Novel germline mutations and unclassified variants of BRCA1 and BRCA2 genes in Chinese women with familial breast/ovarian cancer

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Abstract

Background: Germline mutations in the BRCA1 and BRCA2 genes greatly increase a woman’s risk of developing breast and/or ovarian cancer. The prevalence and distribution of such mutations differ across races/ethnicities. Several studies have investigated Chinese women with high-risk breast cancer, but the full spectrum of the mutations in these two genes remains unclear.

Methods: In this study, 133 unrelated Chinese women with familial breast/ovarian cancer living in Zhejiang, eastern China, were enrolled between the years 2008 and 2014. The complete coding regions and exon-intron boundaries of BRCA1 and BRCA2 were screened by PCR-sequencing assay. Haplotype analysis was performed to confirm BRCA1 and BRCA2 founder mutations. In silico predictions were performed to identify the non-synonymous amino acid changes that were likely to disrupt the functions of BRCA1 and BRCA2.

Results: A total of 23 deleterious mutations were detected in the two genes in 31 familial breast/ovarian cancer patients with a total mutation frequency of 23.3 % (31/133). The highest frequency of 50.0 % (8/16) was found in breast cancer patients with a history of ovarian cancer. The frequencies of BRCA1 and BRCA2 mutations were 13.5 % (18/133) and 9.8 % (13/133), respectively. We identified five novel deleterious mutations (c.3295delC, c.3780_3781delAG, c.4063_4066delAAAGTC, c.5161 > T and c.5173insA) in BRCA1 and seven (c.1-40delGA, c.4487delC, c.469_473delAAGTC, c.5495delC, c.6359C > G and c.7588C > T) in BRCA2, which accounted for 52.2 % (12/23) of the total mutations. Six recurrent mutations were found, including four (c.3780_3781delAG, c.5154G > A, c.5468-1del8 and c.5470_5477del8) in BRCA1 and two (c.3109C > T and c.5682C > G) in BRCA2. Two recurrent BRCA1 mutations (c.5154G > A and c.5468-1del8) were identified as putative founder mutations. We also found 11 unclassified variants, and nine of these are novel. The possibility was that each of the non-synonymous amino acid changes would disrupt the function of BRCA1 and BRCA2 varied according to the different algorithms used.

Conclusions: BRCA1 and BRCA2 mutations accounted for a considerable proportion of hereditary breast/ovarian cancer patients from eastern China and the spectrum of the mutations of these two genes exhibited some unique features. The two BRCA1 putative founder mutations may provide a cost-effective option to screen Chinese population, while founder effects of the two mutations should be investigated in a larger sample size of patients.

Keywords: BRCA1, BRCA2, Germline mutation, Unclassified variants, Founder mutation, Chinese women
Background
In 2009, the morbidity rate of breast cancer was 42.55 per 100,000 Chinese women, and breast cancer ranked first in cancer incidence and fifth in cancer-related deaths among females [1]. The mean age at diagnosis of breast cancer is 45–55 years in Chinese women, which is considerably younger than that in western women [2]. A significant proportion of breast cancer in Chinese women is caused by genetic alterations. Germline mutations in many genes, such as BRCA1, BRCA2, ATM, TP53, RAD51C, and XRCC2, have been identified to be associated with breast cancer [3–5]. Several studies have investigated germline mutations in genes including BRCA1, BRCA2, TP53, BRIP1, PALB2, CHEK2, RAD50, NBS1 and RAD51C in Chinese women with high risk breast cancer [6–21]. We previously summarized the spectrum of the germline mutations in these genes and found that the BRCA1 and BRCA2 tumor suppressor genes are the two most important susceptibility genes and account for nearly 98 % of hereditary breast cancer in China [22]. We found that the spectrum of BRCA1 and BRCA2 germline mutations in Chinese high risk breast cancer patients are much smaller than those in Caucasian patients, and little has been recognized in this field. The overall mutation frequencies in these two genes in Chinese high risk breast cancer patients ranged from 8.3 to 27.8 %, depending on the detection methods and patient inclusion criteria used. These frequencies are much lower than the 25–40 % in BRCA1 and 6–15 % in BRCA2 that have been observed in Caucasian populations [22]. Because germline mutations in BRCA1 and BRCA2 greatly increase a woman’s risk of developing breast and/or ovarian cancer, and the prevalence and distribution of the germline mutations differ in different races/ethnicities, we were interested in identifying the full spectrum of these mutations in high-risk female breast cancer patients in the Chinese population.

In this study, we screened the entire coding regions and exon-intron boundaries of the BRCA1 and BRCA2 genes in 133 familial breast/ovarian cancer patients from eastern China. A total of 23 deleterious mutations, including 12 novel mutations (five in BRCA1 and seven in BRCA2), were detected in these two genes in 31 familial breast/ovarian cancer patients, and the total mutation frequency was 23.3 % (31/133). The highest frequency of 50.0 % (8/16) was found in the breast cancer patients with a history of ovarian cancer. Six recurrent mutations were found, including four in BRCA1 and two in BRCA2. We also found 11 unclassified variants (UVs), nine of which were novel. Additionally, using comparative evolutionary bioinformatic programs, we identified the non-synonymous amino acid changes that are likely to disrupt the functions of the BRCA1 and BRCA2 genes. Our study suggested that BRCA1 and BRCA2 mutations accounted for a considerable proportion of the hereditary breast/ovarian cancer patients in eastern China and that the spectrum of the mutations in these genes exhibited unique features.

Methods
Subjects
All patients were diagnosed between 2008 and 2014 in the Zhejiang Cancer Hospital, eastern China. The criterion for familial breast/ovarian cancer was that at least one first- or second-degree relative of the breast cancer patient had been affected by breast cancer and/or ovarian cancer, regardless of age. Written consent was obtained from all participating patients. The study was approved by the Research and Ethics Committee of Zhejiang Cancer Hospital, China. Peripheral blood samples were drawn from at least one affected person in each family and stored in EDTA tubes at –80 °C. A total of 133 patients from unrelated families were enrolled in this study. For the 62 patients who enrolled before 2012, the BRCA1 gene was analyzed with a polymerase chain reaction (PCR)-sequencing assay as previously reported [13], and the mutations of the BRCA2 gene were screened in this study.

BRCA1 and BRCA2 mutation analysis
Genomic DNA was extracted from the peripheral blood leukocytes of one patient from each family using a ZR Genomic DNA Kit (Zymo Research, Orange County, CA, USA) or a QIAamp DNA Blood Mini kit (Qiagen, Hilden, Germany). The entire coding regions and exon-intron boundaries of BRCA1 [U14680.1] and BRCA2 [U43746.1] were screened using PCR-sequencing assay. Totals of 32 pairs and 40 pairs of primers for BRCA1 and BRCA2, respectively, were synthesized by Invitrogen. The primers and PCR conditions are available on request. The PCR products were verified on standard agarose gels prior to mutation analysis and purified by membrane retention. The purified fragments were sequenced using a BigDye Terminator Cycle Sequencing Kit and an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). All mutations were confirmed by duplicate independent PCR. No screening for large genomic rearrangements was performed.

All of the mutations and variants were named according to the Human Genome Variation Sequence systematic nomenclature (HGVS; http://www.hgvs.org/mutnomen/). The Breast Cancer Information Core (BIC) nomenclature (https://research.nhgri.nih.gov/projects/bic/Member/index.shtml) was also indicated in the tables and text because this system had been widely employed in many studies. All of the mutations and variants were queried against the 1000 Genomes database using the 1000 Genomes Browser (http://browser.1000genomes.org/) to determine whether the mutations and variants had been reported in the Chinese population.
Haplotype analysis

Haplotype analysis was conducted on the unrelated patients with recurrent BRCA1 or BRCA2 germline deleterious mutations. Thirteen microsatellite polymorphic loci were used (BRCA1 D17S855, D17S1322, D17S1323, D17S1326, D17S1327; BRCA2 D13S1304, D13S217, D13S289, D13S1699, D13S1698, D13S171, D13S1695, D13S267) [9, 12]. Primer sequences of all microsatellite polymorphic loci were obtained from the Probe Database (http://www.ncbi.nlm.nih.gov/probe). PCR products fluorescently labeled were size fractioned on an ABI 3730xl Analyzer (Applied Biosystems) using GeneScan 500 LIZ Size Standard. Analysis was performed using the Genemarker v1.5 analysis software.

In silico prediction

To identify the UVs that were likely to disrupt the functions of the BRCA1 and BRCA2 genes, we performed in silico predictions with the following six comparative evolutionary bioinformatic programs: Align-GVGD (http://agvgd.iarc.fr/agvgd_input.php), SIFT (http://sift.jcvi.org/), PROVEAN (http://provean.jcvi.org/index.php), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph/), PMUT (http://mmb2.pcb.ub.es:8080/PMut/), and PANTHER (http://www.pantherdb.org/tools/csnpScoreForm.jsp).

Statistical analysis

Continuous data were presented as the mean ± standard deviation (SD), and the differences between the two groups were evaluated using one-way ANOVA analyses. Frequencies were calculated as the proportion of mutation carriers among all participants. The differences in the overall frequencies of BRCA1 and BRCA2 mutations between groups were evaluated using Chi-square tests and Fisher’s exact tests. The statistics were performed using SPSS version 17.0 software for Windows.

Results

Patient features

A total of 133 unrelated patients with personal and family histories of breast and/or ovarian cancer underwent BRCA1 and BRCA2 germline mutation screening. All of the patients were from the Zhejiang province in eastern China. In our cohort of 133 breast cancer families, there were 2.3 ± 0.7 (mean number ± SD) occurrences of breast cancer per family. The age of breast cancer onset ranged from 22 years to 74 years. The mean age at diagnosis was 43.0 ± 9.3 (mean age ± SD) years. Ovarian cancer was present in 12.0 % (16/133) of all families.

BRCA1 deleterious mutations

In this cohort of 133 familial breast/ovarian cancer patients, 13 deleterious mutations in BRCA1 were found in 18 unrelated patients, including five mutations that were reported in our previous study [13] (Table 1). None of the mutations had been registered in the 1000 Genomes database. The majority of the mutations were either nonsense or frameshift mutations with the exception of c.5467 + 1G > A and c.5468-1del8. Six mutations (46.2 %) were located in exon 11, and others were located in exon 19, exon 20, intron 23 and exon 24. There were five novel deleterious mutations (c.3295delC, c.3780_3781delAG, c.4063_4066delAATC, c.5161C > T and c.5173insA) that had not been registered in the BIC or any other public database. Moreover, two of the mutations (c.5468-1del8 and c.1465G > T) had only been previously reported in Chinese population. In this cohort, we detected four recurrent mutations (c.3780_3781delAG, c.5154G > A, c.5468-1del8 and c.5470_5477del8), which accounted for 30.8 % (4/13) of the total mutations. The mutation c.5470_5477del8 occurred three times, and the others occurred twice. The mean age at diagnosis of these BRCA1 mutation carriers was 39.9 ± 8.1 (mean age ± SD) years (Table 2). No significant differences in the mean age at diagnosis between the BRCA1 mutation carriers, BRCA2 mutation carriers and non-carriers were found.

BRCA2 deleterious mutations

A total of 10 deleterious mutations in BRCA2 were found in 13 familial breast/ovarian cancer patients in this cohort (Table 1). None of these mutations had been registered in the 1000 Genomes database. The mean age at diagnosis of these BRCA2 mutation carriers was 41.1 ± 6.5 (mean age ± SD) years (Table 2). Nine mutations were either nonsense or frameshift mutation, and the remaining mutation c.1-40delGA, which resulted in the deletion of a guanine in intron 1 and an adenine in exon 2, was a splicing site mutation. Sixty percent (6/10) of the all of the mutations were located in exon 11. There were seven novel mutations (c.1-40delGA, c.4487delC, c. 469_473delAAGTC, c.5495delC, c.6141 T > A, c.6359C > G and c.7588C > T) in this cohort, and these mutations represented 70 % (7/10) of the mutations in this gene. Two recurrent mutations (c.3109C > T and c.5682C > G) were detected in this cohort, and both of them were registered in the BIC.

Frequencies of BRCA1 and BRCA2 deleterious mutations

A total of 23 deleterious mutations of BRCA1 and BRCA2 were identified in 31 familial breast/ovarian cancer patients, and the frequency was 23.3 % (31/133; Table 3). The frequencies of BRCA1 and BRCA2 mutations were 13.5 % (18/133) and 9.8 % (13/133), respectively.

In the subgroup analysis, the highest overall BRCA1 and BRCA2 mutations rate was 50.0 % (8/16) in the breast cancer patients with family histories of ovarian cancer. The overall mutation rate of the two genes in the patients who were diagnosed at or before the age of 40 was higher than that of the counterpart group. Compared with the breast cancer patients with fewer than two relatives affected by
breast cancer or unilateral breast cancer, the overall mutation rates were higher in the patients with two or more relatives affected by breast cancer or bilateral breast cancer, but these differences did not reach statistical significance ($P = 0.148$ and $P = 0.115$, respectively).

**Haplotype analysis of recurrent mutations**

Four recurrent *BRCA1* mutations (c.3780_3781delAG, c.5154G > A, c.5468-1del8 and c.5470_5477del8) and two recurrent *BRCA2* mutations (c.3109C > T and c.5682C > G) were identified in unrelated breast cancer patients. As haplotype analysis of *BRCA1* c.5470_5477del8 mutation and *BRCA2* c.3109C > T mutation had been performed in Chinese high risk breast cancer patients [9, 10, 12], we performed haplotype analysis on the other four recurrent mutations in this study. Our results showed that carriers with the recurrent *BRCA1* c.5154G > A mutation shared the same haplotype, as well as carriers with the recurrent *BRCA1* c.5468-1del8 mutation, which suggested that these two putative founder mutations were derived from a common ancestor (Table 4). The three carriers with *BRCA2* c.5682C > G mutation sharing only two alleles (D13S171 and D13S1698) out of eight alleles implied that they might be not derived from a common ancestor (Table 5).

**UVs of *BRCA1* and *BRCA2***

In addition to deleterious mutations, we identified 11 UVs (seven in *BRCA1* and four in *BRCA2*; Table 6). Comparisons with the 1000 Genomes database revealed that only *BRCA1* c.2286A > T (R762S) had been reported in a Pakistani population, and the frequency of the T allele was 0.5 % in that population. None of the UVs had previously been found in the Chinese population. The majority of the variants were novel, with the exception of the mutation c.2286A > T in *BRCA1*, which is registered in the BIC, and c.2726A > T in *BRCA1*, which was recently reported in a

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**Table 1** *BRCA1* and *BRCA2* deleterious germline mutations in 133 Chinese women with familial breast/ovarian cancer

| Gene | No. of patient | Exon | Systematic nomenclature | BIC nomenclature | Amino acid change | References |
|------|----------------|------|-------------------------|------------------|-------------------|------------|
| *BRCA1* | 1 | 11 | c.1465G > T | 1584G > T | E489X | Zhi et al. [7] |
| | 1 | 11 | c.1945G > T | 2064G > T | E649X | BIC |
| | 1 | 11 | c.3295delC | 3414delC | P1099fsX10 | Novel |
| | 2 | 11 | c.3780_3781delAG | 3899_3900delAG | L1260FsX6 | Novel |
| | 1 | 11 | c.4063_4066delAAATC | 4182_4185delAAATC | N1359fsX10 | Novel |
| | 1 | 11 | c.4065_4068delTCATA | 4184_4187delTCATA | N1359fsX10 | BIC |
| | 2 | 19 | c.5154G > A | 5273G > A | W1718X | BIC |
| | 1 | 19 | c.5161C > T | 5280C > T | Q1721X | Novel |
| | 1 | 19 | c.5173insA | 5292insA | E1725FsX7 | Novel |
| | 1 | 20 | c.5251C > T | 5370C > T | R1715X | BIC |
| | 1 | Intron 23 | c.5467 + 1G > A | I4V23 + 1G > A | Splicing defect | BIC |
| | 2 | Intron 23 | c.5468-1del8 | 5587-1del8 | Splicing defect | Zhang et al. [11] |
| | 3 | 24 | c.5470_5477del8 | 5589_5596del8 | I1824FsX3 | BIC |

**BRCA2**

| Gene | No. of patient | Exon | Systematic nomenclature | BIC nomenclature | Amino acid change | References |
|------|----------------|------|-------------------------|------------------|-------------------|------------|
| | 1 | Intron 1 | c.1-40delGA | IVS1-1deGA | Splicing defect | Novel |
| | 1 | 5 | c.469_473delAAATC | 697_701delAAATC | K157SfsX24 | Novel |
| | 1 | 9 | c.755_758delACAG | 983_986delACAG | T251FsX1 | BIC |
| | 2 | 11 | c.3109C > T | 3337C > T | Q1037X | BIC |
| | 1 | 11 | c.4487delC | 4715delC | P1496FsX8 | Novel |
| | 1 | 11 | c.5495delC | 5723delC | S1832FsX8 | Novel |
| | 3 | 11 | c.5682C > G | 5910C > G | Y1894X | BIC |
| | 1 | 11 | c.6141T > A | 6369T > A | Y2047X | Novel |
| | 1 | 11 | c.6359C > G | 6587C > G | S2120X | Novel |
| | 1 | 15 | c.7588C > T | 7816C > T | Q2530X | Novel |

**Table 2** Mean age at diagnosis in different *BRCA1* and *BRCA2* status

| BRCA1 | BRCA2 | Non-carriers | $P^a$ | $P^b$ | $P^c$ |
|-------|-------|-------------|------|------|------|
| Number | 18 | 13 | 102 | |
| Mean age (±SD) | 39.9 (±8.1) | 41.1 (±6.5) | 43.9 (±9.7) | 0.74 | 0.11 | 0.31 |
Discussion

**BRCA1** and **BRCA2** are the most important genetic susceptibility genes for breast/ovarian cancer in both Caucasian and Chinese populations. The spectrum and frequencies of mutations in these two genes in Chinese women with familial breast/ovarian cancer have been insufficiently explored to date. Moreover, the penetrance has not yet been investigated. Due to the limited knowledge on hereditary breast/ovarian cancer, there is no genetic counseling or testing services available in Mainland China.

Our results demonstrated that the frequency of **BRCA1** and **BRCA2** mutations among Chinese women with familial breast/ovarian cancer was 23.3 %. Similar results have been reported in the Korean population [23], Hispanic population [24] and Africa American population [25]. However, the frequency observed in the current study is lower than that reported in an Ashkenazi Jewish population, in which the frequency of **BRCA1** and **BRCA2** mutations was 69 % [25]. Compared with other reports about Chinese populations, the frequency found in our cohort was the highest in patients with familial breast/ovarian cancer. Li et al. [9] used PCR-DHPLC assay to screen for **BRCA1** and **BRCA2** mutations in 241 women with familial breast cancer from northern or southern China and found a frequency of 12.9 %. Although the PCR-DHPLC assay is cost-effective for screening for genetic mutations, a considerable number of disease-associated mutations may have been missed by this indirect detection method [26]. Zhang et al. [11] reported that the frequency of **BRCA1** and **BRCA2** mutations in northern Chinese familial breast cancer patients was 10.5 % (43/409) based on PCR-sequencing assay. The enrolment criteria and mutation detecting assay used in this were comparable with the criteria used in our study, but the reported frequency was much lower than that observed in the present study. In their subgroup analysis, the highest frequency was 23 % in the patients whose tumors had been diagnosed at or before the age of 40 years. However, the frequency reached 33.3 % in this group of patients in our cohort. Moreover, in the study conducted by Kwong et al., [12] the frequency of **BRCA1** and **BRCA2** mutations in high-risk breast/ovarian cancer patients was 15.3 % (69/409).

| Table 3 | Frequencies of **BRCA1** and **BRCA2** germline deleterious mutations in different groups of patients |
| Features | Number of total cases | BRCA1 mutation (%) | BRCA2 mutation (%) | Overall mutation (%) | P-value |
|----------|-----------------------|---------------------|---------------------|----------------------|---------|
| Total    | 133                   | 18 (13.5)           | 13 (9.8)            | 31 (23.3)            |         |
| Age at onset |                       |                     |                     |                      |         |
| ≤40 years | 51                    | 11 (21.6)           | 6 (11.8)            | 17 (33.3)            | 0.031   |
| >40 years | 82                    | 7 (8.5)             | 7 (8.5)             | 14 (17.1)            |         |
| Number of breast cancer cases in a family |           |                     |                     |                      |         |
| ≤2       | 99                    | 12 (12.1)           | 8 (8.1)             | 20 (20.2)            | 0.148   |
| >2       | 34                    | 6 (17.6)            | 5 (14.7)            | 11 (32.4)            |         |
| With a family history of ovarian cancer |           |                     |                     |                      |         |
| Yes      | 16                    | 6 (37.5)            | 2 (12.5)            | 8 (50.0)             | 0.012   |
| No       | 117                   | 12 (10.3)           | 11 (9.4)            | 23 (19.7)            |         |
| Bilateral breast cancer |           |                     |                     |                      |         |
| Yes      | 15                    | 3 (20)              | 3 (20)              | 6 (40)               | 0.115   |
| No       | 118                   | 15 (12.7)           | 10 (8.5)            | 25 (21.2)            |         |

| Table 4 | Haplotype analysis of **BRCA1** recurrent mutations carriers |
| Mutation | Patient No. | D17S855 | D17S1322 | D17S1323 | D17S1326 | D17S1327 |
|----------|-------------|---------|----------|----------|----------|----------|
| c.3780_3781delAG | 1          | 145/147 | 113/116  | 150/152  | 108/110  | 128/130  |
|             | 2          | 141/143 | 119/122  | 156/160  | 86/88    | 158/160  |
| c.5154G > A  | 3          | 143/141 | 122/119  | 156/156  | 90/88    | 154/152  |
|             | 4          | 143/151 | 122/116  | 156/152  | 90/104   | 154/130  |
| c.5468-1del8 | 5          | 147/141 | 116/122  | 152/146  | 104/106  | 128/130  |
|             | 6          | 147/145 | 116/113  | 152/150  | 104/102  | 128/130  |

Shared haplotypes are bolded
As previously extracted, the document discusses the analysis of BRCA1 and BRCA2 mutations in breast cancer patients, particularly focusing on the contribution of founder mutations. The authors report on the discovery of recurrent mutations, including the c.5468-1del8 mutation in BRCA2, which was found to be significant in a diagnostic setting.

The study also highlights the importance of haplotype analysis in identifying founder mutations and suggests that the frequency of mutations in the Chinese population might be underestimated due to technical limitations in the PCR-sequencing assay.

A table is presented, providing data on haplotype analysis of BRCA2 c.5682C > G mutation carriers, with shared haplotypes bolded. The table includes patient numbers, haplotype results, and common allele frequencies.

The document concludes with a discussion on the relevance of these findings for mutation analysis in hereditary breast and ovarian cancer syndrome families and the implications for genetic risk assessment and mutation screening.

Relevant points include:
- The proportion of high-risk breast/ovarian cancer patients, including familial breast cancer patients and early-onset cases, and the frequency of two-gene mutations were much lower in the early-onset patients than in the familial breast cancer cases.
- Large genomic rearrangements account for 4–28% of all BRCA1 and BRCA2 mutations, and such mutations have been found in Chinese women at a high risk for breast cancer.
- Because the PCR-sequencing assay cannot detect these rearrangements, the frequency of mutations in the cohort might have been underestimated, and the frequency of BRCA1 and BRCA2 mutations in the eastern Chinese population could be significant.

Although several studies have reported that the BRCA2 mutations are more frequent than BRCA1 mutations in Asian populations, BRCA1 mutations seemed to be more prevalent in the cohort. This finding might be attributable to two points. First, most studies have reported that BRCA2 mutations predominantly occur in relatively late-onset breast cancer patients compared with BRCA1 mutations, but the patients enrolled in the study were much younger than those in other studies, which might have resulted in an underestimation of the contribution of BRCA2 mutations.

Among the recurrent mutations found, the c.5468-1del8 mutation in BRCA2 was identified as a founder mutation, which might be attributable to geographic differences. The characterization of BRCA1 and BRCA2 mutations in Chinese familial breast cancer patients may be due to geographic differences.

In conclusion, the study emphasizes the importance of identifying recurrent mutations, particularly in the context of founder mutations, and highlights the need for larger sample sizes to better understand the genetic risk assessment and mutation screening.

The document also mentions the contribution of Fanconi anemia family studies to the understanding of BRCA1 and BRCA2 mutations and the functional analysis of the c.1-40delGA mutation in BRCA1, which deletes a guanine in intron 1 and an adenine in exon 2 and causes the loss of the donor site of intron 1, suggesting its pathogenicity.

Furthermore, the document discusses the relevance of these findings for mutation analysis in hereditary breast and ovarian cancer syndrome families, highlighting the need for larger sample sizes to better understand the genetic risk assessment and mutation screening.
| Gene | No. of patient | Exon | Systematic nomenclature | BIC nomenclature | Amino acid change | References | Align-GVGD | SIFT | PROVEAN | PolyPhen-2 | PMUT | PANTHER |
|------|----------------|------|-------------------------|------------------|-----------------|------------|------------|-------|----------|------------|------|----------|
| BRCA1 | 1              | 11   | c.1679A > T             | 1798A > T        | D560V           | Novel      | C0         | Damaging | Deleterious | Possibly damaging | Neutral | Deleterious |
|      | 1              | 11   | c.1537C > G             | 1656C > G        | H513D           | novel      | C0         | Tolerated | Deleterious | Benign | Pathological | Neutral |
|      | 1              | 11   | c.2286A > T             | 2405A > T        | R762S           | BIC        | C0         | Damaging | Deleterious | Benign | Pathological | Neutral |
|      | 1              | 14   | c.4445A > C             | 4564A > C        | R1482A          | novel      | C0         | Damaging | Neutral | Benign | Pathological | Neutral |
|      | 1              | 11   | c.1966A > T             | 2085A > T        | N656Y           | novel      | C0         | Damaging | Deleterious | Possibly damaging | Neutral | Deleterious |
|      | 1              | 11   | c.2340G > T             | 2459G > T        | Q780H           | novel      | C0         | Damaging | Deleterious | Probably damaging | Neutral | Deleterious |
|      | 1              | 11   | c.2726A > T             | 2845A > T        | N909I           | BIC, Thirthagiri et al. [8] | C0         | Damaging | Deleterious | Possibly damaging | Neutral | Neutral |
| BRCA2 | 1              | 10   | c.1568A > G             | 1796A > G        | H523R           | novel      | C0         | Damaging | Neutral | Benign | Pathological | Neutral |
|      | 1              | 11   | c.3904A > G             | 4132A > G        | T1302A          | novel      | C0         | Tolerated | Deleterious | Benign | Neutral | Neutral |
|      | 1              | 11   | c.5590G > A             | 5818G > A        | D1864N          | novel      | C0         | Damaging | Neutral | Benign | Neutral | Neutral |
|      | 1              | 11   | c.6763A > T             | 6991A > T        | T2255S          | novel      | C0         | Damaging | Neutral | Possibly damaging | Neutral | Deleterious |
diagnosis. The inconsistent results implied that these observations did not withstand multiple comparisons in our cohort. Breast cancer patients with family histories of ovarian cancer exhibited the highest overall mutation rate of BRCA1 and BRCA2, which implied that BRCA1 and BRCA2 mutations are more likely to occur in families with a history of both breast and ovarian cancer. This result is consistent with those of other studies [9, 11].

Eleven UVs were found in our study, and the potentials for these variants to disrupt the functions of BRCA1 and BRCA2 varied according to the algorithm program used. The UVs accounted for nearly 1/3 of the total mutations/variants in this study. The risks of breast and ovarian cancer in the UVs carriers might be as high as those in the carriers of the classical pathogenic mutations. A variety of approaches have been used to investigate the clinical relevance of these UVs. Co-segregation analysis is regarded as a robust approach because it is directly related to the disease risk and is not affected by selection bias [37]. The absence of co-segregation provides strong evidence against pathogenicity. Unfortunately, the samples required for us to perform co-segregation analysis of UVs and the deleterious mutations in the multi-tumor families were not available.

Conclusions
In the present study, we found that the frequency of BRCA1 and BRCA2 mutations was 23.3 % in our cohort of 133 Chinese women with familial breast/ovarian cancer, and the frequency of BRCA1 and BRCA2 mutations was 50 % in patients with a familial history of both breast cancer and ovarian cancer. The spectrum of BRCA1 and BRCA2 mutations in the Chinese population are quite different from those in other ethnicities. Six recurrent mutations were detected in this study, in which two recurrent BRCA1 mutations were identified as putative founder mutations, and a larger sample size is required to determine the founder effects of these two mutations in Chinese women. BRCA1 and BRCA2 mutations account for a considerable proportion of Chinese hereditary breast/ovarian cancer patients, and the penetrance of these two genes should be investigated because such investigations will be very important for the development of a preventive treatment strategy in China.

Abbreviations
BIC: Breast cancer information core; PCR: Polymerase chain reaction; SD: Standard deviation; UVs: Unclassified variants.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
WMC designed the study, analyzed the mutational data, performed haplotype analysis and drafted the manuscript. YG and ZWP participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Acknowledgements
This research was supported by the grants from Science and Technology Program offered by Health Bureau of Zhejiang Province, China (Grant numbers: 2007A203, 2012RCB006 and 2014KYYA06) and Zhejiang Province Traditional Medical Science Fund Project of China (Grant number: 2012ZB010).

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Received: 7 December 2014 Accepted: 1 February 2016
Published online: 06 February 2016

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