frameshift	Gemline
35	57	No	IIIb	*BRCA2*	Exon 11	c.4633del	p.(Leu1545Phefs*23)	Frameshift	Somatic
36	59	No	III	*BRCA2*	Exon 11	c.3362C>G	p.(Ser1121*)	Nonsense	Gemline
37	60	No	IIIc	*BRCA2*	Exon 5	c.469_473delAAGTC	p.(Lys157Serfs*24)	Frameshift	Somatic
38	68	No	IIIc	*BRCA2*	Exon 25	c.9281C>G	p.(Ser3094*)	Nonsense	Somatic
39	69	No	IIc	*BRCA2*	Exon 11	c.3163_3166delAATC	p.(Asn1055Lysfs*4)	Frameshift	Somatic
40	72	No	IV	*BRCA2*	Exon 11	c.5851dupA	p.(Ser1951Lysfs*9)	Frameshift	Gemline#

#, novel mutation. FH, family history; AA, amino acid; LGR, large genomic rearrangement.

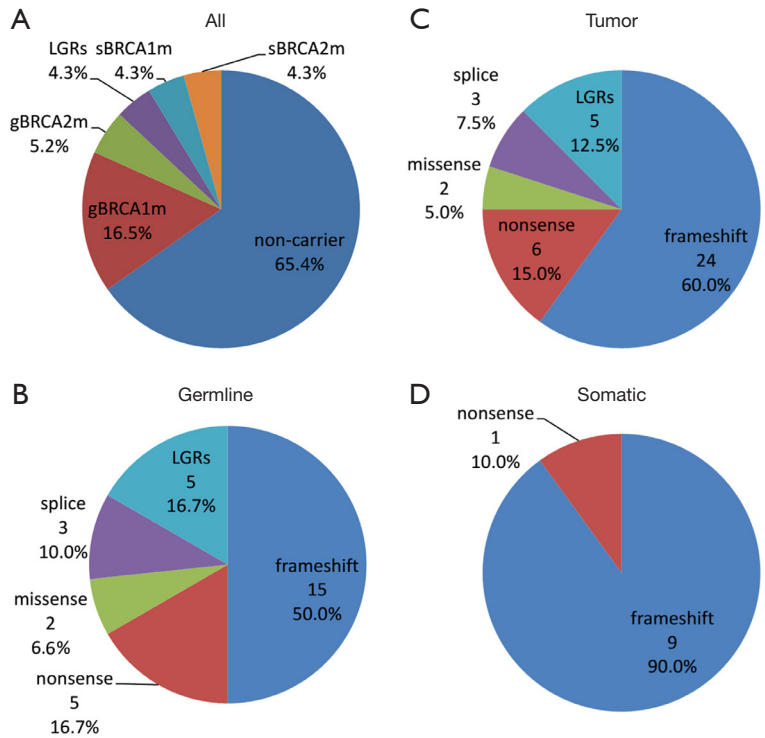

Figure 1 Summary of *BRCA1/2* mutations in 115 Chinese HGSOC patients. (A) Distribution of all mutations and frequencies. (B) Proportion of germline mutation types detected in blood DNA. (C) Proportion of tumor mutation types detected in tumor DNA. (D) Proportion of somatic mutation types detected in tumor DNA. *gBRCA1/2m*, germline *BRCA1/2* SNV/Indel; LRGs, Large genomic rearrangements; *sBRCA1/2m*, somatic *BRCA1/2* SNV/Indel.

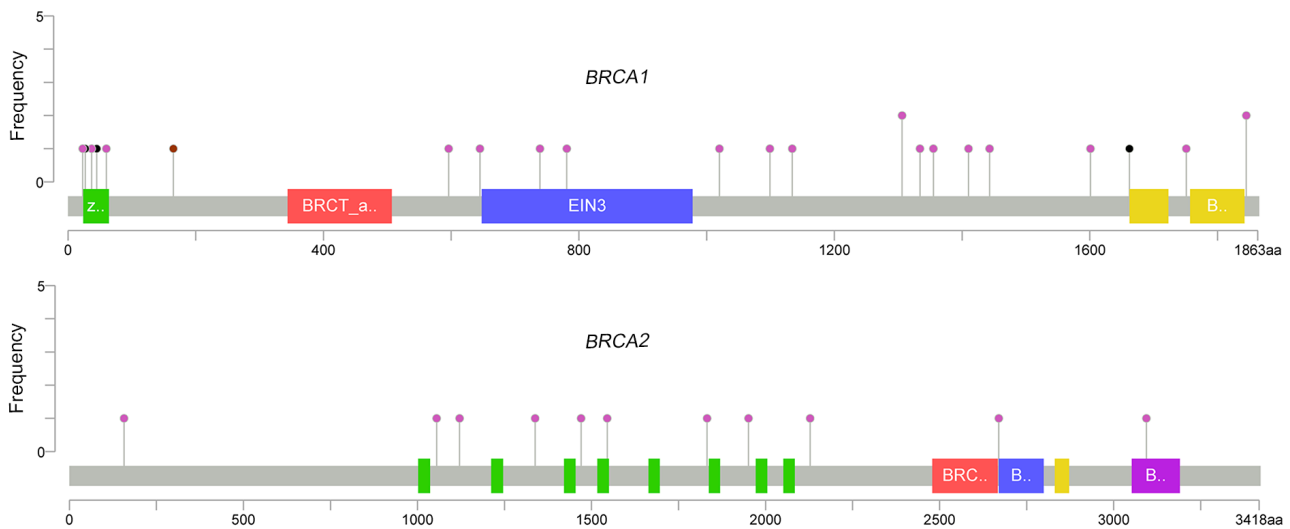

Figure 2 Schematic representation of *BRCA1/2* deleterious mutations in functional domains and protein binding regions.

Table 2 The association between *BRCA1/2* mutations and clinicopathological factors

	Non-carrier	Pathogenic or likely pathogenic *BRCA1/2* mutation						P[d]
		Tumor	P[a]	Germline	P[b]	Somatic	P[c]	
Age								
≤50 [52]	29 (55.8%)	23 (44.2%)	0.076	21 (40.4%)	0.004	2 (3.8%)	0.314	0.009
>50 [63]	46 (73.0%)	17 (27.0%)		9 (14.3%)		8 (12.7%)		
FH of HBOC								
Yes [12]	3 (25.0%)	9 (75.0%)	0.003	8 (66.7%)	0.002	1 (8.3%)	0.4	0.404
No [103]	72 (69.9%)	31 (30.1%)		22 (21.4%)		9 (8.7%)		
FH of other cancer								
Yes [18]	14 (77.8%)	4 (22.2%)	0.287	3 (16.7%)	0.384	1 (5.6%)	0.683	1
No [97]	61 (62.9%)	36 (37.1%)		27 (27.8%)		9 (9.3%)		
Stage								
I–II [12]	10 (83.3%)	2 (16.7%)	0.212	0	0.059	2 (16.7%)	0.628	0.058
III–IV [103]	65 (63.1%)	38 (36.9%)		30 (29.1%)		8 (7.8%)		

[a], tumor *vs.* non-carrier; [b], germline *vs.* non-carrier; [c], somatic *vs*. non-carrier; [d], germline *vs.* somatic. FH, family history.

Table 3 Association of clinicopathological factors with *gBRCA1* and *gBRCA2* mutation

	gBRCA1	*gBRCA2*	P
Age			
≤50 [21]	17 (81.0%)	4 (19.0%)	1
>50 [9]	7 (77.8%)	2 (22.2%)	
FH of HBOC			
Yes [8]	8 (100%)	0	0.155
No [22]	16 (72.7%)	6 (27.3%)	
FH of other cancer			
Yes [3]	2 (66.7%)	1 (33.3%)	0.501
No [27]	22 (81.5%)	5 (18.5%)	
Stage			
I–II [0]	0	0	–
III–IV [30]	24 (80.0%)	6 (20.0%)	

FH, family history; *gBRCA*, germline *BRCA1/2*.

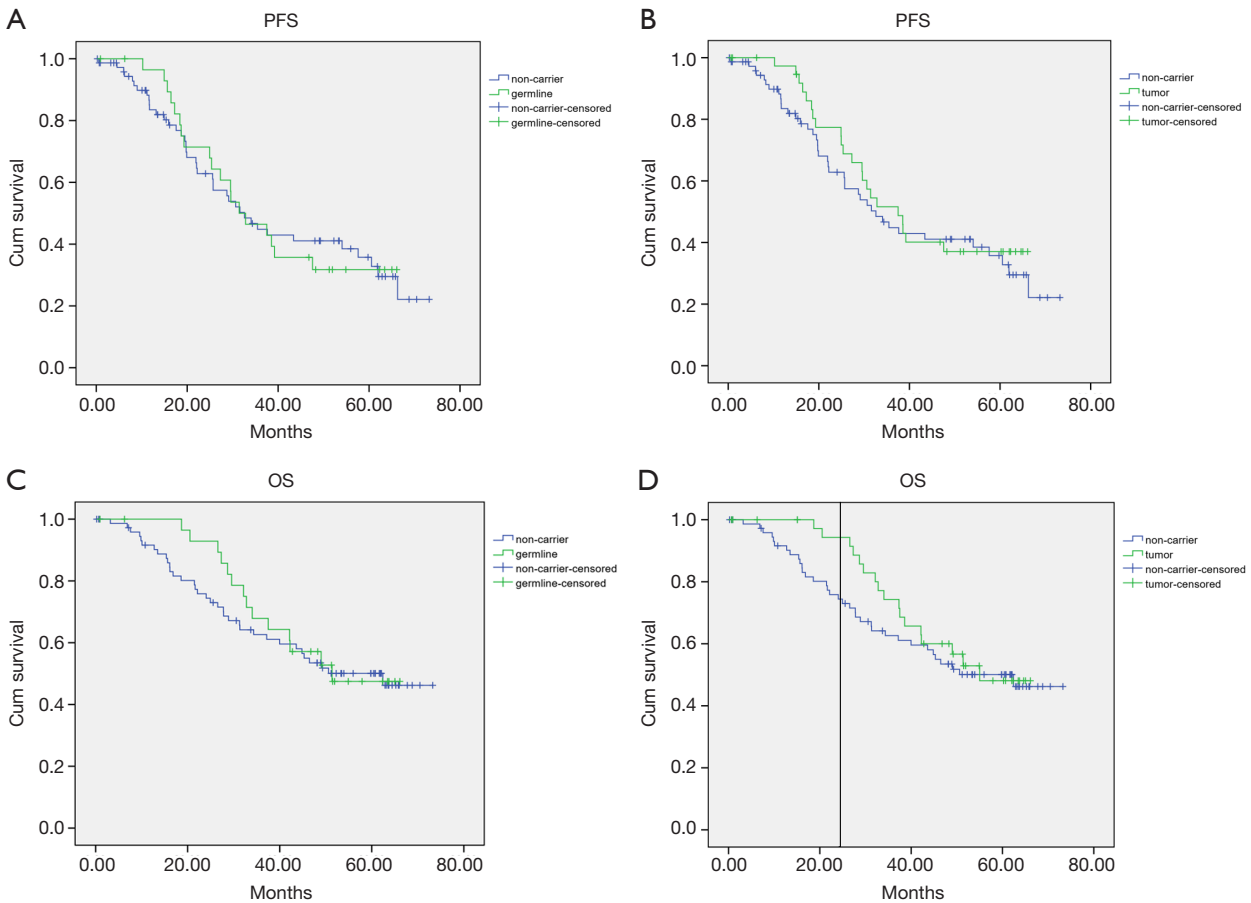

Figure 3 Kaplan-Meier survival analysis on germline and tumor *BRCA1/2* mutations in HGSOC. (A) Progression-free survival (PFS) with and without germline mutation. (B) PFS with and without tumor mutation. (C) Overall survival (OS) with and without germline mutation. (D) OS with and without tumor mutation.

impacts were found between various mutation status in PFS outcomes. No significant differences were found between *BRCA1* or *BRCA2* and non-carriers in PFS or OS (*Figure 4*). The low number of events precluded survival analysis of somatic subgroups.

Discussion

This study specifically focused on Chinese HGSOC patients, and firstly simultaneously clarified the overview of germline and somatic *BRCA1/2* mutations including SNV, indel and LGR, and further investigated the relationship between *BRCA1/2* mutation with clinical pathologic characters and survival outcomes.

Current findings showed that the overall incidence of deleterious *BRCA1/2* mutations in tumor of HGSOC patients was 34.8%, of which germline mutation accounted for the vast majority, and the proportion was 26%. However, this frequency varies greatly in the reports of other countries. One study on germline *BRCA1/2* mutation in 1915 unselected ovarian carcinomas was conducted in the United States, they found the deleterious mutation frequency including SNV, indel and LGRs in 1498 HGSOC patients was 16.0% by targeted capture (11), while another study from the United States showed that 98 out of 433 (22.6%) HGSOC patients were identified with germline *BRCA1/2* mutation (SNV, indel and LGRs) by sequencing and MLPA (12). In a prospectively study mainly focused on the Arab population, a prevalence of 25.7% deleterious germline *BRCA1/2* mutation (SNV and indel) was found in 74 HGSOC patients by sequencing (13). Among Asian countries, a relatively small number screening

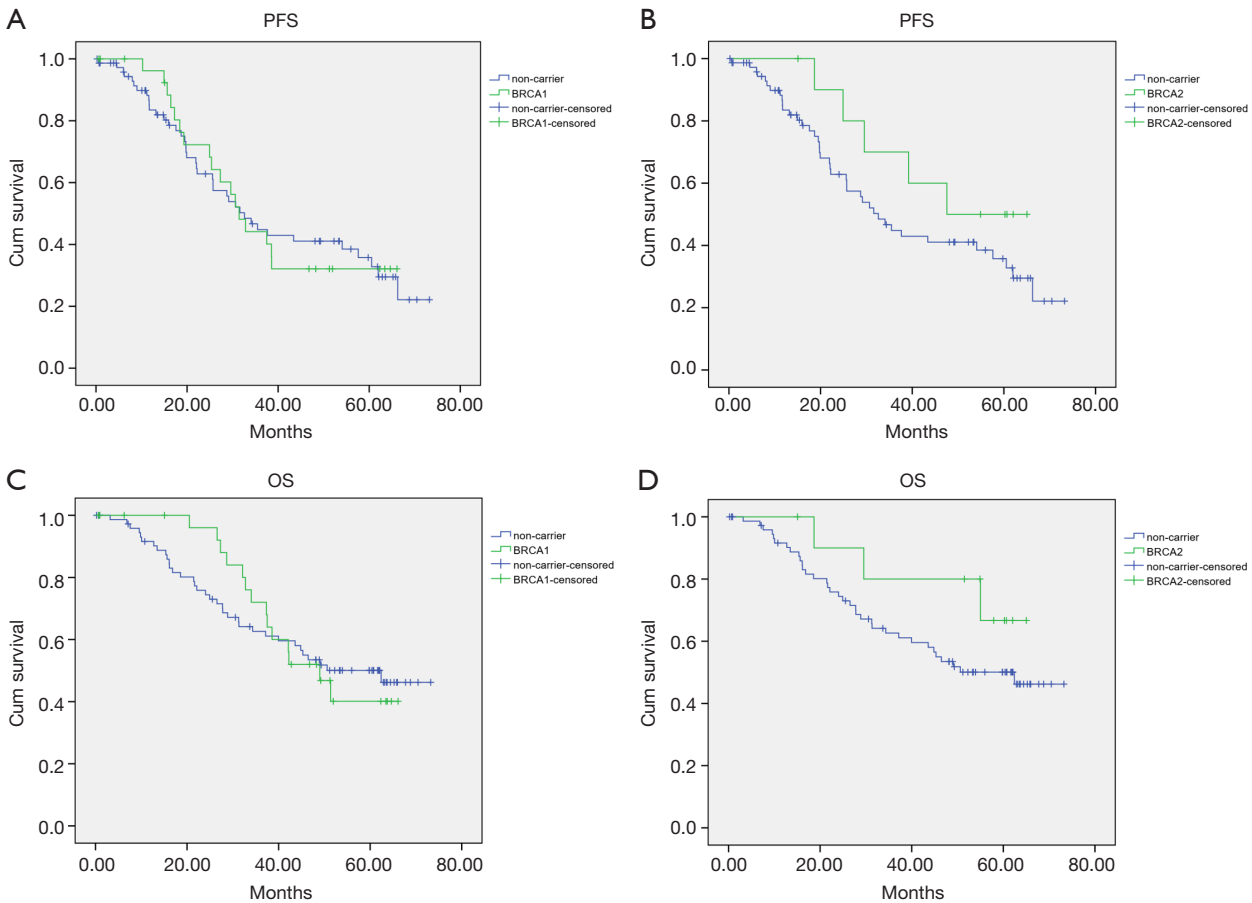

Figure 4 Kaplan-Meier survival analysis on various *BRCA1/2* mutations in HGSOC. (A) Progression-free survival (PFS) with and without *BRCA1* mutation. (B) PFS with and without *BRCA2* mutation. (C) Overall survival (OS) with and without *BRCA1* mutation. (D) OS with and without *BRCA2* mutation.

was performed for germline *BRCA1/2* mutation (SNV and indel) in 230 unselected ovary cancer patients by targeted panel sequencing, they found 22 out of 74 (29.7%) HGSOC patients carrying deleterious mutation (SNV, indel and LGRs) and the result was consistent with a respective study in Japan that reported 28.5% of germline *BRCA1/2* mutation (SNV and indel) by NGS testing (14,15). On the other hand, one study from Thailand found the germline *BRCA1/2* mutation (SNV and indel) frequency in HGSOC was 25.7% by NGS testing (16). In China, a nationwide multicenter germline *BRCA1/2* mutation prevalence study was conducted previously, 601 HGSOC patients were enrolled in this study, they found the deleterious *BRCA1/2* mutation prevalence (SNV, indel and LGRs) was 30.9% by targeted DNA sequencing, which was the high boundary of the previously reported range (17). Although the similar sequencing method for analysis of germline *BRCA1/2* mutations including SNV, indel and LGRs was used in these studies, the incidence possibly varied in differences with geography, ethnicity, population or even economic level. In general, the *BRCA1/2* mutation frequencies in Asian countries seems to be higher than that in Western countries. The deleterious germline *BRCA1/2* mutant rate in our study was similar to some of the above work (13,16). In addition, we identified 4.3% germline *BRCA1* mutation as LGRs, which suggests LGRs detection should be considered in mutation screening and genetic counseling in HGSOC patients, especially for young patients without SNV and indel.

Beyond germline mutations, somatic mutations in HR pathway have been implicated in sporadic ovarian cancer, accounting for approximately 6% of HGSOC (22). Our

results showed that the somatic *BRCA1* and *BRCA2* gene mutation frequencies in Chinese HGSOC patients were 4.3% and 4.3% respectively, which was slightly higher than the previously reported frequencies of 3% and 3% respectively in Western population (4). Currently in China, early screening and routine examination of *BRCA1/2* mutation for ovarian cancer patients is still incomplete, a higher percentage of patients diagnosed with distant metastasis, which may lead to the deviation of somatic mutation frequency. In this study, tumor samples showed high proportion of III and IV FIGO stage as shown before, these might be the reasons of higher somatic *BRCA1/2* mutation frequencies we observed.

From the clinical point of view, the PARP inhibitors efficacy includes both germline mutant and sporadic ovarian cancers with HR deficiency (23). Moreover, an additional important issue of current research is to expand their use in ovarian cancers with HR deficiency or even beyond HR-deficient. Recently, novel combinations of PARP inhibitors with drugs that inhibit HR, such as anti-angiogenics, immune checkpoint, PI3K/AKT/mTOR, WEE1, MEK, and CDK4/6 inhibitors that have also been proposed in patients with *de novo* or acquired HR proficiency to PARP inhibitors (24). However, further clinical practices are required to reduce overlapping toxicities by optimizing dose and schedule, and to utilize the combinations to highly selected patients who would not otherwise benefit from single PARP inhibitors (24). Meanwhile, a comprehensive mutation detection capability would accelerate the identification of cancers appropriate for combined therapy.

Patients with germline *BRCA1/2* mutation are associated with HBOC syndrome, which is characterized by familial clustering of breast and ovarian cancers (25). In this study, we observed significant associations between *BRCA1/2* mutation status and HBOC family history. In patients with family history, the deleterious germline *BRCA1/2* mutation incidence was as high as 66.7%. This result is in concordance with studies that showed 60–70% of patients with family history who had *BRCA1/2* mutation (17,26). Nonetheless, we also observed the deleterious germline *BRCA1/2* mutation rate was 21.4% in patients without HBOC family history in our study. In addition to family history, we also observed approximately 40% of HGSOC patients under 50 years had deleterious germline *BRCA1/2* mutation, which suggests onset age under 50 years is another clinical factor associated with germline *BRCA1/2* status. This finding is consistent with the previous research on young Israeli women which reported about 50% (27). These data conclude the probability to find a mutant rate varies greatly in different clinical subgroups, which leads to the hypothesis that testing for *BRCA1/2* mutations addressed within each specific clinical scenario could be more cost-effective for patients. A recently study also suggested that there is no evidence that delivering a widespread *BRCA1/2* testing for ovarian cancer patients is cost-effective with respect to standard practice for preventive and therapeutic purposes (28). Therefore, how to select candidates for *BRCA1/2* testing is a feasible and challenging work in the future.

In our study, we did not find significant impacts of germline and tumor *BRCA1/2* mutations on PFS, but tumor and germline *BRCA1/2* mutations were all inclined to better outcomes on OS. However, the advantage on OS for those with *BRCA1/2* mutations compared to non-carriers showed a time-dependent decline, as in previous report (11). Specifically, we found this advantage was more significant within 2 years after surgery. The improved survival outcomes were likely caused by a higher sensitivity to platinum in patients with *BRCA1/2* mutations than non-carriers (29).

In summary, there is a high germline (26.0%) and tumor (34.8%) *BRCA1/2* mutation incidences including SNV, indel and LGR in Chinese HGSOC patients. Germline mutations were associated with HBOC family history and age at diagnosis of HGSOC, whereas somatic mutations were not. In our study, tumor *BRCA1/2* mutations predicted increased sensitivity to platinum-based chemotherapy and significantly improved survival outcomes, but the advantage was time-depended. A larger cohort and even multigene panel testing should be examined to clarify the relation between *BRCA1/2* mutation and prognosis.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Medical Ethics Committee of Fudan University Shanghai Cancer Center (NO.: FWA00030121) and informed consent was taken from all individual participants.

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