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De Novo *BRCA1* Mutation in a Patient with Breast Cancer and an Inherited *BRCA2* Mutation

To the Editor:

Individuals with an inherited mutation in both of the breast/ovarian cancer–susceptibility genes, *BRCA1* and *BRCA2*, are rarely described (Ramus et al. 1997; Liede et al. 1998). Despite the large number of variants identified in these genes, there are, in the Breast Cancer Information Core, no published reports of de novo mutations.

During the course of the Australian Breast Cancer Family Study, a population-based study of breast cancer occurring in women at age <40 years (McCredie et al. 1998; Southey et al. 1998, 1999), we identified a proband who developed high-grade breast cancer with axillary nodal metastases. Her father developed prostate carcinoma during his early 50s. Her mother had no history of cancer. The studies outlined below were performed with informed consent from the individuals and were approved by the Peter MacCallum Cancer Institute's review board.

The proband's leukocyte-derived DNA revealed two germline protein-truncating mutations: one in exon 11 of *BRCA2* (6174delT) and a second in exon 11 of *BRCA1* (3888delGA). These mutations were identified by the protein-truncation test and by manual DNA sequencing in our laboratory (fig. 1*A*), and were confirmed, after collection of a further blood sample, by heteroduplex analysis in an outside laboratory (data not shown). The 6174delT *BRCA2* mutation has been reported frequently in individuals of Jewish descent (Neuhausen et al. 1996); however, there was no known Jewish ancestry in this family. The 3888delGA *BRCA1* mutation has not been reported before by the Breast Cancer Information Core. The father of the proband carried only the 6174delT *BRCA2* mutation (fig. 1*A*), and neither parent carried the 3888delGA *BRCA1* mutation. The proband's brother was unavailable for testing. DNA fingerprinting at five informative microsatellite loci supported the genetic relatedness of the parents' and proband's DNA samples (data not shown).

The absence of a germline 3888delGA *BRCA1* mutation in either parent indicated that this variant could represent a de novo alteration that occurred during parental germ-cell gametogenesis. We evaluated this possibility by utilizing a heterozygous polymorphism at nucleotide 1186 (1186 A→G) in the proband's *BRCA1* exon 11. The proband's *BRCA1* exon 11 alleles were separated by cloning, and then were sequenced. The 1186 A→G polymorphism was present on her wild type

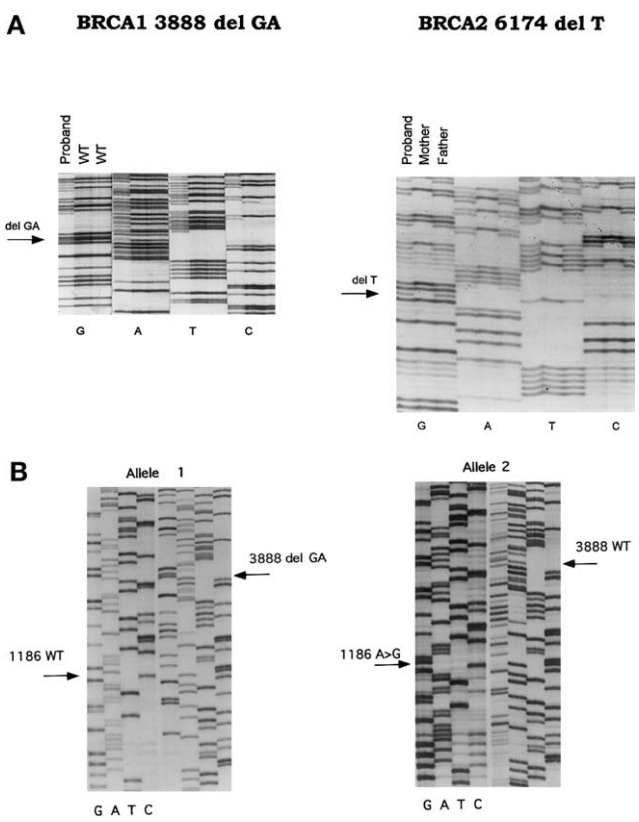

Figure 1 Germline mutations in *BRCA1* and *BRCA2*, and inheritance of *BRCA1* mutation. *A, left panel,* DNA sequence of a segment of exon 11 of *BRCA1* showing 3888delGA mutation in proband, as compared with wild-type sequence (lanes WT). *A, right panel,* DNA sequence of part of exon 11 of *BRCA2* showing 6174delT mutation in the proband and her father, contrasted with the wild-type sequence of the mother. *B, left panel,* DNA sequences of the separate, cloned alleles showing *BRCA1* 3888delGA mutation present on paternal allele (allele 1), with wild-type sequence at 1186. *B, right panel,* wild-type *BRCA1* sequence of the maternal allele (allele 2) at 3888, with the 1186A→G polymorphism. *BRCA1* was sequenced as described elsewhere (Southey et al. 1998), and the primers used to sequence *BRCA2* have been described in the Breast Cancer Information Core. Separation of the proband's *BRCA1* alleles was achieved by cloning a purified PCR-amplified 2.9-kb fragment containing the 1186 polymorphism and the 3888 deletion Bluescript (Stratagene).

3888 allele and in her mother's germline but was not present in her father's germline (fig. 1*A*). These data indicate that the *BRCA1* 3888delGA mutation was on the father's allele and further suggest that this mutation arose as a de novo event in a testicular germ cell.

The presence, in the proband, of heterozygous germline mutations in *BRCA1* and *BRCA2* raised the question of whether dysfunction of both these genes was involved in the development of her breast carcinoma.

Loss of heterozygosity (LOH) studies performed on microdissected archival breast-cancer tissue demonstrated consistent LOH of the maternal (wild type) allele at the *BRCA2*-flanking markers D13S260 and D13S290, in multiple independently dissected areas of invasive ductal carcinoma. Loss of the maternal allele was also seen in three adjacent axillary-lymph-node metastases and in multiple apparently separate foci of high-grade ductal carcinoma in situ, a preinvasive lesion (fig. 2). These data are consistent with clonal expansion and dominance of cells that harbor dysfunction of both alleles of *BRCA2*, through multiple phases of malignant progression. In contrast, evaluation of identical microdissected foci at the intragenic *BRCA1* marker D17S855 showed that both alleles were retained, suggesting that LOH-induced inactivation of the second *BRCA1* allele was not selected for during carcinogenesis (fig. 2).

Interestingly, the sequence adjacent to the *BRCA1* mutation—*AGAGGAGAAT* (where GA constitutes the deleted doublet) is similar to that adjacent to the *BRCA2* 6174delT mutation—*AGTGGAAAAT* (where T denotes the deleted nucleotide). Although this similarity could be due to chance, it is also possible that these sequences are problematic for the germ-cell DNA-replication machinery and that they are thus prone to mutation. In support of this, haplotype studies suggest that the 6174delT *BRCA2* mutation has arisen on multiple separate occasions in individuals of varying ethnic origins (Berman et al. 1996). In an attempt to define a possible common mechanism underlying the development of these two mutations, we used the *mfold* software of M. Zuker to evaluate the areas of sequence similarity, together with 100 bp of their respective wild-type *BRCA1*- and *BRCA2*-flanking regions, for their ability to form secondary structures. The segments of both genes preferentially formed consistent hairpin loops arising from unpaired nucleotides at a wide range of bond energies under physiological conditions. Moreover, in each case, the deleted bases lay <2 bp from an unpaired sequence. It is therefore possible that these frameshift mutations occurred because of deletion of nucleotides adjacent to unpaired DNA sequences arising in these quasipalindromic regions. Frameshift mutations occurring in quasipalindromic sequences have been well characterized in *Escherichia coli* (De Boer and Ripley 1984; Rosche et al. 1998) and have been suggested as a basis of the deletion mutations seen in several human genes (Cooper and Krawczak 1993, pp. 185–188).

There is a further potential explanation for the occurrence of the two mutations occurring in this case: *BRCA1* and *BRCA2* have been implicated in the maintenance of genomic integrity (Rajan et al. 1996; Connor et al. 1997; Scully et al. 1997; Bertwistle and Ashworth 1998; Chen et al. 1998; Marmorstein et al. 1998; Patel et al. 1998). Moreover, in the mouse testes, *Brca1* and *Brca2* are expressed in the mitotic spermatogonia as well as in meiotic spermatocytes (Blackshear et al. 1998). Therefore, the presence, in the father, of one mutated *BRCA2* allele in developing germ cells could confer a dosage insufficiency and thus result in a generally increased genomic mutation rate. Such an abnormality could theoretically affect any gene and would thus have a minimal likelihood of being detected, thus explaining the absence of reports of supporting evidence. However, the serendipitous coexistent *BRCA1* variant described here may indicate the need for more-rigorous searches for de novo germline mutations in other genes arising in the background of inherited *BRCA1/2* mutations. Further studies using in vitro methods can address whether sequences such as those described above are indeed more susceptible to mutation, whether *BRCA2* haploinsufficiency results in an increased mutation rate, and whether these two mutation mechanisms could display synergistic effects. This case is, to our knowledge, the first reported example of a de novo mutation arising in *BRCA1*, and it suggests a potential mechanism underlying the development of recurrent germline mutations occurring in association with specific sequences.

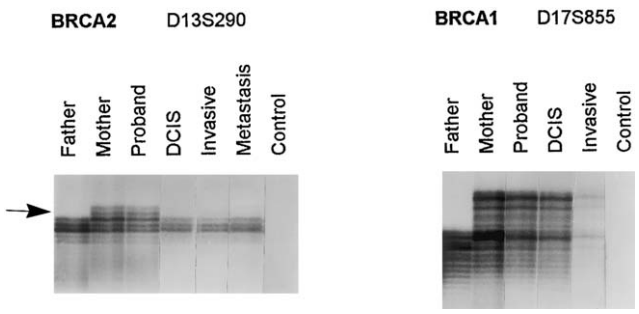

Figure 2 LOH analysis. *Left panel,* Loss of the maternally inherited (wild-type) *BRCA2* allele at the marker D13S290, in ductal carcinoma in situ (lanes DCIS), invasive and metastatic carcinoma from the proband. *Right panel,* Absence of LOH at the intragenic *BRCA1* marker D17S855 in DCIS and in invasive carcinoma. No DNA PCR control lanes are shown. DNA was microdissected from archival tumor samples, was subjected to proteinase K digestion, and was analyzed with standard PCR-based microsatellite-analysis protocols employing published primer sequences for the markers D13S290 and D17S855 (Simard et al. 1994).

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Electronic-Database Information

URLs for data in this article are as follows:

Breast Cancer Information Core, http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/

Institute for Biomedical Computing, Washington University, http://www.ibc.wustl.edu/~zuker/seqanal/

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