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PRINCIPAL INVESTIGATOR: Carolyn W. Broome, Ph.D.

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FOREWORD

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Carolyn Browne

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INTRODUCTION

Mutations in single genes account for 3-8% of all breast cancer (Easton, 1994; Eeles et al., 1994). Early onset disease is common in hereditary breast cancer families. The incidence of breast cancer among blacks is higher than whites below the age of fifty (Ries et al., 1998). Most inherited breast cancer is due to the highly penetrant breast cancer predisposing genes *BRCA1* and *BRCA2*. Specific breast cancer predisposing mutations in *BRCA1* and *BRCA2* have been associated with different ethnic groups; therefore mutations in African Americans are expected to differ from those reported in other populations. Most studies of hereditary breast cancer have included few African Americans (Miki et al., 1994; Futreal et al. 1994; Castilla et al., 1994). In order to determine the spectrum of germline *BRCA1* and *BRCA2* mutations in African Americans, the entire coding regions and flanking introns are being examined in 75 breast cancer patients from families at high-risk of hereditary breast cancer. Thus, this study represents one of the largest collections of high-risk African Americans whose *BRCA1* and *BRCA2* coding sequences are being completely scanned for mutations. Mutations/variations detected in high-risk families are also being tested in controls, unselected for disease. This investigation will provide information for breast cancer genetic testing and genetic counseling in African Americans.

BODY

Task 4: To collect more blood samples from high-risk African American breast cancer patients, low- risk patients and controls. Items listed for the first 12 months have been accomplished. We have now recruited an additional 30 African American breast cancer families at high-risk for inherited breast cancer; the total number of high-risk families is 75. Samples from affected and non-affected family members have been collected. High-risk criteria were multiple cases (including first-degree, second-degree, and distant relatives in the same lineage) of breast cancer or multiple cases of breast and ovarian cancer per family; or breast cancer with early age of onset (≤ 40 years); or bilateral breast cancer; or breast and ovarian cancer in the same individual; or male breast cancer (Table 1, appendix).

In addition, we have 84 low-risk African American breast cancer families, who do not fit the high-risk categories for inherited breast cancer and 108 control subjects, who were not selected for disease. We need to recruit more subjects in these two categories.

Task 1: To identify *BRCA1* mutations in additional high-risk African American families. *BRCA1* mutation analysis using single-stranded conformational polymorphism (SSCP) followed by sequencing of variants in high-risk African American families has been extended from 45 families (Panguluri et al., 1999; Mefford et al., 1999) to 55 families as proposed in Task 1. Of the 10 additional families, one pathogenic mutation, 2495insG, was detected in a family with 4 cases of breast cancer (Table 2). This frameshift mutation is expected to produce a protein termination at amino acid residue 800, presumably deleting 57% of the *BRCA1* protein. Several polymorphisms and a variation in intron 7 were also detected; these are probably not disease associated.

Task 2: To identify *BRCA2* mutations in high-risk African American families. *BRCA2* mutation analysis using the protein truncation test (PTT) and SSCP followed by sequencing of variants has been undertaken in 69 high-risk African American families (Broome, 1998; Whitfield-Broome et al., 1999; Whitfield-Broome, 1999, see Appendix). Analysis of the

entire *BRCA2* gene is about 75% complete in the high-risk patients. Numerous presentations have been made at scientific meetings; see section on Reportable Outcomes.

Six pathogenic mutations have been detected, one mutation in each of the following patients: two male breast cancer patients, one patient from a family with 2 cases of breast cancer, two early onset patients, and one patient from a family with 3 cases of breast cancer and 2 cases of ovarian cancer (**Table 3**). All of these pathogenic mutations are frameshift mutations that are expected to cause protein truncation; 5 of these mutations were detected with the PTT. Four of these pathogenic mutations, 2001del4, 1991del4, 8643delAT, 4088delA, have not been reported previously. One of these protein truncating mutations, 2816insA, has been reported in both African American and Caucasian populations (Gao et al., 1998; BIC). Another mutation, 4075delGT has been reported in Caucasian populations (BIC).

A novel missense variant H2395L of unknown functional significance was observed (**Table 3**). Numerous silent changes, noncoding variations, and polymorphisms, which are probably not disease causing, were also observed (**Table 4**). Many of these variants have been reported previously to occur globally in control populations (Wagner et al., 1999; BIC).

Task 3: To functionally test *BRCA1* and *BRCA2* missense or intron mutations. This task is planned for the second year of the grant.

Discussion. Among 55 high-risk breast cancer patients, we have detected 5 pathogenic *BRCA2* mutations and 3 pathogenic *BRCA1* mutations. Usually the number of *BRCA1* mutations exceeds the number of *BRCA2* mutations; however, this observation could be a function of our high-risk population, which contains multiple case families with a mean age of diagnosis over 40. *BRCA2* mutations occur more frequently in older patients.

Our original hypothesis was that by studying a large number of high-risk African Americans with a family history of breast/ovarian cancer or early-onset breast cancer, we would detect the *BRCA1* and *BRCA2* mutations that are most common in the African American population. We expected that mutations in African Americans would differ from those reported in other populations. Our data support this hypothesis. Considering our results and those of other investigators, there is a broad spectrum of different pathogenic *BRCA1* and *BRCA2* mutations observed in the African American population. Most of these protein truncating mutations have not been detected in other populations; whereas, a few of these pathogenic mutations are observed in both African American and other populations. Many of the polymorphisms and noncoding variants, which are probably not disease related, are widespread among different populations.

One purpose of this research was to provide information for genetic testing and counseling. The numerous, distinct pathogenic mutations in *BRCA1* and *BRCA2* observed in African Americans reflects the high level of genetic diversity in people of African ancestry (Jorde et al., 1998). Because of this broad spectrum of distinct mutations, genetic testing for *BRCA1* and *BRCA2* mutations needs to involve the entire coding and flanking sequences in high-risk patients.

KEY RESEARCH ACCOMPLISHMENTS

- Additional high-risk families have been recruited; there are now 75 high-risk families.
- A novel *BRCA1* pathogenic, protein truncating mutation has been identified in a multiple case family.

- Six pathogenic *BRCA2* mutations have been identified, four of which have not been reported previously.
- A large number of different pathogenic *BRCA1* and *BRCA2* mutations are observed in the African American population; most are not observed in other populations.
- Genetic testing for *BRCA1* and *BRCA2* mutations needs to involve the entire coding and flanking sequences in high-risk patients.

REPORTABLE OUTCOMES

Abstracts and Publications

Whitfield-Broome C, (1999) Inheritance of Breast Cancer in African American Women: How Should We Monitor? Presentation, Howard University Women's Health Institute, Washington DC, April, 1999. The Proceedings of Health Issues and Concerns of Women of Color: A Call to Action, Health and Human Services, in press.

Presentations

Whitfield-Broome, C. (1998) Inherited breast cancer in African Americans. Segment on Heart and Soul, Black Entertainment Television, October, 1998.

Broome, C. (1998) Howard U. Cancer Center Symposium on "Innovative Scientific Advances in Breast Cancer Among African-Americans", Washington, D.C. October, 1998. High risk *BRCA1* and *BRCA2* screening in African American women. Invited presentation.

Broome, C. (1999) Breast cancer mutations in African Americans. Sigma Xi presentation, Howard University.

Publications on *BRCA1* – work performed before this grant

Whitfield-Broome, C., Dunston, G.M., Brody, L.C. (1999) American Association for Cancer Research, Philadelphia, April, 1999 *BRCA2* Mutations in African Americans, Proc. Amer. Assoc. for Cancer Research, 40:269, abstract #1788

Panguluri RCK*, Brody LC, Modali R, Utley K, Adams-Campbell L, Day AA, **Whitfield-Broome C**, & Dunston GM. (1999) *BRCA1* mutations in African Americans Human Genetics 105:28-31 and on line <http://dx.doi.org/10.1007/s004399900085>.

Mefford HC, Baumbach L, Panguluri RCK*, **Whitfield-Broome C**, Szabo C, Smith S, King MC, Dunston G, Stoppa-Lyonnet D, Arena F. (1999) Evidence for a *BRCA1* founder mutation in families of west African ancestry. Am J Hum Genet 65:575-578.

Development of cell lines

Leukocytes from ten of the high-risk patients have been transformed into permanent cell lines.

Training

As provided in the approved budget, a Ph.D. graduate research assistant, Yasmine Kanaan is working on this project.

CONCLUSIONS

In order to determine the spectrum of germline *BRCA1* and *BRCA2* mutations in African Americans, the entire coding regions and flanking introns are being examined in breast cancer patients from families at high-risk of hereditary breast cancer. Additional high-risk families have been recruited so that there are now 75 high-risk families. Thus, this study represents one of the largest collections of high-risk African Americans whose *BRCA1* and *BRCA2* coding sequences are being completely scanned for mutations. A novel *BRCA1* pathogenic, protein truncating mutation has been identified in a multiple case family. Six pathogenic *BRCA2* mutations have been identified; four of which have not been reported previously by other investigators. A missense variant of unknown functional significance was detected. Numerous polymorphisms and noncoding variants were observed in the *BRCA1* and *BRCA2* genes. The polymorphisms and noncoding variants, which are probably not disease related, are widespread among different populations.

A large number of different pathogenic *BRCA1* and *BRCA2* mutations are observed in the African American population; most of these protein truncating mutations are not observed in other populations. The importance of this work is for genetic testing and genetic counseling. The numerous, distinct pathogenic mutations in *BRCA1* and *BRCA2* observed in African Americans reflects the high level of genetic diversity in people of African ancestry. Because of this broad spectrum of distinct mutations, genetic testing for *BRCA1* and *BRCA2* mutations needs to involve the entire coding and flanking sequences in high-risk patients.

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APPENDICES

Table 1 African American breast cancer families at high risk for breast cancer predisposing mutations

Case category for selection	Number of cases (% total cases)
Multiple-case families ($\geq 3^a$)	35 (46%)
Multiple-case families (2^a)	27 (36%)
Early Onset (≤ 40 years) breast cancer	8 (11%)
Bilateral breast cancer	2 (3%)
Male breast cancer	2 (3%)
Breast and ovarian cancers ^b	1 (1%)
Total cases	75 (100%)
Cases tested ≤ 40 years	33 (44%)

^aNumber of breast cancer cases per family, including first-degree, second-degree, and distant relatives.

^bSame individual with both breast and ovarian cancers.

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Table 2 *BRCA1* sequence alterations in African American breast cancer patients. (*BR*, breast; number in *parentheses* indicates number of breast and ovarian cancer cases per family)

Case number	Cancer type	Age at diagnosis (years)	Exon	Nucleotide ^a /codon	Mutation	Amino acid change	Designation
Pathogenic, protein truncating mutation							
BC053	BR(4)	55	11	2495	Insertion G	Frameshift stop at codon 800 ^b	2495insG
Polymorphisms ($\geq 1\%$ of chromosomes in this or other studies) and Noncoding variations							
BC048	BR(2)	40	11	1256/379	T to G	Ile to Met	I379M
BC051	BR(2)	38	11	1256/379	T to G	Ile to Met	I379M
BC052	BR(2)	33	11	1186/356	A to G	Gln to Arg	Q356R
			11	2576/819	C to A	Ser to Tyr	S819Y
			Intron 7	-	C to T	Non-coding	IVS7+553C/T
BC054	BR(6)	76	11	2576/819	C to A	Ser to Tyr	S819Y

^aNumbering starting with the first nucleotide in the 5'-untranslated region of *BRCA1* cDNA (GenBank accession no. U14680).

^bIncluding newly inserted/deleted amino acids and stop codon.

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Table 3 *BRCA2* pathogenic mutations and missense variations in African American breast cancer patients. (BR breast, OV ovarian, number in *parentheses* indicates number of breast or ovarian cancer cases per family)

Case number	Cancer type	Age at diagnosis (years)	Exon	Nucleotide ^a /codon	Mutation	Amino acid change	Designation
Pathogenic Mutations							
BC051	BR(2)	38	10	2001/591	deletion TTAT	Frameshift Stop at codon 612	2001del4 ^C
BC021	Male BR(2)	44	10	1991/588	deletion ATAA	Frameshift Stop at Codon 612	1991del4 ^C
BC002	Early Onset	37	11	4088/1287	deletion A	Frameshift Stop at Codon 1292	4088delA
BC029	Male	59	11	2816/863	insertion A	Frameshift Stop at Codon 880	2816insA ^C
BC061	BR(3) OV(2)	44	11	4075/1283	2 base deletion: GT	Frameshift Stop at Codon 1287	4075delGT
BC001	Early Onset	33	19	8643/2805 8642/2805	2 base deletion: AT T to C	Frameshift Stop at codon 2811	8643delAT
Missense variant of unknown functional significance							
BC011	BR(3)	57	14	7412/2395	A to T	His to Leu	H2395L

^aNumbering starting with the first nucleotide in the 5'-untranslated region of *BRCA1* cDNA (GenBank accession no. U14680).

^bIncluding newly inserted/deleted amino acids and stop codon.

^cWhitfield-Broome et al., 1999.

Table 4 *BRCA2* sequence variations in African American breast cancer patients. (BR breast, OV ovarian, Bi bilateral, number in *parentheses* indicates number of breast or ovarian cancer cases per family)

Case number	Cancer type	Age at diagnosis (years)	Exon	Nucleotide ^a /codon	Mutation	Amino acid change	Designation
Silent changes							
BC038	BR(2)	68	3	459/77	T to G	Silent thr	T77T
Noncoding variations							
BC049	BR(3)	67	2	214	A to C	Noncoding 5' UTR	214A>C
BC025	BR(3)	56	2	218	C to T	Noncoding 5' UTR	218C>T
BC018	Bi	59	8	intron 7	deletion CTTAA	Noncoding	IVS7+611 del5
BC002	Early onset	37	14	intron 14	A to C	Noncoding	IVS14+64A/C
Polymorphisms ($\geq 1\%$ of chromosomes in this or other studies)							
BC051	BR(2)	38	14	7470/2414	A to G	Silent, ser	S2414S
BC015	Early onset	39	14	7470/2414	A to G	Silent, ser	S2414S
BC026	BR(6)	68	14	7470/2414	A to G	Silent, ser	S2414S
BC052	BR(2)	33	14	7470/2414	A to G	Silent, ser	S2414S
BC054	BR(6)	76	14	7470/2414	A to G	Silent, ser	S2414S
BC037	BR(2)	68	14	7625/2466	C to T	Ala to Val	A2486V
BC038	BR(2)	68	14	7625/2466	C to T	Ala to Val	A2486V
BC025	BR(3)	56	2	203	G to A	Noncoding 5' UTR	203G>A
BC002	Early onset	37	14	intron 14	C to T	Noncoding	IVS14+53C/T
BC006	BR(3)	52	14	7663+53	C to T	Noncoding	IVS14+53C/T
BC013	Early onset	37	15	intron 15	C to T	Noncoding	IVS15+5C/T
					C to T	Noncoding	IVS15+7C/T
					N to T	Noncoding	IVS15+28N/T

^aNumbering starting with the first nucleotide in the 5'-untranslated region of *BRCA1* cDNA

**Inheritance of Breast Cancer in African American Women:
How Should We Monitor?**

Carolyn Whitfield-Broome, Ph.D.

Department of Biochemistry & Molecular Biology, Howard University Cancer Center,
Howard University College of Medicine, Washington, DC 20059

Abstract

Inherited mutations in single genes account for 3-8% of all breast cancer. Most inherited breast cancer is due to the highly penetrant breast cancer predisposing genes *BRCA1* and *BRCA2*. Families at high-risk of inherited breast cancer are those with multiple cases of breast cancer in the same (maternal or paternal) lineage, one first-degree (mother, sister) relative with early-onset (<age 50) breast cancer, two first-degree relatives with breast cancer, ovarian cancer, bilateral breast cancer, or male breast cancer. Our data coupled with that of other investigators reveal a large number of distinct pathogenic mutations and variations among African Americans. Most of these variations have not been reported among Caucasians. Preliminary results reveal more *BRCA2* pathogenic, protein truncating mutations than *BRCA1* pathogenic, protein truncating mutations. This diverse spectrum of variations/mutations is consistent with the high level of genetic diversity in people of African ancestry. Due to this genetic diversity, the entire *BRCA1* and *BRCA2* coding and flanking sequences need to be tested in high-risk African American patients.

This manuscript or an edited transcript of my presentation at the conference *Health Issues and Concerns of Women of Color: A Call to Action*, will be published by Health and Human Services, 1999. A clean copy of the edited transcript is not available.

Most breast cancer is sporadic, unrelated to a family history of the disease. In African Americans, 86% of breast cancer occurs over the age of 50 (Ries et al., 1998). Inherited mutations in single genes account for 3-8% of all breast cancer (Easton, 1994; Eeles et al., 1994). Early-onset (< age 50) disease is common in hereditary breast cancer families. The incidence of breast cancer among blacks is higher than whites below the age of 48; whereas mortality is higher in African Americans compared to whites between the ages of 28 and 75 (Ries et al., 1998). The higher mortality among African Americans is primarily due to the late stage of disease on diagnosis, tumor pathogenic differences, and comorbid illnesses (Eley et al., 1994). The pathogenic features of aggressive estrogen-negative and poorly-differentiated tumors are more common in black women than in white women (Moormeier, 1996).

Eighty-four percent of inherited breast cancer is due to the breast cancer predisposing genes *BRCA1* and *BRCA2* (Miki et al., 1994; Wooster et al., 1995; Ford et al., 1998). About half of all cases of hereditary breast cancer and over 80% of families with hereditary breast and ovarian cancer have been linked to germline mutations in *BRCA1* (Ford et al., 1998). Whereas, *BRCA2* mutations account for about one-third of hereditary breast cancer and three-quarters of families with male and female breast cancer (Ford et al., 1998). Mutations in *BRCA1* are also associated with an increased risk of ovarian, prostate, colon, liver and bone cancers (Ford et al., 1994). *BRCA2* mutation carriers are at increased risk of ovarian cancer, pancreatic, prostate, esophageal, and stomach cancers (Wooster et al., 1995; Berman et al., 1996). Most mutation carriers are heterozygous, having a normal and a mutant gene. Loss of heterozygosity for the normal chromosome was observed in tumors from patients with hereditary breast cancer, suggesting that *BRCA1* and *BRCA2* are tumor suppressor genes. Recent investigations demonstrate that *BRCA1* and *BRCA2* function in repairing double strand DNA breaks, homologous recombination, and regulating transcription (Patel et al., 1998; Fuks et al., 1998; Chen et al., 1998; Zhang et al., 1998).

Next to gender and age, the most important risk factor for breast cancer is family history. *BRCA1* and *BRCA2* mutations are highly penetrant. The risk of disease with *BRCA1* or *BRCA2* mutations is 37-84% by age 70 compared to 11% by age 80 for noncarriers (American Cancer Society, 1994; Easton et al., 1993, 1995; Struewing et al., 1997; Thorlacius et al., 1998; Ford et al., 1998). Thus, individual carriers of *BRCA1* or *BRCA2* mutations are at increased risk of disease but some carriers may live to age 80 without developing breast cancer. *BRCA1* and *BRCA2* mutation carriers with breast cancer have a 10-fold increased risk of ovarian cancer (Frank et al., 1998).

Other factors that have been shown to increase breast cancer risk in the general population and may affect risk in mutation carriers are early age of menarche, nulliparity, late age of first birth, late age of menopause (Gail et al., 1989); thus, longer exposure to estrogens increases risk. Additional factors that may affect carcinogenesis are other genes and environmental factors such as diet and alcohol consumption.

Testing for inherited breast cancer mutations. Genetic testing may occur within a research protocol or in a clinical laboratory. It is important that the clinical laboratory is certified as specified by the Clinical Laboratory Improvement Act. When testing is appropriate is still a matter for discussion in the medical community. Genetic testing is considered in families with multiple cases of breast cancer in the same (maternal or paternal) lineage, one first-degree (mother, sister) relative with early-onset (<age 50) breast cancer, two first-degree relatives with breast cancer, ovarian cancer, bilateral breast cancer, or male breast cancer (Rosenthal et al., 1999; Hoskins et al., 1995). It is important to test an affected family member first to identify the potential mutation. If a mutation has been identified in the family, a negative

result is informative. If a mutation has not been identified in the family, a negative result may be due to the absence of a *BRCA1* or *BRCA2* mutation or the inability to detect it by the method used. Most every method of mutation detection may miss certain variations.

Genetic counseling is essential before and after testing because of the pros, cons, and uncertainties of testing, individual risk, and prevention. The advantages of genetic testing are the knowledge that a negative result in a family with known carriers reduces the noncarrier's risk to that for the general population. For women with a positive genetic test, the National Institutes of Health and National Human Genome Research Institute task force recommends monthly breast self-examinations, annual breast and pelvic clinical examinations and mammograms starting at age 25 (Burke et al., 1997). Prophylactic mastectomy or prophylactic oophorectomy may be considered; they reduce but do not eliminate all possibility of disease. It is not known if tamoxifen reduces the risk of breast cancer in women with *BRCA1* or *BRCA2* mutations. As discussed earlier, there is an increased risk of breast cancer in mutation carriers, but no certainty that an individual will present with the disease. The disadvantages of genetic testing are the high costs of commercial testing (\$2500); the negative effects on obtaining health insurance; emotional problems such as anxiety, depression, guilt, family stress; the difficulty in interpreting the effect of certain alterations, such as amino acid substitutions.

***BRCA1* and *BRCA2* mutations in African Americans.** Specific breast cancer predisposing mutations in *BRCA1* have been associated with different ethnic groups. For example, the *BRCA1* 185delAG allele is present in nearly 1% of Ashkenazi Jews (Struwing et al. 1995). Among Dutch breast cancer patients, 36% of *BRCA1* mutations are large deletions (Petrij-Bosch et al., 1997). The specific mutations observed appear to be due to founder effects, rather than to mutational hot spots. Most studies of hereditary breast cancer have included few African Americans (Miki et al., 1994; Futreal et al. 1994; Castilla et al., 1994). Our group at the Howard University Cancer Center is asking the question: What is the spectrum of germline mutations in the breast cancer predisposing genes *BRCA1* and *BRCA2* among African Americans? The answer to this question is important for genetic testing and for determining the frequency of breast cancer mutations in African Americans. The entire coding regions and flanking introns are being examined in 65 breast cancer patients from families at high-risk of hereditary breast cancer. Patients were considered high-risk if they had a family history ($2 \geq$ cases) of the disease, early-onset ($\leq$ age 40) breast cancer, bilateral breast cancer, breast and ovarian cancer, or male breast cancer. Thus, this study represents one of the largest collections of high-risk African Americans whose *BRCA1* and *BRCA2* coding sequences are being completely scanned for mutations.

BRCA1 mutations have been screened using single stranded conformation polymorphism (SSCP). Using specific primers, *BRCA1* DNA is amplified and labeled with radioactive deoxynucleotides, denatured, and the single-stranded DNA is separated on a non-denaturing gel. With a heterozygous mutation, two normal bands and two mutant bands are observed (Orita et al., 1989). Mutant bands are sequenced to determine the alteration. With *BRCA1*, the following alterations were observed on complete screening of 45 high-risk families: two pathogenic, protein truncating mutations, one in a patient with breast and ovarian cancer and one in a patient from a family with five cases of breast cancer; four amino acid substitutions; one amino acid polymorphism; and four substitutions in non-coding regions (introns) (Panguluri et al., submitted). One of the pathogenic mutations (943ins10) has been observed in four additional families; all five families appear to have a common, distant African ancestor (Stoppa-Lyonnet et al., 1997; Arena et al., 1996, 1997, Melford et al., submitted).

Screening of *BRCA2* is about 60% complete. Both SSCP and the protein truncation test have been used to detect mutations. Using specific primers, *BRCA2* DNA was amplified and transcribed into RNA *in vitro*. The RNA was translated *in vitro* into protein with radioactively-labeled methionine. After SDS gel electrophoresis, a normal size protein and a smaller, truncated protein is observed with a heterozygous mutant. The DNA sequence of the mutation is determined. Four pathogenic, protein truncating mutations in *BRCA2* have been identified: two in male breast cancer patients, one in a female with early-onset breast cancer, and one in a female with early-onset breast cancer from a family with 2 cases of breast cancer (Whitfield-Broome et al., 1999, abstract). One of the male breast cancer mutations has been reported previously in an African American with ovarian cancer (Frank et al., 1998; Gao et al., 1998 abstract).

Our data coupled with that of other investigators (Miki et al., 1994; Futreal et al. 1994; Castilla et al., 1994; Gao et al., 1997; Stoppa-Lyonnet et al., 1997; Arena et al., 1996, 1997, 1998, abstracts; Shen et al., 1998, abstract; Ganguly et al., 1998 abstract; Gao et al., 1998 abstract) reveal a large number of distinct pathogenic mutations and variations among African Americans. Most of these variations have not been reported among Caucasians (BIC, 1998). Preliminary results reveal more *BRCA2* pathogenic, protein truncating mutations than *BRCA1* pathogenic, protein truncating mutations. This diverse spectrum of variations/mutations is consistent with the high level of genetic diversity in people of African ancestry (Jorde et al., 1998). Due to this genetic diversity, the entire *BRCA1* and *BRCA2* coding and flanking sequences need to be tested in high-risk patients.

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