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A Somatic Truncating Mutation in BRCA2 in a Sporadic Breast Tumor

To the Editor:

Recently, a second susceptibility gene for hereditary breast and ovarian cancer, BRCA2, was cloned (Wooster et al. 1995; Tavtigian et al. 1996). The subsequent identification of heterozygous germ-line mutations confirmed its role as a predisposing factor in a subset of familial breast and ovarian cancer families (Wooster et al. 1995; Lancaster et al. 1996; Miki et al. 1996; Tavtigian et al. 1996; Teng et al. 1996). The possible involvement of BRCA2 in the sporadic forms of breast and ovarian tumors was addressed in three recent reports analyzing the gene for somatic mutations in 212 primary breast cancers and 55 ovarian cancers (Lancaster et al. 1996; Miki et al. 1996; Teng et al. 1996). Although several alterations were identified, all except two changes were shown to represent germ-line mutations. Moreover, the two somatic BRCA2 alterations were found to be missense mutations resulting in a Asp3095-Glu change (Lancaster et al. 1996) in one case and in a His2415Asn change (Miki et al. 1996) in the other. Given the questionable effect of missense mutations on protein function, the role of BRCA2 in the carcinogenesis of sporadic breast tumors remains unclear.

In order to identify in primary breast cancers the somatic BRCA2 mutations with clear functional consequences, we have analyzed the three large exons—10, 11, and 27—of BRCA2, together constituting 65% of the coding region of the gene (Tavtigian et al. 1996), in 69 unselected samples of frozen breast tumor sections, using the protein-truncation test (PTT) (Roest et al. 1993). Here we report a first case of a truncating somatic mutation in BRCA2 in a primary ductal breast carcinoma with demonstrated loss of heterozygosity (LOH).

PTT was utilized to identify protein-termination mutations in exons 10, 11, and 27 of BRCA2, using genomic DNA and oligonucleotide primers PTT10 f/r (5′-CTTAATACGACTCACTATAGGGAGACCACCATGTATACTTTAACAGGATTTGGAA-3′/5′-ACACAGAAGGAATCGTCATC-3′), the overlapping primer sets PTT11A f/r (5′-CTTAATACGACTCACTATAGGGAGACCACCATGTTTTTATGTTTAGGTTTATTGC-3′/5′-TGCATTCCTCAGAAGTGGTC-3′), PTT11B f/r (5′-CTTAATACGACTCACTATAGGGAGACCACCATGAAACCAAGCTACATATTGCAG-3′/5′-TAATTTCCTACATAATCTGCAG-3′), PTT11C f/r (5′-CTTA ATACGACTCACTATAGGGAGACCACCATGTGGCTTAGAGAAGGAATATTTG-3′/5′-AAAATAGTGATTGGCAACACG3′), and the primer set PTT27 f/r (5′-CTTAATACGACTCACTATAGGGAGACCACCATGACGTTTTCATTTTTTTTATCA-3′/5′-ATAATTTATTGTCGCCTTTGC-3′). SDS-PAGE analysis of translated PCR product PTT11A r/f revealed a truncated protein of ~35 kD in breast tumor tissue but not in blood lymphocytes of patient Sp27 (fig. 1*a*). The genetic identity of tumor and lymphocyte DNA in patient Sp27 was verified by informative markers at D11S524 and D11S554 (data not shown) and was also confirmed at D13S260 and D13S171 (fig. 1*b*). Sequencing of the variant PTT allele in the approxi-

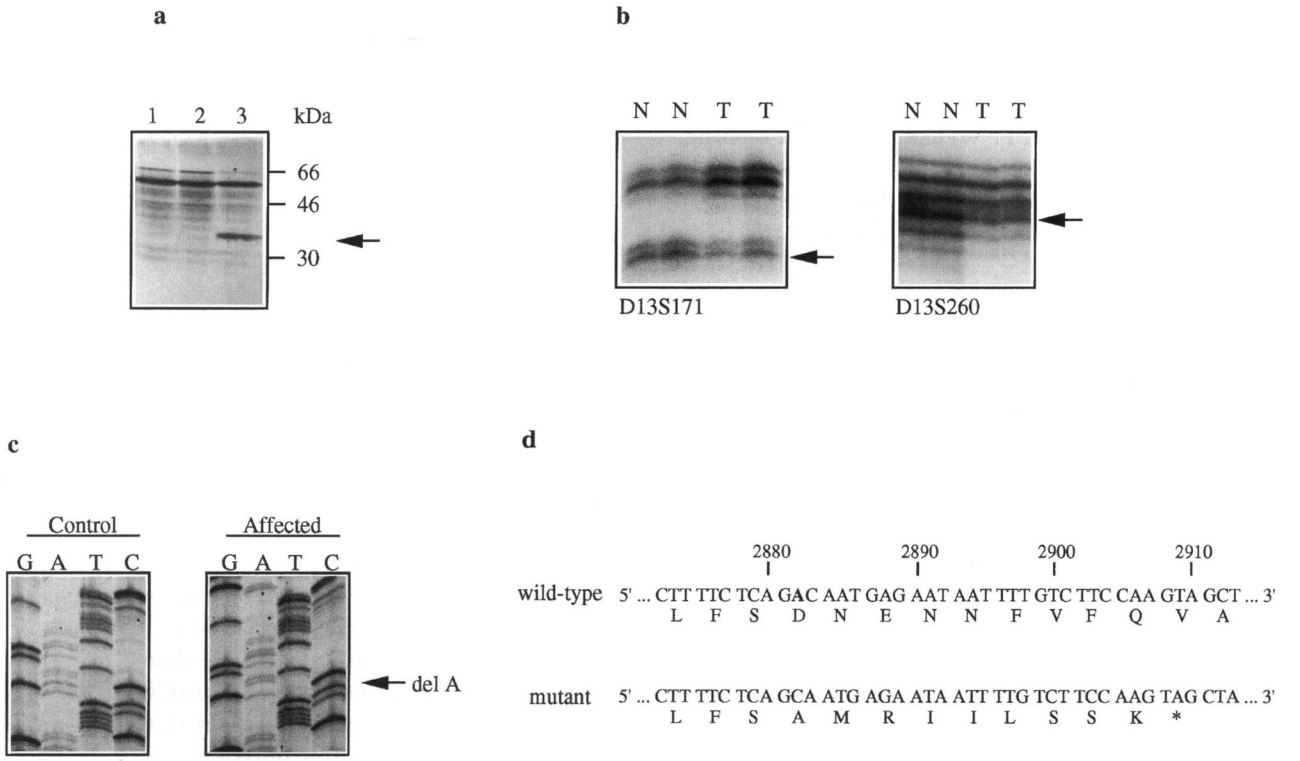

Figure 1 Analysis of BRCA2 exon 11 in ductal breast tumor of patient Sp27. *a,* SDS-PAGE analysis of PTT11A f/r product translated with rabbit reticulocyte lysate. Lane 1, Healthy control individual. Lane 2, Blood sample. Lane 3, Tumor tissue of patient Sp27. The truncated protein product of 35 kD is present only in the tumor, and not in the blood-lymphocyte sample, of patient Sp27 (*arrow*). *b,* LOH at D13S260 and D13S171 in breast tumor DNA (lanes 3 and 4, *arrows*), but not in blood-lymphocyte DNA, of patient Sp27 (lane 1 and 2). *c,* Sequencing of the mutant allele. A 1-bp deletion at nucleotide 2881 of BRCA2 is present. The wild-type sequence is shown for comparison. *d,* 2881delA mutation, which results in a termination codon at position 894 (*asterisk* [*]), with nine novel amino acids at the C-terminus of the truncated protein.

mate region believed to contain the protein-termination mutation demonstrated a 1-bp deletion at nucleotide 2881 (fig. 1*c*), thus resulting in a termination codon at position 894, with nine novel amino acids at the C-terminus of the truncated protein (fig. 1*d*).

To demonstrate LOH at the BRCA2 locus in the breast tumor of patient Sp27, we analyzed markers at D13S260 and D13S171, which were known to closely flank BRCA2 on the proximal and distal side, respectively (Wooster et al. 1994). Whereas in lymphocyte DNA the heterozygous alleles at D13S260 and D13S171 are present in similar intensities, a significant reduction of the respective lower alleles of the two microsatellite markers can be observed in the tumor DNA, thus strongly suggesting that in one allele a chromosomal deletion encompassing the entire BRCA2 gene (fig. 1*b*). Although the minor presence of the wild-type allele in the tumor sample of patient Sp27 was consistently noticed (fig. 1*a* and *b*), we assume this to be due to the presence of contaminating nonmalignant stroma cells in the tumor preparation. Similar technical difficulties are commonly encountered in LOH studies (e.g., see Hosking et al. 1995).

In analogy to the nonrandom distribution of the known BRCA2 germ-line mutations (Wooster et al. 1995; Lancaster et al. 1996; Miki et al. 1996; Tavtigian et al. 1996; Teng et al. 1996), sporadic breast tumors may reveal a similar mutational profile. If this is correct, one can preliminarily extrapolate our data to the entire coding region of BRCA2, suggesting that this gene may be implicated in sporadic breast cancer in ~2%–3% of all cases. In contrast, LOH involving the BRCA2 locus may be present in 30%–40% of sporadic breast tumors, which may suggest a similar number of somatic mutations in the BRCA2 gene (Cletonjansen et al. 1995; Kerangueven et al. 1995). However, it already has been pointed out that LOH in sporadic breast carcinomas may overestimate the involvement of BRCA2 in these tumors. Instead, the BRCA2 locus may be a specific target of LOH in only a minor number of cases (Cletonjansen et al. 1995; Kerangueven et al. 1995). This appears consistent with our and three other reports that identified an additional two somatic missense mutations among 212 breast carcinomas studied (Lancaster et al. 1996; Miki et al. 1996; Teng et al. 1996). Together, these findings show that somatic BRCA2 mutations are

involved in the primary events of breast carcinogenesis, although only in a minor subset of sporadic breast cancers.

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A View of the Neolithic Demic Diffusion in Europe through Two Y Chromosome–Specific Markers

To the Editor:

The farmer economy originated in different places of the world. One of the most important (probably the first) is in the Middle East, in the so-called Fertile Crescent, from which farming spread toward Europe, North Africa, Arabia, East Africa, and southwestern Asia (Indus valley) (Cavalli-Sforza et al. 1994).

Two models, the cultural and the demic, were proposed to explain the neolithic expansion of the early farming to Europe. According to the first, this expansion might have occurred by transmission of new technologies without movements of farmers and then without changes in the genetic makeup of the preexisting populations. According to the second, the spread of the farming economy might have occurred through the migration of farmers who progressively admixed with local paleolithic hunter-gatherers. As a direct consequence, a change of allele frequencies should have taken place for those genes that differentiated the old inhabitants from the newcomers. The model of demic diffusion, called "the wave of advance" by Ammerman and Cavalli-Sforza (1984), is the most accepted, at least for Europe, where it is also supported by archaeological records (Menozzi et al. 1978). It implies clines of the farmers' gene frequencies, which decrease with increasing distance from the area of origin.

By performing principal components analysis on numerous classical markers, synthetic maps were constructed for Europe and the Near East (Menozzi et al. 1978), which show the Near East as the center of concentric clines of decreasing gene frequencies and give value to the theory of demic spread of agriculture.

Further support to this theory is given by a large population survey we carried out on some Y-specific polymorphisms. Two markers have been found, the distribution of which illustrate well the process of "wave of advance."

We studied the *Taq*I Y-specific RFLPs detected by p12f2 (DYS11) and 49a,f (DYS1) probes in ~3,000 subjects of different populations, mainly from Europe (particularly from the Mediterranean basin) but also from Africa and Asia.