Letters to the Editor

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Recurrent Germ-Line *BRCA1* Mutations in Extended African American Families with Early-Onset Breast Cancer

To the Editor:

Genetic susceptibility has been estimated to contribute to 5%–10% of all breast cancer cases but to ~25% of cases diagnosed before age 30 years (Kelsey and Gammon 1991; Skolnick and Cannon-Albright 1992). Among women born and raised in the United States, African American women have a lower risk of breast cancer than Caucasian women. However, the incidence rate of breast cancer in African American women is rising among women <40 years of age (Hankey et al. 1994). It has been observed that the age distribution of disease onset as well as tumor histology is different between Caucasians and African Americans. African Americans have a greater incidence at ages 30–44 years, and medullary carcinoma histology is more frequent in these patients (Eley et al. 1994). Furthermore, breast cancer in younger African American women may be more aggressive, leading to a decrease in the overall survival rates of African American women compared with that of Caucasian women (Eley et al. 1994; Shiao et al. 1995). The vast majority of African Americans originated from western Africa, where breast cancer is considered to be a rare aggressive disease predominantly affecting young women (Parkin 1994). Thus, results of genetic studies in ethnic African Americans are likely to correlate most closely with the results for populations in western Africa and may show that increased genetic susceptibility contributes to a significant percentage of the early-onset breast cancer cases in these populations.

BRCA1 and *BRCA2* mutations are thought to account for 80% of breast cancer in families with a high incidence of early-onset breast or ovarian cancer. Mutation analyses of *BRCA1* and *BRCA2* have been extensively performed in Caucasians with a strong family history of breast cancer (Futreal et al. 1994; Miki et al. 1994; Gayther et al. 1995; Goldgar et al. 1995; Shattuck-Eidens et al. 1995; Struewing et al. 1995; Wooster et al. 1995; Tavtigian et al. 1996). Few of the studies have included African Americans, despite the disproportionate incidence of early-onset breast cancer in this group. Little information exists regarding *BRCA1* and *BRCA2* mutations in ethnic groups other than Caucasians of northern European ancestry. In this study, we screened for *BRCA1* mutations in nine African American families ascertained through The University of Chicago Cancer Risk Clinic. Our findings suggest that *BRCA1* mutations may explain increased susceptibility to breast cancer in a significant percentage of high-risk African American families.

Human Subjects

This study was approved by The University of Chicago institutional review board. Families were identified through The University of Chicago Cancer Risk Clinic, where cancer-risk counseling is currently offered to individuals at high risk for breast cancer. Women tested had been diagnosed with breast cancer and either had a minimum prior probability of 20% of being *BRCA1* gene carriers (Shattuck-Eidens et al. 1995) or were from families with at least two first-degree relatives with breast and/or ovarian cancer at <60 years of age.

DNA Preparation

Genomic DNAs were prepared from blood samples by use of DNA isolation kits from Puregene, according to the manufacturer's instructions.

DNA Sequencing

The entire coding region of the *BRCA1* gene was sequenced by Myriad Genetic Laboratories, using a high-

throughput automated sequencer. Genomic DNAs were PCR amplified by use of M-13–tailed primers. PCR products were thermal cycle sequenced by use of dye primer chemistry, according to the manufacturer's (Perkin Elmer) instructions. Both DNA strands were sequenced; all of the coding sequence and a large portion of intronic sequence were examined for polymorphisms and mutations.

Genotyping of 17q Markers

Genotyping was performed at the University of Utah. The eight markers genotyped were short-tandem-repeat loci assayed by PCR using standard procedures as described by Neuhansen et al. (1996) and include D17S855, D17S1322, D17S1323, D17S1327, D17S1326, D17S1325, D17S1320, and D17S1321.

Allele-Specific Oligonucleotide (ASO) Hybridization

Genomic DNAs from the samples under study were amplified with primers for the corresponding exons containing the mutations. The primer sequences used to detect the 1832del5 mutation were 5′-atcagggaactaaccaaacggag-3′ and 5′-tggtagaagacttcctcctcagcc-3′. The primers used to detect the 5296del4 mutation were 5′-ctccgtgaaaaagagcacgttc-3′ and 5′-gtggtgcattgatggaaggaag-3′. The PCR products then were denatured and applied to Hybond-N+ nucleic acid–transfer membranes (Amersham) with 96-well dot-blot apparatus. Both wild-type (5′-taacccaatcactcgaaaaa-3′ for 1832del5 and 5′-taaagaaagaaaaatgct-3 for 5296del4) and mutant oligonucleotide probes (5′-taacccaatagaatcactcga-3 for 1832del5 and 5′-tattaaagaaaaatgctg for 5296del4) were end-labeled with 50 μCi of γ^{32}P-dATP. Hybridization assays were performed with 30 μM of labeled probe in 5 × sodium/sodium phosphate/EDTA and 2% SDS solution at 45°C overnight. The membranes were exposed to autoradiography, after being washed in 2 × saline sodium citrate at 58°C for 1832del5 and at 52°C for 5296del4. All the mutations identified by ASO were confirmed by direct sequencing. The 3883insA mutation has not been tested by ASO in other family members.

Denaturing PAGE (DPAGE)

Either the forward or backward primer was end-labeled with 20 μCi of γ^{32}P-dATP. Amplification of genomic DNA was performed according to standard PCR procedures, with a labeled:unlabeled primer ratio of 1:4. The PCR products were then mixed with an equal volume of tracking dye and were electrophoresed on standard 6% denaturing polyacrylamide gel. The gels were dried and exposed to x-ray film.

The entire coding region of *BRCA1* in one case in each family was screened for mutations. Five (56%) of the nine probands had germ-line *BRCA1* alterations (table 1). By searching the Breast Cancer Information Core mutational database and the database from *BRCA1* testing at Myriad Genetic Laboratories, we found that three of the mutations (1832del5, 5296del4, and 3883insA) were novel. The majority of the cases listed in the database are of northern European ancestry, and these mutations may not have been seen previously because they are exclusive to African Americans.

The recurrent *BRCA1* 1832del5 mutation was identified first in an individual from family 93-19, who was diagnosed with breast cancer at age 34 years. Subsequently, we extended the screening to other family members, using ASO hybridization and DPAGE. Four additional breast or ovarian cancer cases in this family were confirmed as mutation carriers, by both methods. The DPAGE result is shown in figure 1. All the mutation carriers share the same haplotype, on the basis of haplotype analysis of 625 kb surrounding the *BRCA1* locus. Noncarriers did not share the haplotype. This strongly suggests the cosegregation of the mutation with breast and ovarian cancer in this family. By ASO hybridization, we identified the same 1832del5 mutation in another proband from an unrelated family, 95-95. The ASO result subsequently was confirmed by direct sequencing. There appears to be complete sharing of the 1832del5 haplotype in both families. However, we do not know allele frequencies sufficiently to say whether it may be

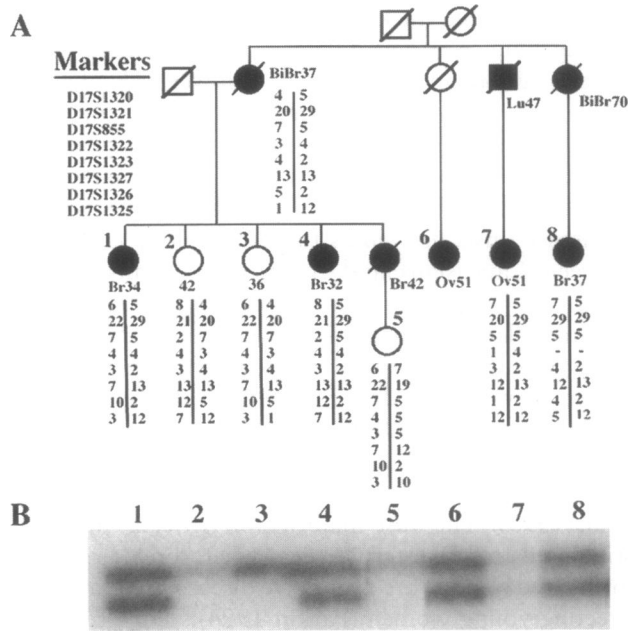

Figure 1 Haplotype and segregation analysis of African American kindred 93-19. *A*, Eight microsatellite markers within or flanking the *BRCA1* gene, which were used for haplotype analysis. Br = breast cancer; Ov = ovarian cancer; and Lu = lung cancer. Ages at diagnosis or testing are indicated. *B*, Representative autoradiogram of segregation analysis of the *BRCA1* 1832del5 mutation in the 93-19 family, by DPAGE. The number above each lane corresponds to the number above each individual in the pedigree in panel *A*. The frameshift mutation was detected first in the proband (lane 1) by sequencing of her entire *BRCA1* coding region.

Table 1

***BRCA1* Mutations in African American Breast Cancer Families**

Family	Mutation	Exon	Effect	No. of Cases (Median Age at Diagnosis)	
				Breast Cancer	Ovarian Cancer
93-19	1832del5	11	Frameshift	7 (35 years)	2 (51 years)
95-95	1832del5	11	Frameshift	5 (36 years)	0
94-49	5296del4	19	Frameshift	6 (34 years)	0
96-45	5296del4	19	Frameshift	3 (40 years)	2 (43 years)
96-75	3883insA	11	Frameshift	2 (29 years)	0
95-69	IVS9+GG	9 and 11	Unknown	5 (45 years)	0
95-19	S1140G	11	Unknown	2 (34 years)	1 (53 years)
95-59	None	...	...	2 (48 years)	0
93-51	None	...	...	10 (50 years)	0

a common haplotype. Women carrying the 1832del5 mutation express a variety of phenotypes, from early-onset bilateral breast cancer and ovarian cancer to later-onset breast cancer (at age 70 years, in family 93-19). There are both seven cases of breast cancer, with a median age at onset of 35 years, and two cases of ovarian cancer, both at age 51 years, in the 93-19 family. In family 95-95, there are five cases of breast cancer, with a median age at diagnosis of 36 years; there were no ovarian cancers. Of the tested individuals, all those testing positive as mutation carriers had developed breast or ovarian cancer.

The second novel mutation, *BRCA1* 5296del4, was first identified in an African American patient from family 94-49, who was diagnosed with a first breast cancer at age 24 years and with a second breast cancer at age 35 years. The mutation presumably was transmitted through her mother, who also was diagnosed with bilateral breast cancer, at ages 34 years and 40 years. In total, there are six cases of breast cancer in this family, with a median age at onset of 34 years. This mutation was found to be recurrent in a second unrelated African American family, 96-45. The proband from this family was diagnosed with breast cancer at age 40 years, and her daughter was diagnosed with bilateral breast cancer at ages 39 years and 45 years. In total, there are three confirmed cases of breast cancer and two cases of ovarian cancer in this family. For the 5296del4 mutation, haplotype analysis is incomplete, but the carriers share a 375-kb region surrounding *BRCA1*.

A third novel mutation, 3883insA, was identified in a 34-year-old woman from family 96-75, who developed breast cancer at age 28 years. Except for her mother, who had breast cancer at age 30, there are no other cases of cancer recorded in the family. Genetic variants of unknown significance were identified in families 95-69 and 95-19, as listed in table 1. The splice-site variant IVS9+GG and the missense variant S1140G may or may not affect *BRCA1* protein function. Therefore, additional family members will need to be sampled to examine cosegregation of the alterations and cancer in the families. The proband from family 95-69 developed bilateral breast cancer at ages 51 and 61 years; two of her sisters, as well as two maternal cousins, also were diagnosed with breast cancer. The median age at diagnosis of breast cancer in the family was 48 years, and there were no reported cases of ovarian cancer on the maternal side. The proband's father had prostate cancer at age 68 years, and there were two cases of ovarian cancer, at ages 55 and 60 years, on the paternal side of the family. Thus, there may be two different alterations segregating in this family. We are currently contacting family members to determine the segregation of the splice variant. A second variant of unknown significance was identified in family 95-19, with two cases of breast cancer (median age at diagnosis 34 years) and one case of ovarian cancer at age 53 years. The proband was diagnosed with ovarian cancer at age 53 years, her daughter had breast cancer at age 33 years, and her father had pancreatic cancer at age 43 years. No *BRCA1* alterations have been identified in two families, including family 93-51, which had 10 cases of breast cancer (table 1).

This study indicates that genetic susceptibility to breast cancer in this limited data set can be explained by *BRCA1* gene mutations in 56% (5/9) of high-risk African American families ascertained through young breast cancer cases referred to an urban cancer-risk clinic. We have not completed *BRCA2* analysis in this cohort, but it is likely that the families with five or more cases of breast cancer and no ovarian cancer (families 95-69 and 93-51) and the family with pancreatic cancer (familiy 95-19) represent *BRCA2* families. None of the previously described African American mutations were

identified in this screen, including the Met1775Arg mutation, which had been detected in two unrelated African American families (Futreal et al. 1994; Miki et al. 1994), and the Cys64Gly mutation, which was identified once in an African American kindred (Castilla et al. 1994). This initial description of a spectrum of *BRCA1* mutations, two of which are recurrent in unrelated individuals from high-risk African American kindreds, should provide initial data for more-intensive genetic epidemiology studies in blacks of African descent.

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BRCA2 Mutations in Hereditary Breast and Ovarian Cancer in France

To the Editor:

BRCA2, a major gene responsible for susceptibility to breast cancer, located on chromosome 13q12-13, recently has been cloned (Wooster et al. 1994, 1995; Tavtigian et al. 1996). *BRCA2* germ-line mutations also have been shown to confer an elevated risk of ovarian and male breast cancer (Wooster et al. 1994; D. F. Eas-