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

A Kwong*, VY Shin, ESK Ma, CTL Chan, JM Ford, AW Kurian, E Tai

KEY MESSAGES

1. A total of 637 blood samples (441 breast, 155 ovarian, and 41 prostate cancers) were obtained in a local Chinese population.
2. The overall prevalence of *BRCA* mutation was 8.05% and the pickup rate of the recurrent panel was 3.52%. Nearly half of the mutations were covered by this panel.
3. We identified three *BRCA* mutations that were seen only in patients with ovarian cancer.
4. Of 79 Chinese breast cancer samples collected from overseas, two recurrent mutations were identified.
5. We compared 84 known mutation cases from overseas (comprising 62 different types of mutations) with our recurrent spectrum. Of which, 15 have been identified in Hong Kong and seven of them were covered in the recurrent panel.

Hong Kong Med J 2018;24(Suppl 3):S4-6
HMRF project number: 01121376

A Kwong, VY Shin, ESK Ma, CTL Chan, JM Ford, AW Kurian, E Tai

Department of Surgery, The University of Hong Kong

* Principal applicant and corresponding author: avakwong@hku.hk

This summary is based on studies first reported in:
(1) Kwong A, Ho JC, Shin VY, et al. Rapid detection of *BRCA1/2* recurrent mutations in Chinese breast and ovarian cancer patients with multiplex SNaPshot genotyping panels. Oncotarget 2017;9:7832-43.
(2) Kwong A, Shin VY, Au CH, et al. Detection of germline mutation in hereditary breast and/or ovarian cancers by next-generation sequencing on a four-gene panel. J Mol Diagn 2016;18:580-94.
(3) Kwong A, Shin VY, Cheuk IW, et al. Germline RECQL mutations in high risk Chinese breast cancer patients. Breast Cancer Res Treat 2016;157:211-5.

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.[1] 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.[2] 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 and 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. Paired-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.[3] 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.

Acknowledgements

This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#01121376). We thank John Ho, Isabella Cheuk, Jiawei Chen, Jennifer Siu, Tommy Au, Fian Law, Donna Ip, Wing Pan Luk, Ling Hui Fung, and Cecilia Ho for their technical support and data analysis. We also thank all the doctors and nurses from the breast cancer research groups in the departments of surgery and oncology of the contributing hospitals in Hong Kong for patient recruitment. We also thank Laura Esserman and Beth Crawford (University of California), Jeffrey Weitzel (City of Hope Cancer Centre), Edmund Tai (Palo Alto Medical Foundation), Mike Field (Royal North Shore Hospital), Susan Domchek (University of Pennsylvania), James Ford and Allison Kurian (Stanford University) and Chiun-sheng Huang (National Taiwan University Hospital) for providing samples and mutation spectrum of Chinese patients with breast cancer.

References

1. Kwong A, Shin VY, Au CH, et al. Detection of germline mutation in hereditary breast and/or ovarian cancers by next-generation sequencing on a four-gene panel. J Mol Diagn 2016;18:580-94.
2. Antoniou A, Pharoah PD, Narod S, et al. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case series unselected for family history: a combined analysis of 22 studies. Am J

Hum Genet 2003;72:1117-30.
3. Nakamura S, Kwong A, Kim SW, et al. Current status of the management of hereditary breast and ovarian cancer in Asia: first report by the Asian BRCA Consortium. Public Health Genomics 2016;19:53-60.
4. Ossa CA, Torres D. Founder and recurrent mutations in BRCA1 and BRCA2 genes in Latin American countries: state of the art and literature review. Oncologist 2016;21:832-9.