Letter to the Editor

cDNA analysis of the BRCA1 unclassified variant c.5194-12G>A

To the Editor:

Germline mutations in BRCA1 and BRCA2 account for a significant proportion of familial breast cancer. Sequencing of BRCA1 and BRCA2 is routinely performed on patients with strong family histories of disease. However, only nonsense, frameshift, splice site and some missense mutations are reported as pathogenic, whereas a significant proportion of variants are of unknown clinical significance, or unclassified variants (UVs). The clinical utility is thus limited by the lack of knowledge about the pathogenicity of these UVs. It has become evident that intronic variants play a role in the regulation of pre-mRNA splicing and can be deleterious (1).

Fig. 1. (a) Pedigree of the breast cancer family with two sisters positive for the BRCA1 c.5194-12G>A variant. Transcript analysis of cDNA fragment, (b) sequencing electropherogram showing the 10 bp insertion, (c) predicted splice effect on BRCA1 expression.
The BRCA1 c.5194-12G>A variant (numbering according to the HGVS nomenclature) is predicted to be an intronic acceptor splice site replacing the normal site (2). Splicing prediction programmes showed that this variant creates a cryptic splice site resulting in a BRCA1 transcript that includes 10 nucleotides from the 3’ end of intron 19 culminating in a premature stop codon (3). The pathological significance of this variant was suggested to be deleterious by in silico analysis. In silico analysis is not completely reliable in predicting RNA splicing (4), and is unable to unequivocally establish pathogenicity. This variant has been reported five times in the BIC database.

This study aimed to ascertain the pathogenicity of the BRCA1 c.5194-12G>A variant detected in two sisters, one of whom has been diagnosed with bilateral breast cancer at 47 and 57 years of age and the other sister diagnosed at 42 years of age. In total there were five early onset breast cancers and two melanomas in the family.

Sequence analysis was performed on reverse-transcribed cDNA derived from the patients’ pure mycin-treated RNA, using primers flanking the region of interest (Invitrogen™ New York, NY). The forward primer (5′-AA CACCACATCACTTTAACTAATC-3′) is located in exon 17 and the reverse primer (5′-ACAGAAGCACC ACACAGCTGT-3′) in exon 22.

cDNA sequence of both patients showed the inclusion of 10 bp intronic sequence due to the creation of a new splice site (Fig. 1) generating a downstream frameshift mutation (p.His1732PhefsX5). This mutation affects the BRCT domain, a highly functional domain associated with the stability and nuclear localization of BRCA1 to DNA-damaged sites (5).

In a substantial proportion of breast cancer families, there are intronic variants in BRCA1 and BRCA2 with unknown pathogenicity. A frequent consequence of intronic variants is aberrant splicing leading to insertions or deletions in the transcript, causing mRNA nonsense-mediated decay or the generation of premature stop codons (6). This study assessed the pathogenicity of BRCA1 c.5194-12G>A intronic UV at the mRNA level and confirms splice-site prediction data (2, 3).

The detection rate of UVs has increased with routine sequencing of BRCA1 and BRCA2, and will be intensified with massively parallel sequencing. Bioinformatics algorithms can be used for initial analyses of intronic UVs and selecting those of interest for analyses at the RNA level (7) but definitive information on the effect of intronic UVs can only be obtained from RNA studies. Our findings and others (8) highlight the importance of studying mutations at DNA and RNA to elucidate the effect of the mutations on mRNA processing, which could be different from that predicted at the genomic level and to provide accurate interpretations of the biological consequences of intronic UVs. This should enable more informed genetic counselling for breast cancer patients and their families.

Acknowledgement

This study was funded from the University of Newcastle’s Research Training Scheme.

References


Correspondence:
Prof. Rodney J. Scott
Director of Centre for Information-Based Medicine
Hunter Medical Research Institute (HMRI)
University of Newcastle
Lot 1 Kookaburra Circuit, New Lambton Heights
NSW 2305
Australia
Tel.: +612 4921 4974
Fax: +612 4921 4523
e-mail: Rodney.Scott@newcastle.edu.au