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Prevalence and clinical significance of pathogenic germline *BRCA1/2* mutations in Chinese non-small cell lung cancer patients

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ABSTRACT

Objective: Germline alterations in the breast cancer susceptibility genes type 1 and 2, *BRCA1* and *BRCA2*, predispose individuals to hereditary cancers, including breast, ovarian, prostate, pancreatic, and stomach cancers. Accumulating evidence suggests inherited genetic susceptibility to lung cancer. The present study aimed to survey the prevalence of pathogenic germline *BRCA* mutations (*gBRCAm*) and explore the potential association between *gBRCAm* and disease onset in Chinese advanced non-small cell lung cancer (NSCLC) patients.

Methods: A total of 6,220 NSCLC patients were screened using capture-based ultra-deep targeted sequencing to identify patients harboring germline *BRCA1/2* mutations.

Results: Out of the 6,220 patients screened, 1.03% (64/6,220) of the patients harbored the pathogenic *gBRCAm*, with *BRCA2* mutations being the most predominant mutations (49/64, 76.5%). Patients who developed NSCLC before 50 years of age were more likely to carry *gBRCAm* (P = 0.036). Among the patients harboring classic lung cancer driver mutations, those with concurrent *gBRCAm* were significantly younger than those harboring the wild-type *gBRCA* (P = 0.029). By contrast, the age of patients with or without concurrent *gBRCAm* was comparable to those of patients without the driver mutations (P = 0.972). In addition, we identified *EGFR*-mutant patients with concurrent g*BRCAm* who showed comparable progression-free survival but significantly longer overall survival (P = 0.002) compared to *EGFR*-mutant patients with wild-type germline *BRCA*.

Conclusions: Overall, our study is the largest survey of the prevalence of pathogenic *gBRCAm* in advanced Chinese NSCLC patients. Results suggested a lack of association between germline *BRCA* status and treatment outcome of EGFR-TKI. In addition, results showed a positive correlation between pathogenic *gBRCAm* and an early onset of NSCLC.

KEYWORDS Germline BRCA mutations; non-small cell lung cancer; prevalence; BRCA1; BRCA2

Introduction

Certain germline alterations predispose individuals to hereditary cancers. Approximately, 5% of breast cancer cases and 20%–30% of ovarian cancers are caused by germline

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alterations in the breast cancer susceptibility type 1 and 2 (*BRCA1* and *BRCA2*) genes[1,2]. Women with germline *BRCA* mutation (*gBRCAm*) have significantly higher lifetime risk of developing breast, ovarian, tubal, and peritoneal cancers. The overall prevalence of *gBRCAm* in women ranged from 1 in 400 to 1 in 800 depending on the ethnicity[3]. In addition to breast and ovarian cancers, results showed that *gBRCAm* additionally conferred increased susceptibility to a spectrum of other cancers, including but not limited to, colorectal[4,5], lung[6], prostate[7], pancreatic[8], and stomach cancers[7,9].

Abnormalities in the DNA repair system are central to the emergence of mutations, which ultimately result in the development of cancer[10]. *BRCA1* and *BRCA2* are tumor suppressor genes that participate in the DNA repair processes through homologous recombination repair (HRR) and thus contribute to the maintenance of genomic integrity. *BRCA1* and *BRCA2* act in concert to repair DNA lesions, which normally stall the DNA replication fork and/or cause DNA double-stranded breaks. Previous studies on *BRCA1/2* have driven the development of therapeutic strategies targeting defects in HRR, such as poly (ADP-ribose) polymerase (PARP) inhibitors, whose function is based on the inability of *BRCA*-deficient cells to repair DNA lesions. Studies showed that cells with complete *BRCA1/2* inactivation exhibit high sensitivity to DNA damaging agents[11,12]. Furthermore, numerous clinical trials have demonstrated an excellent response rate of *gBRCAm* carriers to PARP inhibitors[13,14]. The recent approval of olaparib for ovarian cancer combined with numerous promising results from trials involving other PARP inhibitors either as a single agent or in combination with other regimens for various cancer types has demonstrated the potential use of PARP inhibitors[15]. In addition, along with sensitivity to PARP inhibitors, *BRCA* mutations have been associated with sensitivity to platinum-based chemotherapy *in vitro*[16] and in phase III clinical studies in breast[17] and ovarian cancers[18].

Although environmental factors, such as tobacco smoking, are regarded as the primary causes of lung cancer, increasing evidence has suggested inherited genetic susceptibility to lung cancer[6]. Genome-wide association studies (GWAS) have revealed multiple polymorphic variants as determinants of lung cancer risk[19-21]. However, to date, the prevalence of *gBRCAm* in Chinese lung cancer patients remains elusive. In the present study, we screened 6,220 Chinese advanced NSCLC patients to survey the prevalence of *gBRCAm* by sequencing the entire coding region of *BRCA1/2*. We also investigated the clinical significance of *gBRCAm* in advanced NSCLC patients.

Patients and methods

Patients

Sequencing results of germline *BRCA* were retrieved and retrospectively screened from 6,220 advanced (stage IIIB to IV) Chinese NSCLC patients with various histology subtypes and who underwent somatic mutation profiling for the purpose of treatment selection and genetic testing. The study was approved by the Ethics Committee of Xiangya Hospital (Approval No. 201705818). Patients provided written informed consent for the use of their tissue and/or blood samples for analyzing their germline mutations, as well as their clinical data. The screened cohort was recruited from ten hospitals.

Preparation of plasma cell-free DNA

Peripheral blood (10 mL) was collected, stored in ethylenediaminetetraacetic acid (EDTA) acid tubes, and allowed to stand in 25°C for 2 h. The supernatant was transferred to a 15-mL centrifuge tube and then centrifuged for 10 min at 16,000 *g* at 4°C. Circulating cell-free DNA (cfDNA) was recovered from 4 to 5 mL of plasma using the QIAamp Circulating Nucleic Acid kit (Qiagen).

DNA preparation and capture-based targeted DNA sequencing

DNA quantification was performed using the Qubit 2.0 Fluorimeter with the dsDNA HS assay kits (Life Technologies, Carlsbad, CA). A minimum of 50 ng of DNA is required for NGS library construction. DNA shearing was performed using Covaris M220, followed by end repair, phosphorylation, and adaptor ligation. Fragments with sizes ranging from 200-400 bp were selected using AMPure beads (Agencourt AMPure XP Kit), followed by hybridization with capture probes baits, hybrid selection with magnetic beads, and PCR amplification. The quality and size range of amplified fragments were then assessed by performing bioanalyzer high-sensitivity DNA assay. Paired-end sequencing of the indexed samples was performed on a NextSeq 500 sequencer (Illumina, Inc., USA).

Germline variant calling and filtering

Germline SNVs were identified using Varscan with default parameters. Germline indels were identified using Varscan and GATK. Pathogenic *BRCA* mutations were determined by

a clinical molecular geneticist according to the guidelines of American College of Medical Genetics (ACMG). ClinVar and Enigma were used during manual curation for final confirmation of the results.

Somatic sequencing data analysis

Sequence data were mapped to the reference human genome (hg19) using BWA aligner 0.7.10. Local alignment optimization, variant calling, and annotation were performed using GATK 3.2, and VarScan. Plasma samples were compared to their corresponding white blood cell controls to identify somatic variants. Variants were filtered using the VarScan fpfilter pipeline. Loci with depths of less than 100 filtered out. At least 2 and 5 supporting reads were required for INDELs in plasma and tissue samples, respectively, while 8 supporting reads were required for SNV calling in both plasma and tissue samples. According to the ExAC, 1000 Genomes, dbSNP, ESP6500SI-V2 database, variants with population frequencies higher than 0.1% were classified as SNPs and excluded from further analysis. After filtering, the remaining variants were annotated with ANNOVAR and SnpEff v3.6. DNA translocation analysis was performed using Factera 1.4.3.

Statistical analysis

All data were analyzed using R software. Survival data were analyzed by Kaplan-Meier and log-rank test to compare the differences between the survival groups. Differences in groups were calculated and presented using paired, two-tailed Student's *t*-test. $P < 0.05$ was considered as statistically significant. The *p*-values were adjusted for variables, such as age, gender, and pathological stage, when applicable.

Results

Patient characteristics

We conducted a retrospective nationwide multicenter study across ten hospitals to survey the prevalence rate of *gBRCAm* in advanced (stage IIIB to IV) Chinese NSCLC patients with various histological subtypes. Patients who initially underwent NGS-based molecular testing for treatment guidance were included in the study. The WBCs were additionally sequenced for the purpose of filtering out germline mutations. Sequencing results of WBCs were retrieved and screened for *gBRCAm* status. The screened cohort comprised 3,412 males and 2,809 females with a median age of 63.2 years. The majority of the patients (4,752; 76.4%) were diagnosed with adenocarcinoma, followed by 765 patients (12.3%) with squamous cell carcinoma, 51 patients (0.82%) with large cell carcinoma (LCC), 31 patients (0.5%) with adenosquamous carcinoma, and the remaining 621 patients with other subtypes. A total of 1,089 patients were treatment-naïve, while the remaining 5,131 patients previously received treatment. We identified 64 patients with pathogenic *gBRCAm* and had a median age of 59 years; of these, 26 were females and 38 were males. Fifty-five patients were diagnosed with adenocarcinoma, two with LCC, two with squamous cell carcinoma, and the remaining five patients with NOS; of these, 43 patients were non-smokers, 13 were smokers, and the remaining eight patients had no information regarding smoking history. Thirty-five of them were treatment-naïve. Patient demographics of the cohort with pathogenic *gBRCAm* are summarized in **Table 1**.

Prevalence of gBRCA1/2 mutations

Of the 6,220 NSCLC patients screened, we detected 64 pathogenic and 699 variants of unknown significance

Table 1 Demographics of 64 patients with pathogenic germline *BRCA1/2* mutations

Chrematistics	No. of patients (%)
Gender	
Male	38 (59.4%)
Female	26 (40.6%)
Age, years (median and range)	59 (33-77)
Smoking status	
Yes	13
Non/ever	43
No information	8
Histology	
Adenocarcinoma	55 (86%)
Squamous cell	2 (3%)
Large cell	2 (3%)
Not specified	5 (8%)
Pathogenic/likely pathogenic germline mutations detected	
BRCA1	15 (23.4%)
BRCA2	49 (76.6%)
Treatment history	
Treatment-naive	35 (54.7%)
Previously treated	29 (45.3%)

(VUS) germline *BRCA* variants. Overall, we identified 64 patients harboring pathogenic *gBRCAm*, corresponding a prevalence of 1.03% (64/6,220). Results revealed a g*BRCA2* predominance with 15 (23.4%) g*BRCA1* loss-of-function (LOF) mutation carriers and 49 (76.6%) g*BRCA2* LOF mutation carriers. Consistent with *gBRCAm* findings reported in other cancer types, the mutations were found to be evenly distributed between the two genes, and no particular hotspots were identified. The distributions of g*BRCA1/2* mutations are presented in **Supplementary Figure S1A-S1B**. Among the pathogenic g*BRCA2* variants, we observed four types of mutations, with frameshift mutations being the most predominant mutation type accounting for 57.2% (28/49), followed by nonsense/stop gain point mutations with 24.5% (12/49), splice-site variants with 14.3% (7/49), and the remaining missense variants with 4%. Meanwhile, the majority of the pathogenic *BRCA1* variants were nonsense mutations (8/15), followed by frameshift mutations (5/15) and splice-site mutations. No g*BRCA1* pathogenic missense mutations were identified in our cohort. Furthermore, we detected several mutations, including R1443* in g*BRCA1* and D252fs, Q1037*, R2336L, and exon 16 splice site variant in g*BRCA2*, in at least two patients from our cohort.

Somatic mutation spectrum of patients with *gBRCAm*

In addition to analyzing the *gBRCAm* status of the lung cancer patients in our cohort, we investigated somatic mutations associated with patients harboring *gBRCAm*. Somatic mutation profiling was conducted using a panel consisting of 56 lung cancer-related genes spanning 330 kb of the human genome. Collectively, we identified 164 somatic mutations spanning 31 genes. A total of 47 patients harbored the following classic lung cancer driver mutations: 33 with *EGFR*, 3 with *ALK*, 6 with *KRAS*, 5 with *MET*, 1 with *ERBB2*, 1 with *ROS1*, and 2 with *BRAF* mutations (**Figure 1**). Four patients had multiple driver mutations, and six patients had no mutations detected based on this panel. *EGFR* was most frequently mutated gene, occurring in 53% of patients, followed by *TP53* and *PIK3CA*, occurring in 47% and 12% of patients, respectively. Six patients (9%) harbored somatic *BRCA2* mutations corresponding to two nonsense, one synonymous, one splice site, and two missense mutations. Other frequently occurring somatic mutations included *MYC* (9%), *CDKN2A* (8%), and *APC* (6%). Next, we examined whether there is a difference in the somatic mutation spectra in patients with *gBRCA1* and *gBRCA2* mutations. Our findings revealed that patients harboring *gBRCA1* or *gBRCA2* mutations had comparable mutation profiles (**Figure 1**). Taken together, the somatic mutation profiles of patients with *gBRCAm* were comparable to those of patients harboring wild-type germline *BRCA1/2*. We observed no significant differences in the somatic mutation spectra of patients with the g*BRCA1* and g*BRCA2* mutations.

gBRCAm is more prevalent in patients with early disease onset

Numerous studies have suggested correlations between

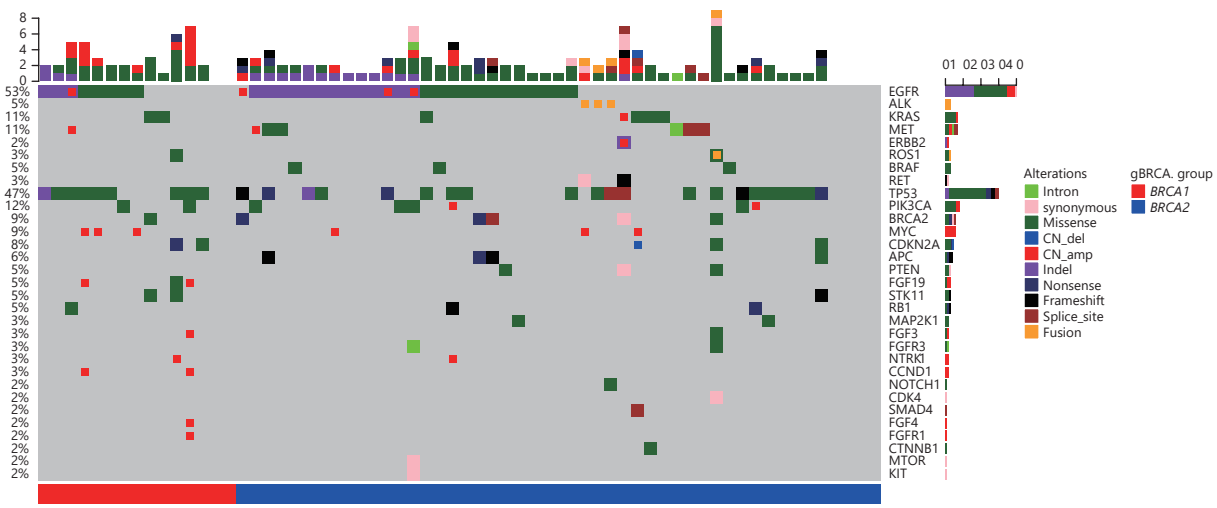

Figure 1 Somatic mutational profiles of 64 patients carrying pathogenic *gBRCAm*. Columns represent patients. Rows represent genes. Top bars represent the number of mutations per patient. Side bars represent the percentage of patients with the mutation. Colored boxes indicate different mutation types.

gBRCAm and the early onset of disease in several cancer types, including lung cancer. First, we investigated whether *gBRCAm* is associated with early onset of NSCLC in Chinese patients. The screened cohort comprised 947 patients diagnosed before age of 50; among them, 16 harbored *gBRCAm*, corresponding to a prevalence rate of 1.69%. A total of 4,945 patients were diagnosed at or after the age of 50; of these, 45 patients were *gBRCAm* carriers, corresponding to a prevalence of 0.91%. Our results revealed that patients with an onset of disease before 50 were more likely to have *gBRCAm* ($P = 0.036$) (**Figure 2A**). Importantly, it is well-established that *ALK* fusions tend to occur in younger patients; therefore, patients with *ALK* fusions were excluded from the analysis. In addition, results indicated that patients with *gBRCAm* tend to carry classic driver mutations for lung cancer ($P = 0.076$, **Figure 2B**). Further analysis revealed that among patients harboring driver mutations, those with concurrent *gBRCAm* were significantly younger compared to patients with wild-type (WT) g*BRCA* ($P = 0.029$, **Figure 2C**). We observed no significant differences in age between patients with *gBRCAm* and WT g*BRCA* among patients without the driver mutations ($P = 0.972$, **Figure 2C**). Furthermore, among the patients with *gBRCAm*, those with concurrent driver mutations were younger than patients without the driver mutations ($P = 0.032$, **Figure 2D**). In addition to driver mutations, we identified a positive correlation between *gBRCAm* with somatic *BRCA2* mutations ($P = 0.034$, **Figure 2E**) and a marginal positive correlation between *gBRCAm* and *PIK3CA* mutations ($P = 0.055$, **Figure 2F**). Somatic *BRCA2* mutations are likely to act as the second hit for tumorigenesis. In our cohort, we identified six patients with germline *BRCA2* mutations who carried concurrent somatic *BRCA* mutations; five of these patients harbored a *BRCA2* mutation and one had *BRCA1* mutations. Taken together, our findings suggested that *gBRCAm* predisposes patients for the development of lung cancer.

Survival analysis of patients with gBRCAm

Previous studies reported that patients with *gBRCA*m are more sensitive to platinum-containing chemotherapy[18,22].

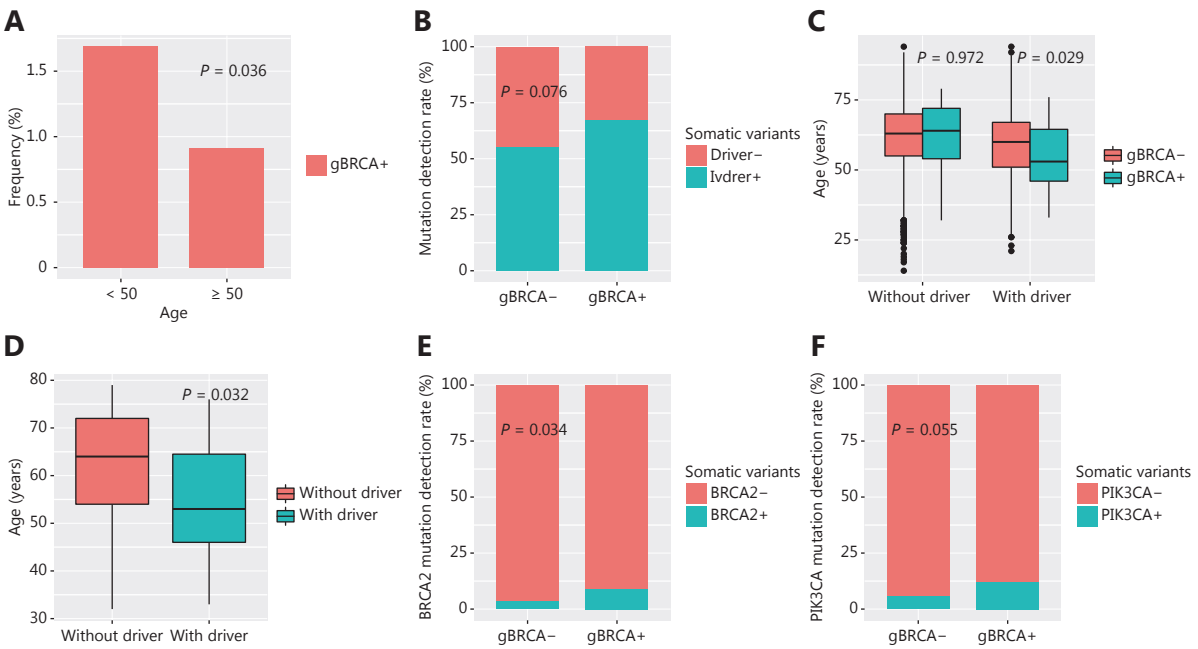

Figure 2 Relationship between *gBRCAm* and clinical and molecular features. (A) Germline *BRCA* mutations are more likely to occur in patients < 50 years of age. (B) Association between germline *BRCA* status and the presence of driver mutations. Blue bars denote the presence of driver mutations; red bars denote the lack of driver mutations. X-axis denotes the status of germline *BRCA*. (C) In the absence of driver mutations, no age difference was observed between patients with (+) or without (–) germline *BRCA* mutations. (D) In the presence of driver mutations, patients with germline *BRCA* mutations are younger. X-axis denotes the presence or absence of classic lung cancer driver mutations. (E) Association between germline *BRCA* status and somatic *BRCA2* mutation. (F) Association between germline *BRCA* status and somatic *PIK3CA* mutation. X-axis indicates the *gBRCAm* status, (–) denotes wild-type status, while (+) represents mutation carriers. Y-axis indicates the percentage of patients with the somatic molecular features being observed. $P < 0.05$ was considered statistically significant.

Next, we investigated the efficacy of chemotherapy in 13 *gBRCAm* treatment-naïve patients who were ineligible for targeted therapy. These patients had a median progression-free survival (PFS) of 9.9 months and median overall survival (OS) of 26 months (**Figure 3A-3B**).

Next, we additionally evaluated the efficacy of EGFR tyrosine kinase inhibitor (TKI) in 20 patients harboring *EGFR* activating mutations and treated with first-generation EGFR-TKI. The study included a cohort of 89 patients harboring *EGFR* mutations but had WT g*BRCA* and treated with EGFR-TKI at the Xiangya Hospital. Patients with *gBRCAm* had a comparable median PFS with patients harboring WT g*BRCA* (9.2 *vs.* 7.3 months), after adjusting for age, gender, and disease stage ($P = 0.098$) (**Figure 4A**). Interestingly, the overall survival (OS) of patients with concurrent *gBRCAm* was significantly longer than that of EGFR-TKI-treated patients harboring WT g*BRCA* (37.5 months *vs.* 17.4 months, $P = 0.002$, **Figure 4B**).

Discussion

In the present study, we analyzed the incidence of *gBRCAm* in advanced Chinese NSCLC patients (stage IIIB to IV). To the best of our knowledge, our study is the first and largest cohort to investigate the prevalence of *gBRCAm* in Chinese NSCLC patients, which was found to be 1.03% of *gBRCAm* with a predominance of *BRCA2* (49/64, 76.5%). This *BRCA2* predominance was previously observed in prostate cancer

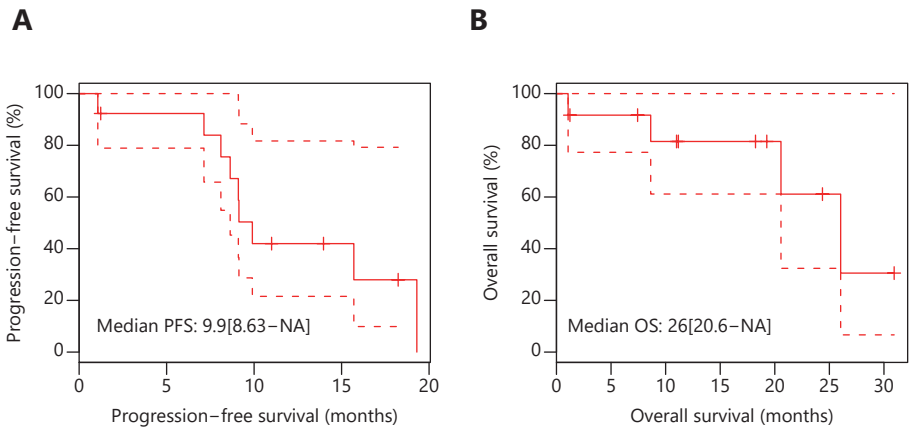

Figure 3 Treatment outcomes and overall survival of patients with pathogenic germline *BRCA* mutations treated with platinum-containing chemotherapy. (A) Progression-free survival (PFS) and (B) overall survival (OS) from the day of treatment of 13 patients who received platinum-containing chemotherapy as first-line treatment.

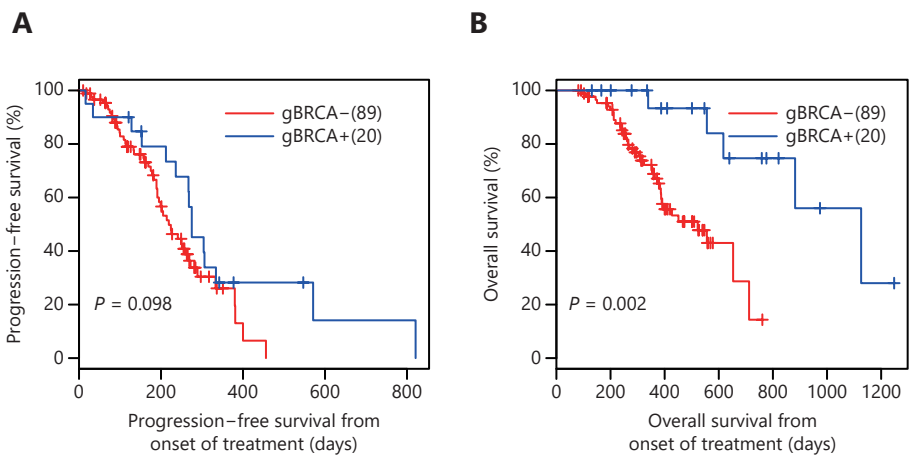

Figure 4 Treatment outcomes and overall survival of *EGFR*-mutant patients with pathogenic germline *BRCA* mutations treated with EGFR inhibitor. (A) Progression-free survival (PFS) and (B) overall survival (OS) from the day of treatment of 20 patients who received first-generation EGFR inhibitor as first-line treatment.

patients[23]. However, the majority of *gBRCAm* in ovarian cancer are predominantly composed of *BRCA1* mutations (172/235, 73.2%)[2]. In prostate cancer, patients with germline *BRCA2* mutations showed elevated global genomic instability and harbored unique mutations that were rarely or not yet been reported in sporadic localized prostate cancer[24,25]. However, our findings revealed that NSCLC patients harboring g*BRCA2* mutations had comparable mutation spectra to those of patients harboring g*BRCA1*, as well as patients with WT g*BRCA*.

Previous studies have established the association between *gBRCAm* and the development of various cancers, including but not limited to breast, ovarian, prostate, pancreatic and stomach cancers. Germline *BRCA* mutation associated cancers present with distinct clinical behaviors often characterized by an earlier onset, improved efficacy of certain treatments, and more favorable survival[26,27] However, the role of *BRCA* mutations in NSCLC remain elusive. Our results revealed a positive association between *gBRCAm* and the early onset of NCSLC. Patients with *gBRCAm* were significantly more likely to develop NSCLC before the age of 50 ($P = 0.036$). Interestingly, patients with concurrent *gBRCAm* and mutations in classic lung cancer driver genes were also found to develop NSCLC at a younger age ($P = 0.029$). However, this phenomenon was not observed in patients without mutations in driver genes.

While the clinical relevance of *gBRCAm* has been well-established in multiple types of cancer, the significance of somatic *BRCA* mutations is beginning to be understood. Our cohort has six *gBRCAm* carriers with concurrent somatic *BRCA* mutations, suggesting that the somatic *BRCA* mutation could act as a second hit for the development of tumorigenesis (**Table 2**). Interestingly, two of these patients, namely, patients 3 and 6, harbored the germline and somatic *BRCA2* mutations on the same amino acid residue (*i.e.* Arg1694 and Ser2984) but with different mutation types.

Despite very poor survival outcomes, platinum-based chemotherapy remains the standard therapy for the majority of heavily-treated advanced NSCLC patients, who have a median progression-free survival (PFS) and overall survival (OS) of 3.6 months and 7.9 months, respectively[28]. Enhanced sensitivity to platinum-containing chemotherapy has been associated with germline *BRCA* mutations based on pre-clinical and clinical studies on ovarian and breast cancers[17,18]. In the present study, we consistently observed the good efficacy of chemotherapy in *gBRCAm* treatment-naïve patients with PFS and OS of 9.9 months and 26 months, respectively. Interestingly, patients with concurrent *gBRCAm* and somatic *EGFR* mutations also showed a tendency to respond well to EGFR-TKI compared to patients without *gBRCAm*. In addition to investigating somatic mutations, studying the germline genetic status of lung cancer patients is also important for understanding their genetic landscape and for guiding clinical decisions.

The present study has certain limitations. Numerous cancer predisposing genes, mostly tumor suppressors, have been identified[29]. This study was only limited to the identification of pathogenic mutations. Further studies are required to elucidate the clinical significance of other pathogenic cancer predisposing genes in NSCLC. Numerous studies revealed that not all *BRCA* germline mutations resulted in the locus-specific loss of heterozygosity (LOH)

Table 2 Mutational profile of the patients with germline and somatic mutations in *BRCA1/2*

Patient number	Gender	Age (years)	Somatic mutations				Germline mutations			
			Gene	Mutation type	Mutation	AF	Gene	Mutation type	Mutation	AF
1	Female	48	BRCA2	Stop gained	c.1688G > A (p.Trp563*)	14.93%	BRCA2	Stop gained	c.6952C > T (p.Arg2318*)	46.92%
2	Male	64	BRCA2	Missense	c.9065G > C (p.Arg3022Thr)	21.20%	BRCA2	Stop gained	c.7718T > G (p.Leu2573*)	50.50%
3	Male	70	BRCA2	Synonymous	c.5082A > G (p.Arg1694=)	4.73%	BRCA2	Stop gained	c.5080A > T (p.Arg1694*)	54.19%
4	Male	65	BRCA2	Stop gained	c.9299T > A (p.Leu3100*)	25.90%	BRCA2	Splice donor	c.7805+1G > A	59.10%
5	Male	68	BRCA2	Missense	c.8026A > T (p.Met2676Leu)	1.11%	BRCA1	Frameshift	c.2143_2155delinsTCTTT (p.Thr715fs)	40.53%
6	Male	60	BRCA2	Splice region	c.8951C > T (p.Ser2984Leu)	0.70%	BRCA2	Stop gained	c.8951C > G (p.Ser2984*)	47.83%

and further reported the differential clinical significance between *BRCA* LOH mutations and non-LOH mutations. Unfortunately, panels used in this study cannot detect LOH. Only patients with advanced disease were screened (stage IIIb to IV) and analyzed. Future studies that include patients in the early stages of the disease are needed to provide a more comprehensive understanding of g*BRCA* status. The lack of family history also limits further investigation.

In conclusion, to the best of our knowledge, we have conducted the largest and most comprehensive survey of the prevalence of pathogenic *gBRCAm* in Chinese NSCLC patients. Results revealed a positive correlation between pathogenic *gBRCAm* and early onset of NSCLC.

Acknowledgements

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Conflict of interest statement

JY, HH-Z, AL, HL, XM and HH are employees of Burning Rock Biotech. The other authors declare no conflict of interest.

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Supplementary materials

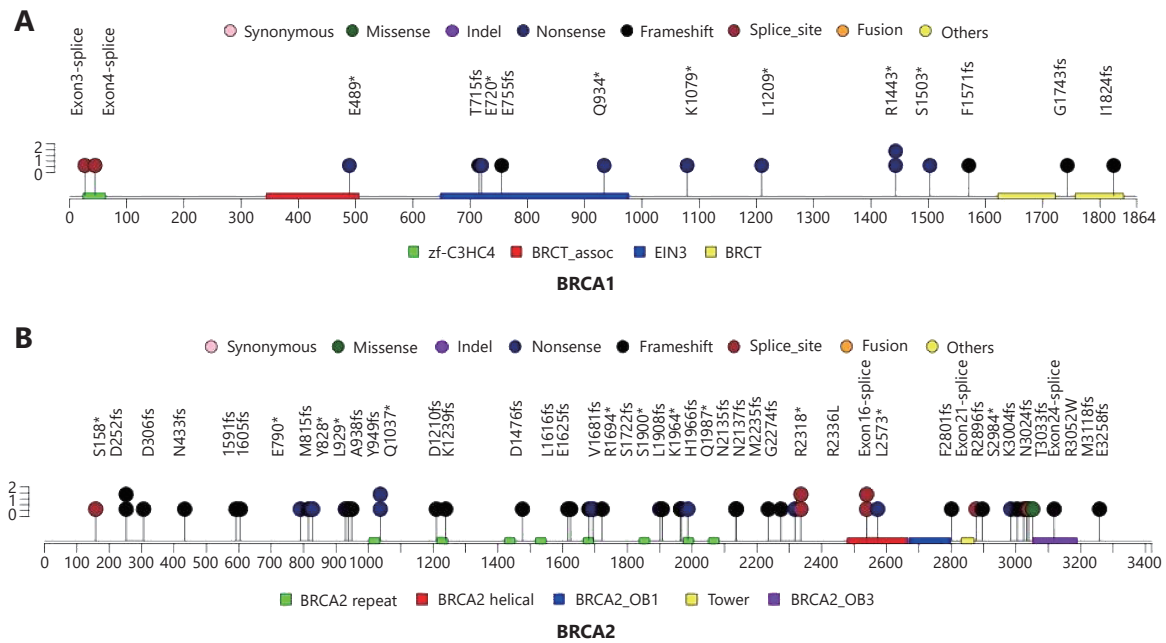

Figure S1 Pathogenic germline *BRCA* mutations found in this cohort.