Multicenter cross-sectional screening of the *BRCA* gene for Chinese high hereditary risk breast cancer populations

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Received August 11, 2017; Accepted October 25, 2017

DOI: 10.3892/ol.2018.8538

Abstract. Due to lack of systematic reviews, BRCA, DNA Repair Associated (*BRCA)* mutations in the Chinese population are not completely understood. The following study investigates the prevalence and type of *BRCA* mutations in Chinese patients with high hereditary risk of breast cancer (BC). Patients Drwere recruited from 14 cities between October 2015 and February 2016, and were selected based on family and personal medical history. *BRCA* mutations were analyzed by collecting blood samples from all participants. 437 BC patients were included. A total of seventy-six (17.4%) mutation carriers were identified with no geographic difference. The mutation rate in the early-onset BC patients was lower compared to family history of breast/ovarian cancer (OC), bilateral BC, male BC, BC&OC or meeting ≥2 criteria (9.2 vs. 21.7, 24.0, 22.2, 16.7 and 24.3%, respectively, P=0.007). A total of 61 mutation sites were identified (*BRCA1* 32, *BRCA2* 29) including 47.5% novel sites and extra 10 variants of uncertain significance. A total of five sites were repeated in more than one unrelated patient. A total of 11 sites were associated with hereditary breast and ovarian cancer syndrome, two of which were confirmed by family pedigrees. Compared with *BRCA*$^{-}$ patients, patients with *BRCA1* mutation tended to be triple-negative BC (P<0.001), whereas patients with *BRCA2* mutation were more likely to be hormone receptor positive

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Key words: breast cancer, *BRCA1*, *BRCA2*, chinese population

BC (P=0.02). The present study provides a general *BRCA* mutation profile in the Chinese population. The prevalence of *BRCA* mutation in BC patients with high hereditary risk is lower compared with Western populations. Chinese mutation type is different with Western people, without obvious founder mutation.

Introduction

Even though, the incidence of breast cancer (BC) in China is lower compered to Western countries, since the 1990s it has increased twice as fast as the global rate (1) with very early onset (2,3). In China, BC patients <50 years old account for 46% of the total cases (4), with the peak prevalence observed in the 45 to 55 years old age (5-8). Meanwhile, the highest mortality is observed in even younger age i.e. in the 30 to 44 years old age (5). Therefore, in order to reduce the number of BC patients, and the growing burden of this disease, it is urgent to promote BC prevention in China.

BRCA (Breast Cancer Susceptibility Gene) is associated with the majority of hereditary BC, which accounts for about 5 to 10% of all cases of breast cancers (9). Meanwhile, the resemblance in clinical and pathologic features between sporadic triple-negative BC (TNBC) and *BRCA1* mutant BC implies that mechanism behind *BRCA* germline mutant tumors is strongly associated with somatic mutation of sporadic BC (10). However, a lack of basic information about the prevalence and spectrum of *BRCA* mutations hinders research progress on the etiology (6) and risk evaluation of model of breast cancer prevention in China. Additionally, because of the large presence of ethnic-specific contexts (11-14), the Western risk evaluation models do not apply well to China. Therefore, the following paper assembled a wide-range clinic-based cross-sectional study of hereditary risk among BC patients, who were representative for *BRCA* mutations study. The patients were from different parts of mainland China, which was important for determining the prevalence and types of *BRCA* mutations, as well as to provide basic information for further studies of *BRCA* and BC prevention models in China.

Materials and methods

Study population. The present study has been approved by center medical ethics committee (Ethics Committee of the First Affiliated Hospital of Third Military Medical University, PLA), and was successfully registered with Chinese Clinical Trial Registry (ChiCTR), which is the international clinical trial registration platform.

In the time period from October 2015 to February 2016, 445 patients diagnosed with BC were recruited from 18 tertiary general clinics located in North, South and Northwest China, which account for 3/4 of Chinese national territory (Fig. 1). Among these patients, three refused to participate in the study, while for other five the recruitment criteria couldn't be confirmed. Pathological examination was used to confirm the cancer diagnoses and breast cancer subtype. Furthermore, twenty-seven participants were additionally excluded from the clinical data analysis due to incomplete or illegible clinical information. Where it was possible, family members of probands with confirmed BC were recruited to participate.

After reading and signing the informed consent, participants were interviewed in order to provide medical details including past medical history, present BC diagnosis age, tumor size, states of axillary lymph nodes and metastasis, and details of family cancer history. Consequently, fresh peripheral venous blood (5 ml) was collected from each participant, and transferred into a coded Ethylene Diamine Tetraacetie Acid (EDTA) tube at 4˚C. During the same day, blood samples were sent to Annoroad Gene Technology (Beijing) Co., Ltd, for further analyses (*BRCA* genetic testing). Each participant received a sealed file filled with *BRCA* test result (excluding their clinical records). Genetic consultation was provided in order to better explain the *BRCA* test results. The participants did not encounter any financial costs (including genetic consultation) for their participation in the present study.

Recruitment criteria. The recruitment criteria adhered to the breast cancer diagnosis and treatment guidelines and specifications (Chinese Cancer Society, V2015). Participants who met one of the following criteria were included in the study: i) BC with onset age ≤35 years old (early-onset BC group), ii) ≥1 relative[a] (either sex) from the same side of the family as BC patient and (or) ovarian cancer (BC/OC) diagnosed at any age (BC/OC family history group), iii) two primary BC cancers[b] [bilateral BC (BBC) group], iv) male BC patients (MBC group), v) BC with OC (BC&OC group) and vi) meeting ≥2 criteria above simultaneously (mixed group). [a]Including first, second and third degree relative with no age limitation. [b]Two primary BC, bilateral BC excluding metastatic contralateral BC or unilateral BC including two or more different types of cancers.

In case the patients failed to sign the informant consent, they were excluded from the present study.

BRCA gene analysis. *BRCA1* (MIM:113705) and *BRCA2* (MIM:600185) testing was performed using Annoroad Gene Technology (Beijing, China) on an Illumina HiSeq 2500 platform (Illumina, San Diego, CA, USA). Genomic DNA was first extracted from peripheral blood white cells using the QIAGEN DNeasy Blood and Tissue Kit (Qiagen, Shanghai, China). The genomic DNA was then fragmented by a bioruptor sonication device (Diagenode, Leige, Belgium). The construction and capture of the DNA library followed the standard protocols from Illumina (Illumina, San Diego, CA, USA) and Roche (Roche, Shanghai, China). A Qubit 3.0 Fluorometer (Invitrogen, San Francisco, CA, USA) and Bioanalyzer (Agilent, Santa Clara, CA, USA) were used to determine the quantity of the library. Finally, the library was sequenced on one lane using 100 paired-end (2x100 bp) strategies.

Variant nomenclature. Reference sequences used for *BRCA1* and *BRCA2* analyses were GenBank NM_007294.2 (*BRCA1*) and NM_000059.3 (*BRCA2*). Mutation nomenclature was described according to Human Genome Variation Society (v2.0) (15).

Statistical analysis. Medians were used with interquartile ranges of abnormally distributed data for continuous variables (diagnosed ages of patients) and rank test for analyses. Proportions were shown for categorical variables. Comparisons of mutation rates and proportions were analyzed by Chi-square

Table I. The geographic mutation rates (n=437).

Area	N	BRCA[+a] (%)	P-value
North China	201	31 (15.4)	0.5
South China	151	30 (19.9)	
Northwest China	85	15 (17.6)	
Total	437	76 (17.4)	

[a]Both *BRCA1* and *BRCA2* mutations.

Table II. The mutation rates in the recruited groups (n=437).

Recruit Criteria	N	BRCA[+a] (%)	P-value
EO[b]	173	16 (9.2)	
BC/OC FH	129	28 (21.7)	
BBC	50	12 (24.0)	
MBC	9	2 (22.2)	0.007
BC&OC	6	1 (16.7)	
Fixed	70	17 (24.3)	
Total	437	76 (17.4)	

EO, early-onset breast cancer; BC/OC FH, breast and/or ovarian cancer family history; BBC, bilateral breast cancer; MBC, male breast cancer; BC&OC, breast cancer with ovarian cancer. [a]Both *BRCA1* and *BRCA2* mutations. [b]Patients were diagnoses with BC onset breast cancer, BC/OC FH, breast and/or ovarian cancer family history; BBC, bilateral breast cancer.

test of unordered categorical variable. Univariate and multivariate logistic analysis were used to examine the relationships between hormone receptors (HRs, including estrogen receptor ER, progesterone receptor PR) and human epidermal growth factor receptor 2 (HER2) and *BRCA* mutation states with odd ratios (OR) and 95% confidence intervals (95% CI).

All P-values were two-sided. P<0.05 was considered to indicate a statistically significant difference. All data were analyzed using PASW Statistics 22.0 (SPSS, Inc., Chicago, IL, USA).

Results

Study population. From a total of 437 BC patients enrolled in the study, almost half came from north China (Table I). Forty percent of early-onset BCs (173/437) and 30% of patients with BC/OC family history (163/437) were observed in more than half of the participants (Table II). The median age of 437 BC patients was 35.0 (31.0, 46.0) years. The median age of mutation carriers was higher compared to non-carriers [41.0 (34.0, 47.0) vs. 35.0 (30.0, 46.0), P=0.002].

Mutation frequency. Seventy-six (17.4%) *BRCA* mutation carriers were identified, 31 (15.4%) of which were from patients from North China, 30 (19.9%) from patients from South China, and 15 (17.6%) from patients from Northwest China. No significant difference in gene mutation rates was found between different region areas (Table I). Furthermore, the early-onset patient rate (9.2%) was significantly different in relation to remaining 5 groups (P=0.007) (Table II); while no significant difference was found between the 5 groups (P>0.05).

BRCA mutation status. According to the American College of Medical Genetics and Genomics (ACMG) (16), a total of 61 deleterious mutation points (29 in *BRCA1*, 32 in *BRCA2*) were observed in 76 carriers (Table III), and consequently classified into the already 'known' (Table IV) and the 'novel' (Table V) mutations. Briefly, 72% of novel variations were found in *BRCA2* (Tables IV and V). Moreover, five mutations of 61 were observed in more than one unrelated patients from different areas (Fig. 2 and Table IV).

Thirty-four points (55.7%) were frame shift, followed by 17 (27.9%) nonsenses, 7 splices (11.5%), 2 pathogenic missenses (3.3%), and 1 synonymous mutation (1.6%). Extra 10 missenses were found as variants of uncertain significance (VUS) (Table VI), accounting for 14.1% of all the variants including 61 deleterious mutation.

Table III. Deleterious and novel mutations (n=61).

Gene	Known (%)	Novel (%)	Total
BRCA1	21 (65.6)	8 (27.6)	29
BRCA2	11 (34.4)	21 (72.4)	32
Total	32 (52.5)	29 (47.5)	61

Figure 1. The regional distribution of subjects in China. The four regions of China's territory are indicated with red lines. Areas where patients came from are indicated with blue.

Eleven different mutations in 10 families were related to hereditary breast and ovarian cancer syndrome (HBOC), four in *BRCA2* were novel (Table VII). Moreover, none of the identified mutations were shared between the families. *BRCA1 c.190T>C&BRCA2 c.9090dup* were found in one same family, and were carried by a proband from maternal and paternal line respectively. The proband's BC was inherited from maternal HBOC (Fig. 3A). In *BRCA1 c.5431C>T* hereditary family, all

Table IV. *BRCA* known deleterious mutation sites (n=32).

Gene	Location	Exon	Mutation type	AA change	Probands
BRCA1	*c.190T>C*[b]	4	M	p.Cys64Arg	HOBC
	c.212G>A[c]	4	M	p.Arg71Lys	HBOC
	c.212+1G>T[b]	Intr	S	-	MBC/BCFH
	c.441+1G>A	Intr	S	-	EO
	c.1660G>T[a]	10	N	p.Glu554Ter	BCFH
	c.1674del	10	FS	p.Gly559fs	MBC
	c.2014A>T[b]	10	N	p.Lys672Ter	BCFH
	c.2572C>T[d]	10	N		BBC
	c.3329dup	10	FS	p.Gln1111fs	EO
	c.3400G>T	10	N	p.Glu1134Ter	BBC/BCFH
	c.3472G>T	10	N	p.Glu1158Ter	EO
	c.3607C>T	10	FS	p.Arg1203Ter	BCFH
	c.3626T>G	10	N	p.Leu1209Ter	BBD
	c.3640G>T[b]	10	N	p.Glu1214Ter	BCFH
	c.4065_4068del	10	FS	p.Asn1355fs	HBOC
	c.4484+1G>A	Intr	S	-	BCFH
	c.4801A>T[d]	15	N	p.Lys1601Ter	HBOC
	c.5251C>T	19	N	p.Arg1751Ter	EO
	c.5278-1G>C	Intr	S	-	BBD
	c.5431C>T	22	N	p.Gln1811Ter	HBOS
	c.5470_5477del[d]	23	FS	p.Ile1824fs	BBD/BCFH
BRCA2	*c.961C>T*	10	N	p.Gln321Ter	EO
	c.1310_1313del[b]	10	FS	p.Lys437Ilefs	BCFH
	c.1399A>T	10	N	p.Lys467Ter	EO
	c.2806_2809del[d]	11	FS	p.Asp936fs	EO
	c.3109C>T[a,d]	11	FS	p.Gln1037Ter	BBD/BCFH
	c.5682C>A[a]	11	N	p.Tyr1894Ter	BCFH
	c.7007G>T[a]	14	S	p.Arg2336Leu	MBC
	c.8504C>G	20	N	p.Ser2835Ter	BCFH
	c.8517C>A	20	N	p.Tyr2839Ter	BCFH
	c.9100C>T	23	N	p.Gln3034Ter	EO
	c.9117G>A	23	Syn	p.Pro3039=	EO

BBC, bilateral breast cancer; BCFH, breast cancer family history; EO, early-onset breast cancer; FS, frameshift; HBOC, heredity breast and ovarian cancer syndrome family; Intr, Intron; MBC, male breast cancer; M, missense; N, nonsense; S, splice; Syn, synonymous. [a]Once found in Chinese people. [b]Hereditary mutation from the same family. [c]Deleterious missense mutation was carried in unrelated probands. [d]Mutation was carried in unrelated probands.

the middle-age females had BC/OC, but all the male carriers of four general relatives were healthy (Fig. 3B).

Clinical analysis. 410 precise tumor node metastasis TNM results were obtained. No significant difference was found among wild type *BRCA* (*BRCA*$^-$), *BRCA1* and *BRCA2* mutation patients (Table VIII).

Univariate analysis demonstrated higher expression of ER negative (ER$^-$) and PR negative (PR$^-$) in *BRCA1* group compared to *BRCA*$^-$ group (72.6% vs. 35.1%, P<0.0001; 82.4% vs. 40.6%, P<0.0001). Conversely, higher expression of ER positive (ER$^+$) and PR positive (PR$^+$) were found in *BRCA2* group compered to *BRCA*$^-$ group patients (93.5% vs. 64.9%, P=0.001; 87.1% vs. 59.4%, P=0.002). Moreover, *BRCA1* and *BRCA2* groups were both more frequently HER2 negative (HER2$^-$) compared to *BRCA*$^-$ group (97.1, 96.8% vs. 74.3%, P=0.003 and 0.005 respectively). Based on the multivariate analysis, PR$^-$ and HER2$^-$ were the independent risk factors for *BRCA1* mutation, HER2$^-$ alone for *BRCA2* (Tables IX and X). Then, compared with *BRCA*$^-$, *BRCA1* mutation tended to be TNBC (68.6% vs. 24.8%, P<0.0001), while *BRCA2* mutation had higher proportion of HRs positive (HRs$^+$) BC (93.5% vs. 75.2%, P=0.002) (Table XI).

Discussion

The results from the present study, which is to our knowledge, the largest screening study ever performed in China, reveal that the total *BRCA* mutation rate is 17.4% for breast cancer

Table V. Novel variations (n=29).

Gene	Location	Exon	Mutation type	AA change	Probands
BRCA1	*c.1934del*	10	FS	p.Ser645fs	EO
	c.2957del	10	FS	p.Ile986fs	HBOC
	c.3294del	10	FS	p.Leu1098fs	BCFH
	c.3621del	10	FS	p.Lys1207fs	BBD
	c.3859del	10	FS	p.Glu1287fs	BCFH
	c.4013del	10	FS	p.Lys1338fs	EO
	c.4676-1G>T	Intr	S	-	EO
	c.5156del	18	FS	p.Val1719fs	BCFH
BRCA2	*c.31del*	2	FS	p.Phe11fs	EO
	c.767_771del	9	FS	p.Thr256fs	EO
	c.988del	10	FS	p.Lys330fs	BCFH
	c.3364del	11	FS	p.Gly1122fs	EO
	c.426-2A>T	Intr	S	-	HBOC
	c.4410_4413del	11	FS	p.Ile1470fs	BCFH
	c.5480del	11	FS	p.Ile1827fs	BCFH
	c.5495del	11	FS	p.Ser1832fs	MBC/BCFH
	c.5599_5602del	11	FS	p.Tre1867fs	EO
	c.5718_5719del	11	FS	p.Asn1906fs	BBC
	c.5753del[a]	11	FS	p.His1918fs	BCFH
	c.6288_6289del[a]	11	FS	p.Pro2096fs	BCFH
	c.6462_6465del	11	FS	p.Tyr2154fs	BBC/EO
	c.6552del	11	FS	p.Glu2184fs	BCFH
	c.6698_6699insTTTT	11	FS	p.Ala2233fs	HBOC
	c.7178_7179del	14	FS	p.Met2393fs	BCFH
	c.8019_8020insAT	18	N	p.Lys2673fs	BBD
	c.8039_8040del	18	FS	p.Asp2680fs	EO
	c.8367_8369 TAC>A	19	FS	p.Tyr2789Ter	EO
	*c.8400_8402delinsAAA*A	19	FS	p.Phe2801fs	EO
	c.9090dup[a,b]	23	FS	p.Thr3030fs	EO

BBC, bilateral breast cancer; BCFH, breast cancer family history; EO, early-onset breast cancer; FS, frameshift; HBOC, heredity breast and ovarian cancer syndrome family; Intr, Intron; MBC, male breast cancer; M, missense; N, nonsense; S, splice. [a]More than one carriers from the same family. [b]Inherited from paternal family, which doesn't have breast or ovarian cancer family history.

patients at risk of hereditary *BRCA* mutation across China with no observed geographical differences.

One of the main components of the present study has to do with 21.7% of mutation rate which lies in BC/OC family history subgroup. This finding is in line with a Korean study conducted across 36-centers (22.3%) (17). The mutation rate appears generally lower compared to Western countries (23~35.3%) (18-22), but higher compared to Peking or Shanghai regions (10.5~18.2%) (23-25). Lower prevalence of *BRCA* mutation is in line with comparisons of BC incidence with the Western countries. It clearly suggests essential distinction in *BRCA* mutation between Asian and Euromerican people that goes well beyond different study design biases. Furthermore, thus far observed domestic inconsistencies may be caused by the limitations related to areas and criteria. The present study covers most of the Chinese regions, i.e. areas with huge concentrations of Chinese populations. Moreover, the present study does not impose the BC onset age limitation for family cancer history, which allows for wider screening rang. However, some families with late-onset hereditary BC/OC that are really in need of *BRCA* testing may be excluded from testing due to young cutoff diagnose age established by *BRCA* testing guideline. Most of all, our results are representative of the real data on hereditary risk for breast cancer patients in China.

The sporadic early-onset (≤35 years) BC mutation rates (9.2%) are nearly twice higher compared to those obtained by the recent studies from China (5%, ≤40 years) (24) and from Western countries (5.9%, <36 years) (26), but lower compared to other 5 risk factor groups in this study. The observed discrepancy may come from different sample sizes and study populations. Moreover, different age limitations for early-onset suggest that cutoff age may impact the mutation rates in younger patients. Currently, the age of 45 is set as upper limitation of young BC for *BRCA* testing according to Chinese BC Treatment Guideline (27). Nevertheless,

Table VI. Information of VUS (n=10).

Gene	Location	AA change	Proband		Family history
BRCA1	*c.446A>C*	p.E149A	p.(Glu149Ala)	EO	-
	c.1669A>C	p.T557P	p.(Thr557Pro)	MBC (TNBC)	Uterine cancer
	c.4580A>T	p.E1527V	p.(Glu1527Val)	BBC	-
	c.5156T>C	p.V1719A	p.(Val1710Ala)	BBC	-
	c.5498T>A	p.V1833E	p.(Val1833Glu)	MBC	-
BRCA2	*c.6875A>C*	p.E2292A	p.(Glu2292Ala)	BBC (TNBC)	-
	c.7811T>C	p.L2604P	p.(Leu260Pro)	EO	-
	c.7967T>C	p.L2656P	p.(Leu2656Pro)	EO	-
	c.8162T>C	p.L2721P	p.(Leu2721Pro)	BBC	one BC sister
	c.9374T>C	p.L3125P	p.(Leu3125Pro)	BBC (TNBCs)	-

EO, early-onset breast cancer; MBC, male breast cancer; BBC, bilateral breast cancer; TNBC, triple negative breast cancer; VUS, variants of uncertain significance.

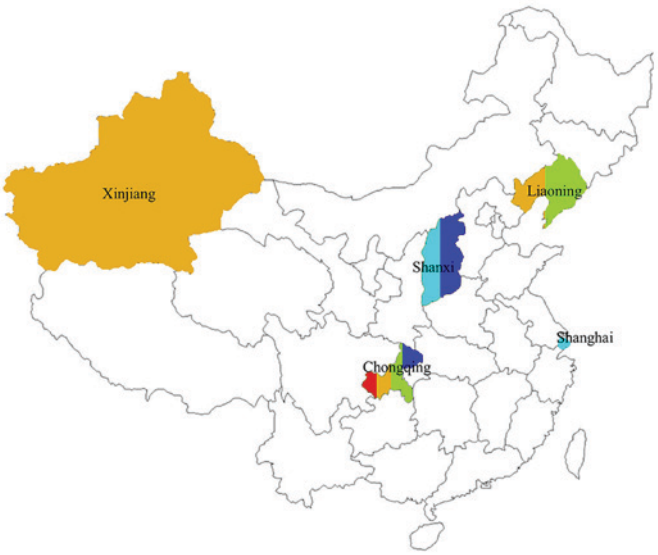

Figure 2. The regional distribution of unrelated subjects carrying the same mutations. The areas of *BRCA1 c.2572C>T* carriers are indicated with dark blue; *BRCA1 c.4801A>T* in green; BRCA1 *c.5470_5477del* in yellow; *BRCA2 c.2806_2809del* in light blue; and the areas of *BRCA2 c.3109C>T* carriers are indicated in red.

increasing trend of breast cancer incidence in younger patients in China (6) may imply more patients with sporadic early-onset from the whole population. Ten years interval, between the ages 35 and 45, may actually double the difference on sensitivity and specificity for *BRCA* screening in Chinese patients with breast cancer. Additionally, besides onset age, family history, bilateral BC, with OC and similar should also be considered in the early-onset BC to improve *BRCA* test indications (24). Consequently, we suggest re-evaluation of the early-onset age for *BRCA* test to increase sensitivity of *BRCA* mutation screening and to fit current BC epidemiology in China.

BRCA mutation sites in our study suggest special features of *BRCA* mutation in China. Four sites are hereditary, and nearly half are novel. Also, 14.1% VUS were slightly higher than Asian data (13.6%) obtained from a study conducted by Hall *et al*. Interestingly, their data were already more than 2.5 times higher compared to European VUS (28). Then, *BRCA1 c.2572C>T* reappeared twice in two unrelated patients from Chongqing and Shanxi Provinces, respectively. Up to now, it has been found in unrelated patients from five different provinces in China (29). Nevertheless, it has not been frequently reported in international studies, meaning it might be one of founder mutation candidates among Chinese populations. *BRCA1 c.5470_5477del* is another highly repeated site found three times in our study. Internationally, the site has been previously identified in Korean sample (30), while nationally it has been reported in Shanghai (31), Beijing (32), Zhejiang, and Liaoning (33). Thus far, it has been found in 12 unrelated carriers from six different areas of China, including HBOC patients (33.3%) (32), sporadic BC patients (33.3%) (31,33) and early-onset BC (33) or BBCpatients (33.3%) (the present study). Also, three of the patients (from Zhejiang, Shanghai, and Liaoning) share the same haplotype (33). To our knowledge, it has only once been reported in native Asian patient (34), therefore, it is strongly associated with Asian founder mutation. However, Kwong *et al* have recognized *BRCA2 c.3109C>T* as a founder mutation in southern Chinese population in Hong Kong (35,36). 81.5% of probands in the study were immigrants from Guangdong. It was repeated in two unrelated patients from Chongqing, southwest of China, but it did not appear in our population sample from southern China. Further large scale unselected population epidemic research is necessary to clarify this.

Two confirmed HBOC families have special features. The proband who carried two mutations is the youngest patient in her family, thus it is not possible to ignore the effects of paternal inherited *BRCA2 c.9090dup* (novel) on the proband given her uncle has lung cancer (Fig. 3A). *BRCA1 c.5431C>T* pathogenicity shows a gender trend: It appears harmful for women, and silent for men (Fig. 3B).

All the information on the mutation sites reported above suggest quite different *BRCA* mutation spectrum and features that exit in Chinese BC patients compared with well-known white populations.

Table VII. Familial mutations information (n=11).

Gene	Location	Family history features	Chinese[a]
BRCA1	*c.190T>C*[b]	HBOC of maternal hereditary	N
	c.1660G>T	BC sisters	Y
	c.2014A>T	BCs of maternal history	N
	c.212+G>T	BCs of maternal history	Y
	c.3640G>T	BC sisters	N
	c.5431C>T	HBOC of paternal hereditary	N
BRCA2	*c.988del*	BC sisters	N (Novel)
	c.1310_1313del	BCs maternal history	N
	c.5753del	BCs maternal history	N (Novel)
	c.6288_6289del	BCs maternal history	N (Novel)
	c.9090dup[b]	Paternal line	N (Novel)

BC, breast cancer; HBOC, heredity breast and ovarian cancer syndrome family; Y, yes; N, no. [a]Whether mutation was once reported in Chinese. [b]Carried by the same proband.

Table VIII. TNM in three *BRCA* groups (n=410).

	TNM (%)				
Gene	I (n=126)	II (n=199)	III (n=67)	IV (n=18)	P-trend
BRCA$^-$	99 (28.9)[a]	171 (49.9)	57 (16.6)	16 (4.7)	0.33
BRCA1	17 (48.6)	14 (40)	3 (8.6)	1 (2.9)	
BRCA2	10 (31.3)	14 (43.8)	7 (21.9)	1 (3.1)	

TNM, tumor metastasis node. [a]Three carcinoma *in situ* patients were included.

Table IX. HRs and HER2 comparisons between BRCA1 and BRCA$^-$ groups (n=376[a]).

Molecular markers	*BRCA1* (%)	*BRCA*$^-$[b] (%)	Univariate P-value	Multivariate P-value	OR (95%CI)
ER					
<1%	24 (70.6)	120 (35.1)	<0.0001	0.73	1.2 (0.4-3.3)
≥1%	10 (29.4)	222 (64.9)	-		
PR					
<1%	28 (82.4)	139 (40.6)	<0.0001	0.003	6.3 (1.9-20.6)
≥1%	6 (17.6)	203 (59.4)	-		
HER2					
-	33 (97.1)	254 (74.3)	0.003	0.01	12.7 (1.7-95.6)
+	1 (2.9)	88 (25.7)	-		

HRs, hormone receptors; ER, estrogen receptor; PR, progesterone receptor; HER2, humane epidermal growth factor receptor 2; OR, odd ratio; 95% CI, 95% confidence interval. [a]One *BRCA*$^-$ and 1 *BRCA1* mutant participants were excluded because of unclear HER2 state. [b]Wild type *BRCA*.

Similar to other studies, we came to the conclusion that *BRCA1* mutation is concerned with TNBC. However, *BRCA2* mutations tend to be HRs$^+$ BC, which is inconsistent with results from other studies (37,38). The different results show heterogeneous of *BRCA2* mutant BC beyond different study populations and regions (39,40). The present study suggests that *BRCA2* mutant BC may responds better to endocrine therapy due to high proportion of HRs$^+$ tumor with the same

Table X. HRs and HER2 comparisons between *BRCA2* and *BRCA*$^{-}$ groups (n=373[a]).

Molecular markers	*BRCA2* (%)	*BRCA*$^{-b}$ (%)	Univariate P-value	Multivariate P-value	OR (95%CI)
ER					
<1%	2 (6.5)	120 (35.1)	0.001	0.09	0.2 (0.4-1.3)
≥1%	29 (93.5)	222 (64.9)	-		
PR					
<1%	4 (12.9)	139 (40.6)	0.002	0.41	0.6 (0.2-2.2)
≥1%	27 (87.1)	203 (59.4)	-		
HER-2					
-	30 (96.8)	254 (74.3)	0.005	0.03	9.4 (1.3-71.0)
+	1 (3.2)	88 (25.7)			

HRs, hormone receptors; ER, estrogen receptor; PR, progesterone receptor; HER2, humane epidermal growth factor receptor 2. [a]One *BRCA*$^{-}$ participant was excluded because of the unclear HER2 state. [b]Wild type *BRCA*.

Table XI. Molecular types comparison in three *BRCA* groups (n=409[a]).

Gene	TNBC (%)	HR^{+b} (%)	P-value
BRCA^{-c}	85 (24.8)	258 (75.2)	-
BRCA1	24 (68.6)	11 (31.4)	<0.0001
BRCA2	2 (6.5)	29 (93.5)	0.02
Total	111	298	

TNBC, triple negative breast cancer; HRs^{+}, Hormone receptors positive. [a]Thirteen participants were excluded because of HER2 over-express type with no BRCA mutation carriers. [b]ER positive or/and PR positive regardless of HER2 state. [c]Wild type *BRCA*.

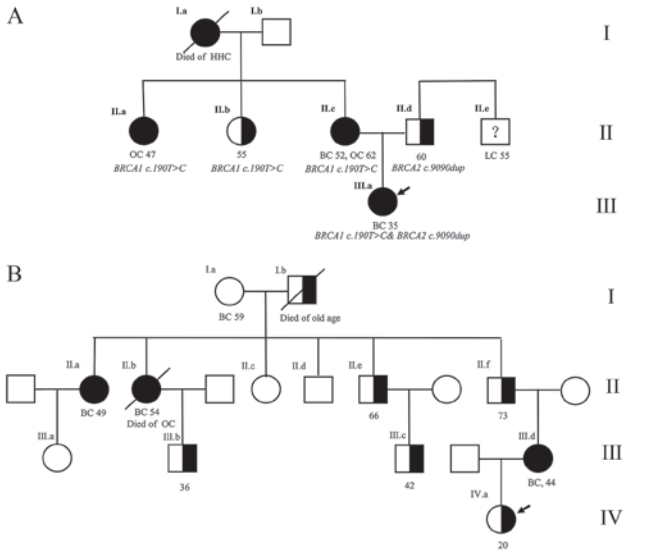

Figure 3. Family pedigrees of BRCA1 c.190T>C&BRCA2 c.9090dup and BRCA1 c.5431C>T. Cancer patients with mutations are indicated with solid symbols, while the healthy carriers are indicated with half solid symbols. The late individuals are indicated with bias crossing symbols. Probands are indicated with arrows. Unknown BRCA state is indicated question mark. Ages under symbols indicate cancer diagnosed age for patients and current age for healthy carriers. BC, breast cancer; OC, ovarian cancer; HHC, liver cancer; LC, lung cancer. (A) Family pedigree BRCA1 c.190T>C&BRCA2 c.9090dup. IIe is a lung cancer patient who rejected to have BRCA testing. (B) Family pedigree of BRCA1 c.5431C>T. Ia is a female breast cancer patient without BRCA1 c.5431C>T.

background of hereditary risk of BC compared with *BRCA*$^{-}$ and *BRCA1* mutant BC.

In summary, this study provides a general *BRCA* mutation profile in China, which enhances the prevalence of *BRCA* mutations in non-white populations. The *BRCA* screening provides a distinguishing *BRCA* mutation profile in China, which compared to the West reveals lower mutation prevalence, and special mutation spectrum. Cutoff ages for diagnosis of early-onset and BC/OC family history should be re-evaluated based on population screening data to improve *BRCA* test indications. *BRCA2* mutation suggests the best response to endocrine therapy among *BRCA* mutant and *BRCA*$^{-}$ BCs in this selected hereditary risk population. However, further studies are necessary to confirm precise *BRCA* mutation situation in China.

Acknowledgements

The authors would like to thank Zefei Jiang, Mengmeng Zhang, Shaohua Wei, Yuanyuan Wang, Dawei Yun, Huiquan Jiang, Yang Li, Yimeng Hu, Yong Yang and other medical staff for their involvement in the study design, BRCA lab test, information collection, data analysis and manuscript writing. The present study was supported by the China Health Promotion Foundation.

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