Letter to the Editor

A germline mosaic BRCA1 exon deletion in a woman with bilateral basal-like breast cancer

To the Editor:

Germline mutations in the BRCA1 and BRCA2 genes account for around half of all hereditary breast cancer cases and the majority of breast–ovarian cancer families. Thousands of BRCA1/BRCA2 mutations have been identified throughout the world since the genes were first discovered in the mid-1990s, but de novo BRCA1/BRCA2 mutations are extremely rare and to date only nine such cases have been reported in the literature, four in BRCA1 (Table 1) and five in BRCA2. Here, we present a case of a de novo BRCA1 mosaic deletion, the first such case to our knowledge.

The proband was diagnosed at 39 years with two synchronous right-sided, grade 3, ER− PR−, HER2−, and CK5+ breast cancers measuring 19 and 21 mm in diameter. Both lesions were invasive ductal type, each with a slightly different morphological pattern and two of 18 lymph nodes contained metastases. Eight years later, she was diagnosed with a 18 mm left-sided, grade 3, ER− PR−, and HER2− invasive ductal breast cancer with lympho-vascular invasion and accompanying ductal carcinoma in situ (DCIS) but no affected lymph nodes (0/17). There was weak focal staining for CK5 and absence of staining for CK14. Aside from a paternal cousin who had breast cancer at 30 years there was no family history of other BRCA-related cancers, with multiple unaffected female relatives in the family (Fig. 1a).

BRCA1/BRCA2 mutation analysis of all coding exons by fluorescent sequencing of DNA extracted from peripheral blood lymphocytes was negative, but analysis by multiplex ligation-dependent probe amplification (MLPA) (MRC Holland, P002-C2, Amsterdam, Netherlands) detected a deletion of BRCA1 exon 16 present at low level (Fig. 1b). Further evidence of mosaicism was seen in the fluorescent sequence analysis of the p.Ser1613Gly polymorphism (c.4837A>G) in exon 16 which showed a reduced electropherogram signal (approximately 33%) of the single-nucleotide polymorphism (SNP) in the proband compared to a heterozygous control. MLPA and c.4837 A>G SNP analysis of DNA extracted from a saliva sample showed a similar level of mosaicism. No other pathogenic BRCA1 or BRCA2 mutations were detected in the proband and both parents tested negative for the deletion. The deletion was confirmed by long range PCR (Expand Long Template PCR System, Roche Applied Science, Penzberg, Bavaria, Germany; primers Forward 5′cattggccacattttctacct 3′, Reverse 5′atagaatggttacctcctgt 3′) and determined to be 3726 bp (Fig. 1c), predicted to cause a frameshift and to result in a truncated BRCA1 protein.

There was 95% sequence homology in a 38 nt motif at the sequence breakpoint target regions (Fig. 1d), suggesting that the deletion was the result of a large genomic rearrangement that most likely occurred through a recombination mechanism (Fig. 1e,f). Dosage analysis of BRCA1 in the right breast cancer showed a reduction in the MLPA peak ratio from 0.64 to 0.36 (Fig. 1g,h), suggesting that the loss of the second BRCA1 allele had occurred in the cancer, with the remaining amount of exon 16 being accounted for by the non-deleted copies of the mosaic allele. Interestingly in the left breast cancer there was a dramatic reduction of the MLPA peak ratio to 0.11, suggesting that both copies of BRCA1 had been lost (loss of the wild type allele with further deletion of the mosaic allele). However, these results need to be interpreted with a degree of caution, given the less uniform MLPA profiles seen in formalin-fixed, paraffin-embedded (FFPE) tissue compared to blood or saliva.

The frequency of de novo BRCA1/BRCA2 mutations may be under-reported as a result of standard clinical referral protocols based on family history. Clinical testing of singletons for BRCA1/BRCA2 mutations is currently only offered in cases with a very suggestive phenotype, although this may change as testing becomes more widely available and the test results directly influence the oncological management of breast cancer.

A recent study by Zhang et al. found no confirmed de novo BRCA1/BRCA2 mutations in 27 families selected, where the proband had a unique (non-founder) mutation or variant of unknown clinical significance and the parents were unaffected. Together with the small number of reported cases (Table 1), this suggests that BRCA1 de novo mutations are a rare event. Interestingly, three of the five published de novo BRCA1 mutations are large deletions which is a considerably higher proportion than that seen when BRCA1/BRCA2 testing is undertaken in breast/ovarian cancer families.

In summary, we report the first case of a germline mosaic BRCA1 mutation in a woman with bilateral breast cancer and provide an insight into the causal mechanism of such mutations. The very strong personal history of breast cancer in this case indicates
Fig. 1. (a) Pedigree of the proband. (CaBr = breast cancer). (b) Detection of a \textit{BRCA1} mosaic deletion in the proband. Analysis by MLPA detected a low level deletion of exon 16 in \textit{BRCA1} in the patient (top and middle left panel) compared to a normal control (bottom panel). Reduced signal for the p.Ser1613Gly SNP confirmed mosaicism in lymphocytes (top right panel) and saliva (middle right panel) compared to a sample heterozygote for the SNP (bottom right panel). (c) Characterization of the breakpoints of the \textit{BRCA1} deletion. Long-range PCR amplified a 1638 bp band in the patient (lines 1–2) compared to a 5365 bp band in a normal control (lines 3–4). (d) Sequence of the deleted PCR band and alignment with the \textit{BRCA1} reference sequence. This showed the presence of a 95% homologous 38 nucleotides motif on both sides of the breakpoint (boxed sequence where highlighted nucleotides differ). The upstream and downstream sequence of the breakpoint is highlighted in pink and green, respectively, on the electropherogram. (e) Likely mechanism of deletion and (f) alignment of the upstream and downstream homologous motifs. Note that the deleted fragment sequence is aligned with the reference sequence corresponding to the region upstream from the breakpoint. The experiment was repeated four times and the average results with range are summarized in 1 h.)
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Table 1. Summary of published de novo BRCA1 mutations

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<tr>
<th>BRCA1 mutation</th>
<th>Phenotype</th>
<th>References</th>
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<tr>
<td>c.3769delG</td>
<td>Breast cancer aged &lt;40 years. Also paternally inherited BRCA2</td>
<td>(1)</td>
</tr>
<tr>
<td>c.5332+1G&gt;A</td>
<td>Bilateral breast cancer at age 38 years (grade II, ER+) and 43 years</td>
<td>(2)</td>
</tr>
<tr>
<td>del exons 1–12</td>
<td>Breast cancer aged 30 years (grade II, ER−, PR−, Her2−)</td>
<td>(3)</td>
</tr>
<tr>
<td>del whole gene</td>
<td>Bilateral breast cancer aged 28 years (grade I, ER+, PR+, and Her2+) and 37</td>
<td>(4)</td>
</tr>
<tr>
<td>g.146232_149957del</td>
<td>Bilateral breast cancer at 39 (grade III, ER−, PR, and Her2−) and 47</td>
<td>This study</td>
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It is likely that mosaic mutations in BRCA1/BRCA2 will be more often seen in the future as clinical laboratories switch to next generation sequencing platforms and testing becomes more widely available in breast or ovarian cases with no family history.

References


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