Profilo dell’mutazione germinale BRCA1: p.Ile1845fs in un grande gruppo di donne cinesi di Han con cancro al seno

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Abstract

Background: Il cancro al seno è una delle principali neoplasie carcinomatose parzialmente causate da fattori di rischio genetici. Le mutazioni germinali del gene BRCA1 sono corpiamente associate con cancro al seno. L’identificazione di mutazioni BRCA1 migliora notevolmente la strategia preventiva e la gestione del cancro al seno. Lo scopo del nostro studio era quello di valutare la frequenza della mutazione deleteria BRCA1: p.Ile1845fs in cancro al seno, nonché la correlazione tra la mutazione p.Ile1845fs e i parametri clinicopatologici e i risultati clinici.

Risultati: Abbiamo selezionato un totale di 23,481 pazienti a rischio clinico con cancro al seno e 6489 controlli sani per la sequenza p.Ile1845fs (che sia di sanger o di sequenziamento a prossima generazione). Identificammo 94 pazienti con cancro al seno (0.40%, 94/23481) e 11 controlli sani (0.17%, 11/6489) portatori della mutazione p.Ile1845fs. La mutazione BRCA1: p.Ile1845fs è stata più frequente nei pazienti con la classificazione molecolare TNBC (20.21%, 19/94) e con una storia familiare (37.23%, 35/94) rispetto ai non-carrier (P = 3.62E-6 e 0.034, rispettivamente). In base ai nostri dati, abbiamo aumentato la frequenza della mutazione p.Ile1845fs e confermato che la mutazione BRCA1: p.Ile1845fs è associata a un rischio aumentato di cancro al seno (OR = 2.36, 95%CI = 1.26–4.89, P = 0.004).

Conclusioni: La mutazione BRCA1: p.Ile1845fs è stata una mutazione frequentemente patologica nel cancro al seno in donne cinesi di Han e i nostri dati possono essere utili per la diagnosi e il trattamento del cancro al seno.

Keywords: Cancro al seno, BRCA1, p.Ile1845fs, Clinicopatologico
Material and methods

A total of 23,481 clinically high-risk breast cancer patients and 6489 healthy controls were recruited at 19 clinical centers in 11 Chinese provinces between 2012 to 2018. Clinicopathological features of the patients, including age, ethnicity, menopausal status, type of tumor, disease stage, lymph nodes and tumor size, were collected. Family history is defined that the breast cancer patients had one or more cancer patients (any kind of cancer) in the first-, second- or third-degree relatives. The control subjects were hospital-based unrelated healthy individuals with no breast cancer or any other cancers. The written informed consents were signed by all participants. The study protocol was approved by the Ethics Committee of all the hospitals involved.

Genomic DNA was extracted from blood specimens using the QIAamp DNA kit (Qiagen). DNA were amplified by multiplex-amplicon PCR and libraries were then prepared using protocols recommended by Illumina. The validated DNA libraries were sequenced on an Illumina sequencing system (Illumina HiSeq X10). Read pairs (fastq data) generated from the sequencing system were aligned with reference sequences (BRCA1: NM_007300.3) and processed for variant calling. The pathogenic variant p.Ile1845fs was validated by sanger sequencing, and we successfully validated the mutation results with 100% concordance.

The statistical analysis were performed using the R program (http://www.r-project.org/). Chi Square test or the Fisher exact test were used to analyze the two-group comparisons and the OR and the corresponding 95% CI were estimated. All data were presented as the mean ± standard deviation (SD). P-values < 0.05 were considered statistically significant.

Results

We analyzed the BRCA1 pathogenic variant, p.Ile1845fs, with breast cancer risk in 23,481 invasive breast cancer cases (46.24 ± 20.11 years) and age-matched 6489 controls (47.33 ± 13.46 years). A total of 94 p.Ile1845fs mutations were identified in 23,481 (0.40%) unselected breast cancer patients and 11 unaffected controls carried p.Ile1845fs mutation (0.17%, 11/6489). In the overall analysis, BRCA1: p.Ile1845fs variant showed a higher frequency in breast cancer cases (0.40%) than in controls (0.17%) with a greater than two-fold increased breast cancer risk (OR = 2.44, 95% CI = 1.12–5.34, P = 0.034, Table 1).

We summarized the clinicopathological characteristics of the 94 patients with BRCA1: p.Ile1845fs variant and 23,387 non-carriers in Table 2. The mean age of these breast cancer patients was 46.16 years (sd = 9.80). The mean age of these non-carriers was 46.25 years (sd = 15.52). Among the 94 BRCA1: p.Ile1845fs variant carriers, 44 (46.81%) patients were diagnosed with estrogen receptor (ER) negative status. 46 (48.94%) patients were detected with progesterone receptor (PR) negative status. 35 (37.23%) patients presented with human epidermal growth factor receptor-2 (HER-2) negative status. 6 (6.38%) patients were classified with Luminal-A molecular typing. 26 (27.66%) patients were classified with Luminal-B molecular typing. 12 (12.77%) patients were classified with HER2 overexpression molecular typing. 19 (20.21%) patients were classified with TNBC (Triple-negative breast cancer) molecular typing. 35 (37.23%) patients had family history. TNBC molecular typing was more frequent in mutation carriers compared with non-carriers (P = 3.62E-6). BRCA1: p.Ile1845fs variant carriers were more likely to have family history of cancer (P = 0.034).

Discussion

In this study we investigated the profiling of the BRCA1: p.Ile1845fs variant in Han Chinese breast cancer. We conducted gene sequencing studies in 23,481 unselected breast cancer cases and 6489 controls and confirmed that BRCA1: p.Ile1845fs variant was associated with increased risk of breast cancer (OR = 2.36, 95%CI = 1.26–4.89, P = 0.004).

BRCA1 is a key factor in the DNA double-strand break repair of other genes that induce human cancers [11, 12]. It plays crucial roles in chromatin remodeling, cell-cycle regulation, and activating DNA repair in response to cellular stress [13, 14]. BRCA1 encodes a 1884-amino-acid-long nuclear protein (NP_009231.2) and is expressed in various tissues including breast tissues. There are more than 1600 known variants in

Table 1 BRCA1: p.Ile1845fs variant in unselected breast cancer cases and controls

| Groups      | Carriers | Non-carriers | Freq (%) | OR     | 95% CI       | P     |
|-------------|----------|--------------|----------|--------|--------------|-------|
| Controls    | 11       | 6489         | 0.17     | 2.36   | 1.26–4.89    | **0.004** |
| Cases       | 94       | 23,481       | 0.40     |        |              |       |

Bold: P<0.05
and its pathogenic variants increase the risks of breast cancer [15, 16]. Our genetic data suggested that BRCA1: p.Ile1845fs was a risk factor for breast cancer with statistically significant OR of 2.36.

Clinicopathological characteristics of BRCA1: p.Ile1845fs variant showed 44 (46.81%) patients were diagnosed with ER negative status, 46 (48.94%) with PR negative status and 35 (37.23%) with HER2 negative status. Based on estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER-2) status, we found 6 (6.38%) Luminal-A molecular typing patients and 26 (27.66%) Luminal-B molecular typing patients. Luminal A and Luminal B share similarities in prognosis, while Luminal B have lower expression of hormone receptors, higher expression of proliferation markers, and higher histologic grade than luminal A [17, 18]. Triple-negative breast cancer is defined by aggressive clinical behavior and occurs in 10–15% of sporadic breast cancers [19, 20]. There were 19 (20.21%) TNBC molecular typing patients carried p.Ile1845fs variant. Family history of breast or ovarian cancer is a high risk factor for breast cancer and genetic testing is recommend for these patients [21]. Among total 94 BRCA1: p.Ile1845fs variant carriers, 35 (37.23%) patients had family history.

Recently, more studies focus on effective detection of informative biomarkers for advanced development of early diagnosis and appropriate treatment in breast cancer. Arason A et al. showed the profiling of BRCA1 c.4096 + 3A > G and found 8 heterozygous carriers (0.44%) in 1820 unselected breast cancer cases, and 3 (0.15%) in 1968 healthy controls [22]. BRCA1: p.Val1833Met variant was genotyped among 3531 breast cancer patients and 1558 healthy controls using sanger and next generation sequencing, with 27 (0.77%, 27/3531) carriers in cases while no carriers in controls [23].

Our study accord with a pathogenic BRCA1 mutation: p.Ile1845fs and identified 94 carriers (0.40%) in 23,481 breast cancer patients, and 11 (0.17%, 11/6489) in controls. Our findings add to the current knowledge of BRCA1, which will be of use in clinical genetic counselling.

In summary, we described the frequency of BRCA1: p.Ile1845fs mutation and its clinical aspects in our cohort. We have found that BRCA1: p.Ile1845fs variant is associated with risk of breast cancer. Further genetic studies and meta-analyses are warranted to derive more precise risk estimates for BRCA1: p.Ile1845fs variant. And such carriers should be counselled accordingly, with clinical recommendations and personalized risk-reduction primary and secondary cancer prevention strategies.

### Abbreviations
BRCA1: Breast cancer susceptibility gene 1; ER: Estrogen receptor; HER-2: Human epidermal growth factor receptor-2; PR: Progesterone receptor; SD: Standard deviation; TNBC: Triple-negative breast cancer

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### Authors’ contributions
HZ conceived and designed the experiments. WY, ZH, WX and WH performed the mutation analysis and validation. ZQ, WY and SY gathered patients’ data. WY wrote the manuscript. All authors read and approved the final manuscript.

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### Availability of data and materials
The authors declare that the data supporting the findings of this study are available within the article.

### Table 2 Clinical characteristics of BRCA1: p.Ile1845fs variant carriers and non-carriers in this study

| Variables          | Carriers | Non-carriers | P    |
|--------------------|----------|--------------|------|
| Age at diagnosis   |          |              | 0.19 |
| ≤ 50               | 52       | 6943         |      |
| > 50               | 22       | 4223         |      |
| na                 | 20       | 12,221       |      |
| ER status          |          |              | 2.21E-08 |
| Positive           | 24       | 11,849       |      |
| Negative           | 44       | 5622         |      |
| na                 | 26       | 5916         |      |
| PR status          |          |              | 1.29E-06 |
| Positive           | 21       | 10,630       |      |
| Negative           | 46       | 6794         |      |
| na                 | 27       | 5963         |      |
| HER2 status        |          |              | 0.011 |
| Positive           | 31       | 10,264       |      |
| Negative           | 35       | 6069         |      |
| na                 | 28       | 7054         |      |
| Molecular typing   |          |              | 3.62E-06 |
| Luminal-A          | 6        | 3726         |      |
| Luminal-B          | 26       | 8061         |      |
| HER2 overexpression| 12       | 2805         |      |
| TNBC               | 19       | 1743         |      |
| na                 | 31       | 7052         |      |
| Family history     |          |              |      |
| Yes                | 35       | 4184         | 0.034 |
| No                 | 41       | 8183         |      |
| na                 | 18       | 11,020       |      |
| Total              | 94       | 23,387       | 23,481 |

Bold: P<0.05
Ethics approval and consent to participate
All patients had been signed on the consent form.

Consent for publication
Written informed consents were obtained from patients for publication of their individual details and accompanying images in this manuscript.

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

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