Abstract

Approximately 50% of individuals thought to have hereditary breast cancer are found to have a pathogenic variant in either the BRCA1 or BRCA2 genes. Of those with a pathogenic variant, between 5-10% involve large deletion or duplication rearrangements. Traditional analysis for these rare events was performed by multiplex ligation-dependent probe amplification, quantitative PCR or comparative genomic hybridization. We report a case of a 38-year-old Christian Arab woman diagnosed with invasive ductal carcinoma at 30 years of age. She underwent bilateral mastectomy at age 36. The family history was suggestive of a hereditary cancer syndrome due to early onset breast and ovarian cancer on the maternal side. Next-generation sequencing (NGS) was performed on 29 genes associated with hereditary cancer syndromes. A duplication involving exons 5-11 of BRCA2 was identified using CNVtai, a new software method of detecting copy number variants (CNV) from NGS read count data. To understand the impact of this duplication on the BRCA2 protein sequence we analyzed sequence reads from this region for split-mappings and were able to confirm that this duplication occurred in tandem within the gene. To determine the precise impact on the protein reading frame, we used PCR amplification on the 5' breakpoint occurring in exon 11 and Sanger sequence analysis across this junction identified a 13 bp insertion at the breakpoint, followed by sequence correlating to the middle of intron 4, that then continued into the middle of exon 11. The duplication is predicted to cause a Met to Arg change at codon 1594 followed by a frame-shift that ends with a premature truncation at codon 1597. The truncated protein is expected to result in a loss-of-function; a well-documented mechanism for BRCA2 inherited breast cancer susceptibility. In addition to being a novel duplication, this is the first clinically reported duplication in BRCA2 using these new methods of detecting CNVs.

Case Study

Patient history: A Christian Arab woman was diagnosed with invasive ductal carcinoma at age 30 years. At 38 years old she underwent screening for the 3 Ashkenazi Jewish founder mutations in BRCA1 & BRCA2 and was negative. Due to cost and turn around time the patient proceeded to be tested for a 29 gene hereditary cancer panel using next-generation sequencing.

Family history:

![Family tree diagram]

Next-generation sequencing reveals a novel duplication in BRCA2

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Results:

Intron 4

Exon 11

Mystery Insertion

Case Study Continued

Methods

Detection of split-reads signal: RQ117 - Split reads (soft-clipped bases shown) and discordant mate pairs (in green) identify ends of the tandem duplication.

Methods Continued

Confirmation of breakpoints

Sequence alignment of PCR product showing duplication breakpoint in intron 4.

Impact of duplication on BRCA2 protein

Conclusions

This is a novel duplication that results in a truncated loss of functional protein; a well-documented mechanism for BRCA2 inherited breast cancer susceptibility. Thus far we have reported 12 additional examples of clinical cases where CNVs were detected using our methods of NGS and CNVtai software. This demonstrates the ability of NGS to not only detect single nucleotide sequence changes but also CNVs in a cost-effective scalable single assay for clinical genetic testing.

References
