MUTATION ANALYSIS OF BRCA1 GENE IN AFRICAN-AMERICAN PATIENTS WITH BREAST CANCER

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An estimated 7% of all breast cancers and 10% of all ovarian cancers are associated with inherited mutations in BRCA1 and BRCA2 genes. The mutations of a breast cancer-susceptible gene, BRCA1, confers increased risk of breast cancer in young women. Numerous studies have reported specific mutations in the BRCA1 and BRCA2 genes in the white population. However, there are very few studies on African-American and other ethnic minority groups. The goal of this study is to identify whether African-American patients with breast cancer carry some common mutations reported in other ethnic groups and whether they carry some novel mutations. We screened hot-region mutations on exons 2, 5, 11, 16, and 20 of BRCA1 gene in 54 African-American patients with breast cancer by NIRCA and SSCP methods. Our data revealed one novel frameshift mutation (3331insG) and three missense sequence variants (A3537G, A3667G, and C4009T) on exon 11. Each sequence change was confirmed by automatic DNA sequencing. One rare sequence variant, A3537G, has been revealed in high frequency (3/54). Our data suggested that African-American patients with breast cancer carry some unique BRCA1 gene mutations. (*J Natl Med Assoc.* 2000;92:29–35.)

Key words: BRCA1 ♦ mutation ♦ African American ♦ breast cancer

Breast cancer is a leading cancer in American women and it is estimated that there will be 175,000 new breast cancer cases diagnosed in 1999, which will account for about 30% of all cancers diagnosed in US women in that year.[1] In the past 20 years, there has been a gradual recognition of a significant disparity in breast cancer survival rates between African-American and white women, especially in younger women.[2–6] According to the data from SEER (Surveillance, Epidemiology, and End Results) cancer statistics review (1973 through 1993), in a population of 100,000, the incidence of breast cancer is lower in African-American women (100.3) than in white women (110.5), but the mortality in African Americans (31.5) is much higher than in whites (25.6). This difference is even more apparent between the younger African-American and white woman (age <50), with their breast cancer incidence and mortality rate 31.3 to 33.1 and 8.6 to 5.2 per 100,000 people, respectively.[5] From 1973 to 1993, the incidence rate of breast cancer in African Americans and whites increased 36.9% and 24.0%, respectively, and during the same time, the mortality rate decreased 4.3% in white patients with breast cancer but increased 18.0% for African-American women. The mortality rate of breast cancer in young whites decreased by 21.5%; however, this rate

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remained almost the same (−0.7%) in young African Americans.[5]

It has been suggested that the breast cancer incidence and survival rate disparity between African-American women and white women might be partly attributed to the low socioeconomic status of the African-American population.[7,8] The Black/White Cancer Survival Study, the most comprehensive study of racial survival differences in breast cancer to date, found that some biological properties of breast cancer tissue, including tumor stage, tumor pathological grade, and hormone receptor status, may also contribute to the racial survival differences.[9] Breast cancers in African-American women generally showed the characteristics of less positive estrogen receptor (ER) and progesterone receptor (PR), less differentiation and later tumor staging.[9,10] The pattern and effect of tumor suppressor gene p53 mutations in the African-American and white patients with breast cancer were also different, and this observation suggested that the type of p53 gene alterations might contribute to the racial difference in breast cancer survival.[11,12]

The BRCA-1 gene was first identified in familial patients with breast cancer and consists of 5592 nucleotides distributed over a genome region of approximately 100 kb in chromosome 17, and its 22 coding exons encode a protein of 1863 amino acids.[13] Currently, more than 500 different mutations have been documented within the coding region of the BRCA1 gene.[14] BRCA-1 gene mutations were thought to be associated with the development of breast cancer in younger women[15,16] and in some defined ethnic groups specific mutations are likely to recur. Three founder mutations, including 185delAG and 5382insC of BRCA1 and 6174delT of BRCA2 have been found predominantly in young Ashkenazi Jewish women with breast cancer.[17–19] The combined carrier frequency of these three founder mutations exceeds 2% in the general Ashkenazi Jewish population.[20–22] In other populations some founder mutations have also recently been found to be related with breast cancers in younger women, such as BRCA1 2595delA in Swedish white,[23] BRCA1 2804delAA in Dutch and Belgian,[24] and BRCA2 999del5 in Icelandic patients with breast and ovarian cancer.[25] The pathological studies of BRCA1-related breast cancer showed that breast cancer with BRCA1 gene mutations generally presented a more malignant histological type, such as histoprognosis grade 3 tumors that were highly proliferating, had less steroid receptor positivity, and had poor differentiation.[26,27] The germline BRCA1 mutation has been suggested to be an adverse prognostic factor in Ashkenazi Jewish women with breast cancer.[28] However, the overall survival in BRCA1-related breast cancer is similar to sporadic cases, as reported recently by Verhoog et al.[29] In comparison to the white women with breast cancer, very little if any information is available on the genetics of breast cancer in minority women like African-American patients with breast cancer. Our medical center in south central Los Angeles serves predominantly African-American (47%) and Hispanic (41%) patients with breast cancer. We examined if our patient population carried BRCA1 mutations similar to those reported for the Ashkenazi and other white populations or if they have novel mutations on the BRCA1 gene. According to the data from the Breast Cancer Information Core, more than 75% of BRCA1 gene mutations were identified on five exons, 2, 5, 11, 16, and 20. In our current study, we use the mutation hot-region screening strategy and examined the germline mutations of BRCA1 in those exons in 54 African-American patients with breast cancer. Hence, the primary objective of our study is to answer:

1. Whether African-American patients with breast cancer express the known BRCA1 mutations, such as 185del AG and 5382insC.
2. Whether there are any recurrent and/or novel BRCA1 mutations in African-American patients with breast cancer not yet reported for the white or other ethnic groups.

MATERIALS AND METHODS

Sample Collection and Processing

King Drew Medical Center is a minority medical institution located in south central Los Angeles, and most of its patients are African American and Hispanic. Most of the investigations on BRCA1 gene mutations in different ethnic groups has been performed in high-risk patients, such as familial breast cancer or early-onset breast cancer. Recent population-based studies, however, indicate that neither age nor family history could independently predict the frequency of BRCA1 gene mutation.[30,31] To investigate the status of BRCA1 mutations in African-

American patients, we screened the BRCA1 hot-spot mutations at exons 2, 5, 11, 16, and 20 in 54 African-American patients with breast cancer. The patients screened in this study were sporadic patients with breast cancer diagnosed and treated consecutively in the Division of Hematology and Oncology of this hospital between 1996 to 1998 and were not selected for family history or age. Written consent was obtained from each patient. Blood samples (5 ml) were collected into heparinized tubes from each patient and subjected to 3000 rpm centrifugation for 10 min. The buffy coat was isolated and kept at −80°C for further DNA extraction.

Genomic DNA Extraction

Polymerase chain reaction (PCR)-ready DNA samples were prepared using the blood DNA-extraction protocol from the whole DNA isolation kit (Puregene; Gentra Systems, Inc, Minneapolis, MN) with modification. Briefly, 100 μl of buffy coat was mixed with 200 μl of phosphate-buffered saline and 900 μl of red blood cell lysis buffer, spun at 14,000 rpm for 20 s, the white cell pellet was lysed in 300 μl of cell lysis solution and incubated at 37°C for 30 min. Samples were treated with RNase A for 15 min and mixed with 100 μl of protein precipitation solution. After centrifugation at 14,000 rpm for 3 min, the supernatant was mixed well with 300 μl of isopropanol and spun at 14,000 rpm for 1 min. The pellet was washed with 300 μl of 70% ethanol once, air-dried for 15 min, and dissolved in 50 to 100 μl of dH_2O at room temperature overnight or at 65°C for 1 h. Typically, we obtained more than 10 μg of PCR-ready DNA from about 100 μl of buffy coat.

PCR and Nested PCR of BRCA1 Gene Exon 11

PCR and nested PCR amplification of BRCA-1 exon 11 were performed using a BRCA1 exon 11 genomic DNA screening module (Ambion, Austin, TX). The BRCA1 exon 11 was amplified into A, B, C, and D—four overlapping fragments by PCR and nested PCR using the primer pair sets corresponding to each fragment of the BRCA1 gene. All the forward- and reverse-nested PCR primers also carried SP6 or T7 promoter sequence, respectively. PCR reaction was set up as follows: 10 mmol/L Tris-HCl (pH 7.3), 50 mmol/L KCl, 15 mmol/L $MgCl_2$, 125 μmol/L of each dNTPs, 250 nmol/L of each primer, 50 to 100 ng of genomic DNA, and 1.5 units of Taq DNA polymerase (Perkin Elmer, Foster City, CA). PCR reactions were carried out in a Perkin Elmer 480 cycler under the following conditions: initial denaturation at 94°C for 2 min; the PCR cycle: 94°C for 1 min, 56°C for 1 min, and 72°C for 2 min; with a total of 30 cycles. After the PCR cycles finished, the reactions were kept at 72°C for 7 min to ensure the complete extension of all PCR products. All PCR products were analyzed with 2% agarose gel (Gibco-BRL, Gaithersburg, MD) in 1×TAE (Tris-Acetate-EDTA).

NIRCA Screening of BRCA-1 Exon 11 Mutation

The protein truncation assay is currently the method most often used for screening of BRCA1 exon 11 mutation. However, this method has not been able to identify missense mutations. In our study we employed a newly developed mismatch detection-based method, Non-Isotopic RNA Cleavage Assay (NIRCA), to screen the BRCA1 exon 11 mutation. Briefly, nested PCR products of BRCA1 exon 11 were subjected to the NIRCA to screen the mutations by the Mismatch Detection II Kit (Ambion). The nested PCR products were in vitro transcribed into sense and antisense RNA; then sense and antisense sample RNA were cross-hybridized with antisense and sense strand of wild type of BRCA1 control RNA, respectively. Hybridized double-strand RNA were digested by three different types of RNase and analyzed on 2% of agarose gel in 1×TBE (Tris-Borate-EDTA) buffer.

PCR-SSCP Screening of BRCA1 Exons 2, 5, 16, and 20

According to the data from the Breast Cancer Information Core (BIC), more than 75% of BRCA 1 gene mutations occur at exons 2, 5, 11, 16, and 20.[14] Hence, in our single-stranded conformation polymorphism (SSCP) screening we used the hot-spot screening strategy to focus on mutation detection on exons 2, 5, 16, and 20. SSCP screening was performed using the protocol obtained from the BIC database.[14] Briefly, first-round PCR was performed with similar conditions described above with various extension time between 30 and 60 s for the amplification of the different exons. PCR products were subjected to re-amplification with [α-^{32}P]dCTP

along with all other reagents for PCR reaction. 2 μl of amplified product was mixed with 8 μl of 95% formamide buffer, denatured at 95°C for 2.5 min, and quickly chilled on ice. The denatured products were separated on 5% acrylamide gel in 1×TBE (pH 8.3) at 4°C at 50-W power. The gel was dried and exposed on Fuji film overnight.

DNA Sequencing

All positive NIRCA screening samples were subjected to automatic DNA sequencing to confirm the nature of each mutation. Custom DNA sequencing was performed by a commercial vender Retrogen (San Diego, CA).

RESULTS

Screening for BRCA1 Exon 11 Mutations by PCR-NIRCA Plus DNA Sequencing

BRCA 1 gene exon 11 accounts for about 60% of BRCA1 cDNA (3426/5711 bp) and codons (1142/1863), and mutations occurring in this region also accounted for about 50% of all the mutations found in BRCA1 gene according to the data from the BIC.[14] In this study we employed a simple new strategy called PCR-NIRCA combined with DNA sequencing to screen BRCA1 exon 11 mutations. This method could detect frameshift mutations as well as nucleotide substitution.[32] We first used PCR and nested PCR to amplify the BRCA1 exon 11 from genomic DNA into four different overlapping fragments and then used an RNA mismatch cleavage assay to screen each fragment. Samples that showed positive changes in the banding pattern were subjected to automatic DNA sequencing to confirm the nature of mutations. As shown in Figure 1, although the amount of first-round PCR product has significant variance, the nested PCR usually provides enough DNA for the next steps of in vitro transcription and RNA mismatch cleavage assay. The cRNA that was transcribed from sample PCR product was cross-hybridized with corresponding wild type control cRNA and subsequently digested with RNase. A sequence variance in the PCR product will lead to a different NIRCA band-shift pattern on an agarose gel (Figure 2). Patient 004 had two extra shifted bands compared with the wild type control, indicative of a mismatch sequence variant in sample DNA. DNA sequencing confirmed a base substitution at nucleotide 3537, which resulted in the codon 1140 change from serine into glycine (Figure 3C).

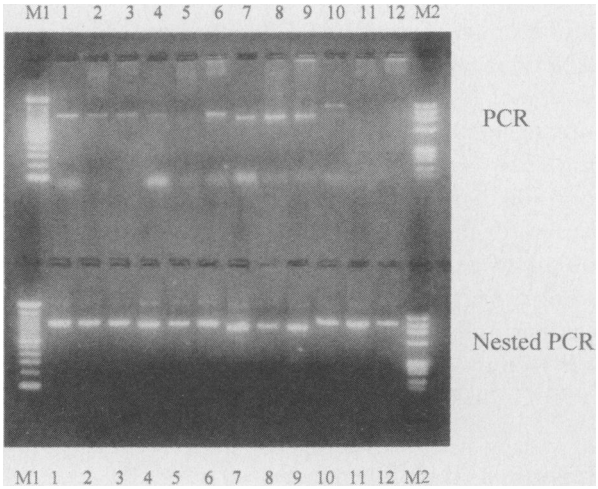

Figure 1. PCR and nested PCR amplification of BRCA1 exon 11. BRCA1 exon 11 was amplified into four fragments according to the protocol in the Materials and Methods. Samples 1 through 3 are fragment A; samples 4 through 6 are fragment B; samples 7 through 9 are fragment C; samples 10 through 12 are fragment D. Samples 1, 4, 7, and 10 are from cell line MCF-7; samples 2, 5, 8, and 11 are from patient 008; and samples 3, 6, 9, and 12 are from patient 013. M1 is a 100-bp DNA ladder; M2 is a φX174-*Hae*III DNA marker.

BRCA1 Gene Mutations and Sequence Variants Found in African-American Patients with Breast Cancer

We did not identify any mutations as well as sequence variants in exons 2, 5, 16, and 20 in the patients tested thus far by PCR-SSCP method. However, one frameshift mutation and five missense sequence variants were detected on BRCA1 exon 11 fragment D by NIRCA plus DNA sequencing (Table 1, Figure 3). The frameshift mutation 3331insG was found in a 43-year-old African-American patient with breast cancer. To the best of our knowledge this mutation has not been reported yet. One sequence variant, A3537G (Ser1140Gly), occurred with high frequency (3/54, 5.6%, Table 1) in our African-American patients with breast cancer. A3537G was considered as a rare sequence variant or missense mutation. We also observed a base substitution, A3667G, and it has been recognized as a polymorphism.

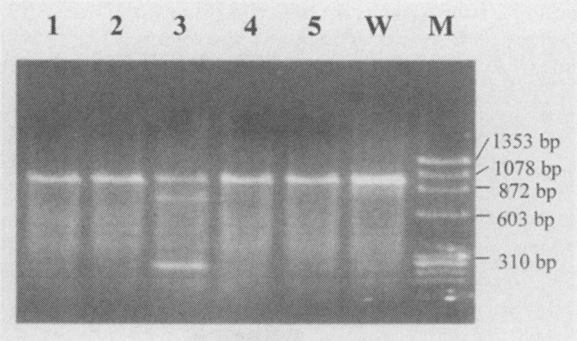

Figure 2. NIRCA screening for mutations of BRCA1 exon11 fragment D. DNA samples extracted from patients' blood buffy coat were subjected to PCR and nested PCR using the BRCA1 exon 11 amplification module (Ambion) and the nested PCR products were in vitro transcribed into sense or antisense RNA and cross-hybridized with antisense or sense RNA from the wild type BRCA1 exon 11 control. Hybridized double-strand RNA were digested by RNase and separated in 2% agarose gel. Samples 1 through 5 are patients with breast cancer samples. Lane 3 represents patient 004. Samples 1, 2, 4, and 5 are negative samples. W, wild type BRCA1 exon11 fragment D control; M, DNA size marker φX174-*Hae*III.

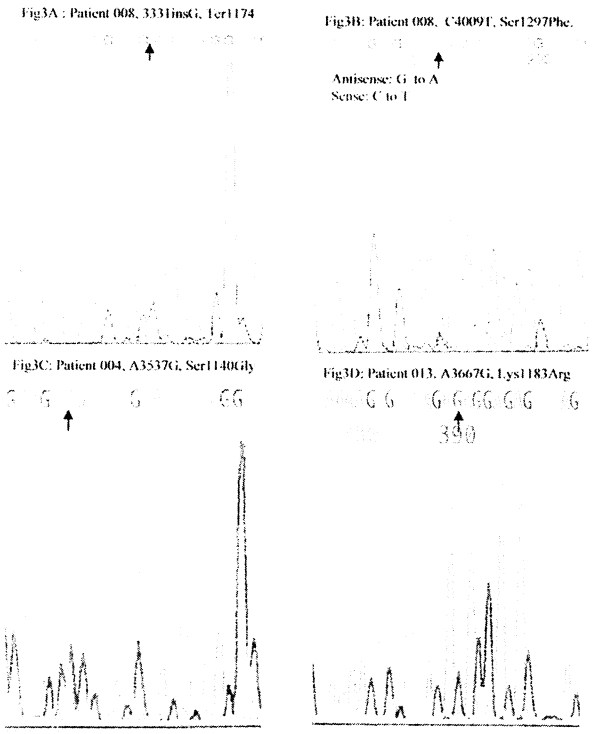

Figure 3. Automatic DNA sequencing of NIRCA-positive samples. *A* and *B* represent patient 008 with 3331insG and C4009T mutation (*B* showed antisense strand sequencing G to A); C represents patient 004 with A3537G mutation; *D* represents patient 013 with A3667G mutation.

DISCUSSION

So far the total entries of BRCA1 gene mutations recorded in the Breast Cancer Information Core (BIC) exceeds 2000, and exon 11 mutations account for about half of all entries, which is compatible with its large size.[14] Generally, the reported sequence alterations randomly distribute across the whole region; however, a trend of increased mutations toward the 3'-end of the gene has been observed.[14] The functional significance of most of missense alterations is not determined because so far there is still no functional test available to check the effect of these changes. In our study, all the sequence alterations were detected in the fourth quarter of exon 11 (Fragment D). Arena et al. screened three African-American patients with breast cancer with strong family history and an additional 42 early-onset patients with breast cancer. They observed that all three familial patients with breast cancer carried previously unreported mutations in exon11 (943ins10, 3888delAG, and 4160delAG). In addition, one mutation (943ins10) and a rare polymorphism (A3557G) were detected from the 42 early-onset patients with breast cancer.[33] Gao et al. investigated BRCA1 mutations in nine early-onset high-risk families with breast cancer. The mutation rate was 56% (5/9) and three novel mutations (1832del5, 5296del4, and 3883insA) were observed.[34] Most of the sequence alterations found in these studies are located in exon 11 and especially in the fourth quarter of exon 11 (3888delAG, 4160delAG, A3557G, and 3883insA). Both studies did not find the founding mutations reported for the Ashkenazi Jewish population. A recent population-based BRCA1 mutation screening in 88 cases of African-American patients with breast cancer observed a few missense variants but no frameshift mutations.[31]

In contrast to our observations on exon 11, we did not identify any mutations in exons 2, 5, 16, and 20 in all our 54 patients with breast cancer. Thus far, we have detected one frameshift BRCA1 gene mutation and three different types of sequence variants in 5 of 54 African-American patients with breast cancer. Patient 008 has a G insertion in codon 1071 and this results in BRCA1 protein truncated at

Table 1. Germline BRCA1 Gene Exon 11 Mutations Detected in African American Patients with Breast Cancer

Patient no.	Age (yr)	Mutation	Consequence	Type
008	43	3331insG	Ter1174	Frameshift
		C4009T	Ser1297Phe	Polymorphism
004	35	A3537G	Ser1140Gly	Rare sequence variant
101	56	A3537G	Ser1140Gly	Rare sequence variant
093	60	A3537G	Ser1140Gly	Rare sequence variant
013	48	A3667G	Lys1183Arg	Polymorphism

amino acid 1074. In addition, this individual also has nucleotide 3890 C to T base substitution, resulting in a change in codon 1297 from serine to phenylalanine. These two genetic lesions have not been reported in the BIC database.[14]

The missense sequence variant A3537G (Ser1140Gly) has been repeatedly detected in our African-American patients with breast cancer (3/54, 5.6%). Szabo et al. found that BRCA1 codon 1140 is conserved among several species, including human, mice, and canine.[35] This sequence variant was originally reported by Merajver et al. in an ovarian cancer patient and was recognized as a missense mutation.[36] This mutation was not observed in at least 80 control chromosomes and to date only two patients have been reported carrying the missense sequence variant Ser1140Gly.[34,36] Both patients were African Americans, one with ovarian cancer and the other with breast cancer. The BIC database, however, has reported 17 entries of this mutation to date.[14] Two of these were identified in African-American patients with breast cancer and the ethnicity of other 15 entries was unknown. The significance of high incidence of this missense mutation in African-American patients with breast cancer is not yet clear. Another missense mutation, A3667G, has been reported before and was recognized as a polymorphism.[37]

CONCLUSION

Our current study identified one novel BRCA1 frameshift mutation (3331insG) and a recurrent rare sequence variant (A3537G). However, we did not identify any founder mutations, such as 185delAG and 5382insC, in our African-American patients with breast cancer. Long-term population-based genetic analysis would determine the epidemiological role of the frameshift mutation 3331insG and the rare sequence variant Ser1140Gly in African-American women.

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REFERENCES

1. Landis SH, Murray T, Bolden S, Wingo PA. Cancer Statistics, 1999. *Ca-Cancer J Clin.* 1999;48:6–29.

2. Chevarley F, White E. Recently trends in breast cancer mortality among white and black US women. *Am J Public Health.* 1997;87: 775–781.

3. Wojcik BE, Spinks MK, Optenberg SA. Breast carcinoma survival analysis for African American and white women in an equal-access health care system. *Cancer.* 1998;82:1310–1318.

4. Ijaduola TG, Smith EB. Pattern of breast cancer among white-American, African American, and nonimmigrant west-African women. *J Natl Med Assoc.* 1998;90:547–551.

5. Ries LAG, Kosary CL, Hankey BF, Harras A, Miller BA, Edwards, BK, eds. SEER cancer statistics reviews, 1973–1993: tables and graphs. Bethesda, MD: National Cancer Institute; 1996: 30–56.

6. Simon MS, Severson RK. Racial differences in breast cancer survival: the interaction of socioeconomic status and tumor biology. *Am J Obstet Glynecol.* 1997;176:5233–5239.

7. Gordon NH, Crowe JP, Brumberg DJ, Berger NA. Socioeconomic factors and race in breast cancer recurrence and survival. *Am J Epidemiol.*1992;135:609–618.

8. Lannin DR, Mathews HF, Mitchell J, Swanson MS, Swanson FH, Edwards MS. Influence of socioeconomic and culture factors on racial differences in late-stage presentation of breast cancer. *JAMA.* 1998;279:1801–1807.

9. Eley JW, Hill HA, Chen VW, et al. Racial differences in survival from breast cancer-results of the National Cancer Institute Black/White cancer survival study. *JAMA.* 1994;272:947–954.

10. Elledge RM, Clark GM, Chamness GC, Osborne KO. Tumor biological factors and breast cancer prognosis among White, Hispanic, and Black women in the United States. *J Natl Cancer Inst.* 1994;86:705–712.

11. Blaszyk H, Vaughn CB, Hartmann A, et al. Novel pattern of p53 gene mutations in an American black cohort with high mortality from breast cancer. *Lancet.* 1994;343:1195–1197.

12. Shiao YH, Chen VW, Scheer WD, Wu XC, Correa P.

Racial disparity in the association of p53 gene alterations with breast cancer survival. *Cancer Res.* 1995;55:1485–1490.

13. Miki Y, Swensen J, Shattuck-Eidens D, et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science.* 1994;266:66–71.

14. Breast Cancer Information Core. Available at: http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/BIC. Accessed Nov. 29, 1999.

15. FitzGerald MG, MacDonald DJ, Krainer M, et al. Germline BRCA1 mutations in Jewish and non-Jewish women with early-onset breast cancer. *N Engl J Med.* 1996;334:143–149.

16. Lanston AA, Malone KE, Thompson JD, Daling JR, Ostrander EA. BRCA1 mutations in a population-based sample of young women with breast cancer. *N Engl J Med.* 1996;334:137–142.

17. Neuhausen S, Gilewski T, Norton L, et al. Recurrent BRCA2 6174delT mutations in Ashkenazi Jewish women affected by breast cancer. *Nat Genet.* 1996;13:126–128.

18. Offit K, Gilewski T, Mcguire P, et al. Germline BRCA1 185delAG mutations in Jewish women with breast cancer. *Lancet.* 1996;347:1643–1645.

19. Tonin P, Weber B, Offit K, et al. Frequency of recurrent BRCA1 and BRCA2 mutations in Ashkenazi Jewish breast cancer families. *Nat Med.* 1006;2:1179–1183.

20. Struewing JP, Abelivich D, Peretz T, et al. The carrier frequency of the BRCA1 185delAG mutation is approximately 1 percent in Ashkenazi Jewish individuals. *Nat Genet.* 1995;11:198–200.

21. Oddoux C, Struewing JP, Clayton CM, et al. The carrier frequency of the BRCA2 6174delT mutation among Ashkenazi Jewish individuals is approximately 1%. *Nat Genet.* 1996;14:188–190.

22. Roa BB, Boyd AA, Volcik K, Richards S. Ashkenazi Jewish population frequencies for common mutations in BRCA1 and BRCA2. *Nat Genet.* 1996;14:185–187.

23. Johannson O, Ostermeyer EA, Hakansson S, Friedman LS, Johansson U, Seilberg G. Founding BRCA1 mutations in hereditary breast and ovarian cancer in southern Sweden. *Am J Hum Genet.* 1996;58:441–450.

24. Peelen T, van Vliet M, Petrij-Bosch A, et al. A high proportion of novel mutations in BRCA1 with strong founder effects among Dutch and Belgian hereditary breast and ovarian cancer families. *Am J Hum Genet.* 1997;60:1041–1049.

25. Johannesdottir G, Gudmundsson J, Bergthorsson JT, et al. High prevalence of the 999del5 mutation in Icelandic breast and ovarian cancer patients. *Cancer Res.* 1996;56:3663–3665.

26. Lakhani SR, Jacquemier J, Sloane JP, et al. Multifactorial analysis of differences between sporadic breast cancers and cancers involving BRCA1 and BRCA2 mutations. *J Natl Cancer Inst.* 1998;90:1138–1145.

27. Armes JE, Egan AJ, Southey MC, et al. The histologic phenotypes of breast carcinoma occurring before age 40 years in women with and without BRCA1 or BRCA2 germline mutations: a population-based study. *Cancer.* 1998;83:2335–2345.

28. Foulkes WD, Wong N, Brunet JS, et al. Germ-line BRCA1 mutation is an adverse prognostic factor in Ashkenazi Jewish women with breast cancer. *Clin Cancer Res.* 1997;3:2465–2469.

29. Verhoog LC, Brekelmans CT, Seynaeve C, et al. Survival and tumour characteristics of breast-cancer patients with germline mutations of BRCA1. *Lancet.* 1998;351:316–321.

30. Malone KE, Daling JR, Thompson JD, O'Brien CA, Francisco LV, Ostrander EA. BRCA1 mutations and breast cancer in the general population: analyses in women before age 35 years and in women before age 45 years with first-degree family history. *JAMA.* 1998;279:922–929.

31. Newman B, Mu H, Butler LM, Millikan RC, Moorman PG, King MC. Frequency of breast cancer attributable to BRCA1 in a population-based series of American women. *JAMA.* 1998; 279:915–921.

32. Dean M. Resolving DNA mutations. *Nat Genet.* 1995;9: 103–104.

33. Arena JF, Smith S, Plewinska M, et al. BRCA1 mutations in African-American women. Am J Hum Genet. 1996;59(Suppl A34):169.

34. Gao Q, Neuhausen S, Cummings S, Luce M, Olopade OI. Recurrent germ-line BRCA1 mutations in extended African American families with early-onset breast cancer. *Am J Hum Genet.* 1997;60:1233–1236.

35. Szabo CI, Wagner LA, Francisco LV, et al. Human, canine and murine BRCA1 genes: sequence comparison among species. *Hum Mol Genet.* 1996;5:1289–1298.

36. Merajver SD, Pham TM, Caduff RF, et al. Somatic mutations in the BRCA1 gene in sporadic ovarian tumors. *Nat Genet.* 1995;9:439–443.

37. Durocher F, Shattuck-Hattuck-Eidens D, Mcclure M, et al. Comparison of BRCA1 polymorphisms, rare sequence variants and/or missense mutations in unaffected and breast/ovarian cancer populations. *Hum Mol Genet.* 1996;5:835–842.