Gynecologic Oncology xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

BRCA germline mutations in an unselected nationwide cohort of Chinese patients with ovarian cancer and healthy controls

Ang Li [a], Rong Xie [b], Qihuan Zhi [a], Yixiao Deng [a], Yangming Wu [a], Weiwei Li [a], Lu Yang [a], Zinan Jiao [a], Jiaqi Luo [a], Yi Zi [a], Gang Sun [a], Jiajia Zhang [a], Yujian Shi [a,*], Jian Liu [b,*]

[a] TopGene Tech Co., Ltd., Guangzhou, 510025, Guangdong, PR China
[b] Department of Medical Oncology, Fujian Cancer Hospital, Fujian Medical University Cancer Hospital, The Teaching Hospital of Fujian University of Traditional Chinese Medicine, Fuzhou, 350014, Fujian, PR China

HIGHLIGHTS

- 22.4% (17.1% BRCA1, 5.3% BRCA2) OC patients and 0.4% (0.3 BRCA1, 0.1% BRCA2) controls carried deleterious variants in Chinese.
- The dense cluster of mutations in BRCA1 exon10 indicated that hypermutation was a characteristic of Chinese OC patients.
- The estimated odds ratio (OR) of OC associated with BRCA1 positive variants was 34.6 (95% CI, 12.5–95.7) in age < 40 group.
- The OR was 42.4 (95% CI, 5.9–305.2) in age ≥ 50 group.

ARTICLE INFO

Article history:
Received 2 May 2018
Received in revised form 30 July 2018
Accepted 31 July 2018
Available online xxxx

Keywords:
Ovarian neoplasms
High-throughput nucleotide sequencing
Case-control studies
BRCA gene
Exon mutation
Chinese genetic testing

ABSTRACT

Objective. To investigate the BRCA status in Chinese patients with ovarian cancer (OC). Though there were two large prevalence studies in Chinese OC patients, this was the first time to observe it in healthy controls.

Methods. We performed BRCA mutation screening using next-generation sequencing to determine the prevalence of BRCA germline deleterious mutations in an unselected cohort of Chinese OC patients (n = 1331) versus healthy controls (n = 1763) and describe the types and spectrum of BRCA deleterious variants.

Results. Among the 1331 patients with OC, 227 (17.1%) carried deleterious variants in BRCA1 and 70 (5.3%) carried deleterious variants in BRCA2. Of 1763 control subjects, 6 (0.3%) and 2 (0.1%) had deleterious variants in BRCA1 and BRCA2. No patient carried mutations in both BRCA1 and BRCA2 simultaneously. Sixty-three novel mutations were identified, and three Chinese specific hot-spot mutations were notified as BRCA1 c.5470_5477delATTGGGCA, BRCA1 c.981_982delAT, and BRCA1 c.3770_3771delAG. Interestingly, all these high-frequency recurrent mutations were distributed on exon 10, which may also be the Chinese OC BRCA mutations' distinct characteristics. In addition, in our study, the estimated odds ratio (OR) of OC associated with BRCA1 positive variants were approximately 34.6 (95% CI, 12.5–95.7) in age group under 40 and 42.4 (95% CI, 5.9–305.2) in group older than 50 in the Chinese population, respectively.

Conclusions. We recommend BRCA testing to all Chinese OC patients and those general Chinese who have family members with hereditary breast and ovarian related cancer (HBOC)-related cancers. Variants carriers would not only benefit from early prevention of OC but also for the medical management.

1. Introduction

Ovarian cancer (OC) was ranked the fifth cancer mortality in US women (14,070 estimated deaths in 2018) due to the late diagnosis stage and the quick resistance to existing medical treatment [1]. In China, there were 52,100 women diagnosed with OC, and about 22,500 OC-related deaths reported in 2015 [2]. Furthermore, no early screening strategy has been proven effective [3]. The unreliable evaluated screening interventions such as transvaginal ultrasound or/and CA-125 level made it difficult to diagnose OC at early stage [4].

Cumulative incidence of OC is 1.4% in general population [5]. Deleterious germline mutations in BRCA1 and BRCA2 have become the most important predisposing factors in OC [6]. The risk of contracting OC by age 70 was estimated to be 39% for women with BRCA1 mutations and 11% for women with BRCA2 mutations [6]. And it has been reported that the prevalence of BRCA mutations in OC patients varies among different ethnic groups [7]. There were hitherto only a few studies

* Corresponding authors.
E-mail addresses: yujian.shi@topgene.cn (Y. Shi), liujianfj@126.com (J. Liu).

https://doi.org/10.1016/j.ygyno.2018.07.024
0090-8258/

investigating its prevalence in Asian population [8–13]. Moreover, the prevalence of BRCA1 mutations of 0.1%, and BRCA2 mutations of 0.2%, is low in other/western population [14], it is necessary to investigate their presentations in Chinese population prevalence. Additionally, the risk of OC is still rising in women with a family history of ovarian or breast cancer but without known mutations in susceptibility genes [15].

According to National Comprehensive Cancer Network (NCCN) guidelines [16], all OC patients are recommended to take BRCA testing for genetic counseling before medical managements, given OC patients with BRCA mutations have higher sensitivity to poly (ADP-ribose) polymerase (PARP) inhibitors [17] and platinum-containing agents [18]. However, the cost is not included in national medical insurance in China. It is essential to accurately find the OC patients who are more likely to benefit from BRCA testing. Wide variations of BRCA gene and full spectrum of BRCA mutations, especially Chinese population-based, have far yet been fully investigated, which also makes genetic counseling even more difficult. Thus, to address these clinical questions and public health concerns, we performed analyses of BRCA germline mutation prevalence in unselected patients with OC and healthy controls to assess the risk of developing OC in BRCA1/2 mutated Chinese women using Next Generation Sequencing (NGS).

2. Material and methods

2.1. Study population and sample collection

Patients with ovarian cancer and control group (all samples were collected in 2017) was included in our study. We recruited incident specimens and controls from Chinese national wide hospitals [19]. Of the 1360 patients with OC and 1783 control subjects recruited, 1331 and 1763 cases and controls, respectively, were contained in this study (see Fig. 1 for exclusion criteria).

2.2. DNA extraction

Extraction of genomic DNA was from each case by the Mag-bind blood and tissue DNA HDQ 96 kit (Omega Bioservices, Norcross, GA, USA) according to the manufacturer's instructions. DNA purity was detected by a UV spectrophotometer (Nano Drop Technologies, Wilmington, DE) and quantification was performed using the Qubit 3.0.

2.3. Library preparation and mutation screening using NGS approach

The BRCA1/2 panel (Morgen, China) was used which covers the entire coding sequences of BRCA1 and BRCA2 including 10–50 bases of adjacent intronic sequence of each exon. According to the manufacturer's instructions, 120 amplicons were divided into two separate multiplex PCR amplification reactions (A/B Pools). 50 ng of sample DNA were added to each of the two reactions. PCR and the purification of PCR products were performed under the conditions according to the manufacturer's instruction. After adding barcode and adaptors for NextSeq (Illumina) using PCR reagents, the quality of PCR products was checked with LabChip GX Touch24 (PerkinElmer). The sequencing process was carried out according to Illumina's protocols (Illumina, San Diego, CA) using NextSeqCN500 (BerryGenomics, China) 300 cycle reagent cartridge corresponding to 2 × 150 bp paired end configuration. The average depth of each run was over 200×. After the sequencing, the FASTQ was used for alignment and variant calling. A variant was called when the minimum depth of coverage is 20×, with allele directional balance of 15%, and mutation threshold of 10%.

2.4. Bioinformatics analysis

Raw (sequence) reads were treated using FASTP (https://github.com/OpenGene/fastp). Hg19, the human genome reference sequence, aligned against the sequence reads with the transformation of Burrows-Wheeler Aligner [http://bio-bwa.sourceforge.net]. With the Genome Analysis Toolkit (GATK; https://www.broadinstitute.org/gatk), we accomplished subsequent local insertion/deletion (indel) realignment and data pre-processing, base quality score recalibration (BQSR). Genetic mutations were called with GATK UnifiedGenotyper using the default parameters except minIndelFrac (set to 0.05). Mutations were annotated by ANNOVAR (http://www.openbioinformatics.org/annovar) and VEP (http://asia.ensembl.org/Tools/VEP).

2.5. BRCA1 and BRCA2 germline mutation classification

Sequence variations were described according to the nomenclature (www.hgvs.org) with the use of reference sequence of NM_007294.3 (hg19) for BRCA1 and NM_000059 (hg19) for BRCA2. For the interpretations of missense mutations, mutation databases such as Breast Cancer Information Core (BIC, https://research.nhgri.nih.gov/bic/), Leiden Open Variation Database (LOVD, http://www.lovd.nl/), and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) were referred. Germline mutations fulfilled the referral condition described above were further classified as benign, likely benign, variants of undetermined significance (VUS), likely pathogenic and pathogenic (Classes 1 to 5, separately) following the classification protocol of American College of Medical Genetics (ACMG) professional practice and guidelines [20]. All annotation processes were performed by at least two genetic experts independently.

2.6. Sanger sequencing

Sanger DNA sequencing was used to confirm identified variants (Classes 3–5) starting from the original samples using the specific gene primers.

2.7. Statistical analysis

Continuous and categorical variables were compared by performing Student's *t*-test and Pearson's χ^2-test or the Fisher exact test, respectively. Each *p* value was based on two-sided hypothesis and $p < 0.05$ was inferred as statistically significant. The results are in case-control condition. Considering that breast cancer could be a cause of risk for the incidence of OC, we excluded the cases with personal history of breast cancer (n = 45) when calculating the odds ratios (ORs) to avoid the biased odds estimates. And the risk of OC incidence could elevate with increasing age, so the estimation of odds was stratified by age. Calculation in this study was based on the following assumption: 1) the hospital-based Chinese OC cases represent the general cases in Chinese OC; and 2) control group represents the general Chinese population. All analyses were performed using the SPSS Statistics software v23.0 and PRISM 7.0.

3. Results

3.1. Prevalence of BRCA1 and BRCA2 mutations and variants of undetermined significance (VUS)

In this study, BRCA mutation carriers were notified as people who have likely pathogenic mutations or/and pathogenic mutations, also called as deleterious variants or BRCA positive. Of the 1331 patients with OC, 227 (17.1%) had pathogenic mutations in BRCA1 and 70 (5.3%) had deleterious mutations in BRCA2. Of 1763 control subjects, 6 (0.3%) carried deleterious variants in BRCA1 and 2 (0.1%) had deleterious variants in BRCA2 (Table 1). VUS in BRCA were presented in 85 cases (6.4%) versus 84 controls (4.8%) (Fig. 2a & b). Cases were excluded if they carried both a BRCA1 and BRCA2 VUS mutation (n = 3) to avoid repeating count.

Please cite this article as: A. Li, et al., BRCA germline mutations in an unselected nationwide cohort of Chinese patients with ovarian cancer and healthy controls, Gynecol Oncol (2018), https://doi.org/10.1016/j.ygyno.2018.07.024