Letters to the Editor

Am. J. Hum. Genet. 62:000, 1998

A *BRCA1* Nonsense Mutation Causes Exon Skipping

To the Editor:

Nonsense mutations can induce the skipping of constitutive exons, as has been shown in a few disease-causing genes (Dietz et al. 1993; Gibson et al. 1993; Naylor et al. 1993; Hull et al. 1994; Santisteban et al. 1995; Pié et al. 1997). Shortened translational reading frames are also sometimes associated with a reduction in mRNA abundance (Maquat 1996). However, few of the premature-termination codons involved in genetic diseases have been comprehensively assayed for induced transcript defects.

The *BRCA1* gene, located on chromosome band 17q21, has a coding sequence of 5,592 nucleotides scattered on 22 exons (Miki et al. 1994). Germ-line mutations in this gene lead to the predisposition to breast and ovarian cancer. More than 1,250 different mutations of the *BRCA1* gene have been reported worldwide, spread over the entire length of the gene (Breast Cancer Information Core 1997 [http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/]); most of them lead to premature-termination codons, as a result of frameshift, nonsense, or splice-site mutations (Couch et al. 1996) or large rearrangements (Puget et al. 1997; Swensen et al. 1997). Most mutation-screening studies are conducted on genomic DNA only, and, as yet, little effort has been made to check for possible induced transcript defects. By contrast, evaluation of the phenotypic risk associated with mutations has received a great deal of attention because it is very important in genetic counseling. A correlation between the location of the mutation in the *BRCA1* gene and the ratio of the incidence of breast to ovarian cancer has been shown (Gayther et al. 1995; Shattuck-Eidens et al. 1995). However, such a trend has not been found systematically. The reason may be that taking into account the location of the mutation rather than its consequences at the level of the transcript could be misleading in such studies, because it is impossible to predict which premature stop codon will lead to a reduction in mRNA abundance or to exon skipping.

We describe here, for the first time, nonsense-mediated exon skipping in the *BRCA1* gene, in one breast and ovarian cancer family (number 3497), recruited by Dr. H. Lynch at Creighton University, Omaha, that contains four breast and four ovarian cancer cases (among which are one case of bilateral breast cancer and one case of bilateral breast cancer and ovarian cancer). A fragment of *BRCA1* cDNA covering exons 16–22 was shown to produce, after amplification by PCR and migration on an agarose gel, two bands of equal intensity in affected family members, one of the expected size (596 bp) and one slightly smaller. Simultaneous direct sequencing of both templates demonstrated a 78-bp in-frame deletion in the smaller fragment corresponding to the entire exon 18. Alternative splicing removing exon 18 does not occur naturally (Xu et al. 1997; authors' unpublished data). In order to identify the basis of exon skipping, we amplified, by PCR, a 352-bp fragment from genomic DNA encompassing 78 bp of intron 17, all of exon 18, and 196 bp of intron 19. We observed no mutation in all the *cis*-acting consensus elements known to be involved in RNA splicing (i.e., the 3′ and 5′ splice sites and the predicted branchpoint flanking exon 18: TTCTAAT at positions −31 to −25). However, a G→T substitution was found in exon 18 at nucleotide 5199 (codon 1694), which changes a glutamic acid to a stop codon (Glu1694ter). To find out whether any transcript with exon 18 containing the stop codon was expressed, we specifically amplified from cDNA a fragment with primers in exons 16 and 18 (thereby preventing amplification from the transcript without exon 18), which was then sequenced. Only a wild-type exon 18 sequence was found in all carriers, which implies that the mutant allele produces only mRNA in which exon 18 had been skipped.

Although the Glu1694ter mutation would be expected to lead to the truncation of 169 amino acids from the BRCA1 protein, the skipping of the in-frame exon 18 removes 26 amino acids (Asp1692–Phe1717). Nevertheless, it leads to the disruption of the first BRCT domain, since it removes part of block D, the most highly conserved motif within this domain (Callebaut and Mornon 1997). The block D motif is organized around a conserved aromatic residue. In the case of *BRCA1*, this residue is Trp, which is changed to Gly because of the junction of exon 17 to exon 19. It is therefore unlikely

that the mutant BRCA1 protein would remain totally functional.

In at least two cases of nonsense-mediated exon skipping (Santisteban et al. 1995; Pié et al. 1997), the mutation was found to be located in a purine-rich sequence that could function as an exonic splicing enhancer (ESE), thereby impairing its function. These ESE sequences have been shown to affect the selection of the splice sites (Cooper and Mattox 1997). In the case of Glu1694ter in *BRCA1* exon 18, exon skipping is probably not the result of the inactivation of an ESE, since the G→T substitution does not take place in a purine-rich sequence. It has also been proposed that point mutations in an exon could lead to disruption of secondary structure, with consequent aberrations in RNA splicing. We cannot exclude a structural effect of Glu1694ter on the splicing of *BRCA1* exon 18, the mutation occurring only six nucleotides downstream of the intron 17 splice acceptor. By contrast, experimental data suggest that maintenance of an open reading frame can serve as an additional level of scrutiny during exon definition; nonsense codons could thus directly alter splice-site selection (Dietz and Kendzior 1994). Additional work is needed to better identify the relevant mechanism and machinery that evaluate the coding potential of nuclear pre-mRNAs.

Glu1694ter has already been reported four times (Breast Cancer Information Core 1997 [http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/]), but on genomic DNA, so the skipping of exon 18 resulting from this mutation has probably been missed. Clearly, it would be interesting to investigate the consequence of each *BRCA1* mutation, found using genomic DNA, at the cDNA level. In addition to the fundamental aspect of finding the mutation, these findings could have implications to draw a genotype-phenotype correlation.

Acknowledgments

The authors would like to thank the family members. We also thank C. Bonnardel, L. Boutrand, T. Conway, J. Lynch, S. Slominski, and P. Watson, for their expert assistance. This work was supported by program grants from le Comité Départemental de l'Ain de La Ligue contre le Cancer, the Council for Tobacco Research (grant 127DR@), the U.S. Department of the Army (grant DAMD17-94-J-4340), and the Nebraska State Cancer and Smoking-Related Diseases Grant Program. N.P. is a fellow of the Ligue contre le Cancer de Haute-Savoie.

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0002-9297/98/6203-0027$00.00