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# Identification of Two Novel Deletions with Breakpoints Located in Exon 11 in the BRCA2 Gene

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## I. Introduction

Pathogenic variants in the BRCA1 and 2 genes have been associated with increased risk for female and male breast cancer, ovarian cancer, and other cancers, such as prostate cancer, pancreatic cancer, and melanoma. The BRCA1 and BRCA2 genes are tumor suppressor genes involved in the repair of damaged DNA. The vast majority of pathogenic variants in the BRCA1 and BRCA2 genes are point changes that lead to the introduction of premature termination codons or change of an amino acid, small deletions and insertions leading to frameshifts with downstream premature termination codons, and splice site changes. Large deletions and duplications of these genes have also been identified and account for ~10% or less of identified pathogenic variants. Large deletions or duplications have been reported more often in the BRCA1 gene compared to the BRCA2 gene.

Comprehensive gene sequencing and deletion/duplication analysis is widely available for individuals with a personal or family history of cancers that are suggestive of BRCA1/2-Hereditary Breast and Ovarian Cancer Syndrome. These analyses are adding to the spectrum of described pathogenic and likely pathogenic variants. In this poster, we describe two novel deletions in the BRCA2 gene that have a deletion breakpoint within exon 11 of the BRCA2 gene.

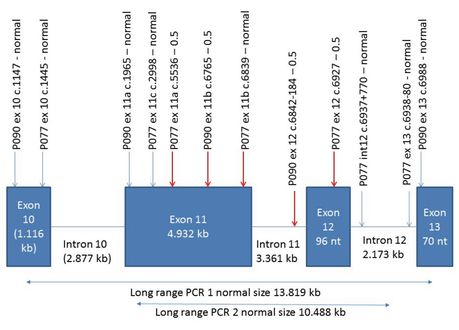
## II. Methodology

Deletion/duplication analysis was performed via Multiplex Ligation Probe Amplification (MLPA) using the P090 Version A4 and P077 Version A3 kits from MRC-Holland. The locations of the probes in the figure represent the left-hand base of the ligation site. Long-range PCR primers were designed specifically for each case, and long-range PCR was performed using the Takara LA Taq HotStart system (Takara). Long range products were visualized through gel electrophoresis and Sanger sequenced.

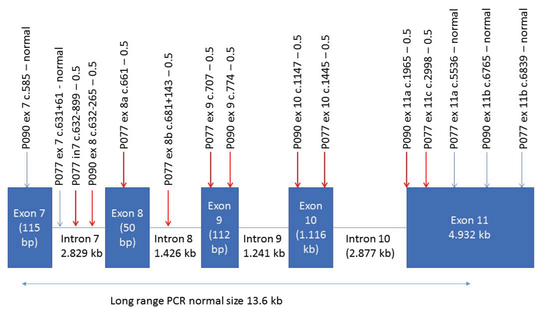
### Figure 1. Diagram of BRCA1 deletions

Red arrows indicate the position of MLPA probes showing decreased signal. Blue arrows indicate the position of the MLPA probes with normal signal. The probe locations are based on the 5' position of the probe ligation site.

### Figure 1A. Patient 1




### Figure 1B. Patient 2




## III. Results

### Patient 1:

MLPA analysis showed a drop of signal in probes located in exons 11 and 12 in both the P090 and P077 panels consistent with a heterozygous deletion (**Figure 1A**). However, two of the exon 11 probes showed normal signal indicating that one of the deletion breakpoints was located within exon 11 and was located between c.2998 and c.5536 and the second breakpoint was located near exon 12 in the flanking intronic sequence. Long range PCR showed a band ~ 7kb smaller than expected. Sequence analysis showed that the deletion spanned from c.3450 in exon 11 (hg19: 32911942) to c.6937+132 (hg19: 329118922) in intron 12 for a total length of 6981 nt.

### Patient 2:

MLPA analysis showed a drop of signal in probes located in intron 7 and exons 8, 9, 10, and 11 in both the P090 and P077 panels consistent with a heterozygous deletion (**Figure 1B**). However, three of the exon 11 probes showed normal signal indicating that one of the deletion breakpoints was located within exon 11 and was located between c.2998 and c.5536 and the second breakpoint was located intron 7. Long range PCR showed a band of approximate 1 kb in size compared to the normal expected size of 13.6 kb. Sequence analysis showed that the deletion spanned c.631+194 in intron 7 (hg19: 32900943) to c.4985 in exon 11 (hg19: 32913477) for a total length of 12534 nt.

The regions around the deletion breakpoints for both cases have microhomology around the breakpoints suggesting that a DNA repair mechanism utilizing microhomology may have been involved in the genesis of these deletions

### Figure 2. Microhomology at the deletion breakpoints

Patient 1.

```
Exon 11      ACCAGATGACTATCTTAA
Intron 12    AATCAAGGACCTCTTTAT
Fusion       ACCAGATGACCTCTTTAT
```

Patient 2.

```
Intron 7     TGGTAGTCCAGTGGTGTCA
Exon 11      CCCTTATTCAGTCATTGAA
Fusion       GTGGTAGTCAGTCATTGAA
```

## IV. Conclusions

While BRCA2 deletions are less common than BRCA1 changes, we identified two unique deletions in BRCA2 with breakpoints occurring within exon 11, adding to the spectrum of deletions identified in BRCA2.

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