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Prevalence of *BRCA1* and *BRCA2* mutations among clinic-based African American families with breast cancer

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Abstract To define the prevalence and relative contributions of *BRCA1* and *BRCA2* mutations among African American families with breast cancer, we analyzed 28 DNA samples from patients identified through two oncology clinics. The entire coding regions of *BRCA1* and *BRCA2* were screened by protein truncation test, heteroduplex analysis, or single-stranded conformation polymorphism followed by DNA sequencing of variant bands. Deleterious protein-truncating *BRCA1* and *BRCA2* mutations were identified in five patients or 18% of the entire cohort. Only 8% (1 of 13) of women with a family history of breast cancer, but no ovarian cancer, had mutations. The mutation rates were higher for women from families with a history of breast cancer and at least one ovarian cancer (three of six, 50%). One woman with a family history of undocumented cancers was also found to carry a deleterious mutation in *BRCA2*. The spectrum of mutations was unique in that one novel *BRCA1* mutation (1625del5) and three novel *BRCA2* mutations (1536del4, 6696delTC, and 7795delCT) were identified. No recurrent mutations were identified in this cohort, although one *BRCA2* (2816insA) mutation had been previously reported. In addition, two *BRCA1* and four *BRCA2* missense mutations of unknown significance were identified, one of which was novel. Taken together with our previous report on recurrent mutations seen in unrelated families, we conclude that African Americans have a unique mutation spectrum in *BRCA1* and *BRCA2* genes, but recurrent mutations are likely to be more widely dispersed and therefore not readily identifiable in this population.

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Introduction

Breast cancer is a major disease in women from most industrialized countries of the world, although the incidence in the USA has dropped from 180,000 to about 176,000 affected women in 1998. It is currently estimated that 5–10% of all breast cancers are caused by mutations in highly penetrant genes such as *BRCA1* and *BRCA2*. However, almost all studies performed to date have defined breast cancer risks and related genetic factors in Caucasian women of European descent. The spectrum of mutations identified in the USA population so far reflects mostly European migrations to North America, but the spectrum of mutations in the African American is beginning to be characterized (Arena et al. 1997; Gao et al. 1997; Mefford et al. 1999).

Although the incidence of *BRCA1* has been reported to be lower among breast cancer patients of African American ancestry (Newman et al. 1998), we previously reported three novel *BRCA1* mutations (1832del5, 5296del4, and 3883insA) in nine extended African American families with early-onset breast cancer (Gao et al. 1997). Two mutations, 1832del5 and 5296del4, were each identified in two unrelated families. In another study, Arena et al. (1996) reported three novel *BRCA1* mutations in African Americans, 943ins10, 3888delAG, and 4160delAG, with 943ins10 being identified in two unrelated families. The recurrent mutation 943ins10 has now been reported in other families from Washington D.C., Florida, South Carolina, Bahamas, and the Ivory Coast, suggesting an ancient origin in Africa for this mutation (Stoppa-Lyonnet et al. 1997; Mefford et al. 1999). A recent report, focused on 45 high-risk African American families, identified only two deleterious *BRCA1* mutations, 943ins10 and 3450del4 (Panguluri et al. 1999). Additional mutations have been identified in African American families who were involved in genetic studies of breast cancer families,

unselected for ethnic background (Futreal et al. 1994; Miki et al. 1994; Couch and Weber 1996; Ganguly et al. 1998), but it is unclear whether they are unique to the African American population. A population-based study of American women with breast cancer identified no disease-related *BRCA1* mutations in 88 black women, but family history and age of onset were not specified for the cases (Newman et al. 1998). Another population-based study by Ostrander and colleagues included only three African Americans, and no mutations were identified. (Langston et al. 1996). To date, there are no published reports on the spectrum of *BRCA2* mutations in African Americans. To further examine the contributions of *BRCA1* and *BRCA2* mutations in African American women with breast cancer, the entire coding regions and flanking exon-intron boundaries of *BRCA1* and *BRCA2* were examined in extended African American families identified from two different geographic locations in the US. This study represents the first report of *BRCA1* and *BRCA2* mutation analysis of clinic-based African American breast cancer families.

Materials and methods

Human subjects

We analyzed DNA samples from 28 African American breast cancer patients for mutations in both *BRCA1* and *BRCA2*. Twenty of the women had been identified because of their family histories of cancer, through high-risk clinics at the University of Chicago or University of Texas, Southwestern, Dallas. The remaining eight patients were African American women with breast cancer and modest family histories of cancer, who provided blood samples for genetic analysis. All gave informed consent after appropriate counseling according to the protocols approved by the different Institutional Review Boards. The women seen in high-risk clinics were either self-referred or referred by a physician and all were counseled about the risks, benefits, and limitations of genetic testing. All women were offered the opportunity to receive positive test results after confirmation in a CLIA-approved laboratory.

Mutation analysis

Screening for recurrent mutations

To estimate the prevalence of recurrent mutations in our cohort, we used allele-specific oligonucleotide (ASO) hybridization to detect five previously identified mutations in African Americans (for *BRCA1* gene: 1832del5, 5296del4, 3883insA, 943ins10, Met1775Arg). Genomic DNA from the samples under study were amplified with primers for the corresponding exons containing the mutations as previously described (Gao et al. 1997). The polymerase chain reaction (PCR) products were then denatured and applied to Hybond-N+ nucleic acid-transfer membranes with 96-well dot-blot apparatus. Both wild-type and mutant probes were end-labeled with 50 μCi of [α-^{32}P]dATP. Hybridization assays were performed with 30 μM labeled probes at 45°C overnight. The membranes were then washed and subjected to autoradiography. No recurrent mutations were identified in this cohort.

Screening for DNA variants

We next screened the entire coding sequence, splice junctions, and neighboring intronic regions of *BRCA1* and *BRCA2*, using a combination of single-strand conformational polymorphism (SSCP) analysis, heteroduplex analysis (HA), and protein truncation test (PTT), followed by DNA sequencing as previously published (Plummer et al. 1995; Gao et al. 1997).

SSCP analysis. For SSCP, genomic DNAs were amplified with primers matched to intron sequences flanking each coding exon except exon 11. Amplifications were performed according to standard PCR procedures, except that 1.0 μCi of [γ-^{32}P]dCTP was added. PCR products were mixed with an equal volume of formamide loading dye, heated to 95°C for 3 min, and chilled on ice. Electrophoreses were performed in 0.5× MDE (FMC Corporation) gels with 4–10% glycerol at 8 W at room temperature or 20 W at 4°C overnight. The gels were dried and exposed against Kodak X-ray films.

Heteroduplex analysis. For heteroduplex analysis, after standard PCR amplification, the PCR products were heated at 95°C for 3 min, then slowly cooled down to 37°C over 40 min, according to the manufacturer's instruction (FMC Bioproducts). After mixing with 6× triple dye loading buffer at 1:5 volume ratio, the PCR products were loaded on 1× MDE gels with 15% urea, and electrophoreses were performed at 400 V at room temperature for 14–20 h. The gels were stained with SYBR green I nucleic acid by diluting the stock 1:10,000 (FMC Bioproducts). The gels were photographed using 300-nm transillumination and Polaroid type 57 film.

Protein truncation test. For the protein truncation test, genomic DNAs of exon 11 of both *BRCA1* and *BRCA2* were screened by PTT with three overlapping PCR fragments, as previously published (Plummer et al. 1995). The forward primers include additional 5´ sequences containing the T7 promoter and a translation start site. PTT using the TNT-T7 coupled wheat germ extract system (Promega) was performed according to the manufacturer's instructions, incorporating [^{35}S]methionine or [^{35}S]cysteine. Samples were electrophoresed on 12.5% acrylamide gels and were exposed to Kodak X-ray film.

DNA sequencing. PCR products showing an electrophoretic variant pattern by SSCP, HA, or PTT were reamplified from the original genomic DNA and both strands were directly sequenced by the fluorometric method with automated sequencing procedures (Dyedeoxy terminator cycle sequencing kit and ABI377 DNA sequencer; Applied Biosystems).

Statistical analysis

Statistical analysis was performed using SPSS software package (SPSS, Chicago). The incidence of both *BRCA1* and *BRCA2* mutations was calculated for the entire sample, and subset analysis was performed by selected characteristics of the individual tested. The prevalence of *BRCA1* mutations in the study was compared with that of *BRCA2* mutations by Fisher's exact test. Logistic regression was used to model the probability of having identifiable mutations. Covariates included age of onset for the individual tested, number of family members with breast cancer, number of family members with ovarian cancer, and number with bilateral breast cancers.

Results and Discussion

A summary of the families studied, the number of relatives affected with breast or ovarian cancer or other types of cancers, the mean age of onset of affected cases, and mutation status, is listed in Table 1. Among the 28 cases examined, 20 women had family histories of a high incidence of breast and/or ovarian cancer among first-degree relatives. For these 20 women with hereditary breast can-

Table 1 Family information and mutation status of African American breast cancer patients with family histories of a high or moderate incidence of breast (*BC*) and/or ovarian (*OV*) cancer

Characteristics	Families (*n*)	Mutations (*n*)
From family history		
Families with 4 or more BC only in the first-degree relatives	1	1
Families with 2 or more BC only in the first-degree relatives	13	0
Families with 1 or more OV in the first-degree relatives	6	3
Families with other types of cancer or breast in second-degree relatives	8	1
From mean age at diagnosis (years)		
<30	2	1
31–40	3	0
41–50	9	2
51–60	4	2
>60	2	0

cer, the median age of onset was 46 years (range 23–65 years); there was a mean of 2.7 breast cancer cases per family and a mean of 0.3 ovarian cancer cases per family. Women with both breast and ovarian cancer were identified in one family, while bilateral breast cancer was reported in at least one woman in five families. The remaining eight cases had family histories of a moderate incidence of different cancers or breast cancers among second-degree relatives.

Five unique frameshift mutations were identified as detailed in Table 2. We found four deleterious mutations in the 20 cases (20%) with family histories of a high incidence of breast and/or ovarian cancer; whereas only one *BRCA2* mutation, 7795delCT, was identified among the eight patients with family histories of a moderate incidence of cancer (Fig. 1). The patient with this mutation developed breast cancer at age 51 years and is currently alive and well; her family history of cancer is undocumented, but her father may have died at a young age of pancreatic cancer. In the women with strong family histories, a deleterious *BRCA1* mutation was identified in 1 of 20 (5%), while deleterious *BRCA2* mutations were identified in 3 of 20 (15%). A *BRCA2* mutation was identified in the single family with more than six breast cancer cases and no ovarian cancer, giving an overall prevalence of 1 in 13 (8%) in the breast cancer-only families. *BRCA1* or *BRCA2* mutations were identified in three of six (50%) families with one or more ovarian cancers among first-degree relatives. The only *BRCA1* mutation identified in this cohort was found in a family with a woman with both bilateral breast cancer and ovarian cancer (Fig. 1). In addition, we identified six missense mutations in this cohort (Table 2).

Neither *BRCA1* S186Y nor S1140G falls within the functionally critical RING finger or BRCT domains. S1140G has been identified in several unrelated patients as well as control samples and probably represents a benign polymorphism (Panguluri et al. 1999). Indeed, the serine at *BRCA1* position 1140 corresponds to an aspartic acid in mouse and a glycine in dog homologs of *BRCA1*, indicating that amino acid substitutions at this position are functionally tolerated. The *BRCA1* S186Y missense mutation occurs in one of 132 normal control chromosomes from unaffected African Americans, suggesting it may represent a benign polymorphism. However, since serine residue 186 is perfectly conserved in rat, mouse, and dog, it is possible that the S186Y allele represents a deleterious allele with incomplete penetrance.

The *BRCA2* H2440R missense allele was detected in 2 of 132 normal control chromosomes from unaffected African Americans, and the *BRCA2* histidine residue 2440 is not conserved between species. Taken together, these observations suggest *BRCA2* H2440R is a benign polymorphism. The *BRCA2* missense alleles N108H,

Table 2 *BRCA1*/*BRCA2* sequence variants identified in African Americans (Age is mean age at diagnosis)

	Patient	Gene/exon	Designation	Effect	Breast cancer		Ovarian cancer		Other types of cancers
					Cases (*n*)	Age (years)	Cases (*n*)	Age (years)	
Protein-truncating mutations	BC581	BRCA1/11	1625del5	Frameshift	4	23	1	37	Lung, pancreatic cancers
	BC306	BRCA2/10	1536del4	Frameshift	6	46.4	0	NA	Prostate cancer
	95-19-1	BRCA2/11	2816insA	Frameshift	2	34	1	53	Pancreatic cancer
	BC351	BRCA2/11	6696delTC	Frameshift	2	56	1	65	Two lung cancers
	96-96-1	BRCA2/15	7795delCT	Frameshift	1	51	0	NA	Unknown cancer
Missense variations	96-97-1	BRCA1/9	S186Y	Unclassified	3	65	0	NA	Brain cancer
	95-19-1	BRCA1/11	S1140G	polymorphism	2	34	1	53	Pancreatic cancer
	BC358	BRCA2/4	N108H	polymorphism	1	40	1	30	Lung, chest cancers
	BC108	BRCA2/14	H2440R	polymorphism	1	27	0	NA	Gastric, liver, lung cancers
	BC123	BRCA2/14	Q2384K	polymorphism	6	57	0	NA	Stomach, colon cancers
	BC234	BRCA2/14	K2339N	Unclassified	5	62	0	NA	Prostate cancer

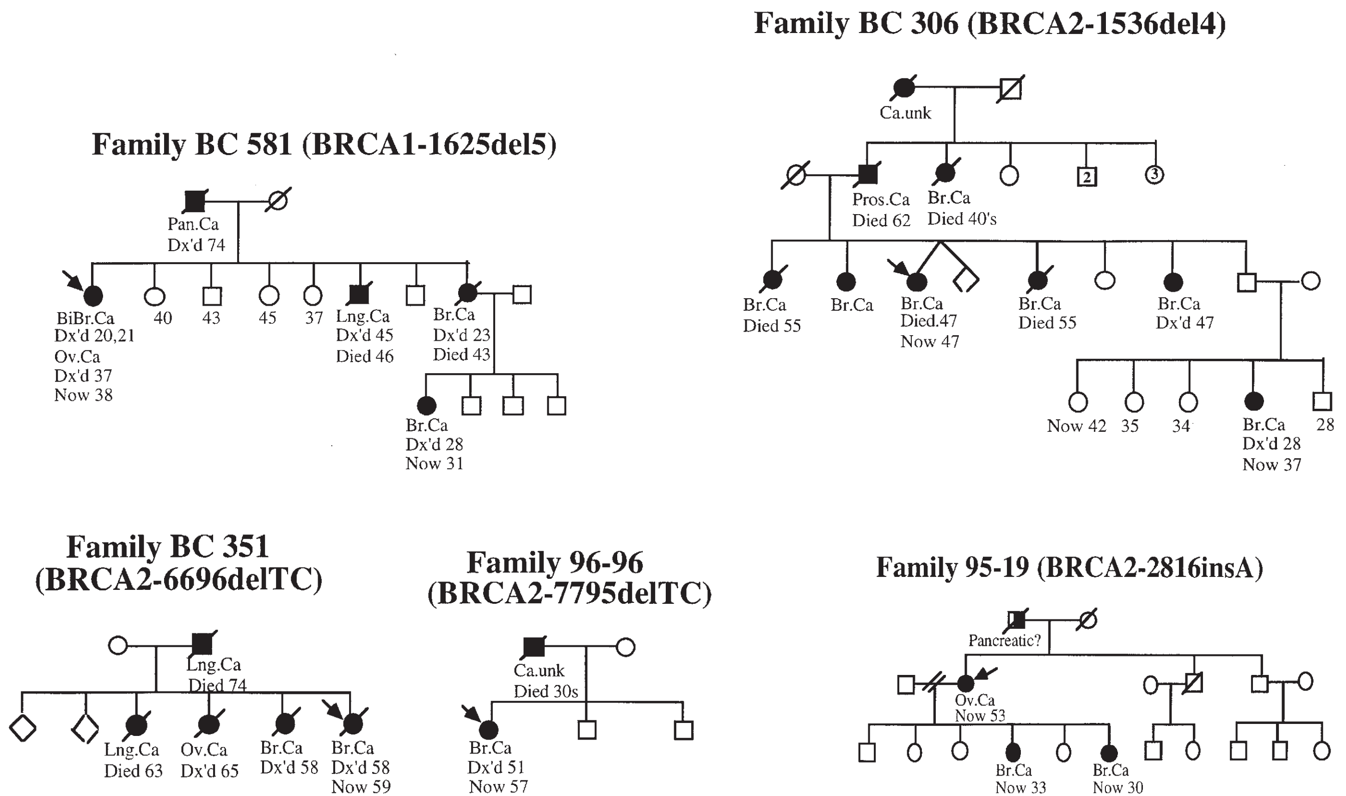

Fig. 1 Pedigrees of families with *BRCA1* or *BRCA2* mutations. *Solid circle*, patients diagnosed with cancers; *open circle*, unaffected relatives (*Lng.Ca* lung cancer, *Br.Ca* breast cancer, *Ov.Ca* ovarian cancer, *Ca.unk* cancer unknown, *Dx'd* diagnosed age)

Q2384K, and K2339N were not observed in the 132 control chromosomes. N108H has been previously classified as a benign polymorphism (Breast Cancer Information Core 1998) and, correspondingly, *BRCA2* residue 108 is not conserved between species. The *BRCA2* glutamine residue 2384 is not conserved between species, again suggesting that amino acid substitutions are tolerated at this position. Thus, the *BRCA2* Q2384K missense allele may represent a benign polymorphism. However, the lysine residue 2339 is perfectly conserved between species, suggesting that the nonconservative amino acid substitution in the K2339N allele may represent a deleterious mutation. While it is not possible to determine the physiological significance of an amino acid substitution by interspecies sequence comparisons, the degree of residue conservation may provide useful insights into the tolerance of a given position to substitutions.

In the present cohort, we identified only one protein-truncating *BRCA1* mutation. This mutation was found in a patient with early-onset bilateral breast cancer at ages 20 years and 21 years and ovarian cancer at age 37 years. In our previous report, we identified five *BRCA1* mutations in nine high-risk families (56%). These families were selected because they had more than a 20% prior probability of being *BRCA1* carriers according to the Shattuck-Eidens model (Shattuck-Eidens et al. 1997), and the individual tested in the family had to have breast cancer diagnosed before age 50 years or ovarian cancer at any age. All of the five *BRCA1* mutations in our previous study were found in early-onset breast and/or ovarian cancer families with mean ages at diagnosis of below 40 years. Four of the five *BRCA1* mutations were also identified in families with three or more cases of breast and/or ovarian cancer. The present cohort included eight families with family histories of moderate incidences of any cancer, and age of onset was not a selection criterion. Thirteen of the families had a mean age of onset of more than 40 years. Not surprisingly, the proportion of families with *BRCA2* mutations was higher in this cohort than the proportion with *BRCA1* mutations. Although the numbers are small, these data are in agreement with studies in other ethnic groups that suggest that *BRCA1* mutations contribute to more cases of early-onset breast cancer than do *BRCA2* mutations (Krainer et al. 1997).

Previous studies have suggested that inherited mutations in the *BRCA1* gene might be less common among breast cancer patients of African American ancestry than other groups studied (Newman et al. 1998). In the population-based study from North Carolina, no *BRCA1* mutations were identified among 88 black women, but age of onset of the cases was not specified. In a recent report focused on 45 high-risk African American families, only two (4%) deleterious *BRCA1* mutations were identified, but *BRCA2* was not analyzed (Panguluri et al. 1999). We have now completely screened a sizable number of clinic-based African American patients for mutations in both *BRCA1* and *BRCA2* genes. As a result, we have identified nine high-risk families (excluding the single case with a *BRCA2* mutation and no family history) with deleterious

Table 3 Family and clinical information in 28 high-risk African American families. All African American families with at least two cases of breast and/or ovarian cancer among first-degree relatives in our data base, who have been completely screened for *BRCA1* and *BRCA2*, were included in this analysis (*BR* breast cancer, *OV* ovarian cancer, *BiBR* bilateral breast cancer, *Pr* prostate cancer, *Panc* pancreatic cancer, *ND* not done, *NA* not available, *neg* negative)

Family (*n*)	BRCA1	BRCA2	Age of onset for proband	Median age of onset	(*n*) of BR in 1st-deg relatives	Total (*n*)			Other cancers
						BR	OV	BiBR	
BC351	neg	6696delTC	58	58	3	2	1	0	Lung
BC306	neg	1536del4	47	40	5	7	0	0	Pr
BC358	neg	N108H	40	35	1	2	2	0	Lung, chest
BC342	neg	neg	38	34	1	1	1	0	Lung
BC292	neg	neg	38	50	2	4	0	0	None
BC372	neg	neg	46	40	2	5	0	0	Lung, colon, Pr
BC79	neg	neg	35	35	2	5	0	0	Colon, Pr
BC39	neg	neg	30	30	2	2	0	0	None
BC33	neg	neg	36	44	2	2	0	0	Lung
BC5	neg	neg	43	46	1	1	1	1	Colon
BC47	neg	neg	NA	NA	3	3	0	1	None
BC123	neg	Q2384K	39	49	2	2	0	0	Colon
BC108	neg	H2440R	51	50	4	5	0	1	Pr
BC472	neg	neg	49	52	2	2	0	0	None
BC234	neg	K2339N	62	62	2	4	0	1	Pr
BC365	neg	neg	52	50	2	3	0	0	Lung, colon
97-73	neg	neg	46	45	2	2	0	0	Panc
96-97	S186Y	neg	58	65	2	3	0	0	None
95-19[a]	S1140G	2816insA	32	34	2	2	1	0	Panc
BC581	1625del5	neg	20	23	3	4	1	1	Lung, Panc
93-19[b]	1832del5	neg	34	35	4	7	2	2	Lung
95-95[b]	1832del5	neg	43	36	5	5	0	1	Pr
94-49[b]	5296del4	neg	24	34	4	6	0	2	Pr, colon
96-45[b]	5296del4	neg	40	40	2	3	2	0	Colon, Pr
96-75[b]	3883insA	neg	28	29	3	3	0	1	None
95-69[b]	neg	ND	51	45	4	5	0	2	None
95-59[b]	neg	ND	50	48	2	3	0	0	None
93-51[b]	neg	ND	47	50	3	10	0	0	None

[a]Family 95-19 was previously published as having no *BRCA1* mutation (Gao et al. 1997)
[b]Previously reported families (Gao et al. 1997)

mutations. This has allowed us to examine the probability of finding a *BRCA* mutation in African American families who meet a minimum eligibility criterion of two cases of breast and/or ovarian cancer among first-degree relatives, i.e., those with at least a 20% prior probability (Table 3). Using this criterion, the observed proportion with identifiable deleterious *BRCA1* mutations is 6 of 28 (21.4%; 95% CI, 8.3–41.0%), and the proportion with identifiable deleterious *BRCA2* mutations is 3 of 24 (12.5%; 95% CI, 2.7–32.4%). Our analysis would not have identified large deletions, noncoding region *BRCA1* and *BRCA2* mutations, or other unidentified genes that may explain familial clustering of breast cancer in the remaining families. We observed significant differences between the nine families with identifiable mutations and the 19 families without. Overall, the median age of onset in the first individual tested was lower in the group with mutations than in the group without (36.6 years versus 46.4 years, P=0.037). The age at onset of the first individual tested in each family was highly correlated with the median age of onset for all cases in the family (r=0.87). In addition, we found that the mean number of breast cancer cases among families with a mutation is 3.4 as compared to only 2.0 for those without a mutation (P=0.005). We observed only a slight relationship (not statistically significant) between mutation status and the familial incidence of ovarian cancer (data not shown). The relationship between mutation status and the familial incidence of bilateral breast cancer was also not statistically significant (data not shown), and the incidence of other cancers was unrelated to mutation status. Unfortunately, our numbers are too small to come to any definite conclusion after examining the joint relationship between the various covariates and mutation status in a logistic regression model.

In certain ethnic subpopulations, founder mutations, rather than mutation hotspots, are responsible for the increased frequencies of some mutations. Such founder mutations have been described in families from ethnic groups that were geographically or culturally isolated, e.g., *BRCA1* 185delAG in Ashkenazi Jews (Struewing et al. 1995) or *BRCA2* 999del5 in Icelandics (Thorlacius et al. 1996). Although we have previously identified recurrent mutations in unrelated families, we did not identify any recurrent mutations in this cohort. This is not surprising, given that only one ancient mutation, 943ins10 mutation, has been identified in families of West African ancestry (Mefford et al. 1999). It is quite likely that founder mutations in individuals of African descent are going to be

harder to characterize because of significant genetic admixture as well as the varied and wide dispersion of the gene pool in the African diaspora, across different geographic and linguistic regions. Nonetheless, our studies provide compelling evidence that *BRCA1* and *BRCA2* mutations are found among a significant proportion of high-risk families of African ancestry. Therefore, a more concerted effort to identify population-based families and to define the spectrum and penetrance of *BRCA1* and *BRCA2* mutations in this ethnic group is warranted. Only then will clinicians have the necessary tools for more accurate risk assessment and more effective cancer-control initiatives among African American women at high risk for breast and/or ovarian cancer.

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