

LETTER TO THE EDITOR

Mutation in the Coding Region of the *BRCA1* Gene Leads to Aberrant Splicing of the Transcript

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To the Editor:

Mutations in the *BRCA1* gene (MIM# 113705) confer an increased risk of developing breast and/or ovarian cancers [Miki et al., 1994; Ford and Easton, 1995]. To date, the majority of mutations identified in breast or breast/ovarian cancer families have been shown to be due to frameshift and nonsense mutations that lead to the truncation of *BRCA1* protein [Breast Cancer Information Core, 1999]. Missense mutations have also been identified; however, the evaluation of the effects of these changes on the *BRCA1* protein has been challenging since the exact function of the *BRCA1* gene is not known.

As a part of a population-based familial breast cancer registry, we have been studying the germline *BRCA1* mutations in families with family history of breast and breast/ovarian cancer in the province of Ontario, using the protein truncation test (PTT) and DNA sequencing approach described previously [Ozcelik et al., 1996, 1997; recently reviewed in Den Dunnen and Van Ommen, 1999]. In the peripheral blood specimen of a patient with family history of cancer, we identified a truncated protein product in a PCR fragment amplified from *BRCA1* cDNA covering exons 12–16 (Fig. 1a). This patient has been diagnosed with ovarian cancer at the age of 57 with a first-degree relative with ovarian and bilateral breast cancer. The sequencing analysis of the region in which the truncated protein has been identified revealed a substitution of a G nucleotide with a T at position 4603 in one of the two copies of the *BRCA1* gene (Genbank accession number, U14680) (Fig. 1b). This 4603

G→T mutation, which results in amino acid change at codon 1495 (A1495M), occurs at the last base of exon 14 of *BRCA1*. This residue coincides with the –1 position of the exon 14 donor splice site.

Since we have observed a truncated product in the analysis of this region by PTT, we further characterized this alteration and investigated the effect of this base substitution on the splicing of *BRCA1* mRNA. PCR analysis using a forward primer (5'-TCTGCCCTTGAGGACCTGCG-3', position 4429–4447) and a reverse primer (5'-CAAATCGTGTGGCCCAGACT-3', position 4741–4760) complementary to exon 13 and 15, respectively, was performed to amplify the cDNA sample from this patient using other patient samples as controls. PCR analysis showed the expected size PCR products in all samples; however, an additional shorter PCR product was detected in the patient with the 4603 G→T mutation (Fig. 1c). The shorter PCR product along with the expected size fragments were cut out and purified from an agarose gel for DNA sequence analysis. Normal *BRCA1* nucleotide sequence was observed in the analysis of the expected size PCR fragments;

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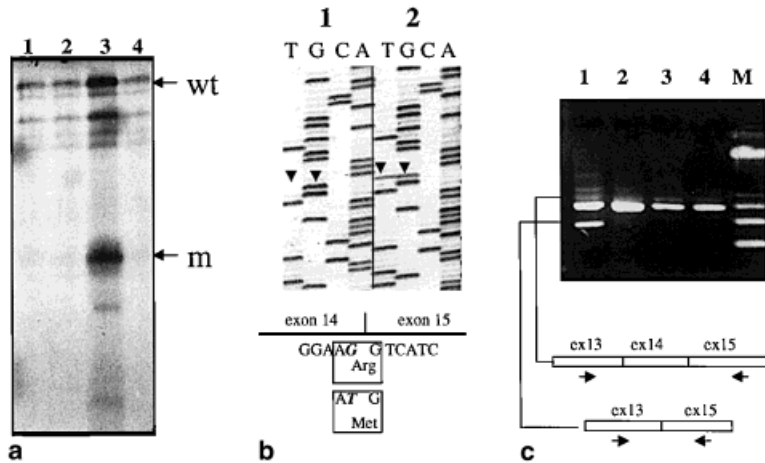


FIGURE 1. Analysis of *BRCA1* 4603 G→T mutation. **a:** PTT analysis and detection of a truncated protein product in the *BRCA1* cDNA region covering exons 12–16. **b:** Sequencing analysis of exon 14 of the *BRCA1* gene of the patient with truncated protein, revealing a substitution of a G nucleotide with a T at the last base of exon 14. **c:** PCR analysis of the cDNA from the patient with the 4603 G→T mutation results in an aberrantly spliced product (lane 1), lacking the entire exon 14.

however, the sequence analysis of the shorter PCR product indicated that the entire exon 14 was deleted (Fig. 1c). The observation of both normal and aberrantly spliced product in peripheral blood derived mRNA of this patient is consistent with the presence of both wild-type and 4603 G→T alleles in the patient's genomic DNA (Fig. 1b). Analysis of the protein reading frame demonstrated that skipping of exon 14 associated with the 4603 G→T mutation would result in a frameshift leading to a stop codon at amino acid 1462 of the BRCA1 protein. This observation is consistent with the detection of the truncated BRCA1 product in our primary analysis of this sample by PTT analysis.

This is an example of a single-base substitution effect, which can be misclassified since the substitution is predicted to cause an amino acid change from arginine to methionine. In fact, this mutation had previously been described as an unclassified variant in the Breast Cancer Information Core [1999]. DNA-based techniques would not be sufficient to identify these types of mutations and complementary methods should also be considered for characterization of these alterations. The incomplete understanding of the association of *BRCA1* and *BRCA2* mutations with the disease may lead to inefficient and improper genetic counseling, as well as inappropriate clinical management of the patient. In this study, we have shown

that the 4603 G→T mutation in the coding region of *BRCA1* leads to a truncated protein. Additionally, we have shown that PTT analysis is useful as an effective and complementary approach to the detection of mutations with potential deleterious effects on the BRCA1 protein.

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