LETTER TO THE EDITOR

Novel BRCA1 and BRCA2 genomic rearrangements in Southern Chinese breast/ovarian cancer patients

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To the Editor,

Breast cancer is the most frequently occurring malignancy in not only Western but also Asian women. Germline mutations in the breast cancer susceptibility genes, BRCA1 and BRCA2, are found in a significant proportion of patients affected by hereditary breast/ovarian cancer [1]. Pathogenic mutations in BRCA1 and BRCA2 are predominantly small deletions, insertions, and point mutations resulting in frame shift, nonsense, premature termination, or splice site alterations, which lead to the formation of a truncated BRCA protein. Owing to the richness of Alu sequences [2] in BRCA1 and BRCA2, albeit to a lesser extent in the latter gene, it is not surprising that BRCA1/2 genomic rearrangements are known to be mediated through Alu repeat sequences. Large genomic rearrangements (LGRs) have been increasingly reported and more than 80 different LGRs have been characterized in BRCA1, but less in BRCA2 [3–5]. One study in the United States reported that genetic testing, as currently carried out, did not provide all available information to women at risk. Their findings indicated that 12 % of those high-risk breast cancer patients with negative genetic test results for BRCA1 and BRCA2 actually carried large genomic rearrangement in one of these genes [3]. These results are consistent with previous studies specifically of BRCA1 in various European populations [6, 7]. Over the past decade, a large number of techniques have become available for detecting large deletions and duplications. Multiplex ligation-dependent probe amplification (MLPA) is one of the most commonly used assays for this purpose [4, 8–11]. The prevalence of LGRs varies between different populations ranging from 0 to 27 % of BRCA1 mutation-positive families from French Canadian and Dutch populations, respectively [10, 12]. Founder LGRs have also been identified. However, in many countries; breast cancer patients without family history are generally not tested for LGRs. Lack of a family history may relate to small family size, non-penetrance, premature death, loss of contact with family members, and inadequate information [13]. Alternatively, lack of family history can also be explained by new germline mutations that found in the probands, but not in any of their family members. De novo mutations are very rare, but reported among BRCA genes [14–17]. Previously, we reported a de novo mutation in which multiple exons were deleted from BRCA1 in a Chinese breast cancer patient [18]. To date, the spectrum of LGR in Chinese population is largely unknown. In this study, MLPA analysis was employed together with full gene sequencing to determine the frequency and spectrum of BRCA1/2 LGRs in a group of Chinese breast cancer patients from Southern China.
A total of 555 clinically high-risk breast and/or ovarian cancer probands (520 female and 35 male), referred to the Hong Kong Hereditary and High Risk Breast Cancer Programme (www.HRBCP.org) from March 2007 to November 2011, were recruited [18, 19]. Based on the lower incidence of breast cancer in Asia cohorts, clinically high-risk patients included in this study were defined as those who (1) had at least one first- or second-degree relative with breast and/or ovarian cancer, regardless of age; (2) were less than 50 years of age at diagnosis; (3) had bilateral breast cancer; (4) had triple negative (TN) or medullary type pathology; (5) had at least one relative with cancers other than breast and ovarian cancer that are known to be related to BRCA mutations; or (6) they were ovarian cancer patients with a family history of breast cancer. The mean age at diagnosis of breast cancer was 45-years (range 18–82) and that of ovarian cancer was 44-years (range 19–64). All probands were from Chinese ancestry and over 90 % were from Guangdong province of Southern China.

MLPA analysis and full BRCA1/2 sequencing of the 555 probands were conducted. Overall, we identified 69 (69/555, 12.4 %) deleterious BRCA gene mutations. Of the 69 deleterious mutations, 29 were in BRCA1 and 40 in BRCA2. Among the 69 mutations, 29 of them were novel in which 12 were in BRCA1 and 17 were in BRCA2. Intriguingly, we also identified 7 out of the 35 male probands who carried only BRCA2 deleterious mutations. Most importantly, among the 29 novel mutations, 4 of them are LGRs (2 in BRCA1 and 2 in BRCA2) and all were only detected by MLPA, but not sequencing. Overall it accounted for 5.8 % (4/69) of all BRCA mutations in our cohort, 6.9 % (2/29) of all BRCA1 mutations and 5 % (2/40) of all BRCA2 mutations. Except for the one we previously reported [18], all remaining LGRs identified in this study are novel mutations and not found in BIC entries.

The characteristics of the probands and characterization of the LGRs are described in Table 1. Based on MLPA analysis, female proband (TWH9701) was found to have a large BRCA1 deletion of exons 1–12. We have previously reported this patient who carried a de novo BRCA1 LGR because none of her parents carried the mutation [18]. Although we could not determine the LGR breakpoints by cDNA sequencing, qRT-PCR analysis has shown that this novel germline mutation resulted in the downregulation of BRCA1 gene expression, suggesting that there is no expression of truncated RNA transcript. Female proband (TWH9701) was found to have a BRCA1 deletion spanning exons 17–20. Sequence analysis of amplified cDNA revealed a deletion of 291 bp with breakpoints located at c.4987_5277. The loss of exons 17–20 caused an in-frame deletion and truncation of the BRCA1 protein (p.M1663_K1759del97). Male proband (HKSH9601) and a family member were identified to carry a BRCA2 deletion of exons 15–16 only by MLPA. Sequencing of amplified cDNA revealed a deletion of 370 bp with breakpoints located at c.7436_7805. This deletion produced a shift in the reading frame and truncation of the BRCA2 protein (p.As2749GlyfsX46). Female proband (HKSH1001) was found to carry a BRCA2 deletion of exons 21 and sequence analysis revealed that a deletion of 122 bp with breakpoints located at c.8633_8754. The loss of exon 21 caused a shift in the reading frame and truncation of the BRCA2 protein (p.Glu2878GlyfsX5).

| Exon deletion | Breakpoints (cDNA) | Predicted amino acid change | Case no. | Gender | Family history of BC | BC and other cancers (age at diagnosis) | Other tumors in proband family | BIC entries |
|---------------|--------------------|-----------------------------|---------|-------|----------------------|----------------------------------------|-------------------------------|-------------|
| 1–12 (BRCA1)  | No transcript      | Uncertain                   | TWH9701 | F     | No                   | BC (30)                                | Bone, leukemia, liver, pancreas | None        |
| 17–20 (BRCA1) | c.4987_5277del291  | p.M1663_K1759del97          | TWH9501 | F     | Yes                  | BC (36); OC (45)                       | Esophagus, stomach             | None        |
| 15–16 (BRCA2) | c.7436_7805del1370 | p.Asp2479GlyfsX46           | HKSH9601| M     | Yes                  | BC (55); GC (54); HCC (54)             | Esophagus                      | None        |
| 21 (BRCA2)    | c.8633_8754del122  | p.Glu2878GlyfsX5            | HKSH1001| F     | Yes                  | BC (39)                                | –                             | None        |

BC breast cancer, GC gastric cancer, HCC hepatocellular carcinoma, OC ovarian cancer, BIC breast cancer information core
* All mutations are named according to the recommendations for the description of sequence variants of Human Genome Variation Society (HGVS)
(2 in BRCA1 and 2 in BRCA2) were detected only by MLPA, which accounted for 6.9% (2/29) of all BRCA1 mutations and 5% (2/40) of all BRCA2 mutations. These findings highlight the LGR spectrum of BRCA1 and BRCA2 genes in Southern Chinese breast cancer patients and the ethnic specificity of these rearrangements. Consistent with the literature, we recommend LGR testing together with BRCA1/2 full gene sequencing for the purpose of comprehensive BRCA1/2 analysis in the clinical setting.

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Conflict of interest None.

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