Screening for founder and recurrent BRCA mutations in Hong Kong and US Chinese populations

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Introduction

In Hong Kong, breast cancer accounts for approximately one-third of all newly diagnosed cancers and 11.1% of all cancer deaths. An exponential increase in the incidence is expected in Hong Kong and other Asian regions, in particular China. Hereditary breast and ovarian cancer (HBOC) syndrome is a genetic disease in which alterations in BRCA1 and BRCA2 genes are common. About 10% of breast cancer cases in Hong Kong are inherited. Patients with a BRCA mutation and their family members have a higher risk of developing breast cancer (45%-65%) or ovarian cancer (11%-39%) by the age of 70 years, compared with those without a BRCA mutation. Screening and risk-reduction intervention for BRCA carriers and their family members are recommended. Identification of ethnic-specific founder and hotspot (recurrent) mutations in Chinese patients would be beneficial for the Chinese population. A genetic testing panel was designed for the population to achieve better risk assessment and preventive measures.

Methods

We recruited high-risk patients who (1) were diagnosed with breast cancer at the age of ≤45 years; (2) had bilateral breast cancer; (3) had triple-negative or medullary type pathology; (4) had ovarian cancer at any age; (5) had male breast cancer; (6) had at least one first- or second-degree relative with breast and/or ovarian cancer, regardless of age; and (7) had one relative with a BRCA mutation. Genomic DNA and RNA samples were extracted from peripheral blood using QIAamp DNA Blood Mini Kit (Qiagen). In the case of splicing variant analysis in transcript level, RNA samples were reverse transcribed to cDNA samples using Superscript III First Strand Synthesis System (Invitrogen).

A total of 25 hotspot mutations, including founder/recurrent mutations reported in a Chinese ethnic group, were identified through a literature search. A single base extension assay, SNaPshot, was adopted for mutation screening. In brief, all 25 mutations were either amplified by polymerase chain reaction (PCR) or multiplex PCR. Single base extension reaction was performed by the SNaPshot kit. After purification, SNaPshot products were run with LIZ120 size standard in a sequencing analyser. Data were processed by Genescan Analysis. Relative intensity of the two alleles in each sample was calculated to confirm heterozygosity. The signal from the normal control DNA was used as reference.

In patients with founder/recurrent mutations,
further validation was performed by full gene sequencing. Mutation analysis was performed by direct DNA sequencing of all coding exons of BRCA1 and BRCA2 and partial flanking intronic sequences. Bi-directional sequencing was performed. Sequencing results were compared with the reference DNA sequences using Variant Reporter software (Applied Biosystems) and then reviewed manually.

The Fluidigm Access Array System (Fluidigm, San Francisco [CA], USA) was used to generate separate pools of 74 PCR amplicons per sample to target all exons of the BRCA genes plus 10 bp from intron-exon boundaries. Dual 8-bp barcode nucleotide sequences were incorporated in the ends of each amplicon for sample identification. Pair-end sequencing of the amplicons (2 x 300bp) was performed on a MiSeq (Illumina, San Diego [CA], USA) with reagent kit v3.

**Results**

All patients underwent BRCA screening with the recurrent panel covering 25 loci. Those who tested negative were then subjected to amplicon-based next-generation sequencing (NGS). The prevalence of BRCA mutations among breast cancer patients was 7.94% (35/441) and the pickup rate of the recurrent panel was 3.4% (15/441). Among ovarian cancer patients, BRCA mutations were identified in 8.39% (13/155) and the pickup rate of the recurrent panel was 3.87% (6/155). Overall, the prevalence of BRCA mutation in the local Chinese cohort was 8.05% (48/596), and the pickup rate of the recurrent panel was 3.52%. Interestingly, we identified three BRCA1 mutations (c.4046, c.212+3A>G, and c.5335delC) by NGS, which were seen only in those with ovarian cancer, not breast cancer.

In 79 blood samples collected overseas, we identified two recurrent mutations (BRCA2 c.3109C>T and BRCA2 c.4965delC) using the recurrent panel and a novel BRCA2 c.3165_3167delinsCC mutation by NGS. Additionally, we compared the spectra of BRCA mutations between Hong Kong and overseas. We received 84 Chinese breast cancer cases that covered 62 different types of mutation (20 BRCA1 and 42 BRCA2). In addition, only 15 types of mutations had been previously identified in Hong Kong. Our recurrent panel covered seven types of these mutations (2 BRCA1 and 5 BRCA2) and was expected to pick up 17 of the 84 cases. The most predominant mutations in this overseas cohort were BRCA2 c.7878G>A and c.5164_5165delAG.

**Discussion**

In this pilot study, <10% of the Chinese patients harboured BRCA1 or BRCA2 mutations, and nearly half of the mutations were recurrent. Consistent with our previous findings,1 this screening method using the recurrent panel could detect 43.8% (21/48) of all BRCA mutations in patients with HBOC. Furthermore, BRCA1 [c.964delG (n=3); c.4372C>T (n=3)] and BRCA2 c.3109C>T (n=7) were the most common mutations in the local cohort. BRCA2 predominance is common among Chinese patients.

The spectrum of BRCA mutation varies across ethnicities, and its prevalence and dominancy also varies among different populations.2 The cost of genetic testing and lack of coverage by the local healthcare system are barriers to mutation screening in Asia and in Hong Kong.3 Development of a screening panel for recurrent mutations offers a simple, rapid, and affordable routine molecular diagnostic method for prevention or management of these high-risk patients and their families with BRCA mutations.4

The bioinformatics and sequencing data analysis of NGS remain challenging, with diverse analysis tools and databases available. Development of a breast cancer genetic screening strategy for Chinese ethnicity could benefit from this new approach and improve cancer risk assessment and management for patients with HBOC.

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**References**
