Letters to the Editor

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An *Alu*-Mediated 6-kb Duplication in the *BRCA1* Gene: A New Founder Mutation?

To the Editor:

Most mutations in the breast/ovarian cancer–predisposing gene *BRCA1* that have been identified to date are point mutations or small insertions and deletions scattered over the whole coding sequence (5,592 nucleotides long) and over the splice junctions (Breast Cancer Information Core). Although ~65% are unique, because of founder effects several mutations have been found in more than one family, both within specific populations and in more-diverse geographic groups (Neuhausen et al. 1996). The other germ-line mutations published so far are five distinct large deletions (Petrij-Bosch et al. 1997; Puget et al. 1997; Swensen et al. 1997), two of which represent 36% of all *BRCA1* mutations in the Dutch population (Petrij-Bosch et al. 1997). The importance of such large genomic alterations is difficult to estimate, because most PCR-based methods that genetic laboratories use on genomic DNA—such as direct sequencing, single-strand conformation analysis (SSCA), heteroduplex analysis (HDA), denaturing gradient gel electrophoresis (DGGE), and the protein-truncation test (PTT)—will not allow their detection.

Here we report the identification of the first large duplication in the *BRCA1* gene in four apparently unrelated families: it comprises exon 13 and extends over 6 kb of intronic sequences. It was initially identified in one family—F3173—originally ascertained by one of us (H.T.L.) and contained one case of breast cancer and four cases of ovarian cancer. Leukocytes of obligate mutation carriers from F3173 had previously been shown to present a great reduction in the amount of the mutant transcript, but no alteration was identified in the *BRCA1* coding sequence when genomic sequencing and cDNA SSCA were performed (Serova et al. 1996). No genomic rearrangement had been found by Southern blot analysis, and no mutation in the promoter or the 5′ and 3′ UTRs was identified by HDA (Puget et al., in press). To look for splicing defects, we amplified, with 11 primer pairs, cDNA synthesized from leukocyte RNA of two patients from F3173, making sure that each exon was entirely contained within one fragment. Because we knew that the mutant allele was poorly expressed, we considered any abnormal PCR fragment visualized on agarose gels to be potentially interesting, irrespective of its intensity. A faint extra band ~170 bp longer than the expected fragment was visualized in the case of patients from F3173 with primers surrounding exons 12 and 13, which was also seen with primers surrounding exon 13 (fig. 1*a*). Sequencing of this extra band revealed the presence of two consecutive exons 13, leading to a frameshift in the mutant mRNA (ter1460). We then performed long-range PCR on genomic DNA, with overlapping primers in exon 13; although, as expected, no PCR product was obtained with control DNA, an ~6-kb fragment was generated in F3173, indicating that an ~6-kb duplication had occurred in the germ line of the F3173 patient (fig. 1*b*). The ~6-kb fragment was then digested by restriction enzymes, which showed that the duplication junction was contained within an ~800-bp *Xba*I fragment (fig. 1*c*). Duplication-specific primers (dup13F/R) were designed, and a 1.1-kb fragment was PCR amplified and sequenced: it revealed that a 6,081-bp region containing exon 13 (nucleotides 44369–50449 [GenBank accession number L78833]) is duplicated in F3173 (fig. 1*c*). Both breakpoints occurred in a 23-bp region of perfect identity, within two *Sx Alu* sequences in the same orientation (86.7% homology)—one in intron 12 and the other in intron 13—which suggests that the duplication is probably the result of a homologous recombination.

To evaluate whether this mutation, which may have previously escaped detection, is present in other families, we screened, by PCR using primers dup13F/R, 52 additional American families ascertained at Creighton University (of which 29 scored negative for mutations in the coding region and splice sites of the *BRCA1* gene when analyzed by HDA and PTT). This resulted in the identification of two more families bearing this duplication: (1) F3653, which contains seven breast cancer cases, and (2) F2773, which contains seven breast cancer cases and three ovarian cancer cases. The three American families with the duplication are of mixed European (English, Dutch, or Irish) descent. Finally, the 6-kb duplication was also found in one Portuguese family with

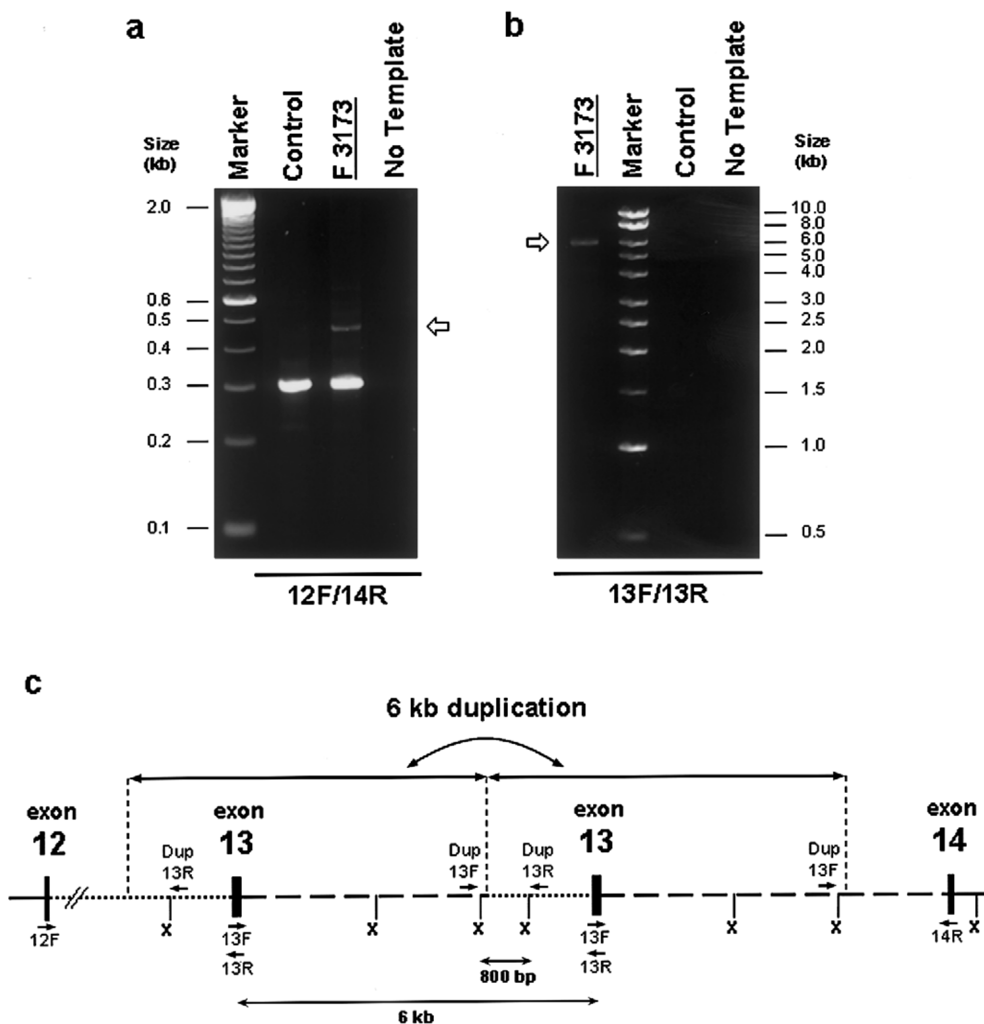

Figure 1 Characterization of the 6-kb germ-line duplication in the *BRCA1* gene in F3173. *a,* Complementary DNA, which was PCR amplified with primers 12F (ACA AGC GTC TCT GAA GAC TGC) and 14R (TGC AGA CAC CTC AAA CTT GTC AGC). Only a 318-bp fragment is generated in the control, whereas in the patient from F3173 a very faint extra band of 490 bp (*unblackened arrow*) containing two consecutive exons 13 as determined by sequencing is also produced. *b,* Genomic DNA, which was PCR amplified with primers 13F (GAT AAA GCT CCA GCA GGA AAT GGC) and 13R (GGC TCC CAT GCT GTT CTA AC). Only the mutant allele in F3173 gives rise to an ~6-kb fragment, shown (*unblackened arrow*), because the wild-type allele cannot be amplified with these primers (see panel *c*). *c,* Duplication of exon 13, schematically represented, with the location and orientation of primers 12F, 14R, 13F, 13R, dup13F (GAT TAT TTC CCC CCA GGC TA), and dup13R (AGA TCA TTA GCA AGG ACC TGT G). The *Xba*I sites (X); introns 12 (*dotted line*) and 13 (*broken line*); and the position and extent of the duplicated region, of the 800-bp *Xba*I fragment generated by the duplication, and of the 6-kb 13F/13R fragment (*two-headed arrows*) are indicated.

three cases of breast cancer, when 69 families (scoring negative for mutations in the coding region and splice sites of *BRCA1* when analyzed by DGGE [Stoppa-Lyonnet et al. 1997]) ascertained in Paris by D.S.-L. were screened. Although these families previously had been subjected to quantitative Southern analysis (Puget et al., in press), this rearrangement was missed because, on the one hand, the extra bands generated by digestions with the selected restriction enzymes were identical or very similar in size to the normal fragments, and, on the other hand, the densitometric analysis does not allow duplications to be identified as easily as deletions (1.5-fold signal-strength difference in duplications, compared with a 2-fold difference in deletions).

All four families were found to bear exactly the same duplication, as revealed by the sequencing of the duplication junction. A founder effect is very likely, since all families could share the same haplotype at nine polymorphic short tandem-repeat markers within or flanking the *BRCA1*locus (D17S776, D17S1185, D17S1320, D17S855, D17S1322, D17S1323, D17S1327, D17S1326, and D17S1329). Of the shared alleles, those

at D17S1185 and D17S855 have a population frequency <15%. Given the geographic diversity displayed by these four families' ancestors, the 6-kb duplication might be relatively old and is therefore likely to be found in other families around the world. Apart from the two frequent *BRCA1* mutations 185delAG and 5382insC, which have been found four and five times, respectively, this duplication is the most frequent mutation identified in the set of American families ascertained by H.T.L. (3 of 40 *BRCA1* mutations).

Although this duplication was identified by reverse-transcription PCR, it should be noted that it could be easily missed, since the mutant allele is poorly expressed, presumably because of premature stop codon–mediated mRNA decay. Given the high concentration of *Alu* sequences in the *BRCA1* gene (Smith et al. 1996), founder rearrangements such as the one reported here could explain a substantial fraction of the estimated 37% of breast/ovarian cancer families whose disease is due to *BRCA1* but for whom no mutation has been identified so far in the *BRCA1* coding sequence (Ford et al. 1998).

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Electronic-Database Information

Accession number and URLs for data in this article are as follows:

Breast Cancer Information Core, http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic (for *BRCA1* mutations)

GenBank, http://www.ncbi.nlm.nih.gov/Web/Genbank (for the *BRCA1* gene sequence [L78833])

References

Ford D, Easton DF, Stratton M, Narod S, Goldgar D, Devilee P, Bishop DT, et al (1998) Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. Am J Hum Genet 62:676–689

Neuhausen SL, Mazoyer S, Friedman L, Stratton M, Offit K, Caligo A, Tomlinson G, et al (1996) Haplotype and phenotype analysis of six recurrent BRCA1 mutations in 61 families: results of an international study. Am J Hum Genet 58:271–280

Petrij-Bosch A, Peelen T, van Vliet M, van Eijk R, Olmer R, Drusedau M, Hogervorst FBL, et al (1997) *BRCA1* genomic deletions are major founder mutations in Dutch breast cancer patients. Nat Genet 17:341–345

Puget N, Stoppa-Lyonnet D, Sinilnikova OM, Pagès S, Lynch HT, Lenoir GM, Mazoyer S. Screening for germline rearrangements and regulatory mutations in *BRCA1* led to the identification of four new deletions. Cancer Res (in press)

Puget N, Torchard D, Serova-Sinilnikova OM, Lynch HT, Feunteun J, Lenoir GM, Mazoyer S (1997) A 1-kb Alu-mediated germ-line deletion removing *BRCA1* exon 17. Cancer Res 57:828–831

Serova O, Montagna M, Torchard D, Narod SA, Tonin P, Sylla B, Lynch HT, et al (1996) A high incidence of BRCA1 mutations in 20 breast-ovarian cancer families. Am J Hum Genet 58:42–51

Smith TM, Lee MK, Szabo CI, Jerome N, McEuen M, Taylor M, Hood L, et al (1996) Complete genomic sequence and analysis of 117 kb of human DNA containing the gene *BRCA1*. Genome Res 6:1029–1049

Stoppa-Lyonnet D, Laurent-Puig P, Essioux L, Pagès S, Ithier G, Ligot L, Fourquet A, et al (1997) BRCA1 sequence variations in 160 individuals referred to a breast/ovarian family cancer clinic. Am J Hum Genet 60:1021–1030

Swensen J, Hoffman M, Skolnick MH, Neuhausen SL (1997) Identification of a 14 kb deletion involving the promoter region of *BRCA1* in a breast cancer family. Hum Mol Genet 6:1513–1517

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