

The New England Journal of Medicine

©Copyright, 1996, by the Massachusetts Medical Society

Volume 334

JANUARY 18, 1996

Number 3

BRCA1 MUTATIONS IN A POPULATION-BASED SAMPLE OF YOUNG WOMEN WITH BREAST CANCER

AMELIA A. LANGSTON, M.D., KATHLEEN E. MALONE, PH.D., JENNIFER D. THOMPSON, B.S.,
JANET R. DALING, PH.D., AND ELAINE A. OSTRANDER, PH.D.

Abstract Background. Inherited mutations in the *BRCA1* gene are associated with a high risk of breast and ovarian cancer in some families. However, little is known about the contribution of *BRCA1* mutations to breast cancer in the general population. We analyzed DNA samples from women enrolled in a population-based study of early-onset breast cancer to assess the spectrum and frequency of germ-line *BRCA1* mutations in young women with breast cancer.

Methods. We studied 80 women in whom breast cancer was diagnosed before the age of 35, and who were not selected on the basis of family history. Genomic DNA was studied for *BRCA1* mutations by analysis involving single-strand conformation polymorphisms and with allele-specific assays. Alterations were defined by DNA sequencing.

Results. Germ-line *BRCA1* mutations were identified in 6 of the 80 women. Four additional rare sequence vari-

ants of unknown functional importance were also identified. Two of the mutations and three of the rare sequence variants were found among the 39 women who reported no family history of breast or ovarian cancer. None of the mutations and only one of the rare variants was identified in a reference population of 73 unrelated subjects.

Conclusions. Alterations in *BRCA1* were identified in approximately 10 percent of this cohort of young women with breast cancer. The risk of harboring a mutation was not limited to women with family histories of breast or ovarian cancer. These results represent a minimal estimate of the frequency of *BRCA1* mutations in this population. Comprehensive methods of identifying *BRCA1* mutations and understanding their importance will be needed before testing of women in the general population can be undertaken. (N Engl J Med 1996;334:137-42.)

©1996, Massachusetts Medical Society.

GENETIC, hormonal, and environmental factors each have a role in the etiology of breast cancer,¹⁻⁵ which is the most common malignant condition and the second most common cause of cancer-related death among American women.⁶ Inherited mutations in *p53*,⁷ *BRCA1*,⁸ and *BRCA2*⁹ are known to confer a predisposition to breast cancer, and heterozygotes for mutations in the ataxia-telangiectasia gene may also be at increased risk for breast cancer.¹⁰ Nevertheless, quantitative information regarding the contribution of inheritance to the overall incidence of breast cancer in the population has been largely inferential.³

The identification and cloning of the *BRCA1* gene offer an opportunity to define further the role of genetic factors in breast cancer. *BRCA1* was mapped to chromosome 17q by linkage analysis of large families that

included multiple women with breast cancer, many of whom had an unusually early age of onset of the disease.⁸ The pattern of breast-cancer cases in families whose disease is linked to the *BRCA1* region is most consistent with an autosomal dominant mode of inheritance and high levels of penetrance, thus fitting the predictions of genetic epidemiologic analyses.^{2,3} Studies have also demonstrated clustering of cases of ovarian cancer in some of these families.¹¹ Women in selected high-risk families who harbor a *BRCA1* mutation appear to have at least an 80 percent lifetime risk of breast cancer, as well as a substantial risk of ovarian cancer.¹² In addition, obligate carriers of mutant *BRCA1* alleles may have an increased risk of both prostate and colon cancers.^{13,14}

The *BRCA1* gene is encoded by 5592 nucleotides distributed over a genomic region of approximately 100 kb.¹⁵ The 22 coding exons of the gene encode a protein of 1863 amino acids. The protein contains a putative RING finger (zinc-binding) domain near the amino terminal, suggesting that *BRCA1* may regulate transcription. One recent study suggests that *BRCA1* may inhibit the growth of breast epithelial cells.¹⁶

A recent collaborative survey describing 80 germ-line mutations summarizes the spectrum and frequency of *BRCA1* mutations identified to date, primarily in high-

From the Divisions of Clinical Research (A.A.L., J.D.T., E.A.O.) and Public Health Sciences (K.E.M., J.R.D.), Fred Hutchinson Cancer Research Center; and the Department of Epidemiology, University of Washington (J.R.D.) — both in Seattle. Address reprint requests to Dr. Ostrander at the Fred Hutchinson Cancer Research Center, 1124 Columbia St., Seattle, WA 98104.

Supported by a contract (NO1 CN 05230) with the National Cancer Institute and by grants (R01 CA63705 to Dr. Ostrander and R01 CA41416, R01 CA63697, and R01 CA 59736 to Drs. Daling and Malone) from the National Cancer Institute. Dr. Langston is the recipient of a Physician Scientist Award (K11 HD00936) from the National Institutes of Health. Dr. Ostrander is the recipient of an American Cancer Society Junior Faculty Award (JFRA-558).

risk families.¹⁷ All classes of mutations are represented — missense mutations, nonsense mutations, deletions, insertions, and intronic mutations. Over 75 percent of the reported mutations result in the production of a truncated protein. Mutations occur throughout the coding sequence, although several common mutations have been found in multiple unrelated families.¹⁷⁻²⁰ There remain a few families whose disease is linked to the *BRCA1* region in which mutations have not yet been found,¹⁸⁻²⁰ implying the existence of mutations in regulatory sequences.

Molecular studies of germ-line *BRCA1* mutations have focused to date on women from high-risk families. Although family studies have proved invaluable for mapping and cloning of the gene, observations in such families regarding the nature and penetrance of *BRCA1* mutations may not reflect the full spectrum of alterations present in the general population. Epidemiologic studies suggest that mutations in highly penetrant dominant genes such as *BRCA1* may account for a substantial proportion of very-early-onset breast cancer in the general population,³ although this prediction has not been directly tested at the molecular level. In order to test this hypothesis, we analyzed DNA samples from women who were enrolled in a population-based study of

Table 1. Characteristics of 214 Women in Whom Breast Cancer Was Diagnosed before the Age of 35 and of the Subgroup of 80 Women Tested for *BRCA1* Mutations.*

CHARACTERISTIC	ALL WOMEN WITH BREAST CANCER BEFORE AGE 35 (N = 214)	WOMEN TESTED FOR <i>BRCA1</i> MUTATIONS (N = 80)
	number (percent)	
Age at diagnosis		
21–30 yr	71 (33)	26 (32)
31–34 yr	143 (67)	54 (68)
Family history of breast cancer†		
None	142 (66)	51 (64)
Mother, sisters, or both	30 (14)	14 (18)
Aunts or grandmothers only	42 (20)	15 (19)
Family history of ovarian cancer†		
None	201 (94)	76 (95)
Mother, sisters, or both	5 (2)	2 (2)
Aunts or grandmothers only	8 (4)	2 (2)
Extent of disease at diagnosis		
In situ	23 (11)	15 (19)
Local	106 (50)	37 (46)
Regional or distant	85 (40)	28 (35)
Vital status		
Alive	164 (77)	75 (94)
Dead	50 (23)	5 (6)

*Because of rounding not all categories total 100 percent.

†Family-history data as reported at the time of the original interview.

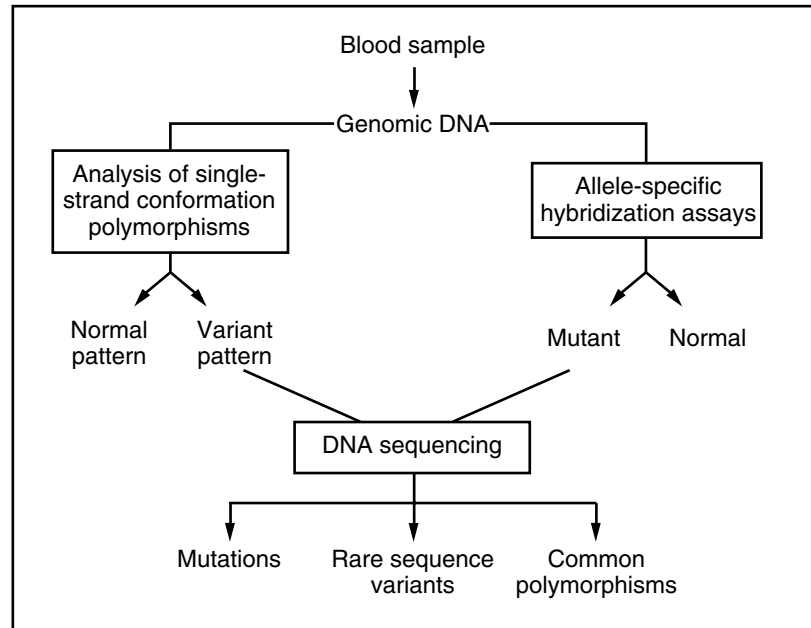


Figure 1. Strategy Used to Screen for *BRCA1* Mutations.

Genomic DNA from a subgroup of 80 women enrolled in a large, population-based study of early-onset breast cancer was analyzed for mutations in the *BRCA1* gene. The screening included single-strand conformation polymorphism analysis and hybridization with allele-specific oligonucleotides. The PCR products that yielded variant patterns on single-strand conformation polymorphism analysis or that were positive for a mutation on allele-specific assay were sequenced directly to ascertain the position and type of sequence variation. The analysis of sequence data permitted each alteration to be assigned to one of the following categories: mutations that affect the structure and function of the gene, rare sequence variants of unknown functional consequence, and polymorphisms that are common in the general population, irrespective of breast-cancer status.

early-onset breast cancer. We assessed the frequency and type of *BRCA1* mutations in women who were given a diagnosis of breast cancer before the age of 35 and who were not selected on the basis of family history.

METHODS

Patient Population

The 80 patients in the study were a subgroup of women from a large population-based study of early-onset breast cancer.²¹ All white women born after 1944 who were given a diagnosis of a first invasive or in situ breast cancer between January 1, 1983, and April 30, 1990, and were residents of King, Pierce, or Snohomish County in Washington State were eligible for the original study. The patients were identified through the Cancer Surveillance System of western Washington, a population-based cancer registry that participates in the Surveillance, Epidemiology, and End Results Program.

Of the 1011 women eligible to participate in the original population-based study, 845 (84 percent) were successfully interviewed; 747 of these women had invasive breast cancer, and 98 had carcinoma in situ. Information regarding potential risk factors for breast cancer was obtained through a structured face-to-face interview. Information on family history (up to the date of diagnosis of the patient's breast cancer) was elicited by asking each patient to identify all first- and second-degree female blood relatives. For each relative identified, the interviewer then asked the year of birth, vital status, year of death (if applicable), history and type of cancer (if any), and the laterality of the cancer if it was breast cancer.

In 1991, blood samples were obtained from 439 of the 845 interviewed women (52 percent); 141 others died before blood samples could be taken, 82 could not be located, 143 were not approached,

Table 2. Definite *BRCA1* Mutations Identified in Six Women with Early-Onset Breast Cancer.

PATIENT No.	AGE AT DIAGNOSIS (YR)*	NUCLEOTIDE CHANGE	CONSEQUENCE	FAMILY HISTORY OF BREAST OR OVARIAN CANCER	FAMILY HISTORY OF OTHER MALIGNANT CONDITIONS	MOST RECENT UPDATE OF FAMILY HISTORY
7	27	4-bp deletion at nucleotide 3875	Premature termination at amino acid 1262	Paternal cousin: breast cancer in her 50s	Paternal aunt: endometrial cancer at age 64 Paternal half-brother: melanoma at age 45	4/95
29	31	T→G at nucleotide 300 in exon 5	Cys61Gly (within zinc-binding motif)	Mother: breast cancer at age 49	None reported	10/94
30	31†	12-bp insertion in intron 20	Unknown	None reported	None reported	10/94
51	32	Insertion of C at nucleotide 5382	Premature termination at amino acid 1829	Paternal aunt: breast cancer at age 40 Paternal aunt: breast cancer at age 60	Maternal uncle: leukemia at age 80	12/92
70	34	C→T at nucleotide 4302 in exon 12	Premature termination at amino acid 1395 (Gln)	None reported	Paternal aunt: malignant condition (primary site unknown) at age 45 Paternal grandmother: malignant condition (primary site unknown) at age 49 Father: colon cancer at age 55	10/94
72	34	4-bp deletion at nucleotide 4184	Premature termination at amino acid 1364	Maternal aunt: ovarian cancer at age 50 Maternal cousin: ovarian cancer at age 32 Paternal aunt: bilateral breast cancer, postmenopausal	Mother: non-Hodgkin's lymphoma at age 50 Maternal half-aunt: leukemia at age 57 Paternal grandfather: stomach cancer, age unknown	4/92

*All six of the women with definite mutations had invasive carcinoma at the time of the initial diagnosis.

†Patient 30 also had a history of cervical cancer, which was diagnosed when she was 26.

and 40 refused to give a blood sample. Of the 214 women in whom breast cancer was diagnosed before the age of 35, 80 provided blood samples that were available for this analysis. Genomic DNA was prepared either directly from blood samples or from lymphoblastoid lines immortalized with Epstein-Barr virus.²²

Renewed contact and follow-up of previously interviewed women were initiated in order to obtain updated information on their female relatives and cancer histories of their male relatives. As of August 29, 1995, updated information had been obtained from 73 of the 80 subjects included in the study (91 percent).

Analysis of Single-Strand Conformation Polymorphisms

Forty-eight primer pairs^{19,23} were used to amplify the *BRCA1* coding sequence, intron-exon boundaries, and the promoter region from genomic DNA of each of the 80 study subjects. The reaction mixtures used for the polymerase chain reaction (PCR) and the conditions of the analysis involving single-strand conformation polymorphisms (SSCP) have been described previously.¹⁹

To characterize the frequency in the general population of each rare allele identified in study patients, we used DNA samples from a reference population of 73 unrelated subjects provided by the Centre d'Etude du Polymorphisme Humain (CEPH). These subjects are the maternal and paternal grandparents of the CEPH families, and their samples are routinely used in genetic-mapping studies. Although these subjects were not part of any population-based study and there is therefore little information on them regarding epidemiologic risk factors, they are not suspected of having any inherited predisposition to malignant conditions.

Screening with Allele-Specific Oligonucleotides

Hybridization assays were performed with oligonucleotide probes corresponding to mutations in exon segments 2, 11Pi, 20, and splice-donor and splice-acceptor sites in intron 5.²³ Genomic DNA from all 80 patients was amplified with the primers for the corresponding exon segment. The PCR products were then denatured and applied to a GeneScreen nylon filter with a 96-well dot blot apparatus. Hybridization with mutant or normal oligonucleotides was performed as previously described.²³ All mutations were confirmed by forward and reverse (bidirectional) sequencing of the PCR product.

DNA Sequencing

For each variant pattern identified by SSCP analysis, the putative mutant allele was eluted from the gel and fragments were reamplified with the original primer pair. In each case the corresponding frag-

ment of DNA was also amplified for direct sequencing with 100 ng of genomic DNA as the template. All PCR products were bidirectionally sequenced with the Applied Biosystems *Taq* DyeDeoxy terminator cycle-sequencing kit according to the manufacturer's instructions. Samples were analyzed with an Applied Biosystems 373A sequencer.

RESULTS

Patients' Characteristics

Table 1 summarizes the characteristics of all 214 women from the population-based study in whom breast cancer was diagnosed before the age of 35 and of the subgroup of 80 women for whom DNA samples were available for analysis of *BRCA1* mutations. The family histories of the subgroup were similar to those of the group as a whole. The subgroup had a higher proportion of women with in situ carcinoma than the group as a whole (19 percent vs. 11 percent), and the women in the subgroup were more likely to have survived their breast cancer (94 percent vs. 77 percent). These differences in the stage of cancer and survival presumably reflect the lag between diagnosis and the drawing of blood from women whose cancer was diagnosed early in the course of the study. One recent report suggests that the survival of women with breast cancer who harbor mutant *BRCA1* alleles may be longer than that of unselected women with breast cancer.²⁴ If this observation is correct, it is possible that our sample of 80 women may have a bias that slightly overestimates the proportion of cases that involve *BRCA1*.

Characterization of Alterations in the Germ-Line *BRCA1* Sequence

Figure 1 shows the strategy we used to screen for mutations in the germ-line *BRCA1* sequence. The alterations we found fall into three categories: definite mutations, each of which either has been associated with breast cancer in previous studies of high-risk families or

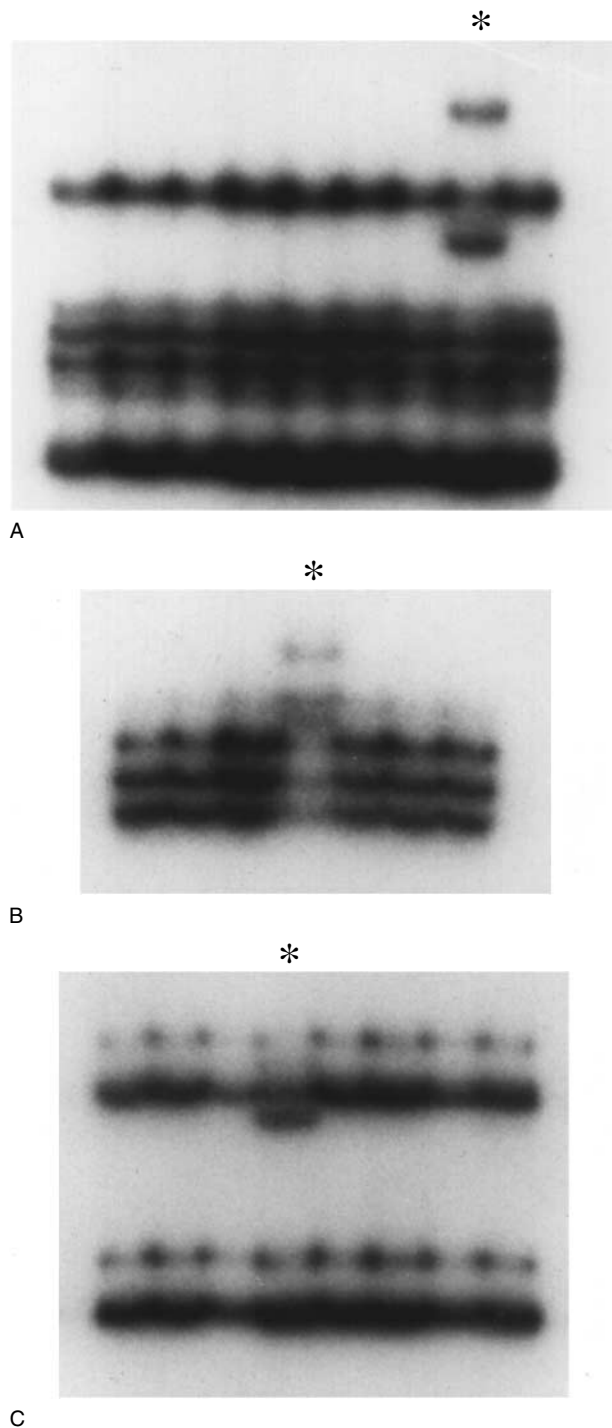


Figure 2. Three *BRCA1* Mutations Identified by Single-Strand Conformation Polymorphism Analysis.

Each autoradiograph of single-strand conformation polymorphism gels shows the normal patterns of migration of denatured PCR products and the mobility shift produced by a mutant allele in the corresponding genomic segment. In each panel, the lane showing the mutant allele (corresponding to a germ-line mutation) is marked by an asterisk. Panel A shows a T-to-G point mutation in exon 5 at the first position of codon 61 (Patient 29); Panel B, an insertion of 12 base pairs in intron 20 (Patient 30); and Panel C, a C-to-T nonsense mutation at nucleotide 4302 in exon 12 (Patient 70).

is predicted to result in protein truncation; rare sequence variants of unknown functional consequence; and polymorphisms that are common in the general population, irrespective of breast-cancer status. Each category is discussed separately below.

Definite *BRCA1* Mutations

The six definite *BRCA1* mutations identified in the study population and the characteristics of the women in whom such mutations were found are shown in Table 2.

Four of the mutations result in premature termination codons and, hence, a truncated protein product (Patients 7, 51, 70, and 72 in Table 2). Three of these four mutations have been described previously in families whose disease is linked to the *BRCA1* region.¹⁷ The nonsense mutation in exon 12 (Patient 70 in Table 2) is novel but qualitatively similar to others already described. The aberrant pattern produced by this mutation on SSCP analysis is shown in Figure 2.

One missense mutation and one large intronic insertion were also identified. The change from cysteine to glycine at amino acid 61 within the zinc-binding motif of the *BRCA1* protein (Patient 29 in Table 2 and Fig. 2) has been reported in at least two high-risk families¹⁷ and has been seen as a somatic mutation in an ovarian tumor.²⁵ The insertion of 12 base pairs (bp) in intron 20 in Patient 30, who had a history of breast and cervical cancer, represents a tandem reduplication of the sequence 5'GTNTTCCACTCC3' that begins 48 bp downstream of the 3' boundary of exon 20. This variant is easily detected by SSCP analysis (Fig. 2) and was not present in any of the 73 subjects in the control population. This mutation has also been identified in a woman with both breast and ovarian cancer who had five maternal relatives with breast cancer.²⁶ This alteration may affect RNA processing, but RNA was not available to test the effect of the mutation.

The family histories of the six women with *BRCA1* mutations are diverse; four of them reported family histories of breast or ovarian cancer. Of the two others, Patient 70 had a paternal aunt and a paternal grandmother with unidentified malignant conditions but had no sisters or maternal aunts, and Patient 30 had no sisters but had two maternal and five paternal aunts, none of whom had a history of cancer (Table 2).

Rare *BRCA1* Sequence Variants

Four rare *BRCA1* sequence variants were identified (Table 3). Such rare variants occur very infrequently in the general population and have not yet been associated with breast cancer in high-risk families. They may confer amino acid changes on the *BRCA1* protein or have the potential to alter RNA processing, but the functional consequence of these changes remains unknown.

Two of the rare sequence variants are single-base substitutions, each producing an amino acid change. The missense change in exon 2 results in the substitution of threonine for methionine at amino acid 18 (Patient 76 in Table 3), which may affect the conformation

Table 3. Rare *BRCA1* Sequence Variants Identified by Analysis of Single-Strand Conformation Polymorphisms in Four Women with Early-Onset Breast Cancer.

PATIENT NO.	AGE AT DIAGNOSIS (YR)	NUCLEOTIDE CHANGE	CONSEQUENCE	FAMILY HISTORY OF BREAST OR OVARIAN CANCER	FAMILY HISTORY OF OTHER MALIGNANT CONDITIONS	MOST RECENT UPDATE OF FAMILY HISTORY	ALLELE FREQUENCIES*
37	32	A→G at nucleotide 4158	Arg1347Gly	Paternal aunt: breast cancer, age unknown Maternal grandmother: breast cancer at age 63	Paternal aunt: gastrointestinal tumor, age unknown	8/92	Patients: 1 in 80 Controls: 1 in 73
65	33	C→T at nucleotide 49 of exon 4†	Noncoding exon	None reported	None reported	9/94	Patients: 1 in 80 Controls: 0 in 73
71	34	C→G at nucleotide 1088 bp upstream of exon 1	Noncoding region	None reported	Paternal grandmother: gastrointestinal tumor at age 59	12/94	Patients: 1 in 80 Controls: 0 in 73
76	34	T→C at nucleotide 172 of exon 2	Met18Thr	None reported	None reported	5/94	Patients: 1 in 80 Controls: 0 in 73

*The control subjects consisted of 73 unrelated subjects for whom DNA samples were provided by the Centre d'Etude du Polymorphisme Humain.

†The GenBank accession number for exon 4 of *BRCA1* is U15595.

of the protein, since a polar hydrophilic residue replaces a residue that is nonpolar and hydrophobic. This alteration was not seen in any of the 73 subjects in the reference population, nor has it been identified in high-risk families. In contrast, the A-to-G missense change at nucleotide 4158 (Patient 37 in Table 3) was also found in one person in the control group. This substitution has been seen previously in a patient with an insertion elsewhere in the gene,¹⁷ suggesting that it represents a rare neutral polymorphism rather than a true mutation.

The other two rare sequence variants were found in noncoding regions, and neither was found in the control group. The single-base substitution in exon 4 found in Patient 65 is unlikely to be of functional consequence, given its location within this noncoding exon. The C-to-G change 1088 bp upstream of the site of the initiation of *BRCA1* transcription (Patient 71 in Table 3) is notable because it lies within exon 1B of another gene, *IAI-3B*, which encodes a putative B-box coiled-coil protein.²⁷ Fewer than 300 bp separate the first exons of *BRCA1* and *IAI-3B*, raising the possibility of coordinate regulation of the expression of these two genes.²⁸

Common Polymorphisms

Eight common polymorphisms of *BRCA1* were identified in the patient population, corresponding to sequence changes in exons 11 (five polymorphisms) and 16 and introns 8 and 16 (data not shown). All alterations within the coding sequences were single-base substitutions, and each polymorphism had an allele frequency of at least 7.5 percent in the study population. These sequence alterations, which have been noted by other investigators in control subjects and patients, are not associated with the penetrant phenotypes seen in families whose disease is linked to the *BRCA1* region.¹⁷⁻¹⁹

DISCUSSION

The identification of six *BRCA1* mutations, as well as several sequence variants with potential functional importance, in this cohort of 80 young women with breast cancer supports the prediction that the *BRCA1* gene has a moderate role in the pathogenesis of breast cancer in this age group.³ The technical limitations of the avail-

able assays may result in a slight underestimate of the true frequency of *BRCA1* mutations in this cohort. For instance, the sensitivity of SSCP analysis, which was the cornerstone of our screening strategy, is approximately 70 to 80 percent under the assay conditions we used.^{29,30} In addition, some noncoding mutations affecting gene expression or RNA processing may not have been detected because complementary DNA was not available for screening. Several of the rare sequence variants we identified may be associated with an increased likelihood of cancer, but we cannot consider them to be definite mutations on the basis of sequence information alone. Such uncertainties illustrate some of the current difficulties in interpreting the results of sequence-based screening for mutations, particularly outside the context of studies of families whose disease is linked to the *BRCA1* region. Nevertheless, the results of family studies in which methods and reagents similar to ours were used suggest that the bulk of *BRCA1* mutations in this patient population should have been detected.^{15,17-20}

Five of the six mutations we identified have been seen previously in high-risk families and include three of the most common mutations identified to date (those in Patients 29, 51, and 72).^{17,26} We did not detect the 2-bp deletion in exon 2 (185delAG) that has been seen in multiple families of Ashkenazi Jewish descent and is estimated to occur at a frequency of approximately 1 percent in this population.³¹ The absence of this mutation in our study population is not surprising, given

Table 4. Distribution of *BRCA1* Mutations and Rare Sequence Variants According to the Family History of Breast and Ovarian Cancer among 80 Women with Early-Onset Breast Cancer.

FAMILY HISTORY	WOMEN	MUTATIONS	SEQUENCE VARIANTS
			number (percent)
Breast or ovarian cancer or both in ≥1 1st-degree relatives	19 (24)	1	0
Breast or ovarian cancer or both in ≥1 2nd-degree relatives but no 1st-degree relatives	22 (28)	3	1
No family history of breast or ovarian cancer	39 (49)	2	3

that Jewish women constituted only 1.2 percent of the study subjects who were under 35 years of age in the original population-based study.

One of the most important findings of this study is that *BRCA1* mutations are not limited to women with strong family histories of breast or ovarian cancer (or both). The distribution of mutations among the study subjects according to the family history of breast and ovarian cancer is shown in Table 4. The absence of correlation between the family history and the genetic risk attributable to *BRCA1* may reflect variations in family structure, incompletely penetrant alleles, the potential influence of additional modifier genes, and differences in patient recall. These observations illustrate the difficulty of making predictions about the presence or absence of *BRCA1* mutations on the basis of a woman's family history.

Our results illustrate several of the technical and scientific reasons why screening for *BRCA1* mutations is relevant but cannot yet be applied to the general population. Similar conclusions are drawn in the accompanying paper by FitzGerald and coworkers, who used different strategies to screen young women from the population at large for *BRCA1* mutations.³² Using currently available mutation assays, including direct DNA sequencing, we are not yet able to detect all alleles that increase the likelihood of disease, nor can we interpret their meaning. In addition, our data suggest that family history is not a good indicator of which women carry mutant *BRCA1* alleles. Further insight into the structure and function of the *BRCA1* gene and its protein product will facilitate the development of more comprehensive mutation-detection strategies and improve the interpretation of alterations in the sequence of the gene. Large population-based screening studies are needed to establish the frequency, importance, and penetrance of the broad spectrum of variations in the sequence of *BRCA1* observed in both affected and unaffected women in the general population. Only then will it be possible to offer reliable results and meaningful counseling to women who choose to have such testing.

Note added in proof: We have recently identified the C-to-T single-base change at nucleotide 49 of exon 4 in 6 of 145 randomly selected men residing in the same geographic area as the 80 women included in this study, suggesting that the variant represents a neutral polymorphism.

We are indebted to the women who participated in the study; to Mary-Claire King and Lori Friedman for providing information on oligonucleotide sequences; to Leigh Francisco for technical assistance with DNA sequencing; and to Eileen Bryant, Brenda Sandmaier, and Jeanne Anderson for thoughtful comments on the manuscript.

REFERENCES

- Newman B, Austin MA, Lee M, King M-C. Inheritance of breast cancer: evidence for autosomal dominant transmission in high-risk families. *Proc Natl Acad Sci U S A* 1988;85:3044-8.
- Claus EB, Risch N, Thompson WD. Genetic analysis of breast cancer in the cancer and steroid hormone study. *Am J Hum Genet* 1991;48:232-42.
- Buell P. Changing incidence of breast cancer in Japanese-American women. *J Natl Cancer Inst* 1973;51:1479-83.
- Brinton LA, Hoover R, Fraumeni JF Jr. Reproductive factors in the aetiology of breast cancer. *Br J Cancer* 1983;47:757-62.
- Kelsey JL, Horn-Ross PL. Breast cancer: magnitude of the problem and descriptive epidemiology. *Epidemiol Rev* 1993;15:7-16.
- Cancer facts & figures — 1995. Atlanta: American Cancer Society, 1995: 10-1.
- Malkin D, Li FP, Strong LC, et al. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science* 1990; 250:1233-8.
- Hall JM, Lee MK, Newman B, et al. Linkage of early-onset familial breast cancer to chromosome 17q21. *Science* 1990;250:1684-9.
- Wooster R, Neuhausen SL, Mangion J, et al. Localization of a breast cancer susceptibility gene, *BRCA2*, to chromosome 13q12-13. *Science* 1994;265: 2088-90.
- Swift M, Reitnauer PJ, Morrell D, Chase CL. Breast and other cancers in families with ataxia-telangiectasia. *N Engl J Med* 1987;316:1289-94.
- Narod SA, Feunteun J, Lynch HT, et al. Familial breast-ovarian cancer locus on chromosome 17q12-q23. *Lancet* 1991;338:82-3.
- Easton DF, Bishop DT, Ford D, Crockford GP. Breast Cancer Linkage Consortium. Genetic linkage analysis in familial breast and ovarian cancer: results from 214 families. *Am J Hum Genet* 1993;52:678-701.
- Arason A, Barkardottir RB, Egilsson V. Linkage analysis of chromosome 17q markers and breast-ovarian cancer in Icelandic families, and possible relationship to prostatic cancer. *Am J Hum Genet* 1993;52:711-7.
- Ford D, Easton DF, Bishop DT, Narod SA, Goldgar DE. Breast Cancer Linkage Consortium. Risks of cancer in *BRCA1*-mutation carriers. *Lancet* 1994;343:692-5.
- 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.
- Thompson ME, Jensen RA, Obermiller PS, Page DL, Holt JT. Decreased expression of *BRCA1* accelerates growth and is often present during sporadic breast cancer progression. *Nat Genet* 1995;9:444-50.
- Shattuck-Eidens D, McClure M, Simard J, et al. A collaborative survey of 80 mutations in the *BRCA1* breast and ovarian cancer susceptibility gene: implications for presymptomatic testing and screening. *JAMA* 1995;273: 535-41.
- Castilla LH, Couch FJ, Erdos MR, et al. Mutations in the *BRCA1* gene in families with early-onset breast and ovarian cancer. *Nat Genet* 1994;8:387-91.
- Friedman LS, Ostermeyer EA, Szabo CI, et al. Confirmation of *BRCA1* by analysis of germline mutations linked to breast and ovarian cancer in ten families. *Nat Genet* 1994;8:399-404.
- Simard J, Tonin P, Durocher F, et al. Common origins of *BRCA1* mutations in Canadian breast and ovarian cancer families. *Nat Genet* 1994;8:392-8.
- Daling JR, Malone KE, Voigt LF, White E, Weiss NS. Risk of breast cancer among young women: relationship to induced abortion. *J Natl Cancer Inst* 1994;86:1584-92.
- Sambrook J, Fritsch EF, Maniatis T. *Molecular cloning: a laboratory manual*. 2nd ed. Plainview, N.Y.: Cold Spring Harbor Laboratory Press, 1989.
- Friedman LS. *Molecular genetics of human breast cancer: the search for BRCA1*. (Ph.D. thesis. Berkeley: University of California, 1995.)
- Porter DE, Cohen BB, Wallace MR, et al. Breast cancer incidence, penetrance and survival in probable carriers of *BRCA1* gene mutation in families linked to *BRCA1* on chromosome 17q12-21. *Br J Surg* 1994;81:1512-5.
- Merajver SD, Pham TM, Caduff RF, et al. Somatic mutations in the *BRCA1* gene in sporadic ovarian tumours. *Nat Genet* 1995;9:439-43.
- Takahashi H, Behbakht K, McGovern PE, et al. Mutation analysis of the *BRCA1* gene in ovarian cancers. *Cancer Res* 1995;55:2998-3002.
- Campbell IG, Nicolai HM, Foulkes WD, et al. A novel gene encoding a B-box protein within the *BRCA1* region at 17q21.1. *Hum Mol Genet* 1994; 3:589-94.
- Brown MA, Nicolai H, Xu CF, et al. Regulation of *BRCA1*. *Nature* 1994; 372:733.
- Orita M, Iwahana H, Kanazawa H, Hayashi K, Sekiya T. Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. *Proc Natl Acad Sci U S A* 1989;86:2766-70.
- Sheffield VC, Beck JS, Kwitek AE, Sandstrom DW, Stone EM. The sensitivity of single-strand conformation polymorphism analysis for the detection of single base substitutions. *Genomics* 1993;16:325-32.
- Struewing JP, Abeliovich 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.
- FitzGerald MG, MacDonald DJ, Krainer M, et al. Germ-line *BRCA1* mutations in Jewish and non-Jewish women with early-onset breast cancer. *N Engl J Med* 1996;334:143-9.