ELECTRONIC LETTER

The *BRCA2* variant 8204G>A is a splicing mutation and results in an in frame deletion of the gene

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Since the identification of the breast and ovarian cancer susceptibility genes *BRCA1* and *BRCA2*, a large number of different germline mutations in both genes have been found.^{1,2} The *BRCA1/2* germline mutations reported to date comprise a broad spectrum of sequence variants, mainly frameshift and nonsense mutations that result in truncated proteins.³ A minor proportion of the documented sequence variants are the result of mutations in sites relevant for correct splicing of the pre-mRNA.

METHODS AND RESULTS

As a participating centre in the German Consortium for Hereditary Breast and Ovarian Cancer in Germany, we have screened families with a strong history of breast and/or ovarian cancer for mutations in *BRCA1* and *BRCA2* by complete sequencing of both genes.⁴ Recently, we detected a mutation at an exon-intron boundary in the *BRCA2* gene of a young woman (B538/300) who was diagnosed with breast cancer at the age of 32. Histological examination of resected breast tissue showed ductal carcinoma (grade I). No other breast or ovarian cancers have been observed in her family. In particular, sequence analysis of this patient showed a G to A transition at position 8204 of exon 17 of *BRCA2* (fig 1). Interestingly, this 8204G>A variant has been reported four times by Myriad Genetics.³ It has been concluded that the indicated sequence variant resulted in the substitution of the amino acid arginine for lysine at codon 2659 (R2659K) and this change was considered to be an unclassified variant. However, since this substitution (8204G>A) is located in the last base of exon 17 of *BRCA2*, eventually involving the donor splice site of exon 17, analogous to similar findings from, for example, exon 23 of *BRCA1*⁵ and exons 7, 13, 21, 23, and 25 of *BRCA2*,³ we were interested to know whether this mutation may affect correct splicing of the *BRCA2* pre-mRNA. Therefore, we isolated RNA from lymphocytes of patient B538/300 and a control specimen (wild type sequence) by using the PAXgene™ Blood RNA Kit (QIAGEN) following the manufacturer's instructions.

Subsequently, 4 µg mRNA of each sample was reverse tran-

Figure 1 Genomic DNA sequence analysis of exon/intron 17 boundary of the *BRCA2* gene in patient B538/300. Transition 8204G>A is located in the last base of exon 17.

scribed using Omniscript™ Reverse Transcriptase Kit (QIAGEN). The resulting cDNAs served as template for a PCR analysis using 5'-TCCAATGATGAAAGGCTGGAA-3' as forward primer and 5'-CTGTACACCTCTTGAAGCCCCA-3' as reverse primer. The expected size of the PCR product from the control sample cDNA was calculated to consist of 546 bp; skipping of exon 17 owing to incorrect splicing would result in a PCR product of 375 bp. As shown in fig 2, PCR analysis of the control sample resulted in only one product of expected size, whereas two products with sizes of 546 bp and 375 bp were amplified from cDNA derived from RNA of patient B538/300 (fig 2). The respective PCR products (375 bp and 546 bp) were

Figure 2 RT-PCR analysis from exon 16 to exon 18 of the *BRCA2* gene. Lane 1, DNA standard. Lane 2, patient B538/300 cDNA. Lane 3, control cDNA.

Key points

- A minor proportion of the documented *BRCA1/2* germline sequence variants are the result of mutations in sites relevant for correct splicing of the pre-mRNA.
- As a participating centre in the German Consortium for Hereditary Breast and Ovarian Cancer in Germany, we have detected a mutation at an exon-intron boundary in the *BRCA2* gene in a young woman and show that this variant 8204G>A is a splicing mutation, which results in an in frame deletion of the gene.
- These results suggest an important role of exon 17 in encoding a relevant domain for maintaining the proper function of the *BRCA2* protein.

Figure 3 Sequence analysis (antisense strand) of RT-PCR products of 375 bp (patient B538/300, upper panel) and 546 bp (healthy control, lower panel).

purified from the agarose gel by using QIAquick® Gel Extraction Kit (QIAGEN) and sequenced. Sequence analysis of the 375 bp product in both sense and antisense direction using amplification primers indicated that the entire exon 17 was deleted (fig 3). Analysis of the protein reading frame showed that skipping of exon 17 associated with 8204G>A transition does not alter the open reading frame but would result in an in frame deletion of 171 bp causing a deletion of 57 amino acids. This in frame deletion comprises amino acids 2602 to 2659 in the C-terminus of the BRCA2 protein, which is a highly (about 93%) conserved region as shown by comparison between human and mouse sequences and based on the overall identity (59%) of both species.⁶ It is important to note that patient B583/300 did not have any other base substitutions within her *BRCA2* gene. In addition, in order to support the impact of the G>A substitution on proper splicing and to exclude the possibility that, for example, an unlinked splice factor can cause such exon skipping, we sequenced the 546 bp

product from the patient. We found a properly spliced sequence identical to that of the wild type control, which was expected to derive from the unaffected allele of the patient.

DISCUSSION

The *BRCA2* deletion described here is located in a protein region which has been described to be most likely responsible for an in vivo interaction between the *BRCA2* and *DSS1* (deleted in split hand/split foot) proteins in mammalian cells.⁷ It has been suggested that the *DSS1* protein is required for normal cell growth and has an important cellular function in eukaryotes. Both the strong conservation and the interaction with other cellular proteins suggest a important role of exon 17 in encoding a relevant domain for maintaining the proper function of the *BRCA2* protein.

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