

A novel de novo *BRCA1* mutation in a Chinese woman with early onset breast cancer

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Abstract Germline mutations in the two breast cancer susceptibility genes, *BRCA1* and *BRCA2* account for a significant portion of hereditary breast/ovarian cancer. De novo mutations such as multiple exon deletion are rarely occurred in *BRCA1* and *BRCA2*. During our mutation screening for *BRCA1/2* genes to Chinese women with risk factors for hereditary breast/ovarian cancer, we identified a novel germline mutation, consisting of a deletion from exons 1 to 12 in *BRCA1* gene, in a patient diagnosed with early onset triple negative breast cancer with no family history of cancer. None of her parents carried the mutation

and molecular analysis showed that this novel de novo germline mutation resulted in down-regulation of *BRCA1* gene expression.

Keywords Breast cancer · *BRCA1* gene · De novo mutation · Early onset

Abbreviations

DCIS	Ductal carcinoma in-situ
TN	Triple negative cancer
ER	Estrogen receptor
BIC	Breast cancer information core
qRT-PCR	Quantitative reverse transcription-polymerase chain reaction

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Introduction

Breast cancer is the most frequently occurring malignancy in women. Germline mutations in the two breast cancer susceptibility genes, *BRCA1* and *BRCA2* account for a significant portion of hereditary breast/ovarian cancer [1]. *BRCA* mutations are distributed over all exons and therefore genetic testing for these *BRCA1/2* genes require a mutation screening method of the entire coding regions of the genes. In our laboratory in Hong Kong, we currently offer mutation screening for *BRCA1/2* genes to Chinese women with breast or ovarian cancer at age 50 years and younger, or with a family history of *BRCA* related cancers such as breast, ovarian, colon, and prostate, using direct full gene sequencing. However, in many countries, breast cancer patients without family history are generally not tested. For example, in the United Kingdom it is estimated that up to 6% of the *BRCA1* and *BRCA2* mutation cases are

missed because they lack a family history of relevant cancers. Lack of a family history may relate to small family size, non-penetrance, premature death, loss of contact with family members, and inadequate information [2]. Alternatively, lack of family history can also be explained by new germline mutations found in the probands but not in any of their family members. Such de novo mutations have been reported in diseases such as hemophilia A [3] and thalassaemia [4]. De novo mutations are very rare among *BRCA* genes [5–8]. Moreover, most of the reported disease-associated mutations in *BRCA1* and *BRCA2* result in frame shift, nonsense, insertions, deletions, or splice site alterations, which lead to formation of a truncated *BRCA* protein. However, large genomic rearrangements in *BRCA1* and *BRCA2* are rarely seen, and a similar *BRCA1* deletion (from exon 1 to 13) has been reported in Finland [9]. Hereby, we describe a de novo mutation in which multiple exons were deleted from *BRCA1* in a Chinese breast cancer patient. Furthermore, we demonstrate that significant reduction in *BRCA1* RNA levels in the proband by qRT-PCR assays, suggesting the absence of truncated *BRCA1* RNA products. The results from this case report illustrate the importance of gene rearrangement testing in addition to full gene sequencing in detecting *BRCA1* and *BRCA2* mutations in breast or ovarian cancer patients, even in the absence of a strong family history.

Materials and methods

Patient history and paternity confirmation

We report a 33-year old Chinese woman who at age 30 was diagnosed with grade 2 invasive ductal NOS breast carcinoma and confirmed to be a triple negative cancer (ER, PR and Her2/Neu negative). No family history of cancers was reported. Due to a complicated family history (Fig. 1), familial relationship of this group was confirmed by paternity test (Identifiler™ System, 15+1 markers, Applied Biosystems Foster City, CA). The results of paternity test were shown in Supplementary Tables 1–3.

Conventional DNA sequencing

BRCA1 and *BRCA2* mutation detection was performed on genomic DNA extracted from peripheral blood samples or paraffin embedded tissue. DNA was extracted using QIA-GEN DNA Mini Kit (Qiagen, Hilden, Germany) according to manufacturer instructions. Mutation analysis was performed by direct DNA sequencing of all coding exons of *BRCA1* and *BRCA2* and partial flanking intronic sequences as described previously [10]. PCR conditions and primer sequences are available upon request. Bi-directional

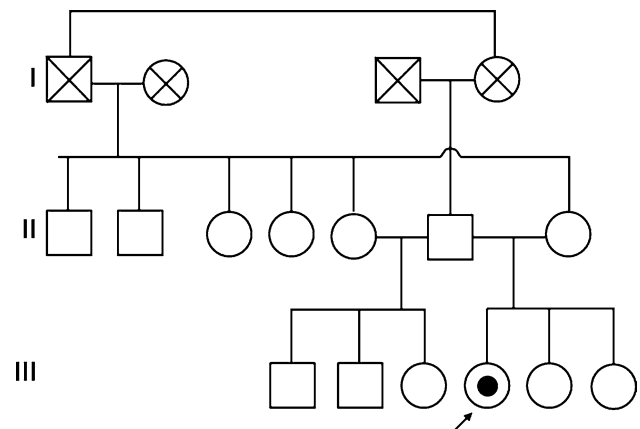


Fig. 1 Family pedigree of proband family. The proband (III-4) is indicated by an *arrow*

sequencing was performed using BigDye Terminator v3.1 Cycle Sequencing Kit and analyzed on an ABI 3130xl genetic analyzer (Applied Biosystems). Sequencing results were compared with the reference DNA sequences (NM_007294.3) using Variant Reporter software (Applied Biosystems) and then reviewed manually. Computational analysis for potential cryptic splice site mutation was performed using splice site prediction programs such as NNSPLICE and ESEF finder when sequence changes were identified. All mutation and sequence variants are named according to the recommendations for the description of sequence variants of Human Genome Variation Society (HGVS).

Multiplex ligation-dependent probe amplification

Multiplex Ligation-dependent Probe Amplification (MLPA) was performed to determine the presence of large genomic rearrangements in the DNA of the proband. The MLPA kit P002 and P045 (MRC-Holland, Amsterdam, Netherlands) was used for *BRCA1* and *BRCA2* gene copy number determination respectively [10–14]. DNA samples were processed according to manufacturer protocol. Each MLPA analysis was carried out with 5 normal control samples. Amplification products were electrophoresed on an ABI 3130xl genetic analyzer and interpreted using GeneMapper software (Applied Biosystems). Results were exported to the Coffalyser program (MRC-Holland) for further analysis.

RNA extraction and quantitative RT-PCR

Total RNA was extracted from blood samples of patients and controls. 0.5 µg of total RNA was reversely transcribed into cDNA with High Capacity cDNA Reverse Transcription kit (Applied Biosystems). Real-time qPCR was

performed using QuantiTect SYBR Green PCR Kit (Qiagen) in ABI PRISM 7900 HT system (Applied Biosystems). All primer sequences are listed in Supplementary Table 4. The expression levels were normalized to house-keeping genes, Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*). ΔC_t was calculated by subtracting the C_t values of *GAPDH* genes from the C_t values of *BRCA1*. $\Delta\Delta C_t$ was then calculated by subtracting ΔC_t of the proband from ΔC_t of non-proband. Fold change of gene was calculated by the equation $2^{-\Delta\Delta C_t}$.

Statistical analysis

Statistical significance was determined by Mann–Whitney test. All *P*-values are two-sided and <0.05 was considered statistically significant by GraphPad PRISM 4 software (GraphPad Software, La Jolla, CA).

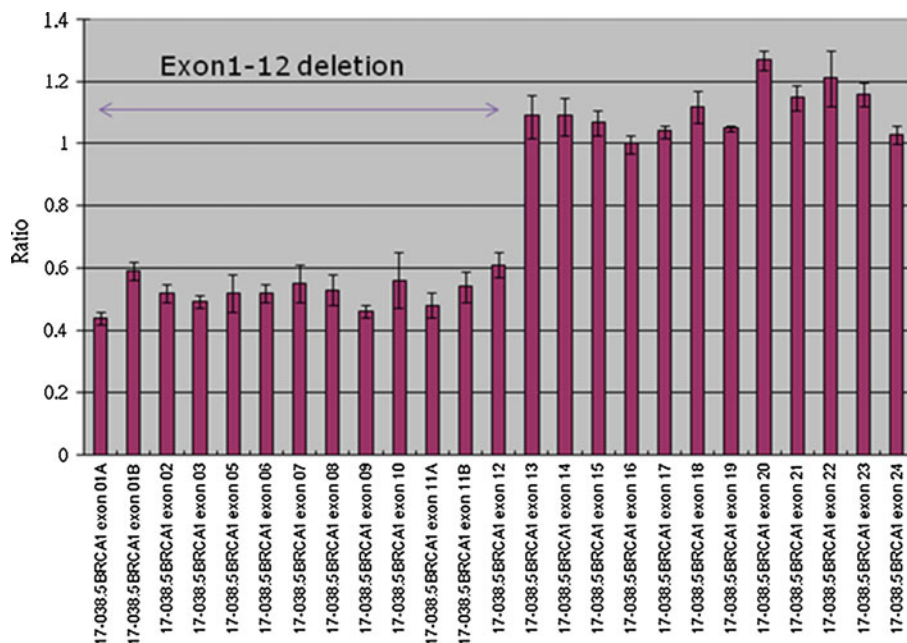
Results

Based on the lower incidence of breast cancer in Asia cohorts [10, 15], we offer *BRCA1/2* genetic testing for clinically high risk female patients; high risk being defined as having (1) at least one first- or second-degree relative with breast and/or ovarian cancer, regardless of age; (2) a diagnosis made when less than 51 years of age; (3) bilateral breast cancer; (4) triple negative or medullary type pathology; (5) at least one relative with cancers other than breast and ovarian cancer that are known to be related to *BRCA* mutations; or (6) ovarian cancer with a family history of breast cancer. Between March 2007 to November

2010, a total of 530 Chinese female patients with breast and/or ovarian cancer were screened by *BRCA1/2* genetic testing. Genomic DNA extracted from peripheral blood samples were sent to Hong Kong Hereditary Breast Cancer Family Registry. *BRCA1* and *BRCA2* mutation screening was performed by direct sequencing of all exons in *BRCA1* and *BRCA2* and partial flanking intronic sequences [10, 16, 17]. Our sequencing results identified 68 (12.8%) deleterious *BRCA* gene mutations. A total of 29 (29/530, 5.5%) were in *BRCA1* and 39 (39/530, 7.4%) in *BRCA2*. During this period of *BRCA1/2* genetic testing, this 33 year old Chinese patient was also eligible for such genetic testing. Based on our sequencing results, nucleotide changes in both *BRCA1* and *BRCA2* from the proband were listed in Supplementary Table 5. In addition to DNA sequencing, MLPA was used to determine if large genomic rearrangements existed for the proband. As a result, this is the only case so far we identified a large deletion of exon 1 to exon 12 of *BRCA1* by MLPA (Fig. 2). Analysis of *BRCA2* did not show any signs of large deletions or duplications. MLPA was also conducted on 5 family members of the proband: mother, father, biological sister, maternal aunt and half sister (daughter of proband's maternal aunt and the same biological father) (Fig. 1). No signs of this or other large deletions in either *BRCA1* or *BRCA2* in any of the family members were detected, suggesting that there are no carriers of the reported large deletion in the family. Therefore the reported large deletion appears to be a de novo mutation.

To investigate whether the defective *BRCA1* allele in the proband could produce protein product, we first attempted to perform RT-PCR to detect whether the presence of

Fig. 2 MLPA probes signals and result of the analysis. Coffalyser Sample Plate Generator (S.P.G.) was used to automatically create sample plate files for MLPA analysis. Results shown that probe signals from exon 1 to 12 were significantly decreased indicating the presence of deletion across those exons



truncated *BRCA1* RNA products for protein truncation testing, but our attempts were not successful. Therefore, we postulate such truncated RNA product is not expressed in the proband. To prove our postulation that such truncated *BRCA1* RNA product do not exist, one of the indirect ways is to compare *BRCA1* RNA levels between proband and non-proband. If the amount of *BRCA1* RNA levels expressed from both wild-type and defective alleles in the proband are significantly lower than control, it would be reasonable to conclude the absence of the truncated RNA products from the defective allele. Thus, we need to design four sets of qRT-PCR primer pairs in which two sets of primer pairs targeting region within the putative deletion (PCR amplicons cover exon 2–7 and exon 10–11, respectively). Thus, these qPCR assays only amplify *BRCA1* RNA from wild-type allele). The other 2 set of primer pairs target the region outside the putative deletion (PCR amplicon cover exon 17–20 and exon 23–24, respectively). Therefore these qPCR assays amplify *BRCA1* RNA from both wild-type and deleted alleles. As a result, *BRCA1* RNA levels from each amplicon region (within or outside deleted regions) were compared between proband and non-proband (non-*BRCA* mutation carriers) including family members ($n = 5$) and normal controls ($n = 18$). Our results indicated that for RT-PCR targeting the deleted region (either E2-7 or E10-11), *BRCA1* RNA levels in the proband were significantly lower than that in non-probands (All P -values < 0.05 , Mann–Whitney test; Fig. 3a). Similarly, for RT-PCR targeting outside the deleted region (either E17-20 or E23-24), *BRCA1* RNA levels in the proband were also significantly lower than that in non-probands (All P -values < 0.05 , Mann–Whitney test; Fig. 3b). Combining all this data, it demonstrates that overall *BRCA1* RNA expression levels in the proband (defective allele carrier) was significantly lower than that in controls, suggesting the existence of truncated *BRCA1* RNA products is unlikely.

Discussion

In this study, we report a novel de novo *BRCA1* mutation encompassing deletion from exons 1 to 12 in a Chinese breast cancer patient of early onset with no family history. Such de novo mutation is extremely rare and has not been reported so far. As the large deletion was identified only by MLPA, it underlines the importance of pursuing gene rearrangement testing if full gene sequencing fails to detect mutations or deletions in *BRCA1/2* genes. Furthermore, significant reduction in *BRCA1* RNA expression levels in the proband is confirmed by RT-PCR assays targeting both PCR products within and outside deleted region, suggesting no truncated *BRCA1* RNA transcript was expressed. Thus, our unsuccessful attempts to amplify the suspected

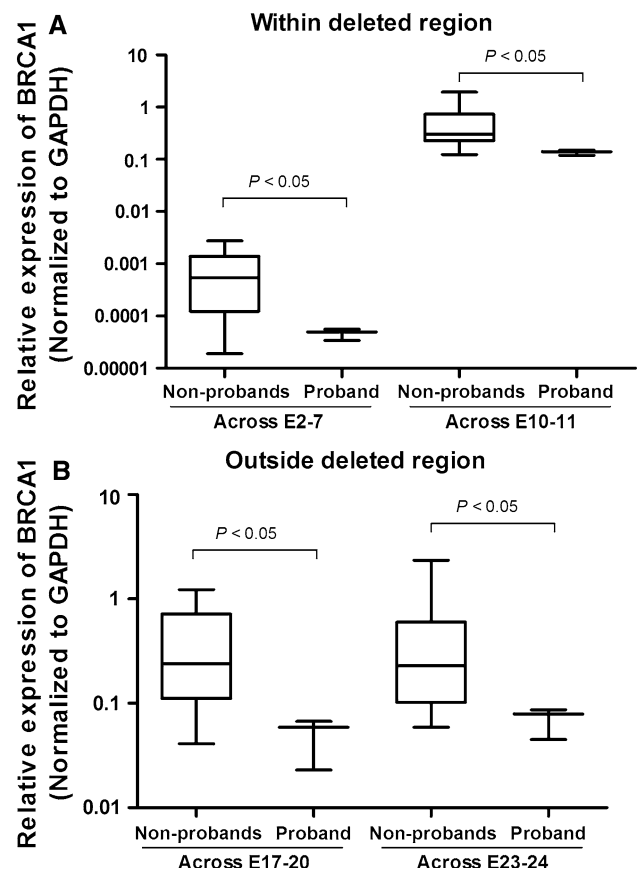


Fig. 3 *BRCA1* RNA level comparison between proband and non-probands (family members and normal controls). Box plots of *BRCA1* RNA expression levels **a** targeting the deleted region **b** targeting outside the deleted region of the proband and non-proband ($n = 23$). Expression levels (Log10 scale at Y-axis) are normalized to *GAPDH*. The lines inside the boxes denote the medians. The boxes mark the interval between the 25th and 75th percentiles. The whiskers denote the interval between the 10th and 90th percentiles. Statistically significant differences were determined using Mann–Whitney tests

truncated product for protein truncation test were expected. Such deletion results in suppression of *BRCA1* protein production in the defective allele. Unfortunately, the breakpoint location of the deletion is still being mapped. We postulate that the deletion breakpoint is located more than 10 kb upstream from the *BRCA1* transcriptional start site.

Although most of the identified deleterious mutations in *BRCA1/2* are truncating mutations resulting in a non-functional protein, large gene rearrangements related to deletion may not be detected by non-quantitative techniques [18]. The emerging technologies such as the use of high density probes combined with array comparative genomic hybridization (aGCH) has identified novel large genomic deletions [19]. Mutation screening is important in early onset breast cancer patients even if there is no family history.

Many factors might lead to the observed mutation in the studied family. This could be due to sporadic germline

BRCA1 mutations, or the mutation could have been inherited from an asymptomatic mosaic parent. Consanguineous marriage in this family could be another factor. The rate of consanguineous marriages in Asian countries such as China, Singapore and Hong Kong was below 10% [20]. This was lower than Iran in which the rate is around 40% and the first cousin marriages are the most common type can affect the pattern of genetic disorders in the population [21]. Another report estimated that 20% of cases of breast cancer diagnosed in women in Pakistan aged below 40 years are attributable to first-cousin marriages [22].

In conclusion, we report a novel de novo mutation in the *BRCA1* gene, resulting in breast cancer in a very young Chinese woman, consisting of a deletion from exon 1 to 12. Such de novo mutation is extremely rare and to the best of our knowledge this is the first case reported in Asian population so far. This patient presents significantly low expression levels of *BRCA1* RNA, indicating the existence of truncated *BRCA1* RNA products is unlikely.

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