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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 arrangement 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.

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Table 1 *BRCA* genomic rearrangements of the probands

Exon deletion	Breakpoints[a] (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	TWH5901	F	Yes	BC (36); OC (45)	Esophagus, stomach	None
15–16 (*BRCA2*)	c.7436_7805del370	p.Asp2479GlyfsX46	HKSH9601	M	Yes	BC (55); GC (54); HCC (50)	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

[a] All mutations are named according to the recommendations for the description of sequence variants of Human Genome Variation Society (HGVS)

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 (TWH5901) 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 BRCA2 protein (p.Asp2479GlyfsX46). 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 BRCA2 protein (p.Glu2878GlyfsX5). Importantly, we have recently confirmed by haplotype analysis that the recurrent LGR (c.7436_7805del370) found in the male proband (HKSH9601) and his family member is a founder mutation [20]. Thus, we are the first to report that male breast cancer in this Chinese family has the *BRCA2* founder LGR.

In conclusion, overall *BRCA1/2* mutation prevalence among this cohort was 12.4 % (69/555). Four novel LGRs

(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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