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PRINCIPAL INVESTIGATOR: Selina A. Smith, Ph.D.

CONTRACTING ORGANIZATION: University of Miami
School of Medicine
Miami, Florida 33136

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Selina A. Smith, Ph.D.

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Miami, Florida 33136

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An increased rate of breast cancer has been observed in African-American women under 50 years of age. Few epidemiologic studies of breast cancer focus explicitly on African-American women, and it is not established whether standard risk factors apply. This study seeks to determine relationships between risk factors and genetics in breast cancer etiology among pre-menopausal African-American women. Breast cancer cases and their primary female relatives (PFRs), were administered several questionnaires (dietary, psychosocial, reproductive, genetic and lifestyles) related to disease risk. Cases were matched by ethnicity and age to two cancer-free women participating in a screening mammography program . DNA samples were extracted from the blood of all cases and PFRs. Data on selected reproductive risk factors indicate that cases and controls are similar except with a difference in age at first birth. The diets of cases and their PFRs were similar. More cases than controls reported taking Vitamins A, C & E. Ninety four (94) percent of cases are *pleased with emotional support provided by family/friends.* Further work is in progress to identify BRCA1 gene mutations in women with affected relatives with breast/ovarian cancer.

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Selena A. Sirois 12/4/98

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ABSTRACT

After increasing rapidly from 1973-90, breast cancer incidence leveled from 1990 to 1995. Mortality, also previously on the rise, has dropped over the past five years, but only for white women. Rates have declined in younger black women; although they are still higher than those of white women have and are improving more slowly. African American women under 50 years of age have the highest rate of new cases of breast cancer in the nation, and they tend to present at an earlier age with larger tumors and a more advanced stage disease. This excess in breast cancer incidence among young black women may be due to increased exposure to known risk factors, exposure to new risk factors, and/or a decreased exposure to protective factors, or due to genetic factors. Studies designed to detect a possible molecular basis for this difference have not been reported. The presence of specific mutations within defined race/ethnic groups, and the paucity of data on the African American population, raises intriguing questions regarding the existence of specific mutations in African Americans. The present project is aimed to begin to answer these questions, specifically the statistical and potentially functional importance of polymorphic variants in these genes in at-risk African American women. The results of these first studies conducted in three families indicated that each proband had characteristic SSCP differences, which were confirmed to be mutations in exon 11 of BRCA1, which were previously unreported at that time (*BIC*). The proband from family 94003 had a 10 bp insertion at nucleotide position 943, causing a premature termination codon at amino acid 289. The proband from family 93004 had a GA deletion at codon 1257, resulting in premature termination of protein synthesis, and the proband from family 94027 had an AG deletion at codon 1354, causing a frame shift and premature stop codon. The initial screening of the BRCA1 locus, including all exon and intron boundaries, has been completed in the first twenty families. Only one deleterious mutation was detected. This alteration, a GTCT deletion at position 3785, was detected in the proband of family 96017, which is classified as a moderate-risk family, with a woman and her paternal grandmother affected . The mutation is predicted to truncate the protein at amino acid 1263. An additional two different polymorphisms in linkage disequilibrium were detected in two high-risk unrelated families. The first, detected in family # 96019, was a linked polymorphism 67 bases into intron 22 and a 3' UTR variant described previously in African Americans. In this family, the polymorphism was detected in 4/4 affected women, and 4/5 at-risk women who were too young to evidence disease symptoms, which is itself an important point. No other abnormality was detected. In the second family, #97001 (High Risk; Category I) a novel polymorphism was detected in the 8th position of intron 22, linked to the same 3' UTR variant in the proband; other family members are being recruited and analyzed for the presence of this variant. The predicted effect of this variant on BRCA1 splicing is unclear, since very few variants in this proximity to the 5′ donor splice site for this, or other genes, have been reported. These combined findings strongly suggest that specific African American BRCA1 mutations and variants exist. These variants may play a causal, but unexplained role in the progression of breast cancer in African American patients and their families and a low germ-line frequency of BRCA1 mutations exists in African American breast cancer patients and families, implicating additional, yet unknown genes.

INTRODUCTION

Cancer incidence and death rates for all cancers combined and for most of the top ten cancer sites declined between 1990 and 1995. According to the latest report from the American Cancer Society (ACS), National Cancer Institute (NCI) and the Centers for Disease Control and Prevention (CDC), this reversed an almost 20-year trend of increasing cases and deaths in the United States (1). After increasing rapidly from 1973-90, breast cancer incidence leveled from 1990 to 1995. Mortality, also previously on the rise, has dropped over the past five years, but only for white and Hispanic women. The breast cancer death rates remain leveled for black women. According to NCI, the data suggest the trend is starting to move in a positive direction for African American women. Rates have declined in younger black women; although they are still higher than those of whites have and are improving more slowly.

Among women under 50 years of age, the breast cancer incidence in African Americans exceeds that in whites (2,3). African American women under 50 years of age have the highest rate of new cases of breast cancer in the nation (4). African American women tend to present at an earlier age with larger tumors and a more advanced stage disease. This excess in breast cancer incidence among young black women may be due to increased exposure to known risk factors, exposure to new risk factors, and/or a decreased exposure to protective factor (5), or due to genetic factors. Studies designed to detect a possible molecular basis for this difference have not been reported.

Epidemiologic studies have shown risk factors such as age, socioeconomic class, race/ethnicity, lifestyle and reproductive factors increase a woman's chance of developing breast cancer. There have been few epidemiologic studies of breast cancer focusing explicitly on African American women, and it is not established whether the standard risk factors apply. Evidence relative to younger versus older African American women is also scarce. In work by Mayberry (6), risk of breast cancer among black women younger than 40 years of age was nearly three times greater for those who used oral contraceptives for more than 10 years relative to never-users (odds ration, 2.8; 95% confidence interval, 1.2 to 6.8). The risk was more than four times greater for severely obese women (body mass index $\geq$ 32.30 km/m^2) relative to women whose relative weights were less than 24.90 kg/m^2. The report also indicated similarities among younger and older black women with regards to additional breast cancer risk factors (i.e., surgical menopause, age at first full term pregnancy and multiple births).

In 1990, linkage studies localized an inherited susceptibility gene for breast cancer aggregates in certain families to chromosome 17q (7). Generally, women who inherit mutations of the BRCA1 gene are more likely to develop cancer than those who do not are. Female carriers of BRCA1 mutations are estimated to have an 85% lifetime risk of developing breast cancer, with more than 50% of breast cancers occurring before the age of 50 years (8). It is however, very likely that other genes may be responsible for inherited breast cancer. The BRCA1 gene has 24 exons and encodes for a protein of 1,863 amino acids. A second breast cancer gene, BRCA2, located on chromosome 13 q12-13, has been cloned (9). The BRCA2 gene has 27 exons and encodes for a protein of 3,418 amino acids (Figure 1) (10).

These genes were originally considered to be classical tumor suppresser genes. Recent work, however, is beginning to provide evidence indicating function similarities among the two genes and also that the proteins made by BRCA1 and BRCA2 genes play a critical role in enabling cells to repair their DNA when damaged (11-14). Recently another gene, PTEN (localized on chromosome 10q23) was cloned and found to be responsible for Cowden disease (OMIM 158350), a dominant disorder involving multiple hamartomarous lesions, macrocephaly, and mental retardation. Since breast cancer develops in approximately 30-50% of women affected with Cowden disease, PTEN, considered a tumor suppresser, may play a role in inherited cases of breast cancer.

The majority of the known mutations in both BRCA1 and BRCA2 are small insertions or deletions, which result in a frame shift and the premature termination of protein synthesis. The mutations are usually scattered throughout these genes, as can be viewed in the *Breast cancer Information Core Database (BIC) (http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/).* However, within defined ethnic groups, specific mutations are likely to recur. For example, in Iceland, the BRCA2 999del5 founder mutation occurs in most breast cancer families and in 0.4% of Icelandic controls (15). In Ashkenazi Jews (Jews of Eastern European ancestry), three founder mutations have been described in breast cancer families: I85delAG and 5382insC in BRCA1 and 6174delT in BRCA2 (16-18). One of these mutations was found in 31% of 80 Ashkenazi women with early-onset (<42 years of age) breast cancer and in 41% of 27 Ashkenazi women with a positive family history who were diagnosed with breast cancer at 42-50 years of age (18-20). In a series of 220 North American Ashkenazi breast cancer families, one of the founder mutations accounted for 45% of all families. It also accounted for a significantly higher percentage (73%) of families with a history of ovarian cancer (21). The combined population frequency of these mutations in a number of large series of young Ashkenazi controls approaches 2.5%: the BRCAl I85delAG, 1.0%; the BRCA1 5382insC, 0.1%; and the BRCA2 6174delT, 1.4% (22, 19, 23, 24).

The actual incidence of germ-line BRCA1 and BRCA2 mutations in the general population, as compared to high-risk groups, remains a point of controversy. According to *Myriad Genetics*, the largest commercial BRCA1/BRCA2 DNA testing operation, thirty-nine percent of women who meet minimal testing requirements harbor a deleterious mutation in either BRCA1 (26%) or in BRCA2 (12%) (25). Interestingly, another 23% of these women do not have detectable deleterious mutations, but rather have gene "variants of unknown functional and clinical significance", including missense and intronic mutations. It is important to note that in this highly selected group, 38% of these women had no detectable BRCA1/BRCA2 alteration! Recent studies conducted by other groups suggest that the prevalence of these germ-line alterations, even in high-risk families, may actually be as low as 13-16%. All the recent data agrees that the vast majority of BRCA1 mutations are detected in individuals from moderate- to high-risk families *(ibid.).* When selecting for an early age of breast cancer, a positive family history is required to exceed a greater than 3% probability that a BRCA1 mutation will be detected *(ibid.).* Together, this data strongly suggests the presence of additional genetic factors, possibly unidentified genes, which contribute to the etiology and occurrence of breast cancer in families.

To date, most of the molecular analyses of BRCA1 and BRCA2 mutations in affected women with a family history of breast cancer have been performed in white families, and very little information, until recently, has been published concerning possible mutations in African Americans (26,27). The BIC Database, accessible through the Internet, gives no information on patient race/ethnic origin, although the vast majority of data is known to represent analysis of white samples. Nonetheless, a recent study published in JAMA (28), reports that in a North Carolina cohort of African American breast cancer women, ranging in age from 20-74 years and unselected for family history, zero of eighty-eight patients had a detectable BRCA1/BRCA2 mutation. Yet, gene "variants of unknown functional and clinical significance" were detected in a substantial number of African American women, with some of these appearing either specific, or in increased frequency, in African Americans patients *(ibid.)*.

The presence of specific mutations within defined race/ethnic groups, and the paucity of data on the African American population, raises intriguing questions regarding the existence of specific mutations in African Americans. The present project is aimed to begin to answer these questions, specifically the statistical and potentially functional importance of polymorphic variants in these genes in at-risk African American women.

BODY

Recruitment: Recruitment for Study Year 4 was hampered by many factors, as discussed under separate cover. To-date, the study has enrolled 130 breast cancer cases of the 200 targeted. The project is now on target with efforts to enroll one (1) family group (breast cancer case and primary female relative) per week (n=52). The remaining 18 cases are currently being identified through intense physician referrals. Each family group member has completed the Breast Cancer Risk Appraisal (BCRA), Health, History and Habits Questionnaire (HHHQ), 24-Hour Dietary Recall, Psychosocial Questionnaire. Blood collection for DNA extraction was also collected for all participants. Family history forms were administered to capture information related to illnesses and causes of death within family groups.

With the one-year, no-cost extension granted by the funding agency, recruitment is back on track.

Objectives: The overall objectives and specific aims of the study remained unchanged and are as follows:

1. **Analyze multigenerational reproductive and lifestyle risk factors in African American family groups.**

   - Conduct a breast cancer risk factor appraisal
   - Evaluate the role of food choice and food practices in breast cancer risk
   - Examine the role of obesity and adipose distribution in breast cancer risk

2. **Determine psychosocial profiles of breast cancer family groups.**

    - Examine the impact of psychosocial factors on breast cancer

3. **Construct pedigree data and bank samples for conducting linkage studies to map breast cancer genes by analyzing the co-transmission of candidate markers, breast cancer and risk factors in multiple-case families.**

    - Identification of families carrying the BRCA1 gene

        - Identify common risk factors in high risk families
        - Determine if breast cancer is familial due to specific genes or because risk factors themselves are familial

RESULTS

While data collection related to the epidemiologic portion of this research continues this reports focuses on the molecular or genetic portion of the study. The results that follow are related to our on-going investigations of BRCA1 and BRCA2.

With the discovery of a second gene associated with increased risk for breast cancer, BRCA2, we have had the opportunity to begin to analyze the distribution and prevalence of alterations in these genes in twenty at-risk African American families, as well as control samples. These families are divided into three arbitrary categories, based on their relative risk: high-risk (HR; three affected 1st degree relatives; 10/20 families), moderate-risk (MR; two affected 1st degree relatives, 7/20 families) and undetermined risk (UR; single affected with medical information being updated, 3/20 families).

Our preliminary data, largely derived from analysis of the BRCA1 locus, suggest that African American women with a family history of breast cancer have characteristic mutations and polymorphic variants not so far observed in whites. One of these mutations (943ins 10bp) is present in 3 out of 87 cases of African American women diagnosed with early onset breast cancer, suggesting a possible founder effect. In addition, the frequency of BRCA1/BRCA2 germ-line mutations in the study population to date has been quite low, indicating the presence of yet-unidentified genes which may contribute to the specific form of breast cancer reported in African American women.

This project began with a limited study of three African American breast cancer patients with a positive family history. An initial interview was conducted with the proband of each family, and a detailed family history, as well as pathological verification of a reported breast cancer, was obtained. Patients were provided with a pretest educational session, where they also reviewed and signed the consent form. Blood samples were collected and genomic DNA was prepared. In these initial studies, the actual molecular investigations were performed at ONCOR MED (Bethesda, MD), however, by 1996, all molecular investigations were being completed in Dr. Arena's (former Co-Principal Investigator) laboratory at University of Miami. The approach has been the same at both laboratories. Methods included:

1. To PCR amplify all (including splice junctions), or a portion of, the BRCA1 and/or BRCA2 genes from genomic DNA from the family proband (and in some cases, other family members);

2. To screen these gene regions for possible mutations using Single Strand Conformational Polymorphism (SSCP) analysis, and

3. To determine the DNA sequence of any SSCP variants.

The results of these first studies conducted in the three families indicated that each proband had characteristic SSCP differences, which were confirmed to be mutations in exon 11 of BRCA1, which were previously unreported at that time *BIC*. The proband from family 94003 (Figure 2A) had a10 bp insertion at nucleotide position 943, causing a premature termination codon at amino acid 289. The proband from family 93004 had a GA deletion at codon 1257, resulting in premature termination of protein synthesis, and the proband from family 94027 (Figure 2B) had an AG deletion at codon 1354, causing a frame shift and premature stop codon.

To further evaluate the specificity of these alterations in the African American population, the presence of these newly described BRCA1 mutations in a second group of 59 African American women with histologically confirmed breast cancer, aged 50 or younger was investigated (Figure 3). In 42 patients, the breast cancer represented a sporadic event in the family, however, 14 of them had at least one first, second, or third degree relative with breast cancer and were classified as familial cases. For each family, blood samples were collected from the proband and from family members, including at least two generations. Family members of the index cases included mother, sister(s), and/or female offspring. The search for possible mutations in these 59 women was directed only to exon 11 of BRCA1 and was performed in Dr Arena's laboratory. Four regions of exon 11, surrounding the above-described mutations, were PCR amplified, screened for possible mutations by SSCP (Figure 4), and variants DNA sequenced to determine location and nature of a possible alteration. These studies resulted in the detection of the 10 bp insertion at codon 943 in another family unrelated to family #94003 discussed above. In addition, a previously undescribed polymorphism in exon 11 (A3557G) was detected in 4% of African American controls, but not in 46 white chromosomes analyzed. These results were presented at the American Society of Human Genetics as a platform presentation in October, 1996 (26).

These studies were followed by an analysis for the presence of the 943ins10bp in additional 25 African American women with histologically confirmed breast cancer, aged 50 and younger. In this sample, another case of the same 943ins10bp mutation was found. All three families with this mutation were recontacted and claimed not to be related. An analysis of the haplotypes showed that these three families shared the same haplotype in a region of 700kb in chromosome 17q21.

The initial screening of the BRCA1 locus, including all exon and intron boundaries, has been completed in the first twenty families. Only one deleterious mutation was detected. This alteration, a GTCT deletion at position 3785, was detected in the proband of family 96017 (Figure 5), which is classified as a moderate-risk family,

with a woman and her paternal grandmother affected. The mutation is predicted to truncate the protein at amino acid 1263. Additionally, two different polymorphisms in linkage disequilibrium were detected in two high-risk unrelated families (Figure 6 for pedigrees). The first, detected in family # 96019, was a linked polymorphism 67 bases into intron 22 and a 3' UTR variant described previously in African Americans.

In this family, the polymorphism was detected in 4/4 affected women, and 4/5 at-risk women who were too young to evidence disease symptoms (Figure 6), which is itself an important point. No other abnormality was detected. In the second family, #97001 (High Risk; Category I) a novel polymorphism was detected in the 8th position of intron 22, linked to the same 3' UTR variant in the proband; other family members are being recruited and analyzed for the presence of this variant. Together, these constitute V97001 for the purposes of this proposal. The predicted effect of this variant on BRCA1 splicing is unclear, since very few variants in this proximity to the 5' donor splice site for this, or other genes, have been reported (*HGMD, Human Gene Mutation Database, http://www. uwcm.ac.uk/uwcm /mg/search/).*

The frequency of these BRCA1 linked polymorphisms has been examined in several control groups. The first control group is that of 50 randomly selected African American samples obtained from Jackson Memorial Hospital. There are no specific demographic indices available for these samples, and there is no information as to their previous history of breast, ovarian, or any other cancer. Nevertheless, all 50 subjects (100 chromosomes) were examined for the presence of the two-above described BRCA1 variants,V96019 and V97001, and their frequency was detected at 3% and 1%, respectively.

The second set of control samples analyzed were 24 African Americans control individuals representing three different, unrelated kindreds with another disorder (available in Dr. Baumbach's (current Co-Principal Investigator) laboratory), who did not have a known history of breast cancer. None of these individuals had either V96019 or V97001. The third set of controls was that of Caucasian samples. The first set analyzed represented 25 random samples (50 chromosomes) - neither of these BRCA1 variants was detected. The second set of Caucasian samples consisted of 21 individuals representing 10 unrelated Caucasian families without histories of breast cancer - again, neither V96019 or V97001 (or their individual variants in intron 22 or the 3'UTR) were detected. In total, 35 white controls (70 chromosomes) have yielded normal SSCP patterns at these positions. Therefore, we believe that these combined findings strongly suggest that specific African American BRCA1 mutations and variants exist. These variants may play a causal, but unexplained role in the progression of breast cancer in African American patients and their families and a low germ-line frequency of BRCA1 mutations exists in African American breast cancer patients and families, implicating additional, yet unknown genes.

In 1997, the rather onerous task began of trying to identify possible mutations in BRCA1 and/or BRCA2 in the proband from each of these 20 families. The strategy, although labor-intensive and time-consuming, was relatively

straightforward. The entire BRCA1 and BRCA2 loci were to be analyzed using a series of exon-specific PCR primers, followed by SSCP analysis as previously described. All SSCP variants were evaluated for the presence of mutations by direct genomic sequencing using bands eluted from SSCP gels, as well as the purified original PCR product.

Similar analyses (as for BRCA1) are ongoing for BRCA2 in these twenty families, as well as any newer families recruited into the study. To date, 39 PCR primer sets, amplifying exons 2-11, 23 and 25, have been optimized and are being used in testing. Exons 9, 23, 23, and most of exon 11 have been analyzed in the 20 families, without any abnormal SSCP (Figure 6) patterns detected. The remainder of exon 11 is being completed next, followed by the remaining BRCA2 exons.

SIGNIFICANCE

The relevance of this project can be viewed in light of the increasing incidence of breast cancer in African Americans women under age 50 in the United States, which now exceeds the incidence in white women. In this age group, African American women have the highest rate of incident breast cancer in the nation. In addition, African American women of all ages have a larger percentage of poorly differentiated breast cancer, are more likely to occur at an earlier age, and have tumors which are estrogen and progesterone receptor negative, all factors associated with more aggressive tumors.

This increased risk for more malicious breast cancer in African American women may be due to increased exposure to known risk factors, increased exposure to new risk factors, or decreased exposure to protective factors or interactions between any of these and specific mutations. Few epidemiological studies of breast cancer have focused explicitly on African American women, and it is not established whether the standard risk factors apply.

The previous finding of a BRCA1 founder mutation in African Americans, as well as our recent findings of unique mutations and variants, should have strong implications to improving and optimizing genetic counseling in high-risk African American women. It would also stimulate more studies within this population, generating needed data. Early identification of potentially high-risk patients may lead to better primary and secondary prevention and earlier intervention. Analysis of cases within families, as well as studies of the type of genetic defect, could be helpful in designing preventive strategies (mammography, chemoprevention, or prophylactic surgery), as well as helping select appropriate therapeutic interventions.

Lastly, the recent observations concerning the occurrence of African American polymorphic variants (in at least BRCA1), and the opportunity to further investigate the inheritance, and ultimately, the functional significance of these genetic alterations may provide important new insights into the molecular basis of familial breast cancer. Based on our findings thus far, it is expected that as an end result to the proposed studies, a cohort of high- to- moderate-risk African American families will remain without detectable BRCA1 or BRCA2 gene abnormalities. A number of these will certainly be suitable for future studies focused on the identification of

new genes and/or genetic alterations which contribute to breast cancer in African American women, as well as the possible functional significance of selected BRCA1/BRCA2 polymorphic varaints.

SUMMARY & CONCLUSIONS

In summary, similar to data for the Ashkenazi Jewish patients, this data indicated that the 943ins10bp represented a unique founder mutation in African Americans with early onset breast cancer. We have identified twenty at-risk African American breast cancer families, who have been and continue to be under intense molecular investigation. These families sort into three categories, based on their relative risk:: high-risk (HR; three affected 1st degree relatives; 10/20 families), moderate-risk (MR; two affected 1st degree relatives, 7/20 families) and undetermined risk (UR; single affected with medical information being updated, 3/20 families). Additional families continue to be identified and recruited, such that the current number is 24 families, with another fifty families expected by the end of 1998.

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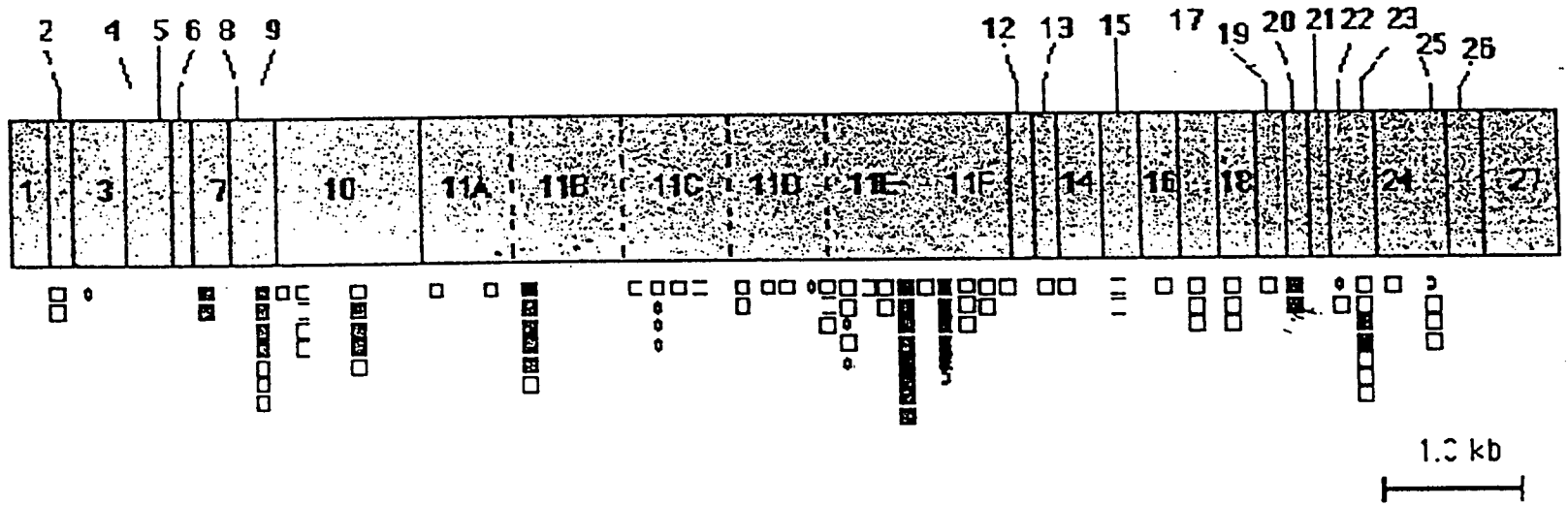


Figure 1. SCHEMATIC DIAGRAM OF THE BRCA2 GENE.

Displayed in the figure is a diagram of the BRCA2 locus indicating positions of exons (by numbers), with various reported mutations shown in shaded squares below the gene structure. Scale as shown is equivalent to 1.0 kb. (Diagram obtained from Breast Information Core)

Figure 2A. FAMILY 94003.

Haplotype analysis of BRCA1 reveals a shared haplotype (shown in shading) with family 95027 shown in Figure 2B. Patient III-2 and her affected mother II-2 carry the same 10bp insertion mutation at BRAC1 cod 943.

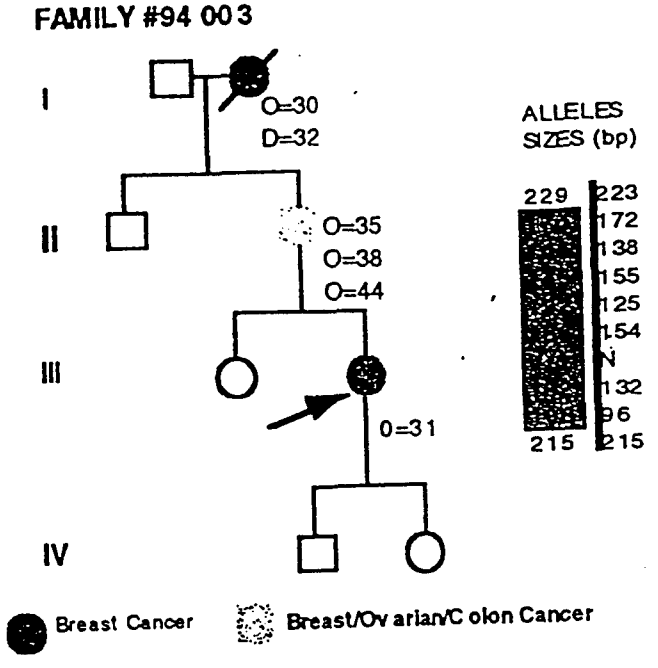


Figure 2B. Family 95027.

Haplotype analysis of BRCA1 reveals a shared haplotype (shown in shading) with family 94003 shown in Fig. 2A. Patient III-2 and her affected mother II-2 carry the same 10bp insertion mutation at BRCA1 codon 943.

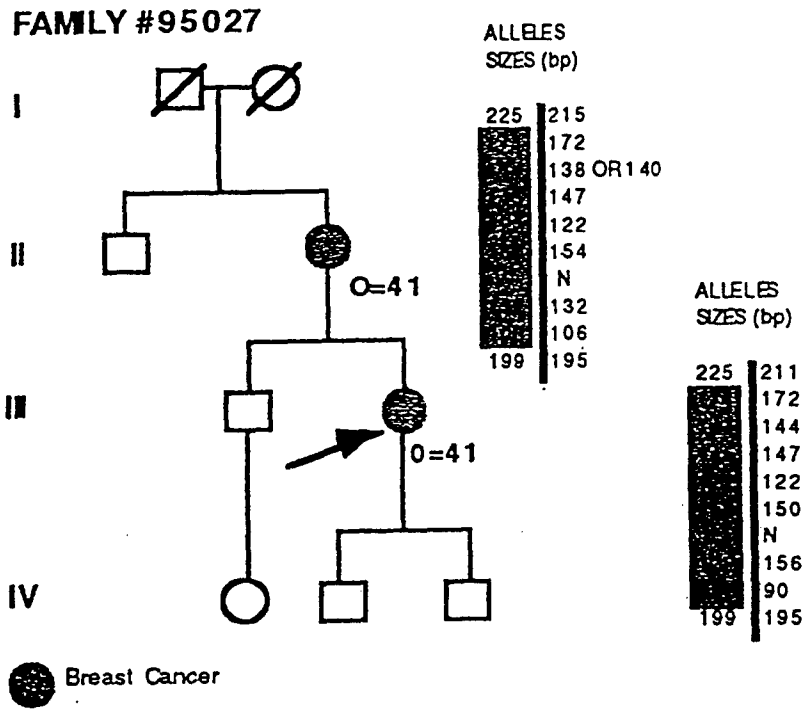


Figure 3. Representative Pedigrees of the Twenty-Four at Risk Families.

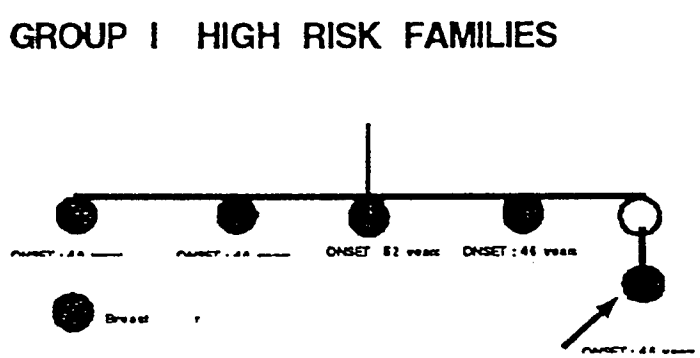


Figure 3. Representative Pedigrees of the Twenty-Four at Risk Families.

GROUP II – MODERATE RISK FAMILIES

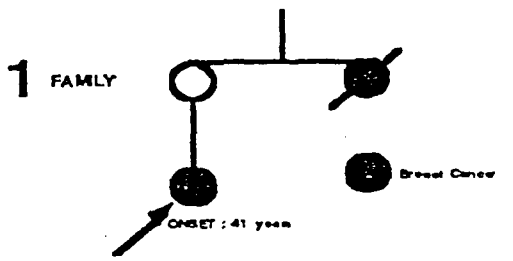


Figure 4. Representative SCCP Analysis of BRCA1.
SCCP Pattern using primers to amplify exon 22 in patients (Lanes 7,13,20) and control samples. Note SCCP variants in the patient lanes.

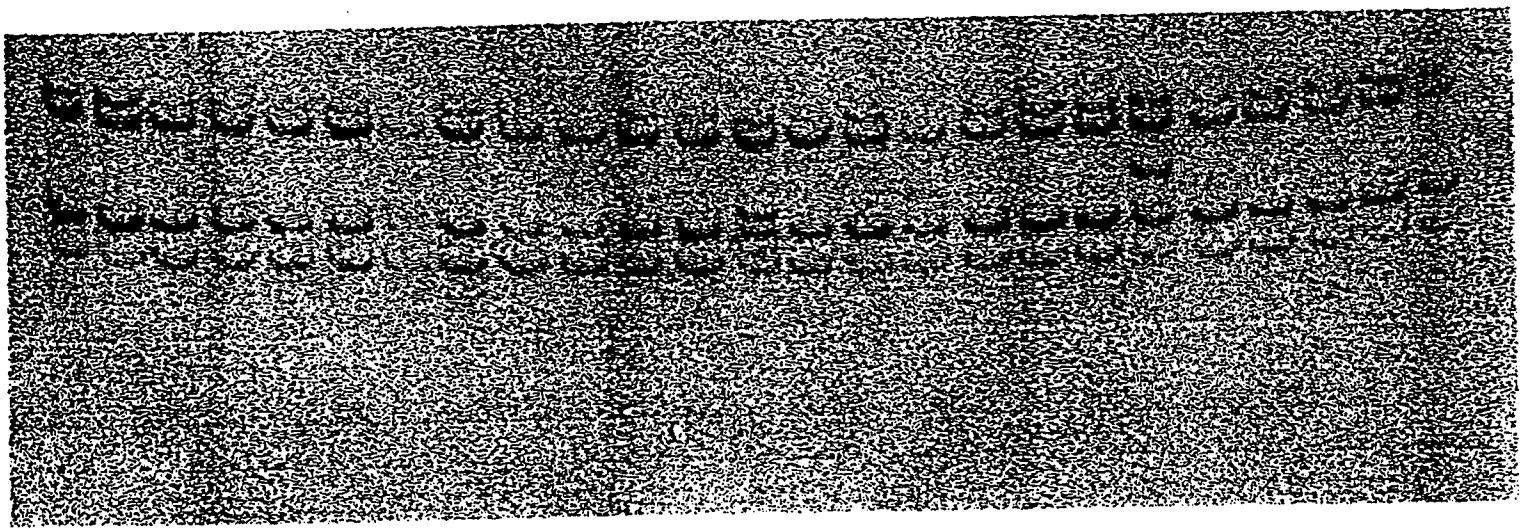


Figure 5. Pedigrees of Three Families Where BRCA1 alterations were detected.

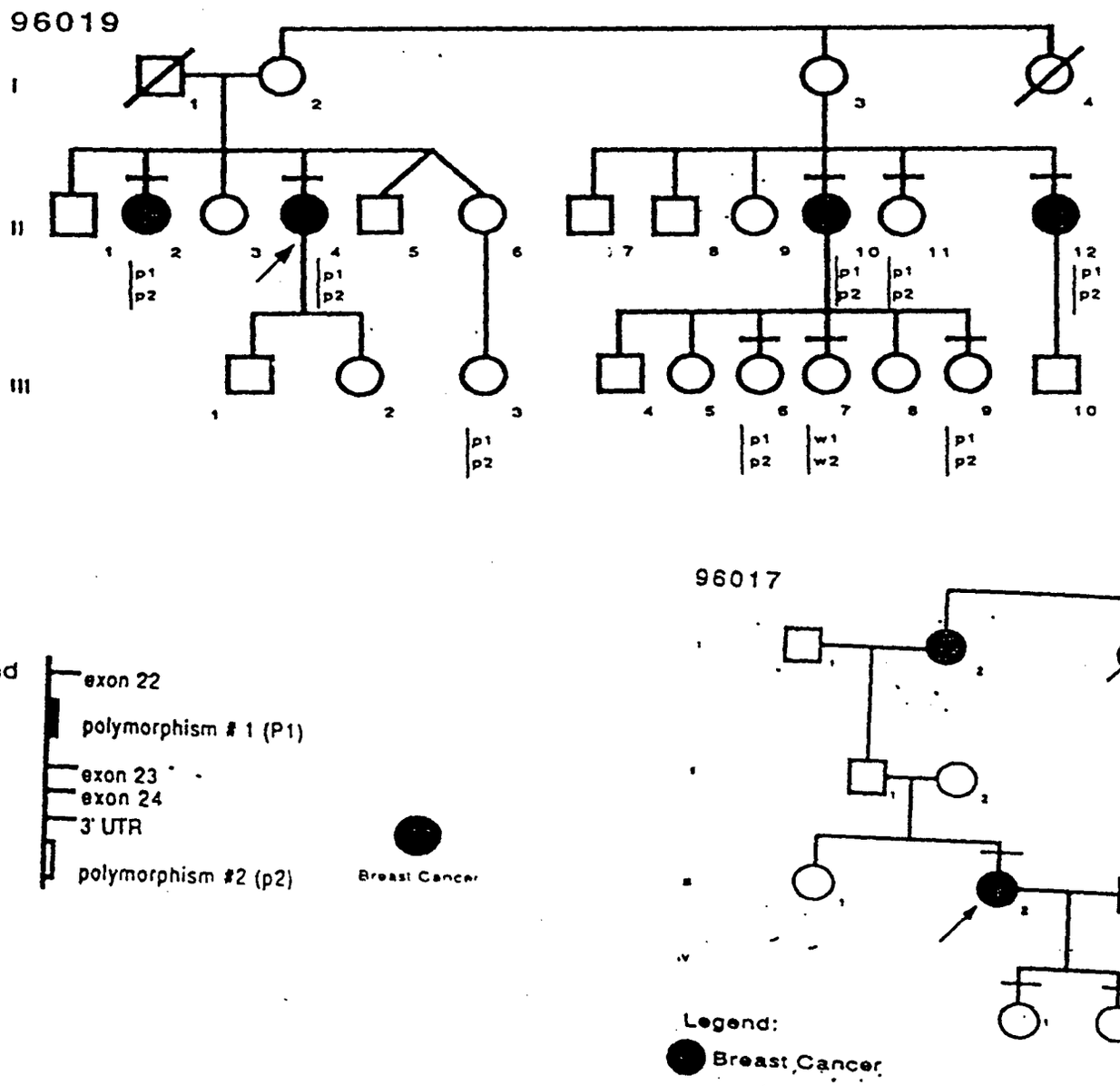
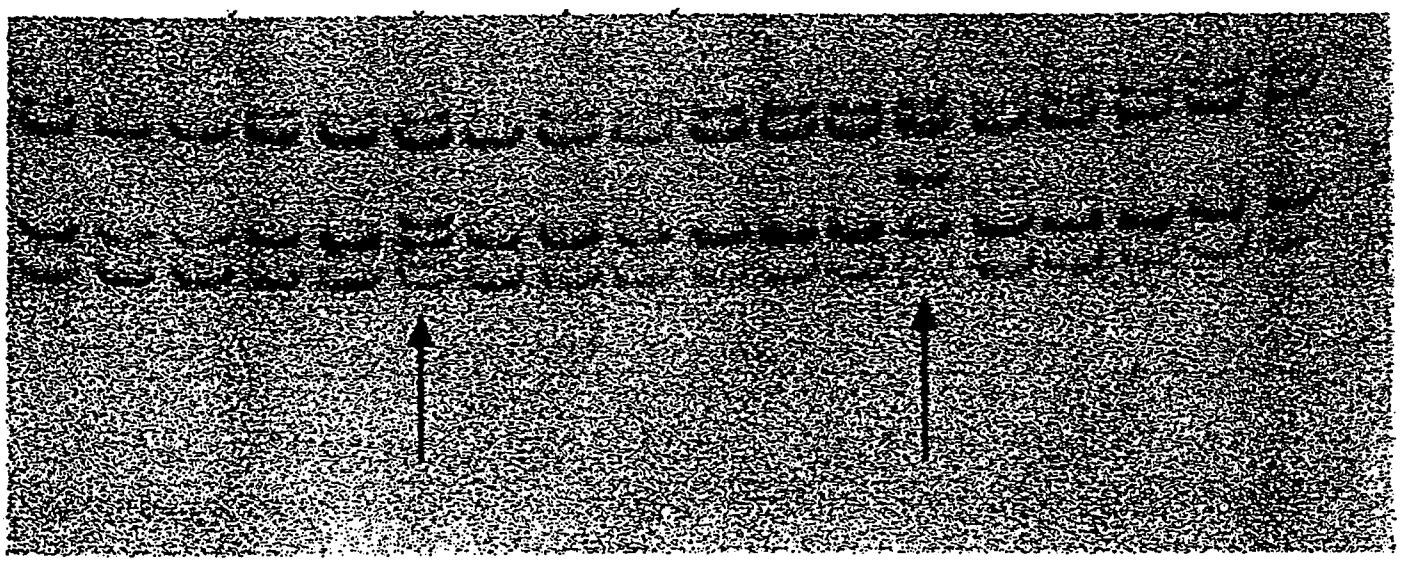


Figure 6. Two Distinct Variants Concerning a Linked Polymorphism are Shown. The polymorphisms are located between intron 22 and the 3′ UTR region in a set of African American non-selected controls. Lane 6 identifies a base change at the intronic 8th position while lane 13 identifies a base change at the 67th position of intron 22.



INFORMED CONSENT
Multigenerational Breast Cancer Risk Factors in African American Women

PURPOSE:
You are being asked to participate in a project to study risk factors in breast cancer patients and their families. This is a screening program and will not include a complete examination. If you agree to participate in this study, you will be asked questions related to your medical history, your diet, and your family history.

PROCEDURES:
The interview will be conducted in your home or over the phone and will take approximately 1 hour. Some participants will be asked to take part in a research study to see if known and suspected risk factors for breast cancer are present in patients with breast cancer and their family groups. A family group will be defined as mother, sister(s), and/or female offspring. In order to participate in the study, the breast cancer patients and at least two members of her family group must agree to participate in the study. If agreed, you will be asked to complete a breast risk appraisal form. This form will be administered to you by a health researcher and will take 20 to 30 minutes to complete. Information on your usual and present diet will also be obtained. A laboratory technician may draw 20 ml (about 1 tablespoon) of blood from you in order to examine the DNA content of your blood. The DNA content of the blood sample will be frozen and stored for future testing.

Genetics research is an increasingly important way to try to understand the role of genes in human disease. You have been given this consent form because University of Miami investigators want to include your tissue, cell or blood sample in a research project, or because they want to save such biological samples for future research. There are several things you should know before allowing your tissues, cells or blood to be studied or to be stored for future study.

1. Your tissue, cell or blood sample will be stored under your name or other unique identifier. If there is a medical reason to seek specific information from you in the future, your doctor will tell you about this. A process called "genetic counseling" is often appropriate in such cases; you should ask your doctor or nurse about this if you have any questions.

2. Your confidentiality will be protected to the extent permitted by law. Your records might be reviewed by government officials or by corporate research sponsors. The University of Miami collaborates with many other organizations, and data are generally shared among them. No data shared with other investigators will include your name or other public identifier, however.

I.R.B.
APPROVAL DATE: 10/9/98
INITIALS: M.A.

3. Genetic research may affect your insurability. For instance, information about your DNA might result in discrimination that would make it difficult for you to obtain certain health insurance coverage in the future. You will still be responsible for paying for health care, however. The University of Miami will not be responsible for such costs, even if care is needed for a condition revealed during research or clinical testing.

4. You have the right to refuse to allow your tissue or blood to be studied or saved for future study. You may withdraw from a study at any time, and remove from research use any samples that contain identifiers after the date of your withdrawal. This means that while the university might retain the identified samples--the law often requires this-- they would not be used for research. Samples without identifiers might still be retained for research; a different consent form is usually used in such cases.

5. Genetic information about you will often apply (in one degree or another) to family members. It is not generally the University's policy to provide genetic information about you to your family members. However, certain studies, called "pedigree studies," share such information among family members. For this and related research, you will be asked if you are willing to share your genetic information with your family members.

6. Your tissue samples might be used to develop commercially valuable medical products. By signing this form you agree not to seek a share of any proceeds that might result; that is, you waive any claim to share in the commercialization of products developed from your tissue or blood samples.

7. In addition to your name, other information about you might be connected to your blood or tissue sample. For instance, information about race, ethnicity, sex, your medical history, and so forth might be available to investigators studying your tissue or blood. Such information is important for scientific context and sometimes for public health. It is possible that genetic information might come to be associated with your racial or ethnic group.

8. It is possible that more tissue or blood samples will be obtained than are necessary for your treatment. That is, investigators might take samples purely for study purposes.

9. Genetic research raises difficult questions about informing you and other subjects of any results, or of future results. Some people want to know what is found out about them; others do not. The risks of knowing include anxiety and other psychological distress, and the possibility of insurance discrimination. The risks of not knowing what is found include not being aware of the need for treatment. These risks can change depending on whether there is a treatment or cure for a particular disease.

10. **In this study, investigators will *not* tell you what they find out about you, nor will they contact you if a test becomes available to diagnose a condition you might have or later develop.**

    For instance, suppose the investigators discover that your tissue sample carries a gene for a disease. Neither the university nor your doctor will try to contact you or find you to tell you about this gene. While we might not know how to test for a particular disease gene today, we might be able to test for it in the future. The number of genes for which this will be possible in the future is quite large—and a policy that required contact at a later date would be overwhelming.

11. The presence of a genetic marker does not necessarily mean that a patient will develop a disease. Informing people of all such markers independently of medical need can cause unnecessary anxiety. Conversely, the absence of a marker does not mean that someone will not get the disease. "Genetic diseases" appear as a result of a complex mixture of hereditary, environmental, behavioral and other factors.

12. There are alternatives to notification by investigators. If you are concerned about a potential genetic malady, you and your doctor might choose to test specifically for it; this would require additional blood or tissue samples. You should discuss this option with your doctor or genetic counselor.

13. With respect to the future use of tissue samples, you agree that: 1) you waive the right to consent to or be notified of any future research, test or analysis which might be performed using these tissue samples, and 2) you waive any rights to any results or information generated by any future research, tests or analysis using those tissue samples and assign any such rights to the University of Miami.

These are the best-known risks and challenges of genetic research. There might be other risks we do not know about yet. No direct benefits can be promised from your participation, though some people find satisfaction in contributing to scientific knowledge about genetic problems and their medical consequences. It is very important that you talk to your doctor, nurse or genetic counselor if you have questions or concerns about the research study or any of the information in this document.

RISKS:
There are some risks involved in having your blood drawn. There is the risk of temporary discomfort and/or bruising at the site of puncture and fainting. On rare occasions infection or the formation of a small clot or swelling to the vein and surrounding area may occur.

BENEFITS:
Although no benefit is promised to you from your participation in this study, the knowledge gained could prove beneficial to you and your family members specifically, and to the African American community in general. There will be no costs associated with your participation.

COMPENSATION:
In any screening program diseases or medical conditions may be uncovered that are not related to the medical condition under study, it is agreed that any conditions that are uncovered will be the ultimate responsibility of you to follow-up. If any abnormal findings are uncovered, you will be referred to a physician for further diagnosis and follow-up. The responsibility of seeking this follow-up is up to you. You are authorized all necessary medical care for injury or disease which is the proximate result of your participation in this research. Other than medical care that may be provided, you will not receive any compensation for your participation in this research study.

CONFIDENTIALITY:
Your consent to participate in the study includes consent for the investigator and her assistants to review all your medical records as may be necessary for the purpose of the study. The investigator and her assistants will consider your records confidential to the extent permitted by law. Your records and results will not be identified as pertaining to you in any publication without your expressed permission. Your stored blood samples will not be used in any other studies without your expressed permission. In rare circumstances, the U.S. Food and Drug Administration (FDA) or the U.S. Department of Health and Human Services (DHHS) may request copies of your records. If this happens, the FDA or DHHS request will be honored. Representatives from the U.S. Army Medical Research, Development, Acquisition and Logistics Command are also eligible to inspect the records of this research project as a part of their responsibilities to protect human subjects in research. Your records may also be reviewed for audit purposes by authorized University of Miami employees or other agents who will be bound by the same provisions of confidentiality.

ALTERNATIVE:
You have the right not to participate in this study.

RIGHT TO WITHDRAW:
Participation in this research project is voluntary and you have the right to withdraw from or refuse to participate in this study at any time with no prejudice to you seeking future medical care at the UM/JMMC. You may ask and will receive answers to any questions concerning your rights as a research subject by contacting Maria Arnold, Institutional Review Board Director, University of Miami, at (305)243-3327. A copy of the signed consent form will be provided to you. If you agree to participate in this study, please initial the front page of the consent form and sign below.

I have read and understand the Informed Consent and agree to participate in the project and I agree to have blood drawn for the DNA testing.

CONSENT FOR SUBJECTS:

_____ _____
Signature of patient Date

_____ _____
Signature of witness Date

For specific questions about the research study or to report any research related problems, you may contact Selina A. Smith, Ph.D., Principal Investigator (305)243-6599. For specific medical related questions about this study you may contact Dr. Stephen Richman, M.D. (305) 243-4909.

Revised 9/15/97

BRCA1 MUTATIONS IN AFRICAN-AMERICAN WOMEN.

J. F. Arena, S. Smith, M. Plewinska, L. Gayol, V. Vincek, F. Villegas, E. Perera, P. Murphy, MC. King, C. Szabo and H. Lubs. University of Miami School of Medicine, Miami Florida. OncorMed Inc., Gaithersburg, Maryland.

ABSTRACT:

Breast cancer in African-Americans is associated with a poorer prognosis than in Caucasians. African-American women tend to present at an earlier age with larger tumors and a more advanced stage disease. Studies designed to detect a possible molecular basis for this difference have not been reported. As part of a pilot project investigating the presence of BRCA1 mutations in the South Florida population, 3 African- American patients with a strong family history of breast cancer were investigated. Mutations were found in all three cases and all were previously unreported mutations in exon 11 (943 ins 10bp, 3888 del GA and 4160 del AG). Because of these findings, we investigated fourty-two additional African-American patients with early onset breast cancer for the presence of mutations in exon 11 of BRCA1. Eight patients (19%) had a positive family history for breast/ovarian cancer with at least one more affected relative, but were not as suggestive of BRCA1 in terms of an early age of onset and number of affected relatives. All others cases (81%) were sporadic. Our search, using four sets of PCR primers, was directed to the regions in exon 11 surrounding these mutations. Only one mutation was found, a second 943 ins 10bp mutation in an unrelated family. However, we found a previously undescribed polymorphism in exon 11(3537 A to G), which was present in 4 of 100 chromosomes from African-American controls and in none of 46 chromosomes of White controls. We conclude that, at least in our series, African-Americans with early onset breast cancer and strongly positive family histories may carry BRCA1 mutations different from other ethnic groups and that further studies are urgently needed to elucidate the possible role of genetics in producing a worse breast cancer prognosis in this ethnic group.

INTRODUCTION

Breast cancer in African-Americans is associated with a poorer prognosis than in Caucasians (Elledge et al, 1994; Coates et al, 1992; Zaloznik 1995); Elias et al, 1994; Wells 1992; Gordon et al, 1992; Caplan et al, 1995). African-American women tend to present at an earlier age with larger tumors and more advanced disease stage (Hunter et al, 1993; Zaloznik 1995). Many reasons, not all of them biological, have been advanced for the difference in stage at presentation between Caucasian and African-American women (Gordon et al, 1992; Wells 1992; Coates et al, 1992), but several studies (Gordon et al, 1992; Wells 1992; Coates et al, 1992) have found that the excess breast cancer mortality among African-Americans cannot be totally accounted for by factors such as education, family income and access to health care (Hunter et al, 1993). It has been estimated that as much as 50% of the excess risk for late stage at diagnosis is related to intrinsic biological properties of the tumor (Hunter et al, 1993). In fact, tumors in African-Americans are more likely to occur at an earlier age, to be estrogen and progesterone receptor negative and to have a higher proliferative fraction (Elledge et al, 1994), all factors associated with more aggressive tumors (Clark et al, 1989; Fisher et al, 1988; Clark et al, 1983; Sigurdsson et al, 1990). The prognosis is worse for African-Americans even when stage at presentation is controlled for (Elledge et al, 1994).

The relationship between stage at presentation and ethnic/genetic background is a pertinent question to address in South Florida. In 1986, a study of breast cancer patients in Dade County, FL for the years 1981 to 1983, showed that 49% of white women and 41% Hispanic women were diagnosed with a local stage disease, while only 26% of black women were diagnosed at this early stage (Zavertnik et al, 1992; Zavertnik 1993). Factors associated with poverty were initially thought to be responsible for the high frequency of advanced disease among blacks. However, as pointed out above, socioeconomic factors do not completely account for the difference in prognosis between the Caucasian and African-American populations and environmental and/or genetic factors must be considered.

The BRCA1 gene encodes a protein of 1,863 amino acids. Aside from a RING finger motif near the N-terminal (Miki et al, 1994) and a granin consensus sequence at amino acid residues 1214-1223 (Jensen et al, 1996), there is little homology with other known proteins. Based on functional studies (Thompson et al, 1995; Holt et al, 1996), the protein is believed to be a tumor suppressor, although its role is not yet well understood. It seems to serve as a negative regulator of mammary epithelial cell growth whose function is compromised in breast cancer. About 70% of known BRCA1 mutations are small insertions or deletions which result in a frame shift and the premature termination of protein synthesis (Simard et al, 1994; Castilla et al, 1994; Friedman et al, 1994). Recently the correlation between specific BRCA1 mutations and the clinical phenotypes was observed suggesting that the size of the truncated BRCA1 protein is clinically important. The C-terminal end of the BRCA1 protein appears to be essential to normal BRCA1 function in breast cells, as patients with the 1853 stop mutation, which truncates only eleven amino acids at the C-terminal, develop very early onset breast cancer. This part of the protein seems to be less important in the ovary since mutations in the 3' portion of BRCA1 are less likely to lead to ovarian cancer than are mutations in the 5' portion of the gene (*reference).

Ethnic differences in rate and type of BRCA1 mutations have been found. The estimated carrier rate in the general population is 1 in 300 to 1 in 800. Struewing et al (1995) found that the carrier rate of a single mutation, 185delAG, may be as high as 1 in 100 among the Ashkenazim, thus putting this population at a significantly increased risk of breast/ovarian cancer. Most of the molecular studies have been carried out in Caucasian families and very little has been published about the mutations in African-Americans. The BIC Database, accessible through the Internet, gives no information on patient ethnic origin. Of 150 families in the BRCA database, only one is identified as African-American.

African-American women under 50 years of age have the highest rate of new cases of breast cancer in the nation (Love 1991). Among women under 50 years of age, breast cancer incidence in African-Americans now exceeds that in Caucasians (Gray et al. 1980, Horm et al. 1984). This excess in breast cancer incidence among young African-American women may be due to increased exposure to known risk factors, exposure to new risk factors and/or a decreased exposure to protective factors (Dupont 1985) or it may be due to genetic factors. In this paper we begin to address the question of differences between the BRCA1 mutations in breast cancer patients of African-American and of Caucasian descent. In molecular studies of the BRCA1 gene in a group of the former, we found several mutations clustered in exon 11. We undertook a systematic screening of these regions of exon 11 among other African-American breast cancer patients in order to determine whether mutations in this region of BRCA1 occurred more frequently in African-Americans than in Caucasians.

Our study provide same information on the analysis of BRCA1 gene mutation in so far under represented African-American population with positive family history of breast cancer. It also suggest that the explanations for a poorer prognosis of breast

cancer in African-Americans is at least partially related to different nature of BRCA1 mutations that cause more aggressive biological properties of the tumor.

METHODS:

Patient Ascertainment:

Patients were ascertained in two groups: In the first group patients were ascertained as part of a pilot project investigating for the presence of BRCA1 mutations in affected women with strong family history of breast cancer in the South Florida population. In this group, 9 patients (3 African-American, 1 Hispanic and 5 White non Hispanic) were tested for BRCA1 mutations. Pedigrees of the three African-American families are displayed in Figure 1. An initial interview was conducted by one of us (J. F. Arena) with the proband in each family. A detailed family history as well as histoligic verification of breast cancer was obtained. Patients were provided with a pretest educational session, where they also reviewed and signed the consent form. The study was conducted in strict accordance with regulations set forth by the University of Miami Committee for the Protection of Human Subjects and the protocol was approved by the Institutional Review Board. Blood samples were collected and sent to OncorMed, a company located in Gaithersburg, Maryland; the entire BRCA1 coding sequence was screened for mutations. A DNA sample from each patient was also stored in our laboratory for future analysis. The results found at Oncormed in this first group prompted us to extend our investigation to a second group of women. The second group was tested, this time in our laboratory, for the presence of mutations in four regions of BRCA1 exon 11.

In this second group, Group 2, 42 African-American women with histologically confirmed cancer of the breast (ICD9 codes 174.0-174.9) between 1989-1994, aged 50 and younger, were identified by one of us (Selina Smith) as part of a research project to evaluate cancer risk factors in two or more generations of African-American women (Grant DOD #DAMD 17-94-J-4245). In each family, blood samples were collected from the proband and from family members so as to include at least two generations of each family. Family members of the index cases included mother, sisters, or daughters. Eight patients (19%) had at least one first degree relative with breast cancer and were classified as familial cases. In 34 families (81%), the breast cancer appeared to be a sporadic event.

Using four sets of PCR primers directed to the regions of exon 11 surrounding mutations discovered by us in the familial breast cancer patients, these regions were screened by SSCP and mutations were confirmed by sequencing.

Acquisition of Control Samples

Residual blood samples were obtained from the Clinical Hematology Laboratory at Jackson Memorial Hospital in Miami, Florida. 249 samples, representing all the samples collected on a single day from African-American inpatients, were procured. The samples were selected for race but not for diagnosis; all identifying information except for sex and age was destroyed. Sufficient DNA was obtained from 110 samples and 50 samples were used in the present study.

Molecular Methods

DNA extraction

Genomic DNA was extracted from whole blood by standard methods.

In Group 2, four pairs of oligonucleotide primers (table 3, Friedman 1994) were used for polymerase chain reaction (PCR) to cover four regions of exon 11 in segments of between 250 and 500 base pairs (bp) in length. PCR was carried out in a 50 ml reaction volume using cycling conditions appropriate for the primer pair. Three sets of PCR primers were developed to screen for mutations in this region (nucleotide 3357-3627, 3857-4170, 4105-4215) and one set of primers was developed to screen for mutations in the region surround the 10 bp insertion in nucleotide 943 (nucleotide 789-1090) as shown in table 3.

Table 3 - PCR primers used to screen three portions of exon 11 surrounding mutations 943 ins 10bp, 3888 del GA and 4160 del AG.

Primer	Region of cDNA amplified	Size of product (bp)	Sequence 5'-3'
11Ai forward	789-1090	473	GGA ATT AAA TGA AAG AGT ATG AGC
11Ai reverse			CTT CCA GCC CAT CTG TTA TGT TG
11M forward	3357-3612	270	TTG AAT GCT ATG CTT AGA TTA GGG G
11M reverse			GAC GCT TTT GCT AAA AAC AGC AG
11P forward	3857-4170	314	CGT TGC TAC CGA GTG TCT GTC TAA G
11P reverse			AGC CCG TTC CTC TTT CTT CAT C
11Pi forward	4105-4215	280	AAA GCC AGG GAG TTG GTC TGA G
11P1 reverse			GTG CTC CCA AAA GCA TAA A

PCR

PCR was carried out in the Perkin-Elmer GeneAmp PCR System 9600. Each reaction contained 10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl, pH 8.3, 200 mM each dNTP, 1 mM each primer, 100 ng genomic DNA, 1.25 U Taq Polymerase (BMB) in a total volume of 25 uL. The cycling conditions were as follows: initial denaturation, 94 C for 5 minutes; 94 C for 45 seconds, 55 C for 30 seconds, 72 C for 30 seconds for 35 cycles; final extension 72 C for 10 minutes.

SSCP

SSCP was carried out in 0.6X MDE gels (J.T. Baker, Inc., Phillipsburg, NJ). Gels were 1 mm thick and 20 cm long. 1-2 μL of PCR product was mixed with loading buffer to a final concentration of 88% formamide, 9 mM EDTA, 0.05% bromphenol blue, 0.05% xylene cyanole FF. The sample was heated to 94 C for 5 minutes, quenched on ice for 5 minutes and immediately loaded onto the gel. Each gel contained a normal and a mutant control. Electrophoresis was at 15 C. The time and wattage were adjusted according to the length of the PCR product as follows: fragment size 200 bp: 2W for 16 hours; fragment size 300 bp: 4W for 16 hours; fragment size 470 bp: 5W for 20 hours. Following electrophoresis, the DNA was silver-stained using the BioRad Silver Stain Kit. Stained gels were photographed, then transferred onto Whatman 3MM paper and dried. Samples which differed from the normal control in their band pattern were sequenced.

Sequencing

The band of interest was gel-isolated on low-melting-point agarose and the DNA was column purified (Wizard PCR Preps System, Promega) following the manufacturer's instructions. The purified PCR product was sequenced by the University of Miami Core Sequencing Facility. The primers used to amplify the segment were also used in sequencing. The sequencing method was Sanger dideoxy chain termination using The ABI Prism Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA Polymerase, FS. The samples were run and read on the ABI Model 373A DNA Sequencer with the Stretch upgrade.

RESULTS:

The pedigrees of the first three families investigated at Oncormed are shown in in figure 1 and the results are shown in Table 1.

FIG. 1 Pedigrees of three African-American families with breast cancer in which three previously undescribed BRCA1 mutations were found. Arrows indicate probands in which the mutations were originally found. In family 93004 the age of onset of breast cancer ranged from 29-34 years.

Table 1 - Mutations in the BRCA1 gene in three African-American patients with early onset breast cancer, from the South Florida population.

FAMILY #	EXON	NT	CODON	BASE Change	AA Change
94003	11	943	275	ins 10bp	STOP 289
93004	11	3888	1257	del 2bp	STOP1265
94027	11	4160	1347	del 2bp	STOP1354

NT - nucleotides AA - amino acids

The proband from family 95027 has 10 bp insertion at nucleotide position 943 causing a premature termination codon at amino acid 289. The proband from family 93004

(see Table 1) has GA deletion at codon 1257 resulting in premature termination of protein synthesis, whereas the proband from family 94027 has AG deletion at codon 1347 causing the frame shift and the stop codon. These results promped us to investigate for the presence of newly described BRCA1 mutations (see Table 1) of the exon 11 in the second group of 42 African-American women with histologically confirmed breast concern between 1989-1994, aged 50 and younger. In 34 of them the breast cancer patient was a sporadic event in the family and eight of them had at least one affected first degree relative with breast cancer and were classified as familial cases. Among the familial cases, we found one mutation (10 bp insertion at codon 943) to be present in one more African-American family unrelated to the one in which we originally described the insertion. Insertion was found in two members of the family both affected by disease (see Figure 3). Two other mutations (GA and AG deletions) were not found in additionally tested individuals, but were found in affected family members of proband in which they were discovered.

Fig. 2 - SSCP study of family # 95027. Patient III-2 and her affected mother II-2 carry the same 10 bp insertion at codon 943.

Among the sporadic cases, we found a new, previously undescribed polymorphism in exon 11 at codon 3537 (fig. 4). It causes a substitution of serine by glycine. This is a conservative change and is not likely to alter BRCA1 protein function.

Fig. 3 - SSCP study of family #95012. Patient and her unaffected sister and unaffected daughter (I-2 and II-2) carry the same base pair substitution.

To verify it we examined other family members of the proband. As we predicted, the substitution was found but did not segregate with cancer. We also investigate 100 chromosomes from African-American controls and 46 chromosomes of Caucasian controls. This polymorphism was present in 4 of 100 chromosomes from African-American controls and in none of 46 chromosomes of Caucasian controls.

Fig. 4 - Mutations found in exon 11 of the BRCA1 gene. Note position of mutation in relation to the granin motiff. The mother of the patient with mutation 5' prime of the granin motiff had the same mutation and had breast, colon and ovarian cancer. The two patients with mutation 3' prime of the granin motiff had only cases of breast cancer in their family.

Discussion:

Of the 22 coding exons of the BRCA1 gene, exon 11 is the largest and has 3426 base pairs covering 57% of the mRNA (5711 bp). The initial investigation in three African-American patients with positive family history for breast cancer lead us to the discovery of three new mutations in exon 11 of the BRCA1 gene. A recent search

of the Breast Cancer Information Core (BIC) database at the following Internet address: http://www.nchgr.nih.gov/dir/lab_transfer/bic// revealed 74 distinct mutations (36 deletions, 11 insertions and 27 single base pair substitutions) in exon 11. Our finding constitutes 4% of the previously reported mutations in the in the BIC database. Information is not available on the genetic background of patients in this database.

Mutations found in families 93004 and 94027 cause a stop codon leading to truncated protein products differing from each other by only 90 amino acids. On the basis of this finding we hypothesize that the truncated protein predicted in these cases should be similar in function (or dysfunction) and we decided to investigate whether mutations in this region of exon 11 could be found more frequently in African-Americans than in the general population. The fact that we did not find mutations in this region in our series of 42 patients may not exclude this possibility in larger series mainly because 81% of our cases were sporadic.

The detection of novel mutations is especially important in our population as the ethnic profile in South Florida is significantly different from other areas where investigations have been carried out. In all three of our African-American patients in whom mutations were found, they were all previously undescribed.

Among women under 50 years of age, the breast cancer incidence in African-Americans exceeds that in Caucasians (Grey et al, 1980; Horm et al, 1984). African-American women under 50 have the highest rate of new cases of breast cancer in the nation (Love 1991). This excess in breast cancer incidence among young black women may be due to increased exposure to known risk factors, exposure to new risk factors, and/or a decreased exposure to protective factors (Dupont 1985) or it may be due to genetic factors.

There is no reported systematic analysis of the BRCA1 mutations in African-American with the family history of breast cancer although it is to be expected that ethnic differences will be found in the type of the BRCA1 and the BRCA2 mutations. Those differences could in part explain the more aggressive clinical presentation and higher rate of breast cancer in African-American. This could also provide a molecular basis for counseling high-risk African-American women.

Our preliminary data suggest that African-American women with family history of breast cancer have characteristic mutations not so far observed in Caucasians. Our hypothesis is that the sequence analysis of the BRCA1 gene in a representative number of African-American women with a family history of breast cancer will confirm ethnic differences in rate and type of BRCA1 mutations. Furthermore, we believe that it will be possible to correlate with the clinical phenotype and more aggressive biological properties of breast tumor in African-Americans.

In summary, we can conclude that our preliminary data suggest that African-Americans with early onset breast cancer and strongly positive family history may carry the BRCA1 mutations different from other ethnic groups and that further studies are urgently needed to elucidate the possible role of genetics in producing a worse breast cancer prognosis in this ethnic group

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Institutions:

1. Dept. of Pediatrics, Univ. of Miami Sch. of Med., Miami, FL
2. Dept. of Epidemiology, Univ. of Miami Sch. of Med., Miami, FL

Abstract

The prevalence of BRCA1 germ-line mutations in African-American (AA) breast cancer patients is controversial. Although such mutations have been reported in several at-risk AA families, recent evidence suggests that the prevalence in unselected AA patients is low. We have completed BRCA1 mutation screening in 20 at-risk AA families categorized for risk: high-risk (HR; three affected 1st $^{\circ}$ relatives; 10 families), moderate-risk (MR; two affected 1st $^{\circ}$ relatives, 7 families) and undetermined-risk (UR; single affected with information being updated, 3 families). The BRCA1 locus was screened for mutations using a series of exon-specific PCR primers, followed by SSCP analysis. SSCP variants were further analyzed by direct genomic sequencing using bands eluted from SSCP gels, as well as the purified original PCR product. We identified only one deleterious mutation, 3875 del GTCT, which is predicted to truncate the BRCA1 protein. Additionally, two different polymorphisms in linkage disequilibrium were detected in two unrelated HR families. The first, a polymorphism 67 bases into intron 22, linked to a 3' UTR variant (described previously in African-Americans) was detected in 4/4 affected women, and 4/5 at-risk women too young to evidence disease. The second, a novel polymorphism, was detected in the +8 position of intron 22 linked to the same 3' UTR variant. The predicted effect on splicing is unclear since very few variants in this proximity to the 5' donor splice site have been reported. Each of these linked polymorphisms was detected in AA controls with respective frequencies of 3% and 1%. The significance of these variants is being investigated. Our results support previous reports of the low incidence of AA germ-line BRCA1 mutations, and suggest a possible role for BRCA1 polymorphisms in an increased risk of early-onset breast cancer.

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