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Evidence for a BRCA1 Founder Mutation in Families of West African Ancestry

To the Editor:

Inherited mutations in the BRCA1 gene (MIM 113705; GenBank U14680) (Miki et al. 1994) are less common among breast cancer patients of African American ancestry than among those of white ancestry. For example, in a population-based series of breast cancer patients from North Carolina, the prevalence of BRCA1 mutations was 3.3% among white women and 0% among African American women (Newman et al. 1998). Nonetheless, inherited BRCA1 mutations have been identified in families of African and African American ancestry at high risk of breast cancer (Gao et al. 1997; Stoppa-Lyonnet et al. 1997; Panguluri et al. 1999 [in press]). To provide effective genetic testing for African American families at high risk for breast and ovarian cancer, it would be helpful to identify ancient BRCA1 mutations of African origin analogous to ancient mutations in other populations (Simard et al. 1994; Peelen et al. 1997; Petrij-Bosch et al. 1997). Here we have described one apparently ancient, African BRCA1 mutation.

BRCA1 mutation 943ins10 was detected in breast cancer patients from the Ivory Coast (Stoppa-Lyonnet et al. 1997), the Bahamas, and the United States (Arena et al. 1997; Panguluri et al. 1999 [in press]) (fig. 1). To confirm the identity of the mutation for the five probands and their relatives, the critical region of BRCA1 was genotyped by fluorescent sequencing with dRhodamine-dye terminators (Applied Biosystems). Primers 5′-GGAATTAAATGAAAGAGTATG-AGC-3′ and 5′-CTTCCAGCCCATCTGTTATGTTG-3′ revealed the heterozygous frameshift mutation 943ins10, a 10-bp insertion in exon 11, leading to a stop at codon 289. The mutation is a tandem duplication, in a repeated-sequence motif, that could have occurred at any site between BRCA1 nucleotides 926 and 943 (fig. 2). The notation "943ins10" designates the most-3′ site of insertion possible (Antonarakis et al. 1998). The 943ins10 variant can be easily detected on agarose gel by amplification of genomic DNA or cDNA with BRCA1 primers 5′-CTGCTTGTGAAT-TTTCTGAGACGG-3′ and 5′-TGCTGTAATGAGC-TGGCATGAG-3′ under standard conditions. Wild-type BRCA1 sequence yields a product of 184 bp, and 943ins10 yields a product of 194 bp.

Genotypes of relatives in these five families were consistent with BRCA1 943ins10 being a founder mutation of African origin. Nine markers within and flanking BRCA1 were genotyped (Genome Database): D17S1325, D17S1326, and D17S1327 (5′ of BRCA1); D17S1323 (intron 12), D17S1322 (intron 19), and D17S855 (intron 20); and D17S1321, D17S1320, and D17S1185 (3′ of BRCA1) (Neuhausen et al. 1996; Smith et al. 1996). The 943ins10 mutation occurred on a single haplotype spanning D17S1320–D17S1326 (fig. 1), a distance of ~700 kb.

The families inheriting BRCA1 943ins10 were from widespread locales of Africa and the African diaspora: the Ivory Coast, the Bahamas, the southeastern United States, and Washington, DC. The families are not recently related, and the four families in North America can trace their history in this hemisphere to the slavery period. The length of the 943ins10 nonrecombinant BRCA1 region is similar to the length of the shared region flanking the BRCA1 mutation 185delAG. Hence, the ages of these mutations may be comparable (Bar-Sade et al. 1998). The shared BRCA1 region flanking 943ins10 is shorter than the BRCA1 regions flanking 5382insC or 2800delAA, so the African mutation is probably older than these European mutations (Neuhausen et al. 1996; Friedman et al. 1995). West Africans were brought to North America as slaves between 1619 and 1808. Hence, the social history of the families studied indicates that the mutation is >200 years old and could be much more ancient.

Figure 1 indicates additional, known cases of breast and ovarian cancer in each family. In families UM94003 and UM95027, mothers of probands were affected. In families UM96034 and HU003, in which mothers were not affected, the 943ins10 allele was inherited from the father. Age at breast and ovarian cancer diagnosis was $\leq$50 years for all probands and affected relatives. Family IC564 includes four women with breast or ovarian cancer, all of whom live in the Ivory Coast, where breast and ovarian cancer are rare (Parkin et al. 1997). In this family, the mother of a patient with ovarian cancer remains unaffected at age 83 years, though she is likely to carry the mutation. That there are elderly carriers without cancer suggests that nongenetic factors may influence the penetrance of BRCA1 alleles in geographic regions with a low background risk for breast cancer.

The geographic distribution of 943ins10 in North America is intriguing and is not completely known. BRCA1 943ins10 occurred in 3 of 96 African American patients seen at the University of Miami, who had breast cancer diagnosed at an early age, and in 1 of 55 African American patients seen at Howard University in Washington, DC, who had breast cancer diagnosed at an early age or who had families with a high incidence of breast cancer. However, in the population-based Carolina Breast Cancer Study, the mutation did not appear among 263 African American breast cancer patients, 50% of whom were aged <50 years and 50% of whom were aged $\geq$50 years at diagnosis (Newman et al. 1998). The 943ins10 allele has not been observed in any patients

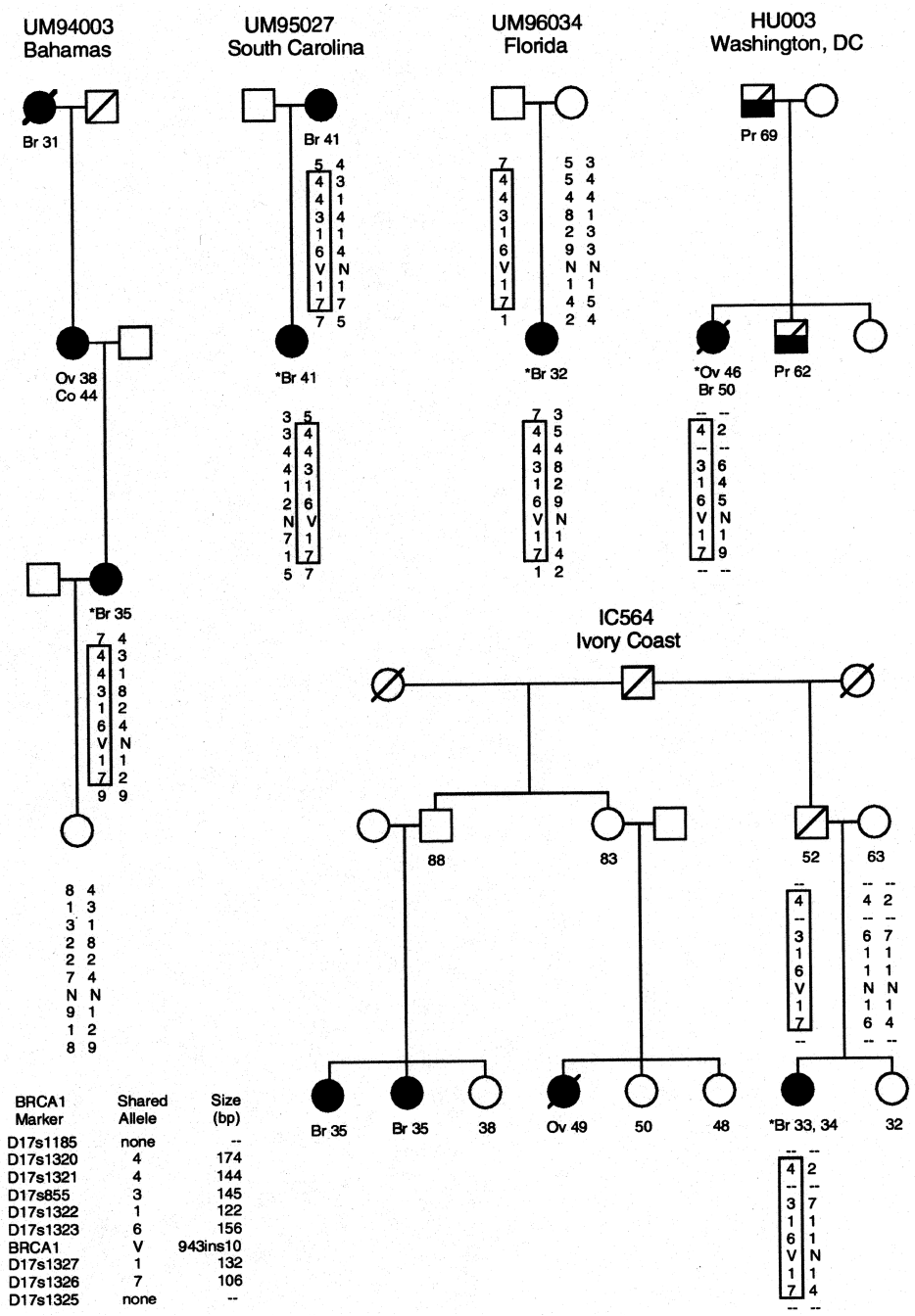

Figure 1 Pedigrees of families carrying the BRCA1 943ins10 mutation. Affected individuals are indicated by a blackened symbol, and probands are denoted by an asterisk (*). The shared haplotype segregating with 943ins10 is boxed. Haplotypes of the fathers of probands in families UM96034 and IC564 have been reconstructed.

with breast cancer who identify their ancestry as solely European.

The migration patterns of African Americans and, hence, the current areas of residence of African American families, may explain the difference, among clinical centers, in the prevalence of the mutation. To determine, among African American women, the proportion of inherited breast or ovarian cancer attributable to BRCA1 943ins10, we would like to encourage testing for this mutation among African American breast and

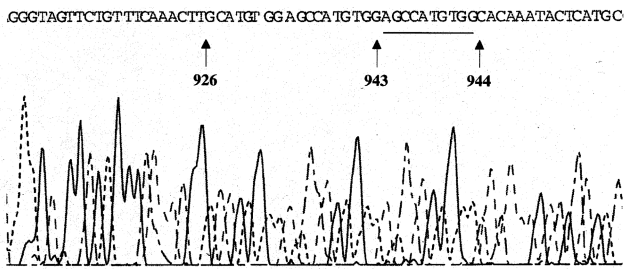

Figure 2 Sequence of the BRCA1 943ins10 mutation. A duplication and insertion of 10 bp causes a frameshift and premature truncation at amino acid 289.

ovarian cancer patients from various regions of the United States. Given the increasing incidence of and higher mortality from breast cancer among African American women, it would be useful to obtain as much information as possible about the roles of BRCA1 and BRCA2 in this population.

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HEATHER C. MEFFORD,[1] LISA BAUMBACH,[2] RAMESH C. K. PANGULURI,[3] CAROLYN WHITFIELD-BROOME,[3] CSILLA SZABO,[1] SELENA SMITH,[2] MARY-CLAIRE KING,[1] GEORGIA DUNSTON,[3] DOMINIQUE STOPPA-LYONNET,[4] AND FERNANDO ARENA[2]

[1]*Departments of Genetics and Medicine, University of Washington, Seattle;* [2]*Sylvester Cancer Center, University of Miami, Miami;* [3]*Departments of Microbiology and Molecular Biology, and Cancer Center, Howard University, Washington DC; and* [4]*Unité de Génétique Oncologique, Institut Curie, and Unité INSERM U.434, Paris*

Electronic-Database Information

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

GenBank, http://www.ncbi.nlm.nih.gov/Entrez (for BRCA1 [U14680])

Genome Database, http://gdbwww.gdb.org (for D17S1325, D17S1326, D17S1327, D17S1323, D17S1322, D17S855, D17S1321, D17S1320, and D17S1185)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for breast cancer, BRCA1 [MIM 113705])

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Address for correspondence and reprints: Dr. Mary-Claire King, Departments of Genetics and Medicine, University of Washington, Box 357720, Seattle, WA 98195-7720. E-mail: mcking@u.washington.edu

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Power Comparisons of the Transmission/Disequilibrium Test and Sib–Transmission/Disequilibrium-Test Statistics

To the Editor:

Several recent papers have considered the extension of the transmission/disequilibrium test (TDT) to families in which parental DNA is not available but in which unaffected siblings can be sampled. Each of these tests compares the alleles in the affected offspring with those in the unaffected offspring. The tests differ both in the precise statistics used and in the numbers of affected and unaffected offspring included. Spielman and Ewens (1998) have developed the sib TDT (S-TDT) for families with an arbitrary number of affected and unaffected members (including at least one of each). Curtis (1997) has used families with a single affected offspring and an arbitrary number of unaffected offspring but has analyzed only that unaffected offspring who has the genotype most different from that of the affected offspring. Boehnke and Langefeld (1998) have used a discordant-sib-pair approach. The S-TDT is a test of linkage, but it is also valid as a test of allelic association in which precisely one affected sibling and one unaffected sibling are used, as is the case in the tests that have been described by Curtis (1997) and Boehnke and Langefeld (1998).

These authors have considered power in different contexts—for example, across offspring genotype configurations (Spielman and Ewens 1998) and across genetic models (Boehnke and Langefeld 1998)—but none of the approaches used was intended to provide an overall assessment of the power of a sibling-based TDT statistic compared with that of the original formulation of the TDT. Here we derive a relationship between power for the S-TDT and the TDT, which shows that, to achieve similar power, considerably more genotyping is required for the S-TDT than for the TDT. This is intuitively clear, for the following reason. For both tests, a family is informative only if at least one parent is heterozygous. The S-TDT requires an additional condition to be true: both alleles from the heterozygous parent must be present in the offspring. This implies that the informativeness of the S-TDT statistic increases with the number of siblings genotyped. Because of the variation associated with the alleles inherited by the n unaffected siblings, we expect that, for finite n, the S-TDT will be less powerful than the TDT, with the power of the S-TDT tending toward that of the TDT as $n \to \infty$. Below we formalize this argument. Our results extend the power calculations of Spielman and Ewens (1998): in table 5 of their paper, they give the power of both the S-TDT and the TDT, for families with one heterozygous and one homozygous parent, a single affected child, and two to four unaffected children. Their power calculations are conditional on both alleles from the heterozygous parent being present in the offspring, which, as the authors acknowledge, covers only a small proportion of possible family genotype configurations. This conditioning on the offspring genotypes implies that all families are informative for the S-TDT, and therefore it crucially affects the power of the S-TDT. With this conditioning, the power of the S-TDT is almost as great as that of the TDT; without it, the power of the S-TDT may be considerably reduced.

For the sake of simplicity, we consider a sample of k families, assuming that in each family there are a single affected offspring and n unaffected offspring. All individuals have been genotyped at a diallelic marker locus with alleles M and m; let the numbers of M alleles in the offspring in the ith family be X_i for the affected sib and Y_{ij}, $j \in \{1,2,\ldots,n\}$, for the unaffected sibs. We condition on the parental genotypes in the sample and compare the TDT and S-TDT for this sample. The difference between the two statistics can be summarized as follows. The TDT compares $X_\cdot = \Sigma_{i=1}^{k} X_i$, with $\mathbf{E}(X_\cdot \mid H_0)$, where this expected value is calculated from the parental marker information, under the assumption that the null hypothesis is true—that is, either of the two alleles in a heterozygous parent is equally likely to be transmitted to an affected child. The S-TDT, however, is designed for use when this parental information is unavailable; instead, $X_\cdot$ is compared with $Y_\cdot/n$, where $Y_\cdot = \Sigma_{i=1}^{k}\Sigma_{j=1}^{n} Y_{ij}$ is the total number of M alleles in the unaffected offspring.

Our test statistics for the TDT and the S-TDT (T_{TDT} and $T_{\text{S-TDT}}$, respectively) are obtained by the method described, by Spielman and Ewens (1998), as the Z-score procedure: test statistics are standardized to mean 0 and variance 1 and are assumed to follow a standard normal distribution. This gives $T_{\text{TDT}} = (X_\cdot - \mu_0)/\sigma_0$, where μ_0 and σ_0^2 are, respectively, the mean and variance of $X_\cdot$, under the null hypothesis of no linkage.