Germline mutations in the tumor suppressor gene BRCA1 predispose to breast and ovarian cancer [1,2]. Most germline BRCA1 mutations are small or large insertions and deletions, or single base pair substitutions. Single point substitutions may result in nonsense or missense mutations, polymorphisms, or splice variants.

Mutations occurring in intron-exon boundaries of BRCA1 have been described in some studies, either in the exonic [3,4] or in the intronic consensus sequence [5–8]. However, only a few of them have been documented to cause abnormal RNA splicing, whereas some of them are classified as benign polymorphisms [9].

During the screening of our genomic DNA bank from breast and/or ovarian cancer patients, we identified a mutation in the last base of exon 23, (5586G→A, accession number U14680) (Fig. 1) in a patient who developed breast and ovarian cancer at ages 40 and 50 years, respectively. This mutation has been reported only once in the Breast Cancer Information Core (BIC) [10] by Myriad Genetics and has been postulated to result in an amino acid change (A1823T) without affecting RNA splicing.

To investigate whether this mutation causes abnormal splicing of RNA, we amplified the region from exon 20 to 24 in this patient and in a control cDNA by using reverse-transcribed RNA from peripheral lymphocytes. The expected length of the normal cDNA fragment is 268 bp. Amplification of the control cDNA showed a single band of 268 bp, whereas a second product of 208 bp was observed as well when using cDNA from the patient (Fig. 2). Direct sequencing of both bands revealed that the longer one corresponds to the normal sequence of BRCA1 cDNA (268 bp), whereas the lower one corresponds to an alternatively spliced form (208 bp) where exon 23 is deleted (Fig. 3). The skipping of exon 23 causes an open reading frame change for exon 24 resulting in a premature stop codon at position 1813. Thus, the length of the predicted protein sequence will be 1,812 amino acids. This protein will be missing amino acids corresponding to exons 23 and 24 replaced with 10 amino acids of a different open reading frame.

This splicing pattern is exactly the same as the one reported by Laskie Ostrow et al. to be caused by mutation IVS23+1G→A [8]. The authors report that the premature stop is at codon 1833. We suppose that this corresponds to the BRCA1 full-length cDNA numbering. According to the genomic sequence (accession number L78833) 5586G→A corresponds to nucleotide 81094 (−1 position of the exon 23 donor splice site) and IVS23+1G→A, which is the next base in the genomic sequence, to nucleotide 81095 (+1 position of the intron 23 5′ splice site).

Greater than 90% of mutations causing aberrant splicing are found in the consensus splice site sequence, with mutations at the 5′ splice site being more frequent than those at the 3′ splice site [11]. Mutations at positions −1 and −2 in the donor splice site consensus sequence, as is the case of 5586G→A, are much less frequent into the genome than mutations in the intronic invariant splice site consensus sequence (positions +1 and +2) as is the case of IVS23+1G→A [12].

In conclusion, reverse transcriptase polymerase chain reaction-based (RT-PCR) analysis of point mutations in the splice site consensus appears necessary to avoid misclassification of such cases leading to inappropriate genetic counseling and patient management.

Genomic DNA was prepared from peripheral blood lymphocytes. Procedures for mutation detection analysis in BRCA1 coding sequence and intron-exon boundaries have been described [13]. RNA also was isolated from peripheral blood lymphocytes, and reverse transcription was performed according to standard protocols. Primers used were GAAGAAACCAC CAAGGTCCA (exon 20, sense) and CACAGGTGCCTCA CACCATCT (exon 24, antisense). The same primers were used for direct DNA sequencing in both directions. We gel-purified RT-PCR products and sequenced directly. Mutation 5586G→A was identified later on in another unrelated breast/ovarian cancer patient, but cDNA analysis was not possible because a fresh sample was unavailable.

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Fig. 1. Genomic DNA sequence analysis of exon 23/intron boundaries. Mutation 5586G→A is located in the last base of exon 23 (IVS23+G→A is the next base in the genomic sequence). Upper panel: control genomic DNA. Lower panel: patient genomic DNA.

Fig. 2. Reverse transcriptase polymerase chain reaction (RT-PCR) analysis of BRCA1 exons 20–24 region. RNA was isolated from peripheral blood lymphocytes of a breast/ovarian cancer patient and an unrelated healthy female. Lane 1, DNA standards. Lane 2, patient cDNA. Lane 3, control cDNA.

Fig. 3. Sequence analysis of RT-PCR of 208 (upper panel) and 268 bp (lower panel).
References


